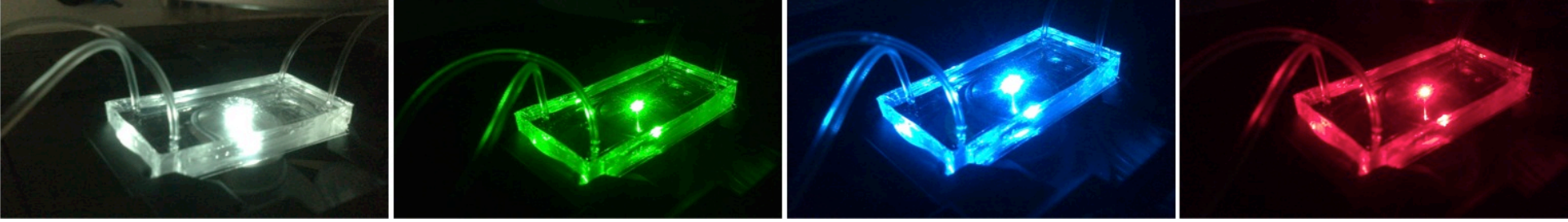


Telomere length and senescence heterogeneity: when size matters!

Zhou Xu
CR2 CNRS
Teixeira lab
Institut de Biologie Physico-Chimique

06.07.16

PDE and Probability for Life Sciences
CIRM, Marseille

Outline of the talk

Luria-Delbrück experiment: The foundation of modern biology is a concerted effort in maths and biology.

Telomere length and senescence

Escaping senescence: an interdisciplinary approach?

The Luria-Delbrück experiment (1943): an early fruitful interaction between mathematics and biology

Photo courtesy of AP/Wide World Photos

Salvador E. Luria in his Massachusetts Institute of Technology Laboratory October 16, 1969, after word that he shared the 1969 Nobel Prize with two other bacteriologists for research on viruses.

Photo courtesy of AP/Wide World Photos

King Gustaf Adolf, right, presents the Nobel Prize in Physiology or Medicine to German-born American biologist Max Delbrück in Stockholm, Sweden, December 10, 1969. Delbrück, of the California Institute of Technology, shares the prize with American biologist Alfred D. Hershey and Italian-American biologist Salvador E. Luria for their discoveries concerning the replication mechanism of viruses and their genetic structure.

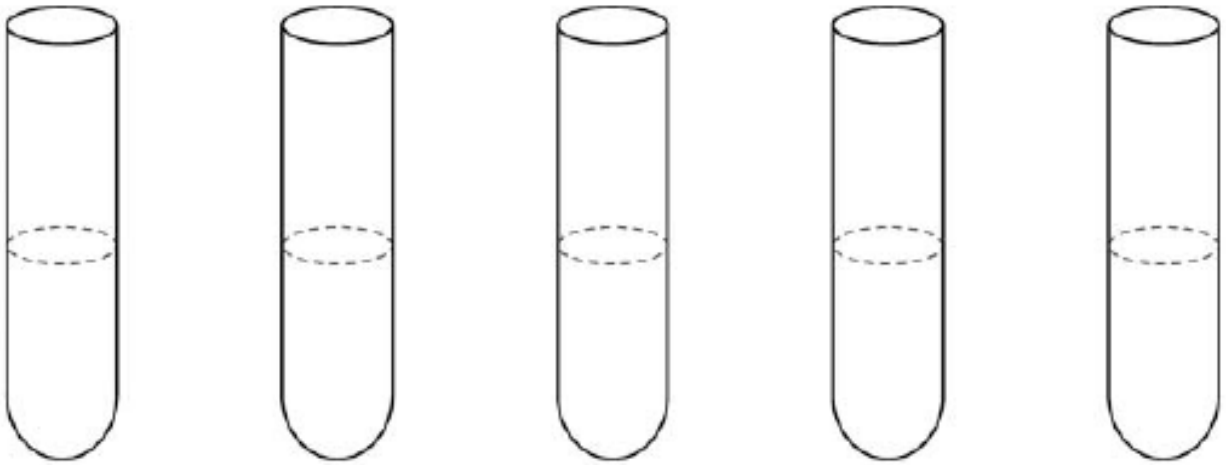
Mutations in bacteria that confer survival advantages arise over time.

Two hypotheses:

1. Directed/induced mutations

2. Random/spontaneous mutations
= Darwinism

N independent cultures starting with one normal (wild-type) cell



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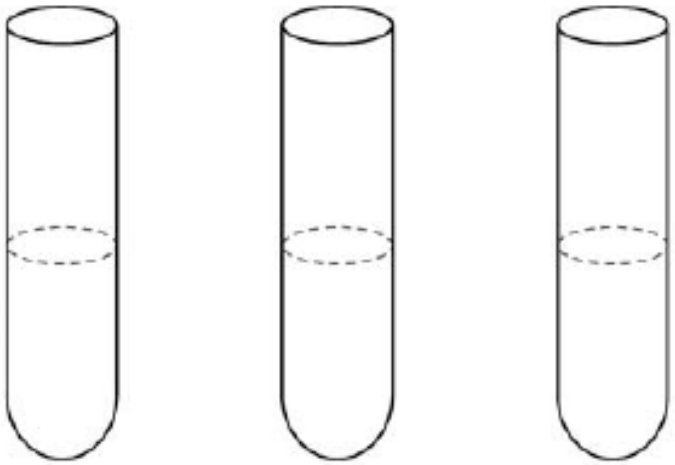
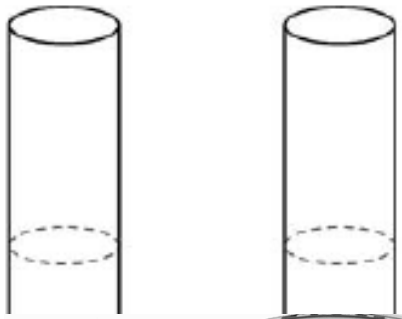
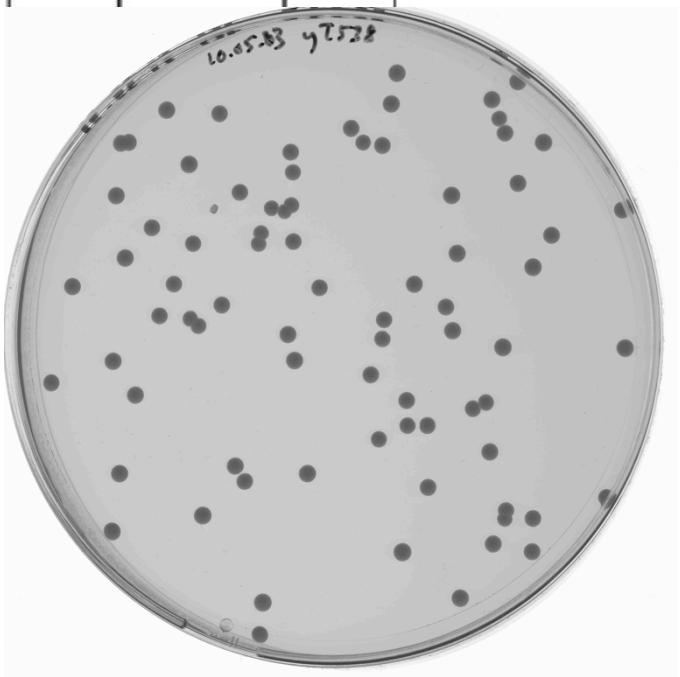
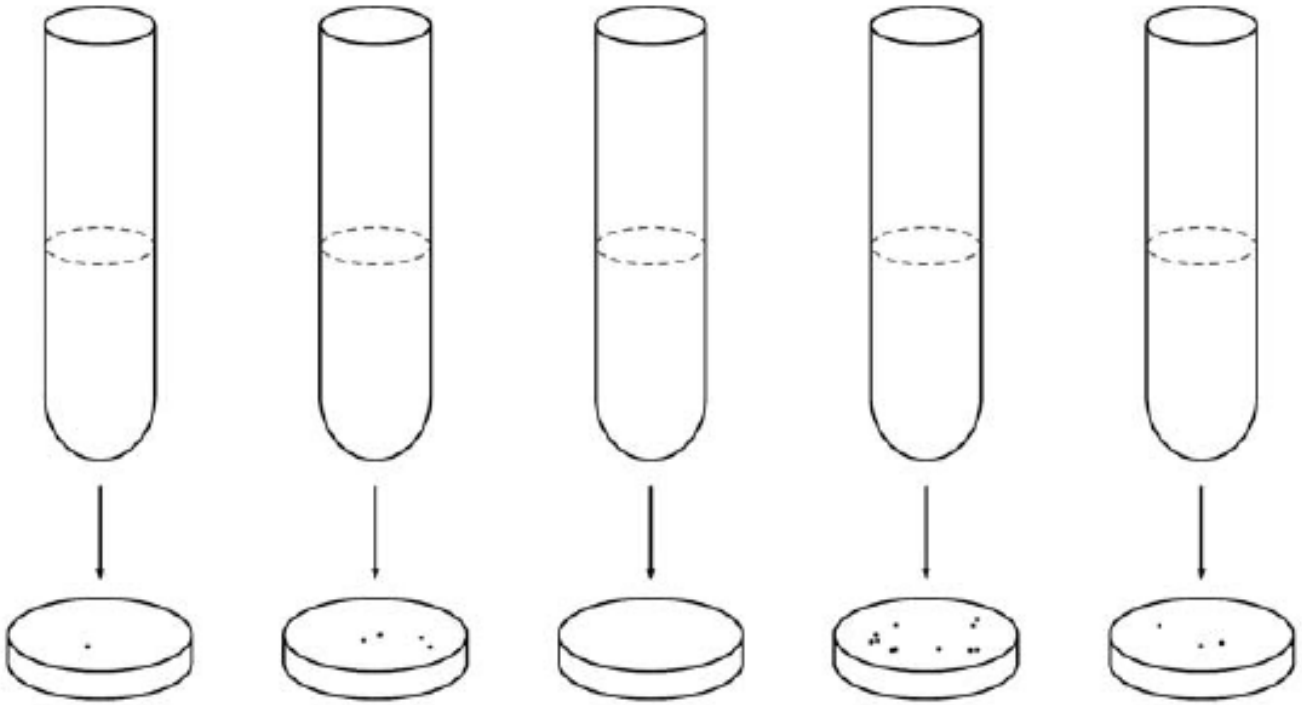






After growth to ~10^9 cells, plating on **selective** medium to count the number of mutants



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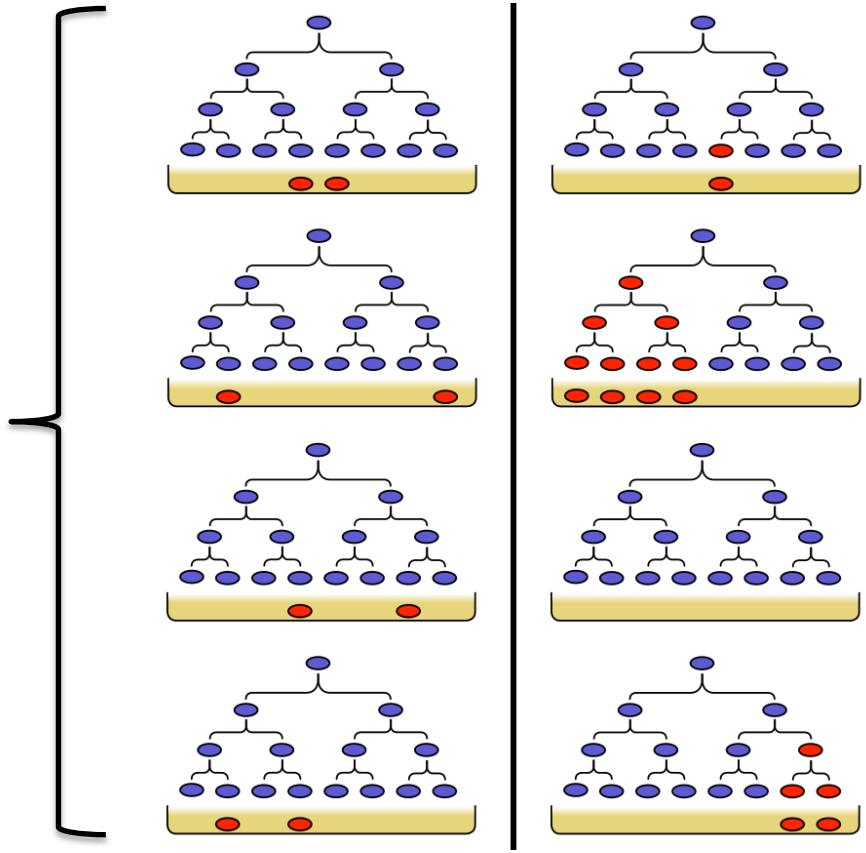
= Darwinism

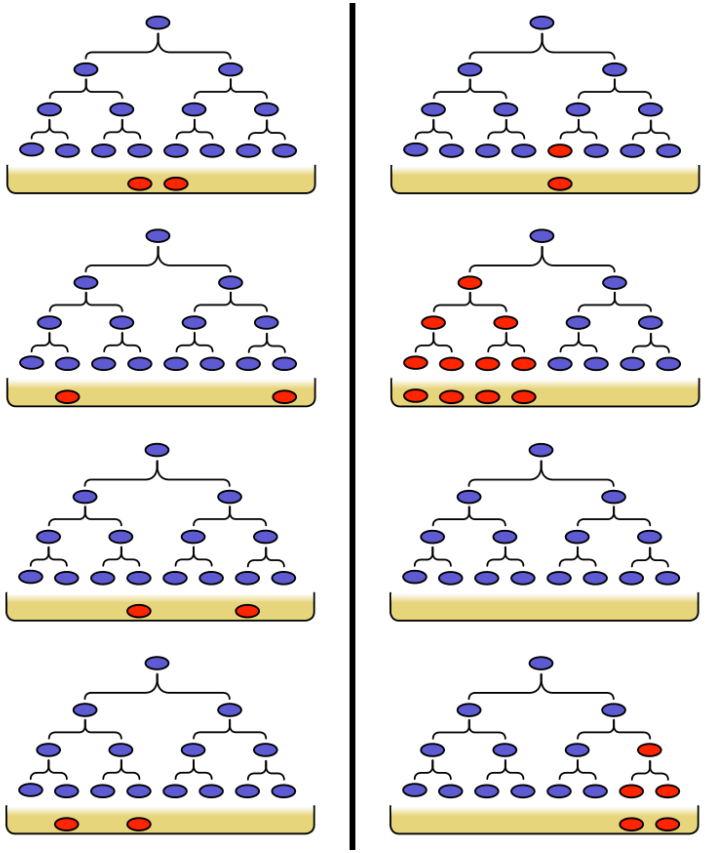
N independent cultures starting with one cell



1. Directed/induced mutations

2. Random/spontaneous mutations
= Darwinism

Poisson distribution



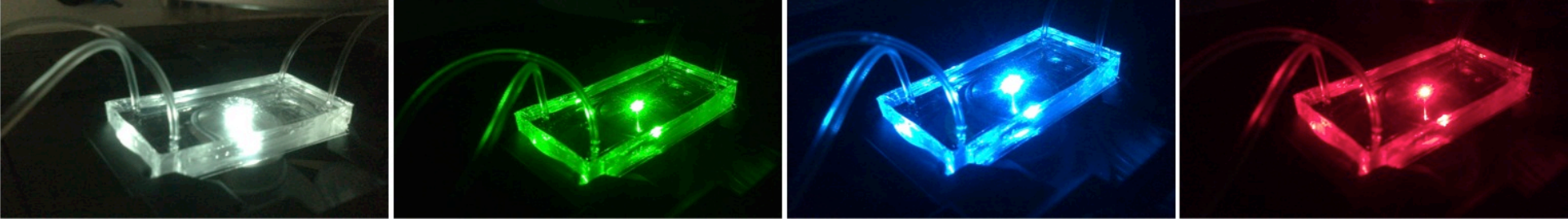
Distribution with much greater variance
= Luria-Delbrück distribution

70 years of progress in the study of the Luria-Delbrück distribution

Assumptions in the Luria-Delbrück model:

- The process starts with one wild-type cell and no mutant,
- Deterministic growth for wild-type and mutant cells,
- Exponential growth (to infinity),
- Mutations occur randomly at a rate proportional to the population size.

Luria and Delbrück	Mean and variance
Lea and Coulson	Approximate pgf, stochastic model
Haldane	Combinatorial approach
Armitage	Exact pgf
Kendall	Arbitrary distribution for cell cycle
Bartlett	Exact pgf, fully stochastic model
Crump and Hoel	Filtered Poisson process theory
Ma and Sarkar	Algorithm for probability function
Many others…	But no closed expression!



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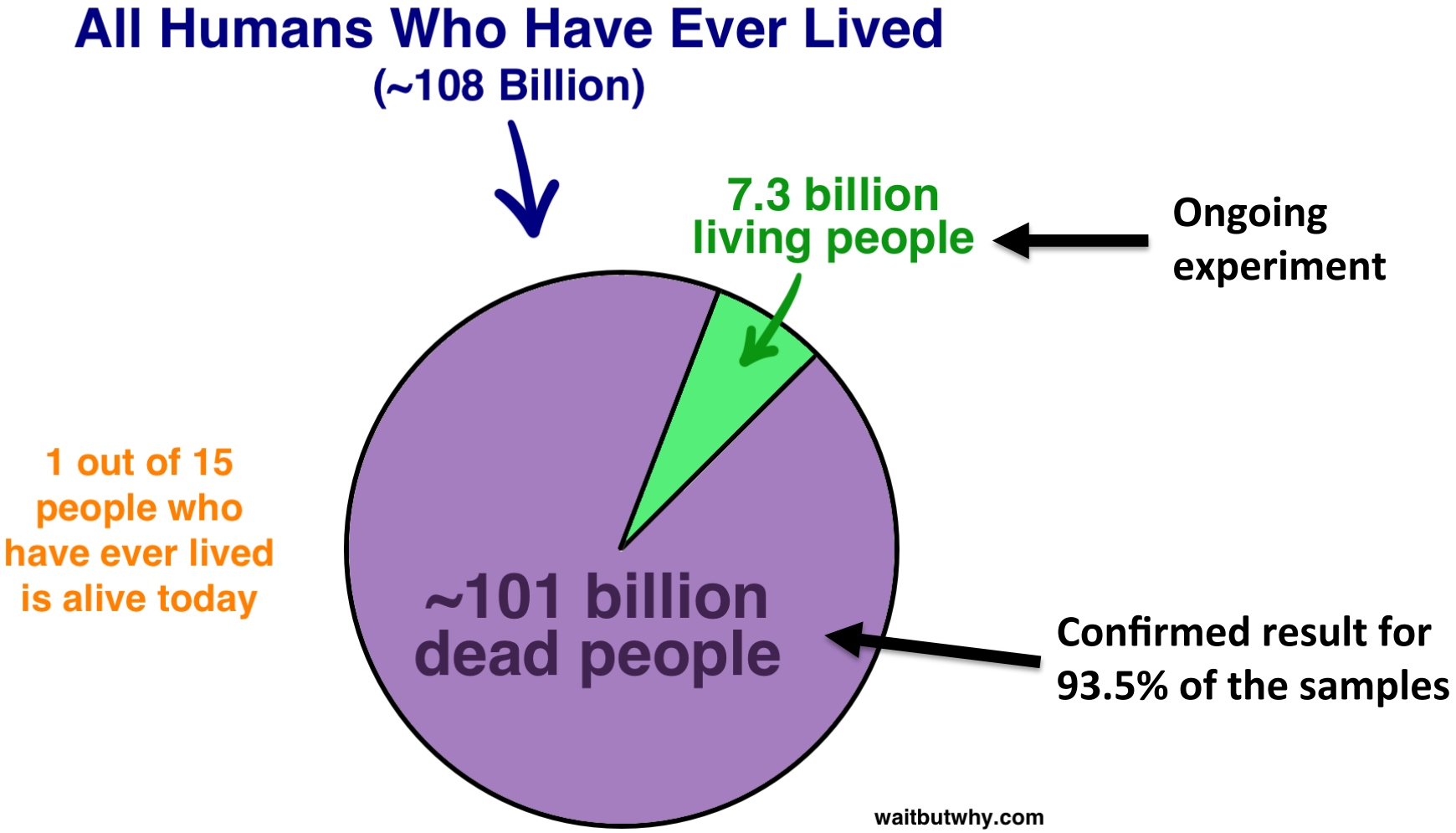
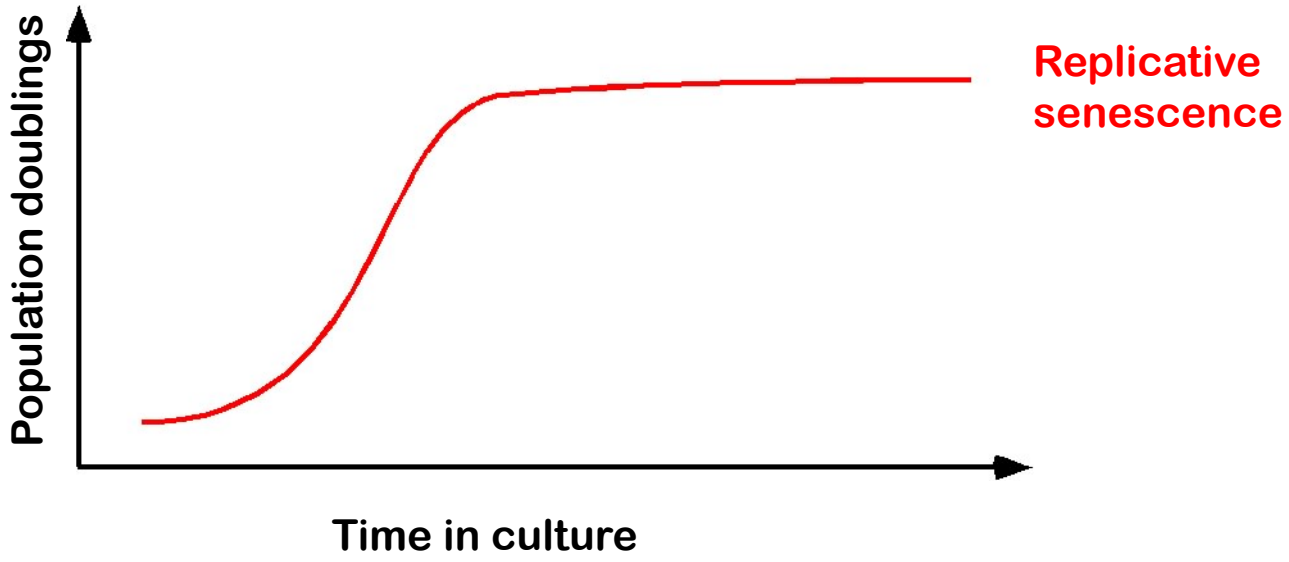
All of us will die one day…



An intrinsic barrier to cell proliferation:

Replicative Senescence

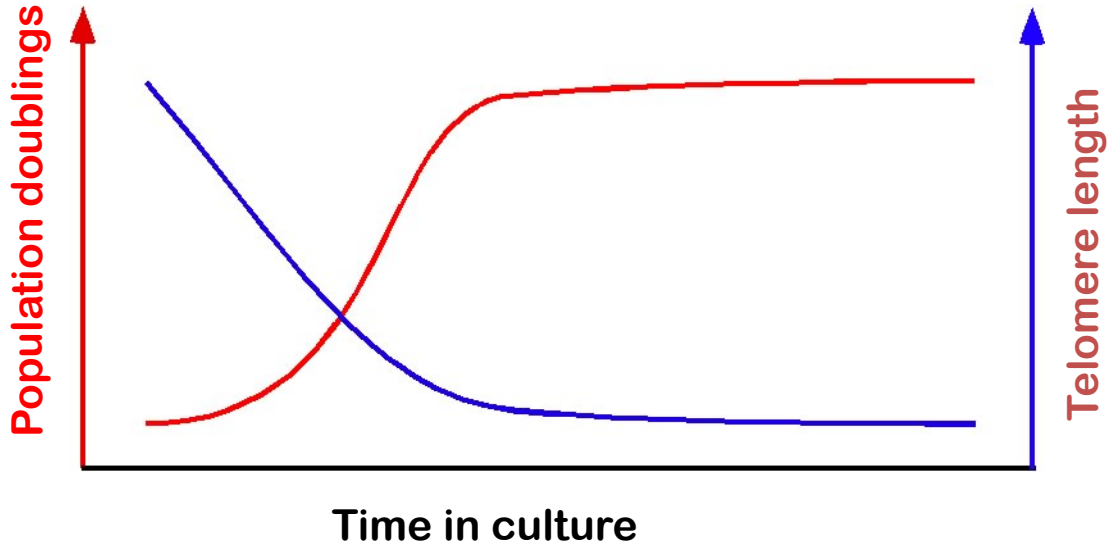
What is replicative senescence?



Replicative senescence is the ultimate and irreversible loss of replicative capacity occuring in primary somatic cell culture

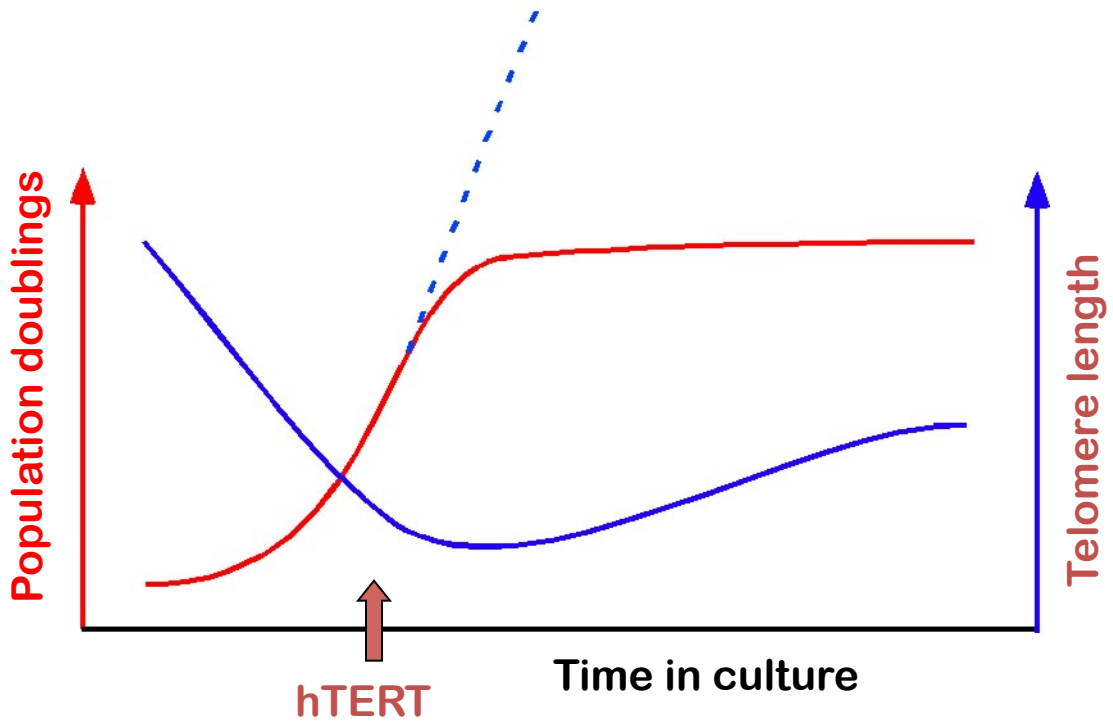
Hayflick and Moorehead 1961 The serial cultivation of human diploid cell strains Exp. Cell Res.

Replicative senescence correlates with telomere attrition

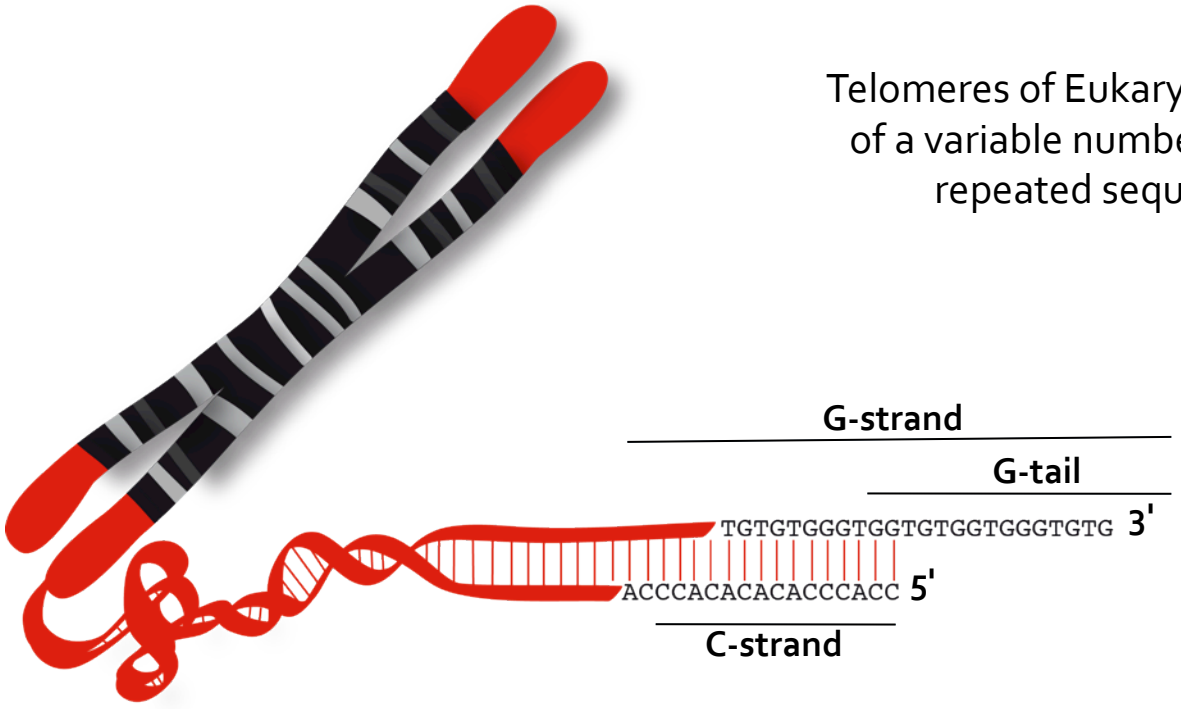


Telomeres shorten during ageing of human fibroblasts
C. B. Harley, A. B. Futcher and C. W. Greider (1990)
Nature 345(6274): 458-460

Telomere elongation is required for unlimited cell proliferation



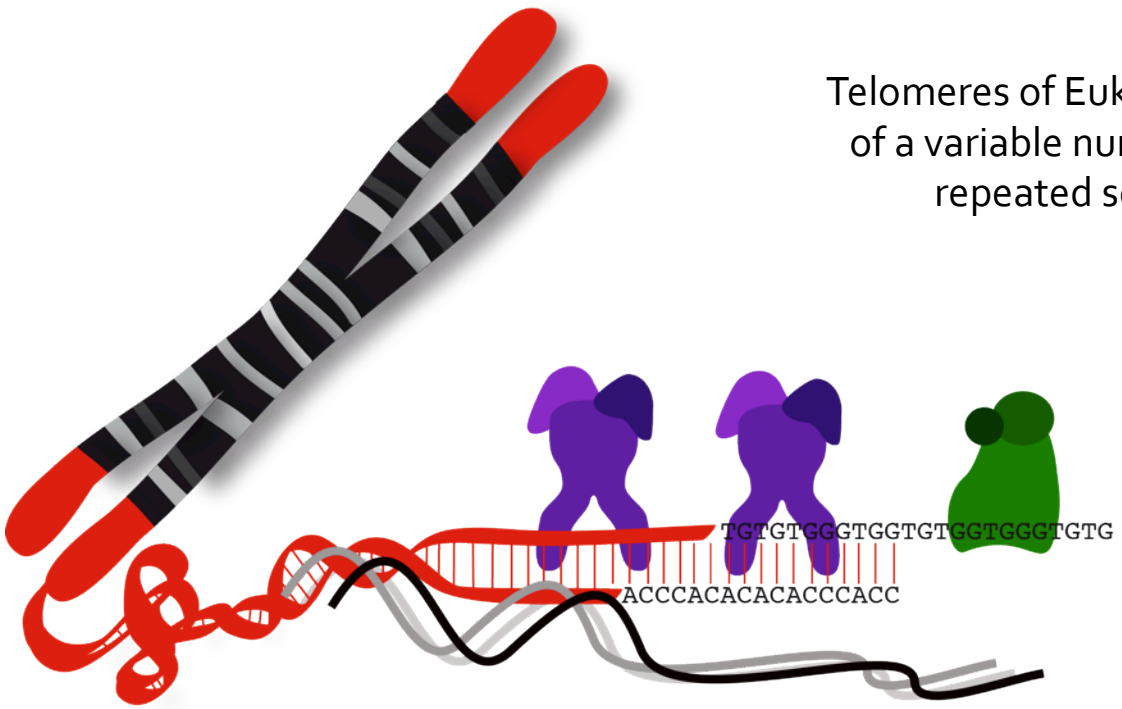
Telomeres



Telomeres of Eukaryotes consist of a variable number of G-rich repeated sequences

Palm, W. & de Lange, T. Annual review of genetics (2008)

Telomeres



Telomeres of Eukaryotes consist of a variable number of G-rich repeated sequences

Telomeric repeats bind specific proteins

Telomeres also bind a telomeric encoded RNA - TERRA

Palm, W. & de Lange, T. Annual review of genetics (2008)

Structure

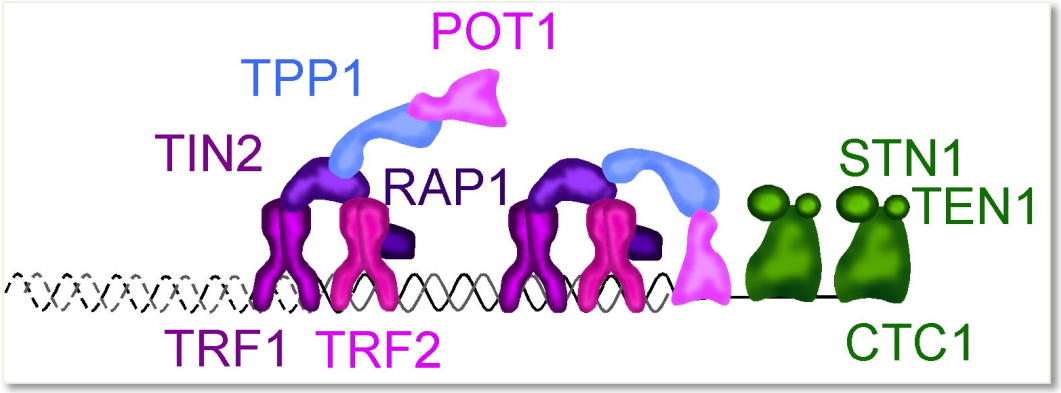
- **Proteins on the double-stranded and single-stranded parts of telomeres**

Yeast telomeres:

250-400 bp of TG1-3 repeats

Overhang <10 nt

Mammalian telomeres:



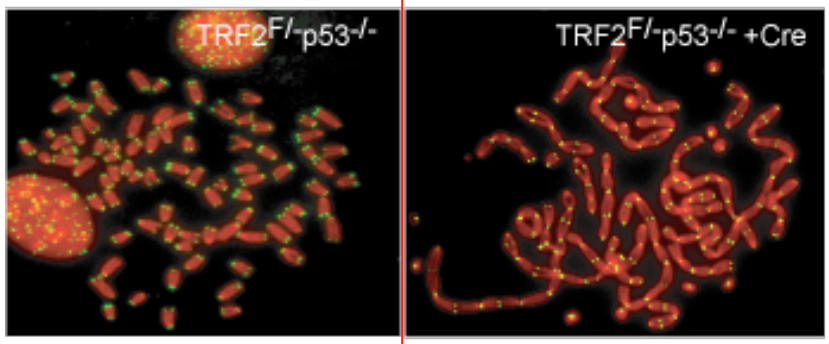
2-100 kb of TTAGGG repeats

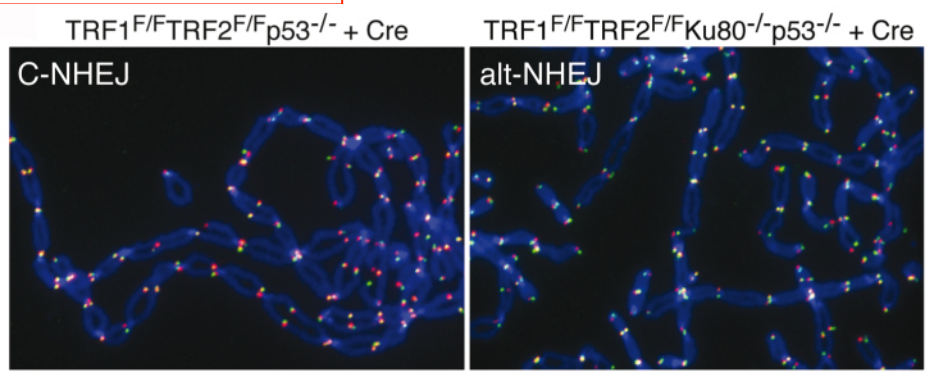
Overhang 50-500 nt

Functions of telomeres

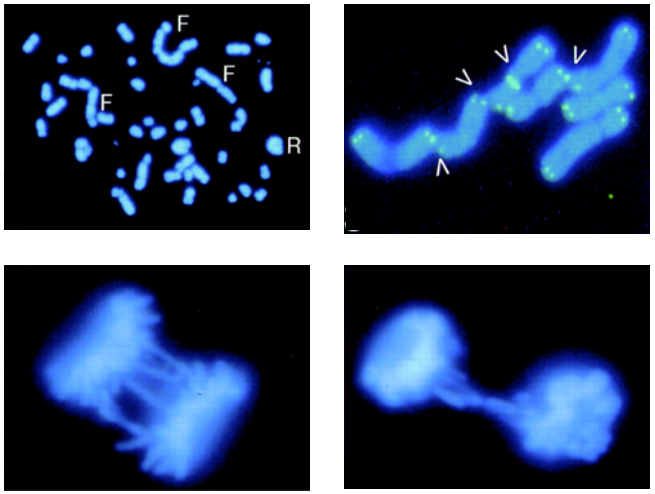
- If dysfunction of telomeric proteins, GENOME INSTABILITY!

Chromosome of a normal cell



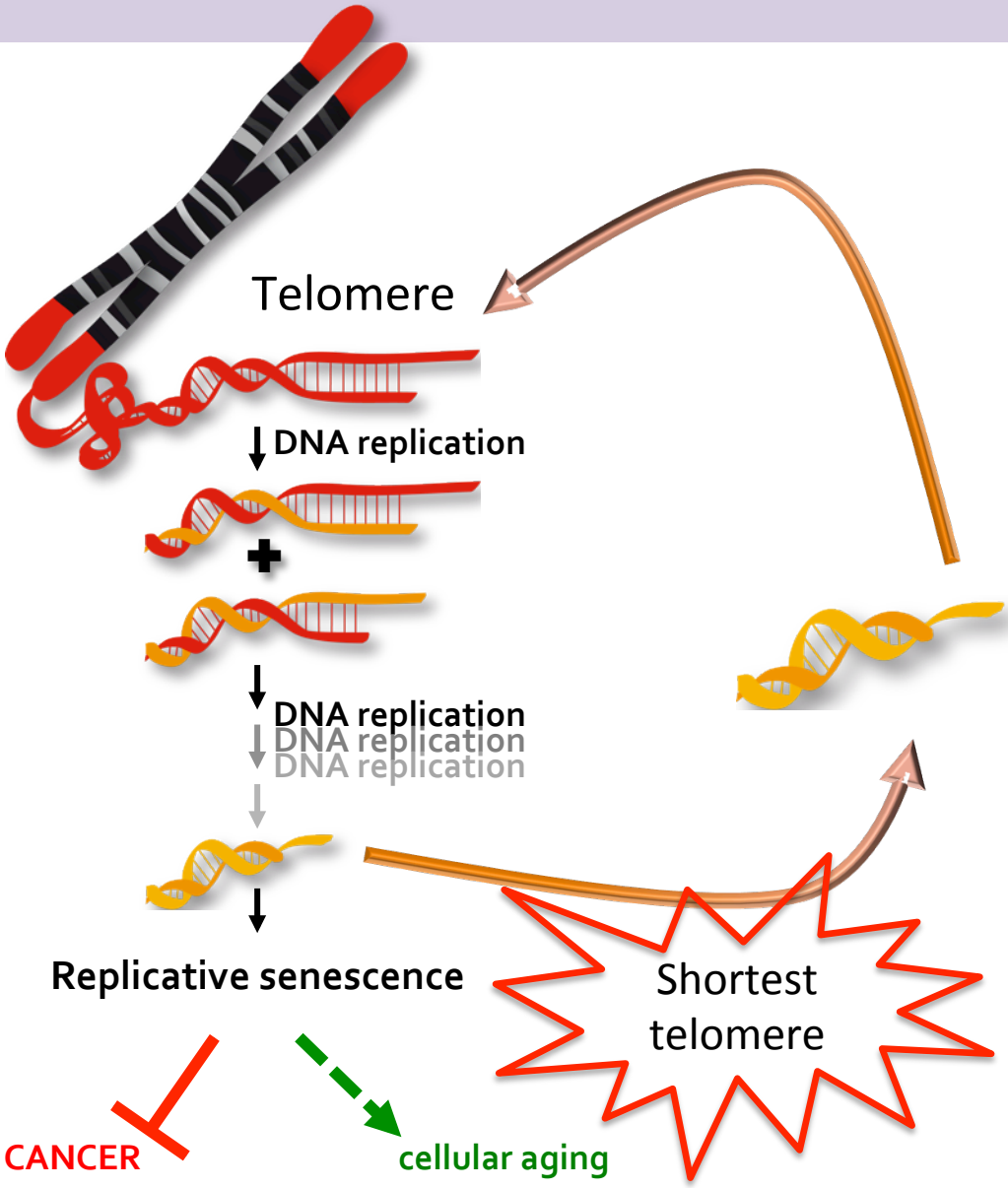


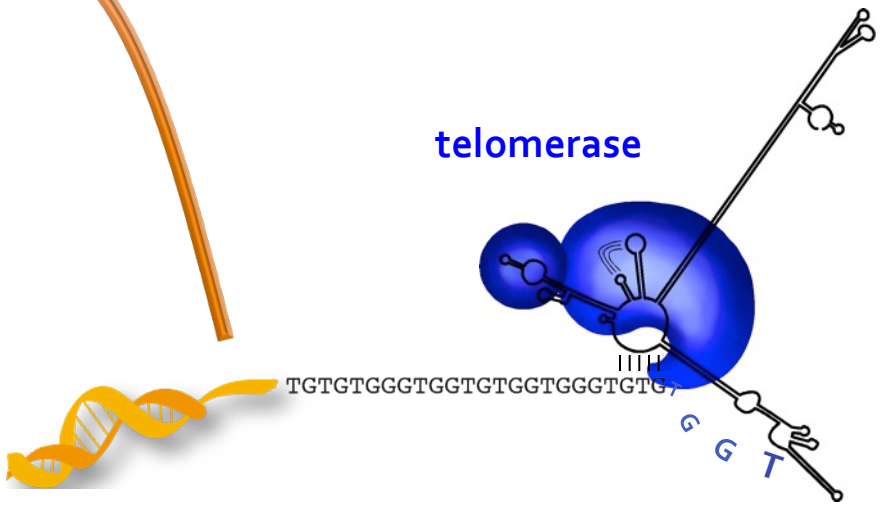
Titia de Lange's work



Anaphase bridge

Telomeres shorten and can be elongated

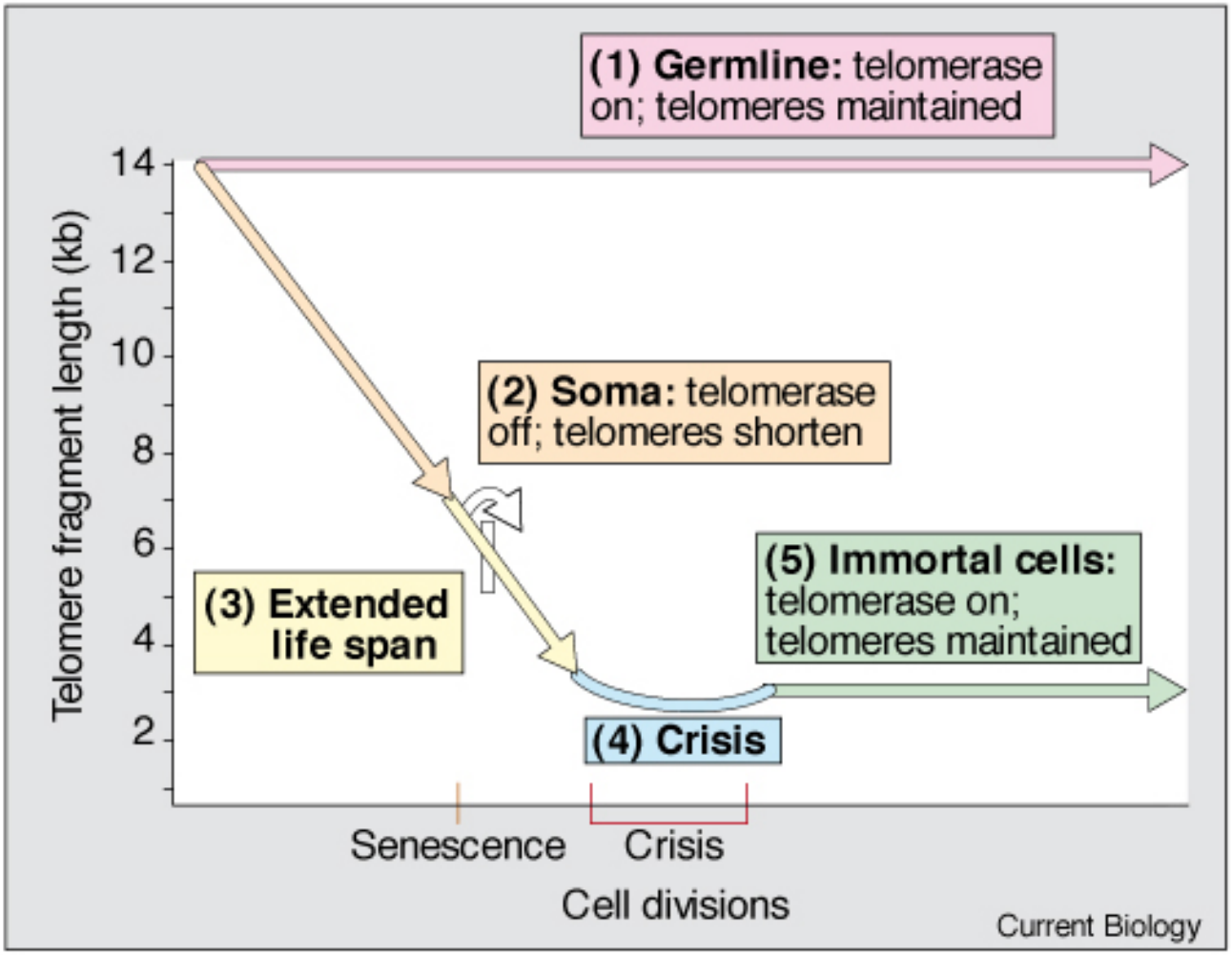




Telomerase is expressed in many unicellular eukaryotes, some stem cells, germ cells and embryonic cells
... and in 90% of cancer cells

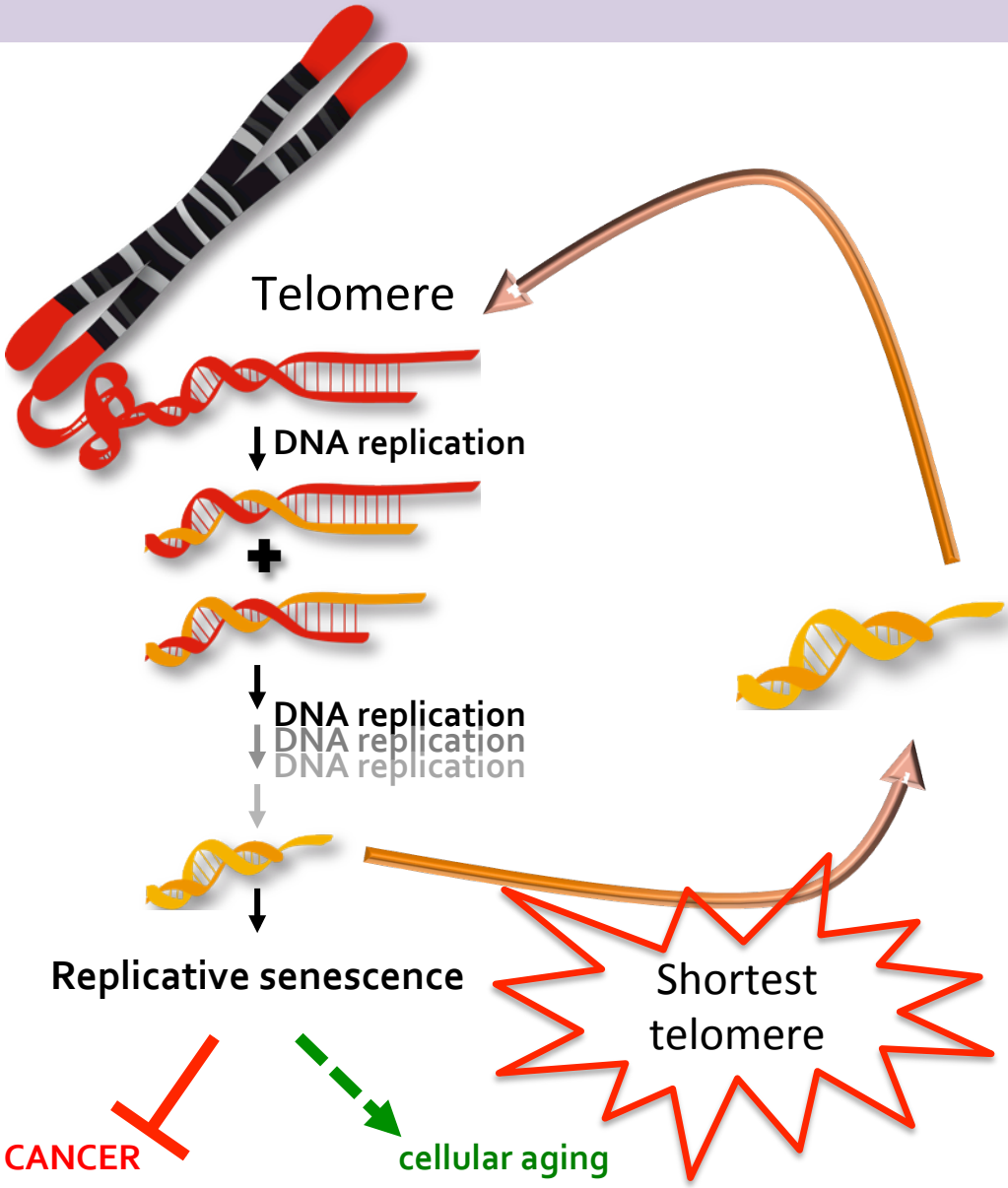
Reviewed in Sahin and DePinho. Nature (2010)

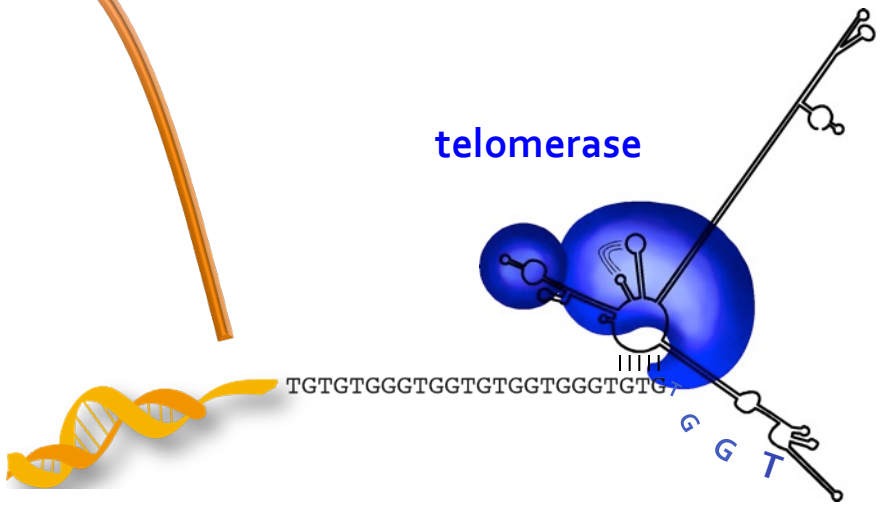
Senescence, crisis and escape



Greider, Current Biology, 1998

Telomeres shorten and can be elongated





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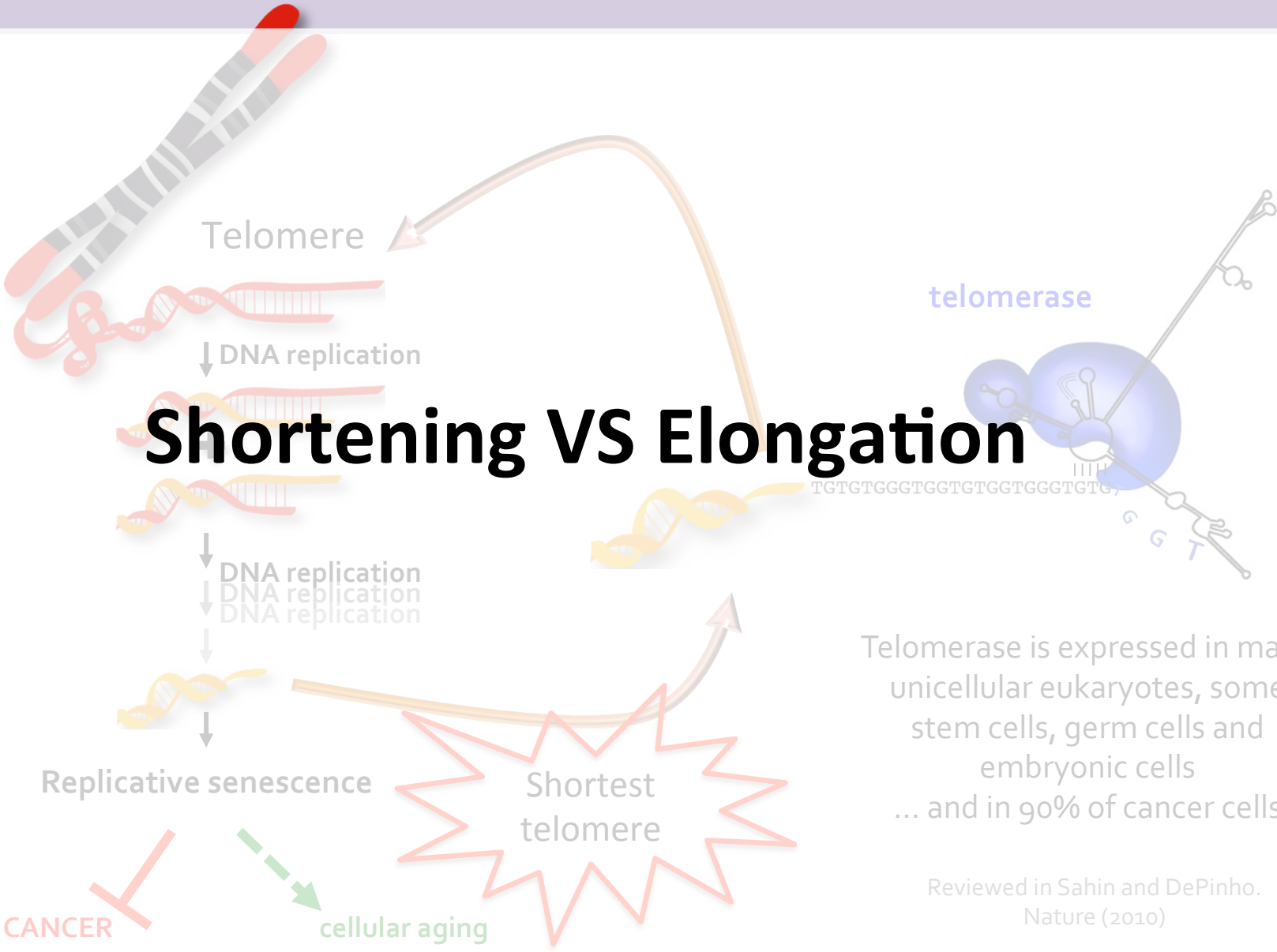

Shortening VS Elongation

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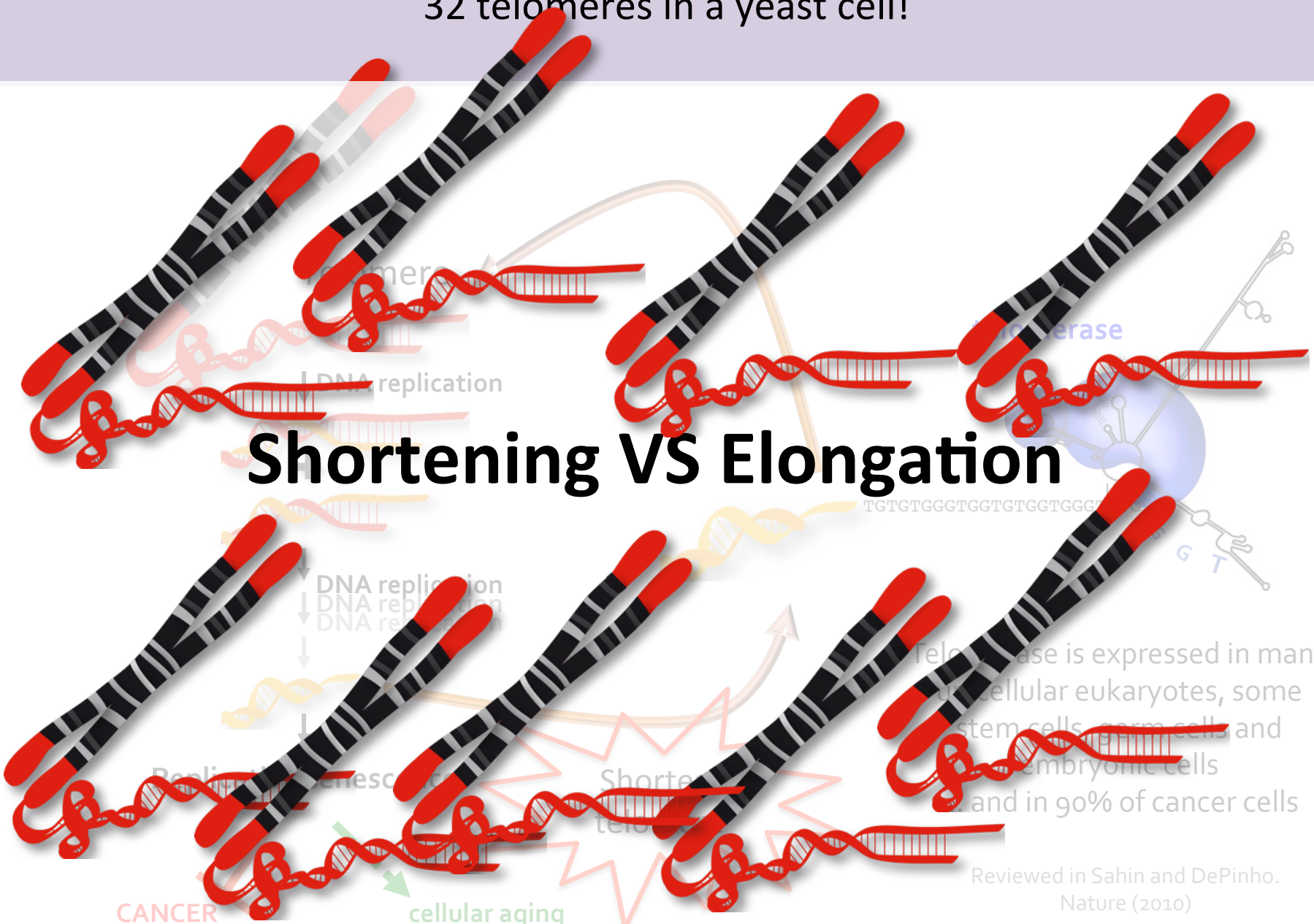
Reviewed in Sahin and DePinho. Nature (2010)

32 telomeres in a yeast cell!



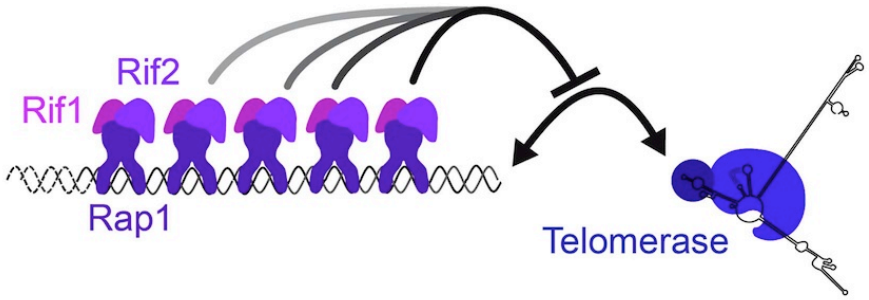
Shortening VS Elongation

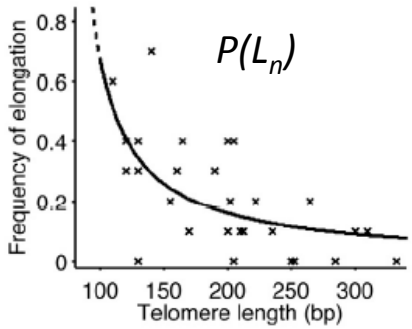
What is the **steady-state distribution** of telomere lengths?

Mathematical model of telomere distribution

Collaboration with Khanh Dao Duc and David Holcman (ENS Paris)

Protein-counting mechanism





Teixeira *et al.* 2004 Cell

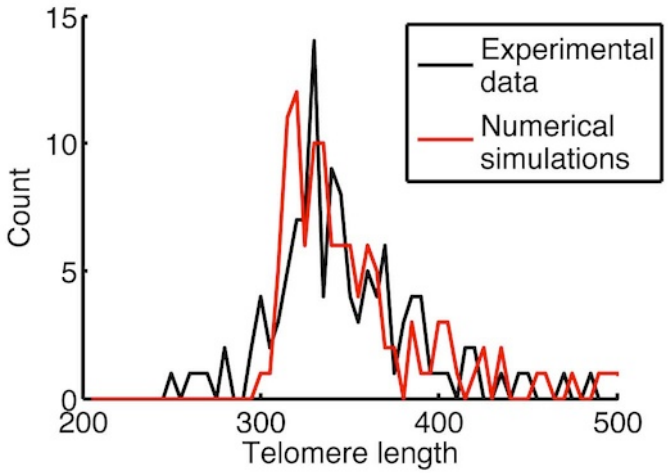
At each cell division, $L_{n+1} = \begin{cases} L_n - a \\ L_n - a + b \end{cases}$, with probability $\begin{matrix} 1 - P(L_n) \\ P(L_n) \end{matrix}$ $\begin{matrix} \text{= shortening} \\ \text{= elongation} \end{matrix}$

- Model of telomere dynamics based on the protein-counting mechanism

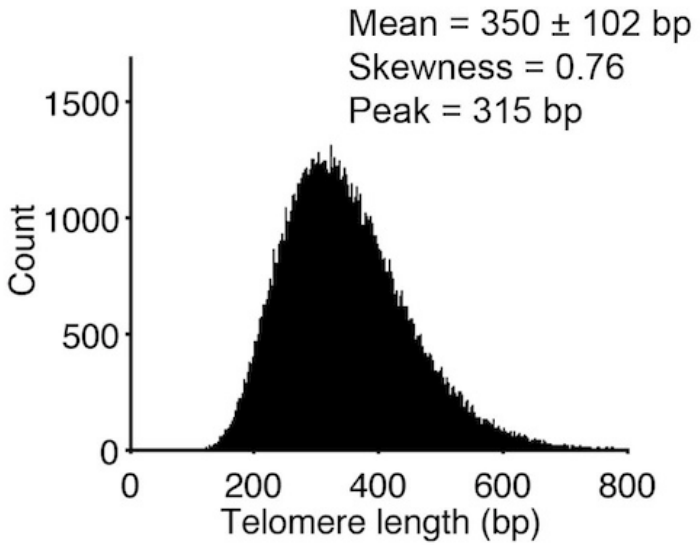
Xu *et al.* 2013 Genetics

Telomere length distribution

Comparison with experimental data



Modeled distribution

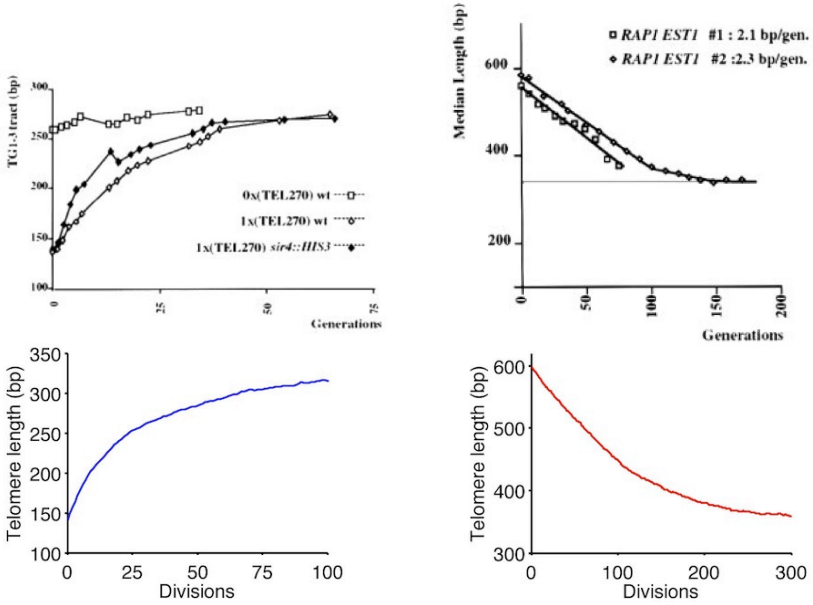


- Consistent with experimental measurements of telomere length

The dynamics of telomere length results from the protein-counting mechanism

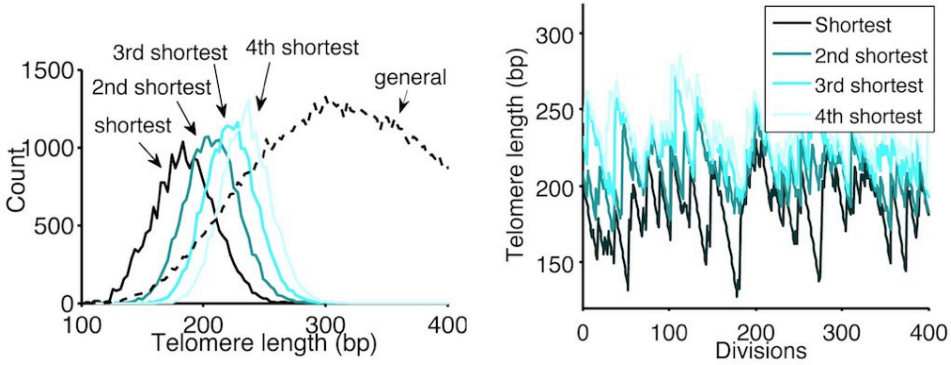
Return to equilibrium after perturbation

Marcand *et al.*, 1999



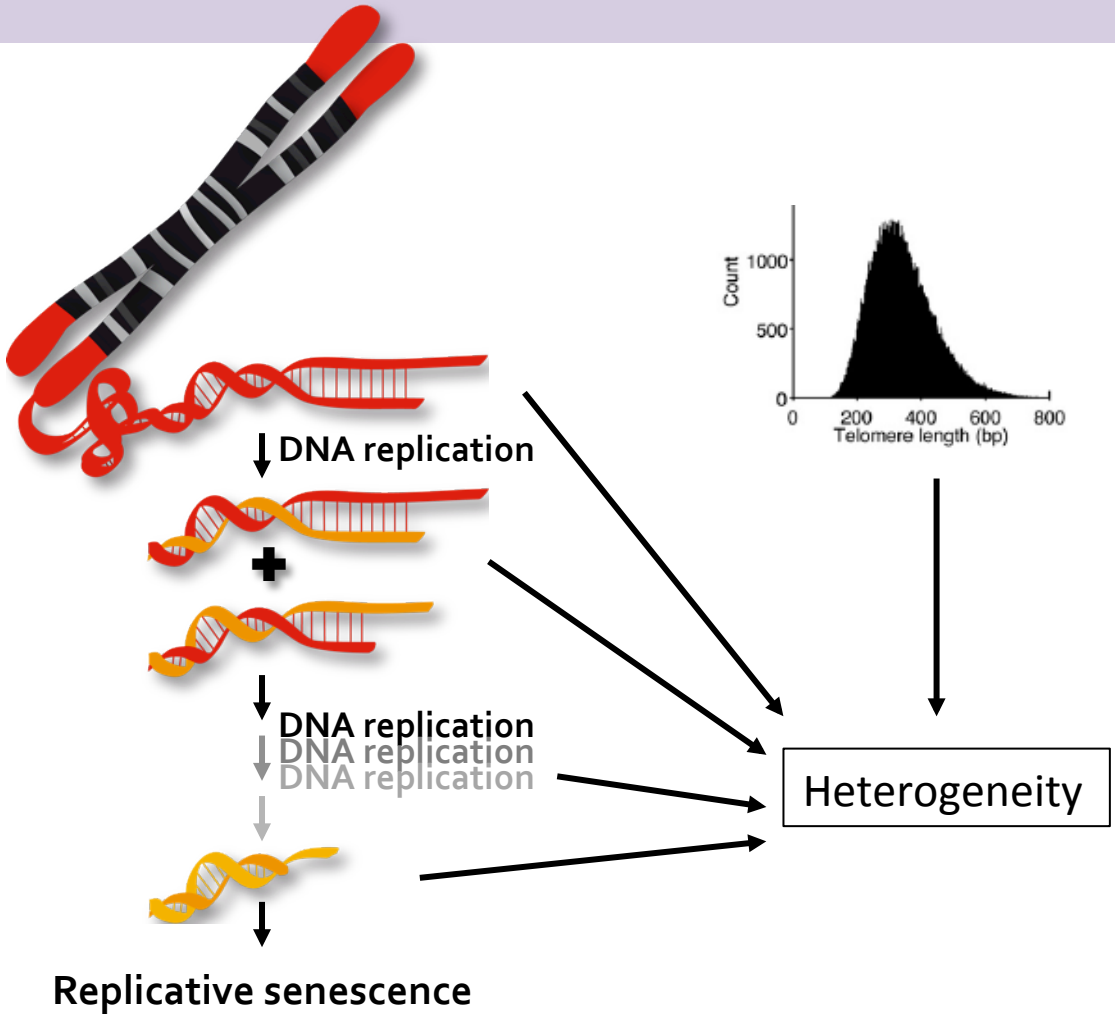
Our work

Dynamics of the shortest telomeres

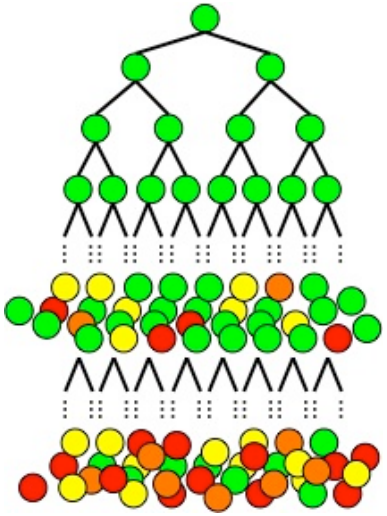


➢ The model reproduces the dynamic behaviour of telomeres

Replicative senescence: a heterogeneous process.

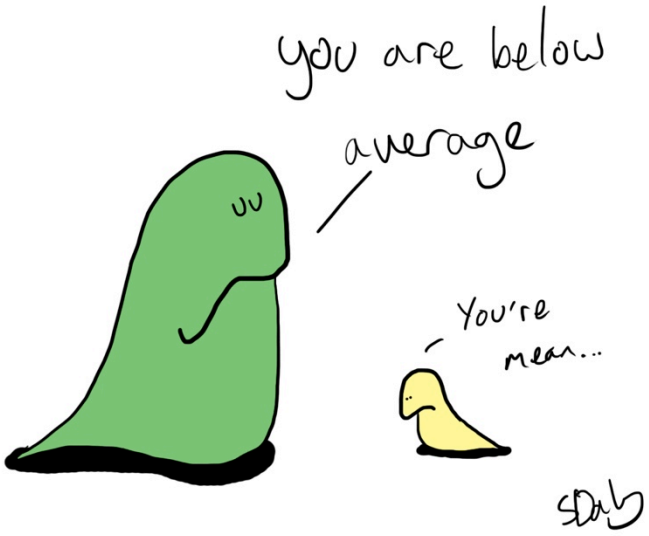


Telomerase-negative cells



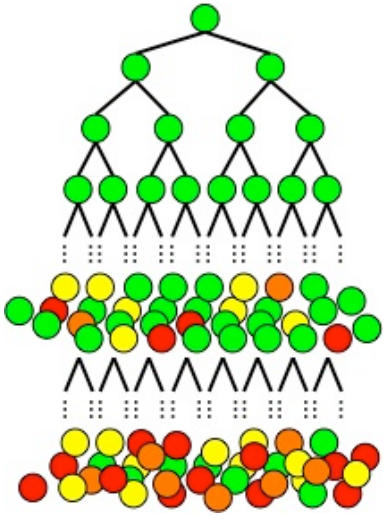
Two caveats of population studies

Average

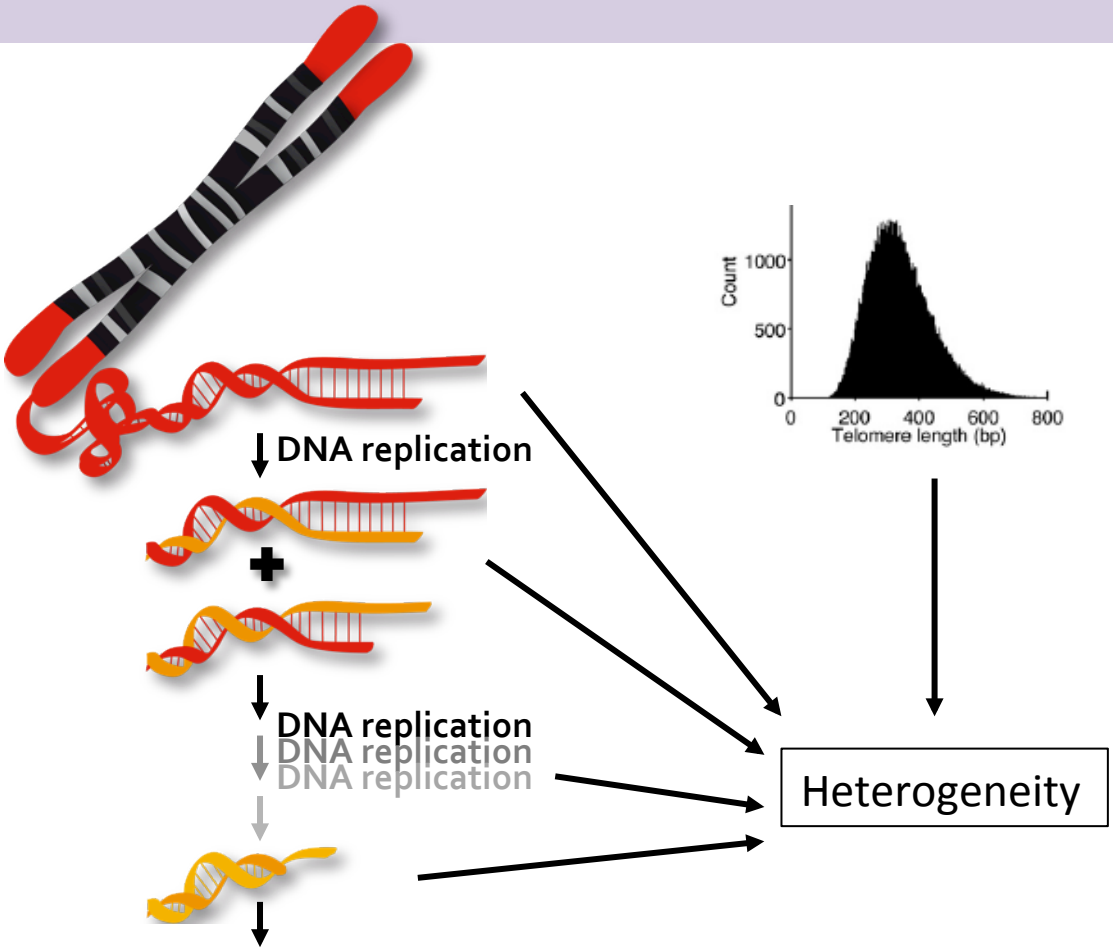


Competition

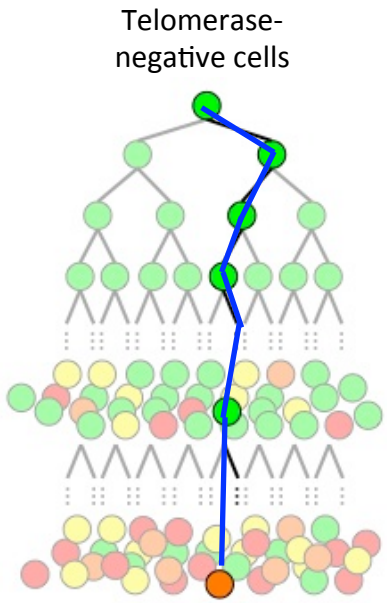
Telomerase-negative cells



A change of scale to study a complex phenotype



Heterogeneity

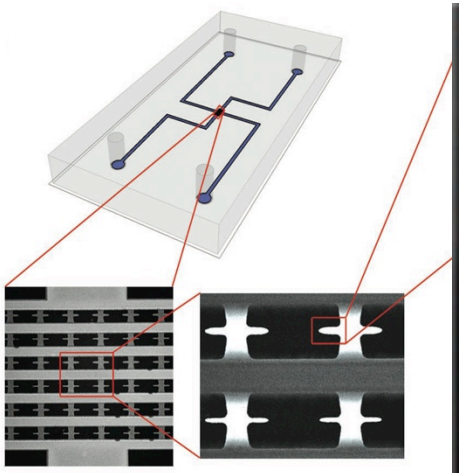


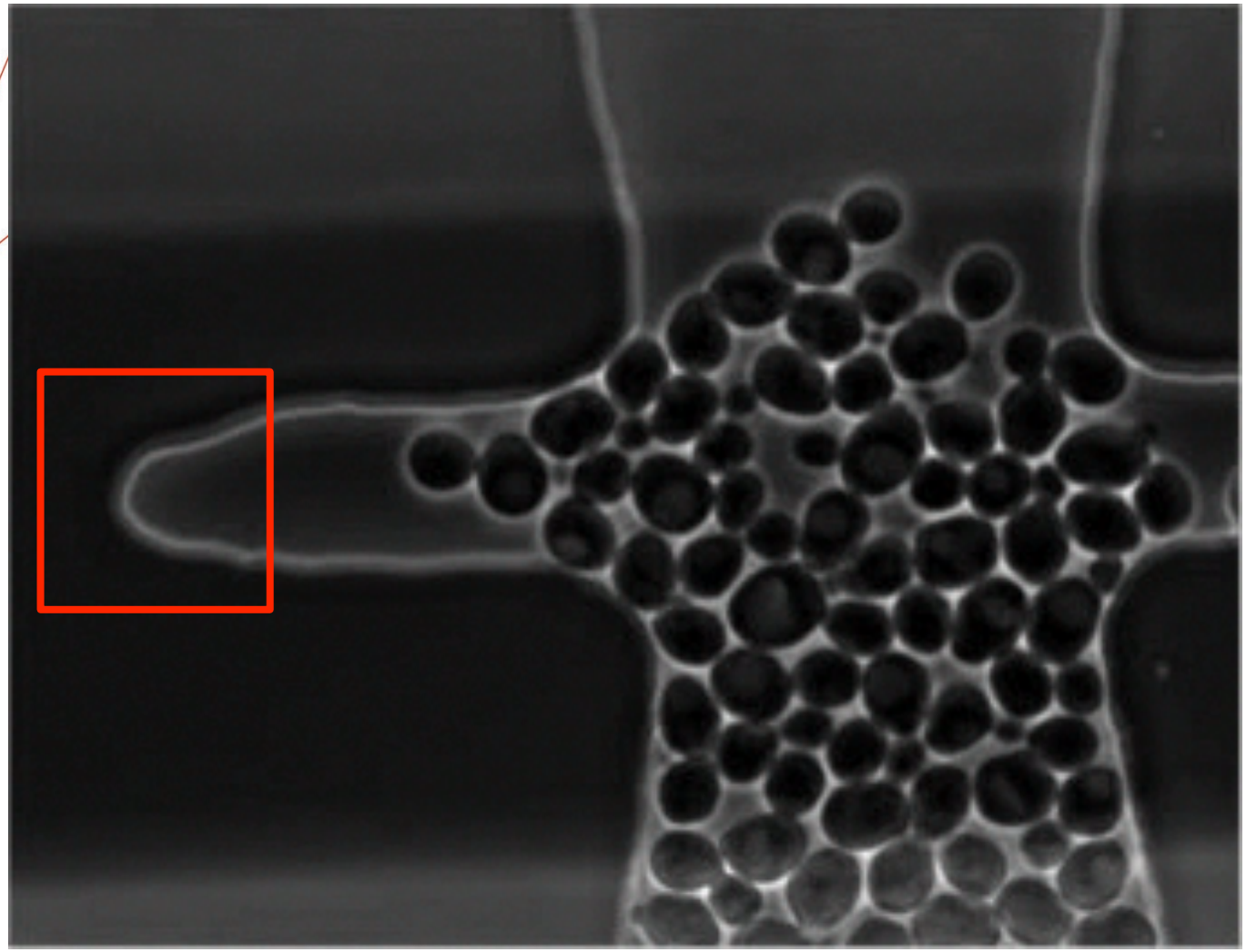
- Sequence of events leading to senescence?
- Dynamics of entry into senescence? Progressive? Sharp?

Study at the level of individual lineages.

Experimental strategy:

Real-time single-cell analysis by microfluidics coupled with live-imaging microscopy



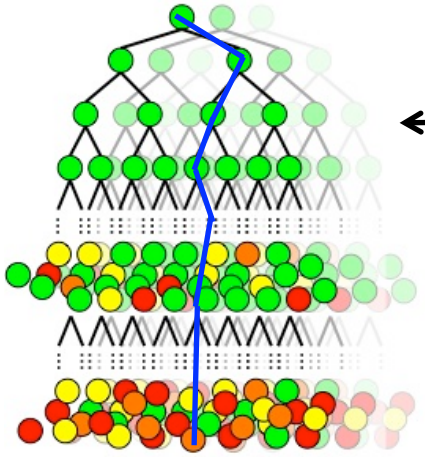


Microfluidics:

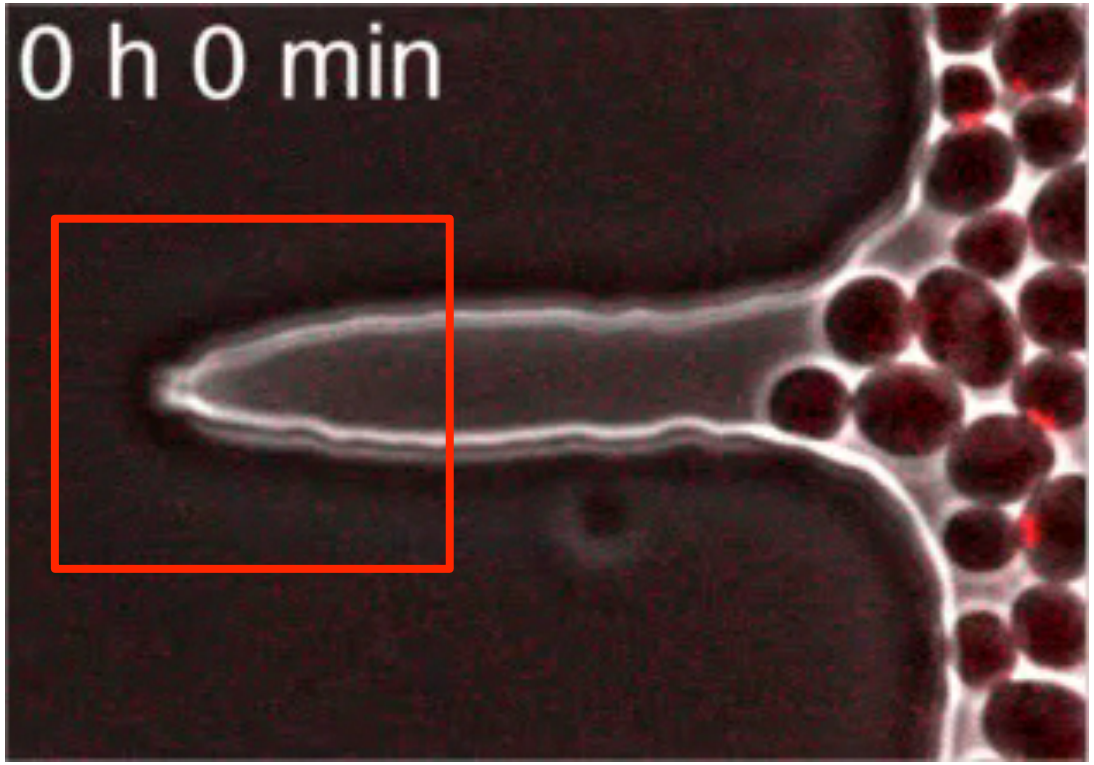
Gilles Charvin
(IGBMC, Strasbourg)
Fehrmann *et al.* 2013
Cell Rep

Experimental strategy

TetO2-TLC1: Repressible telomerase expression by doxycycline addition, to induce senescence.

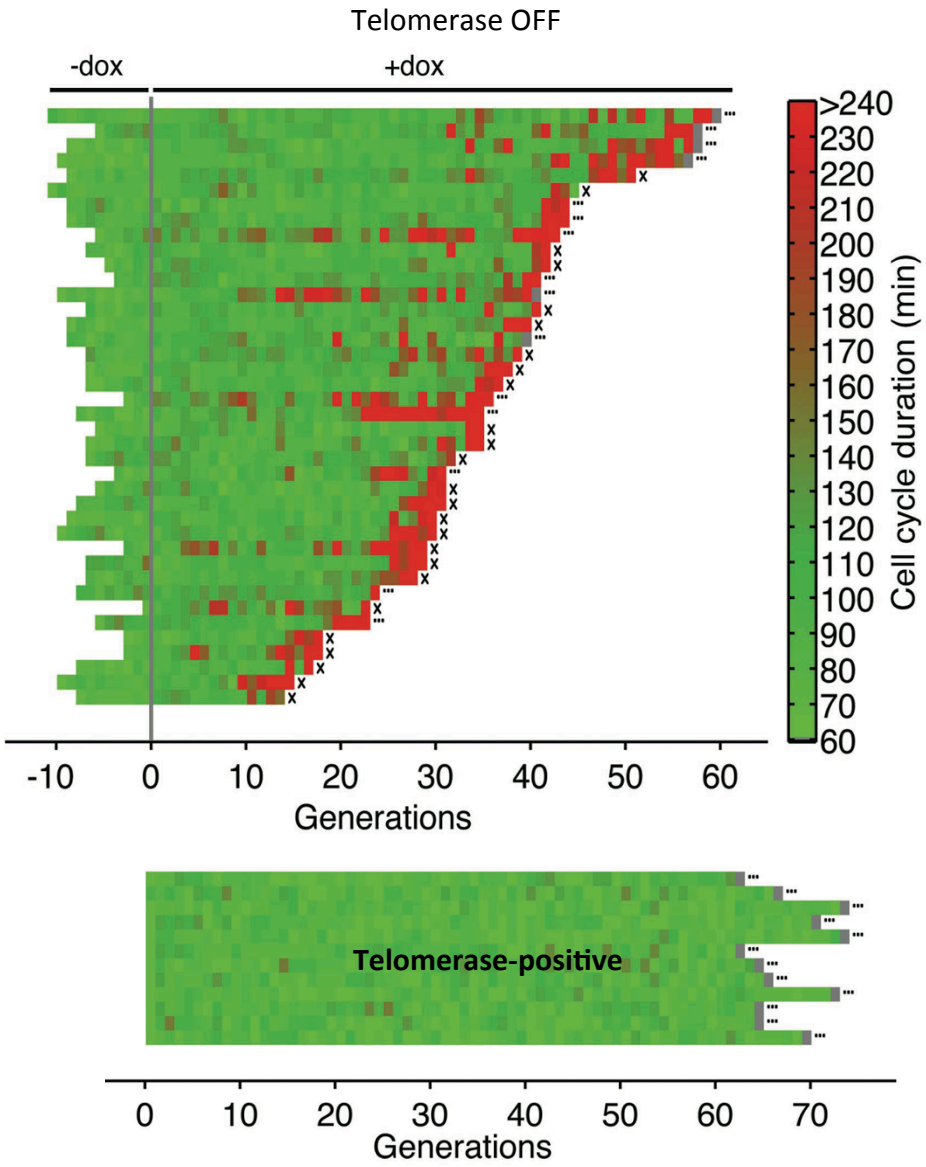


Doxycycline addition
=
Telomerase inactivation



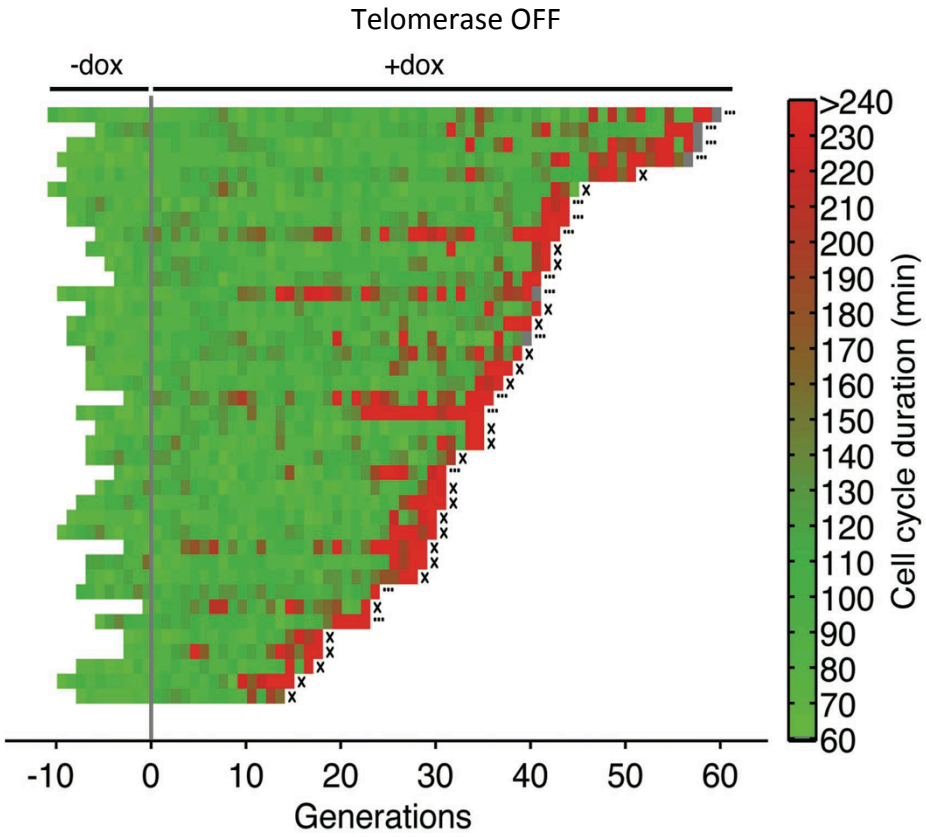
Xu *et al.* 2015 Nature Communications

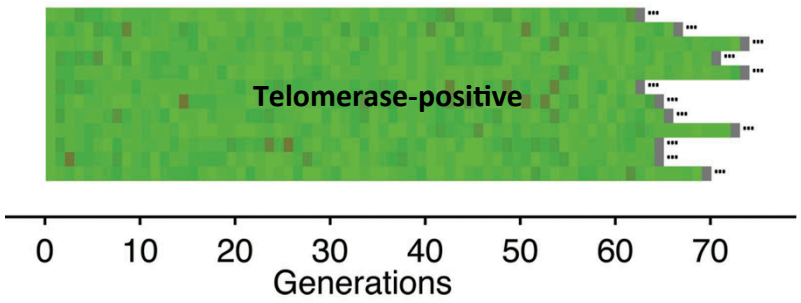
Senescence dynamics in individual lineages

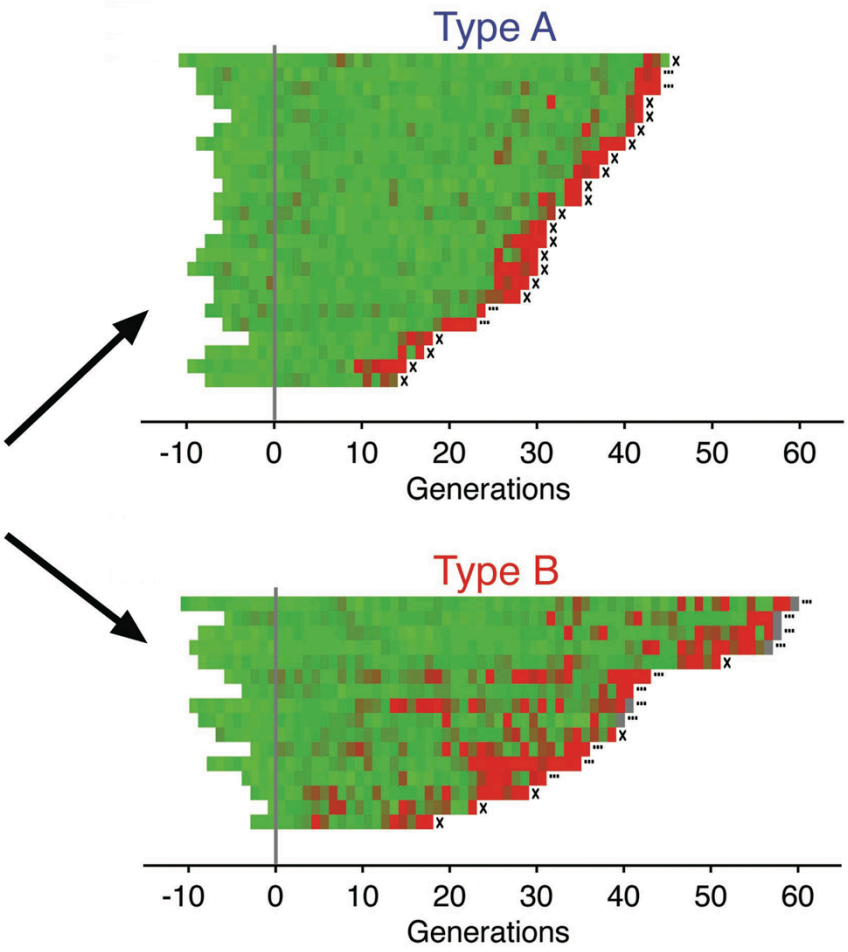


(Xu *et al.*, 2015, *Nature Communications*)

Two types of senescence dynamics

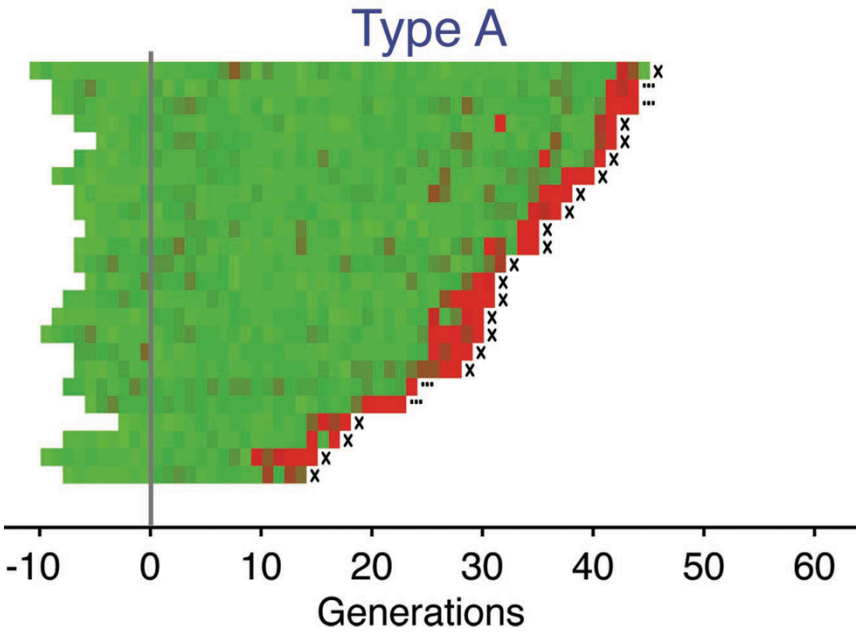






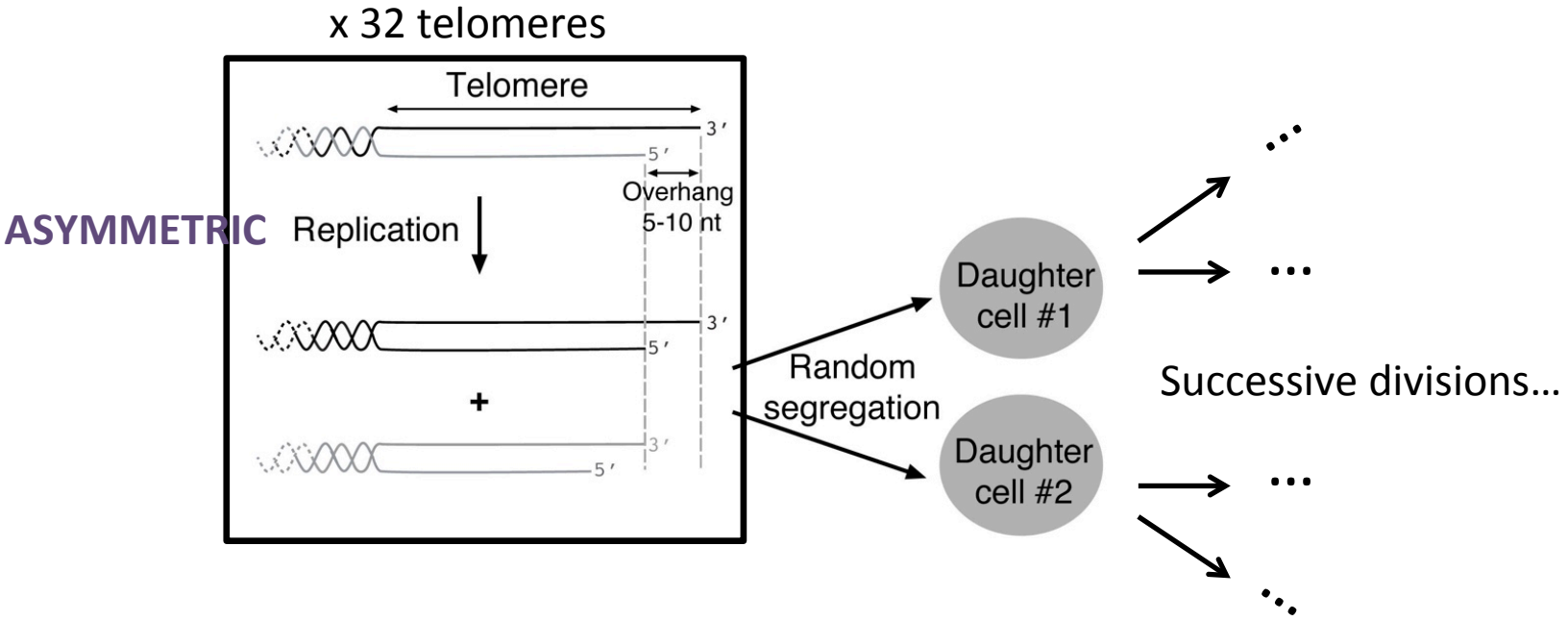
(Xu *et al.*, 2015, *Nature Communications*)

Type A lineages correspond to a canonical model of telomere shortening



Model of senescence:
from the molecular structure of telomeres to senescence onset

Collaboration with Thibault Bourgeron and Marie Doumic (INRIA)

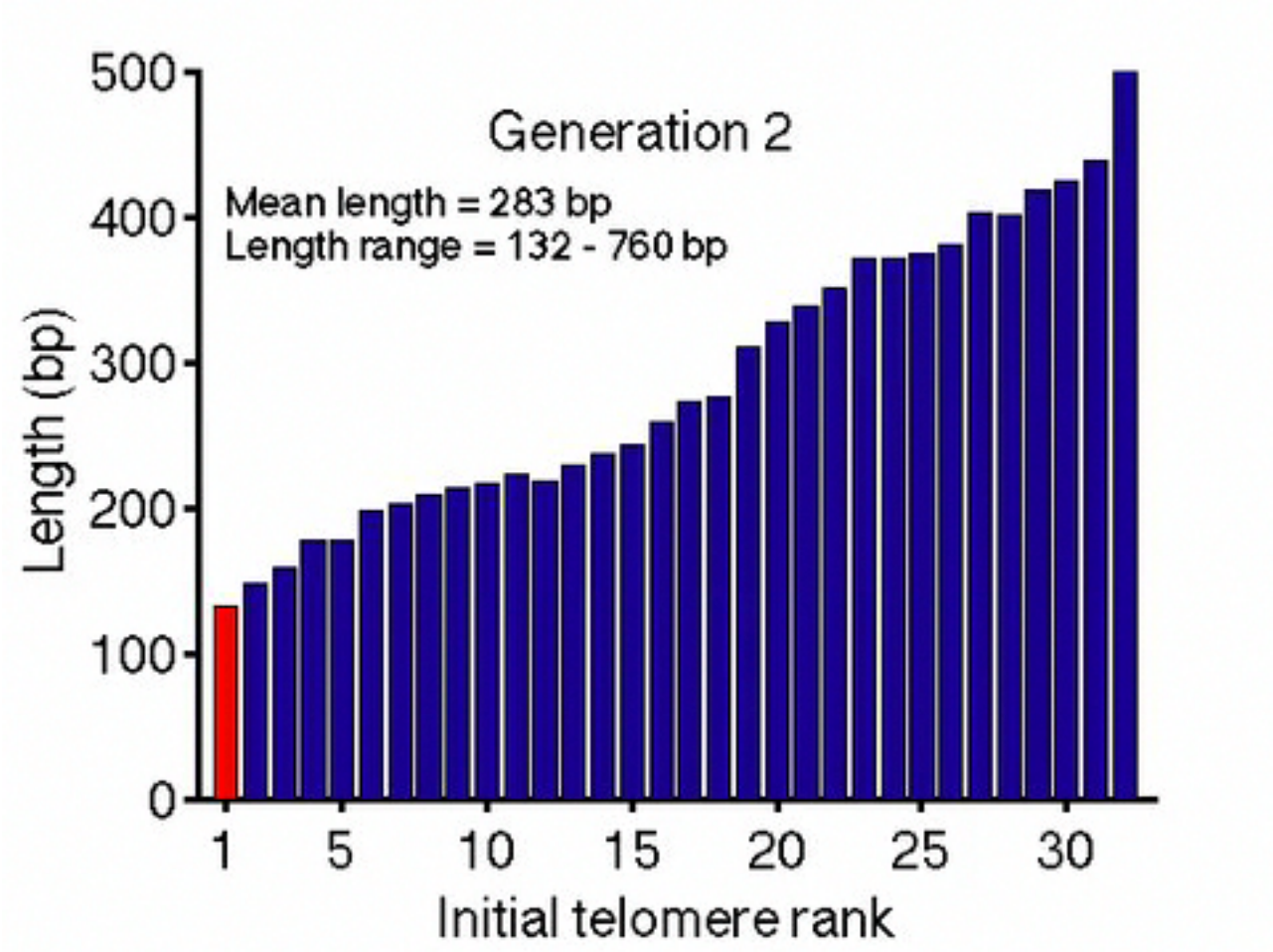


$$L_k^{n+1} = \begin{cases} L_k^n \\ L_k^n - s_k \end{cases}, \text{ if } \begin{matrix} k \in E \\ k \in E^C \end{matrix} \text{ where } E = \sigma(\{1, \ldots, 16\})$$

- Stochastic model of telomere shortening, followed in individual lineages

Bourgeron *et al.* 2015 Sci Rep

Simulation of telomere shortening in a cell lineage

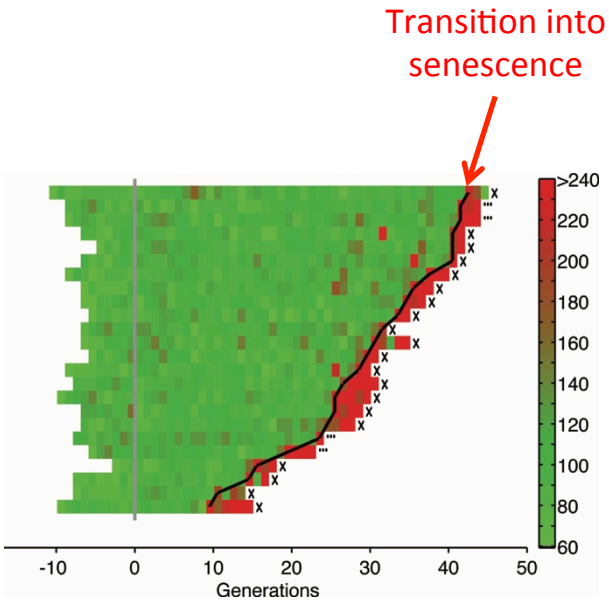


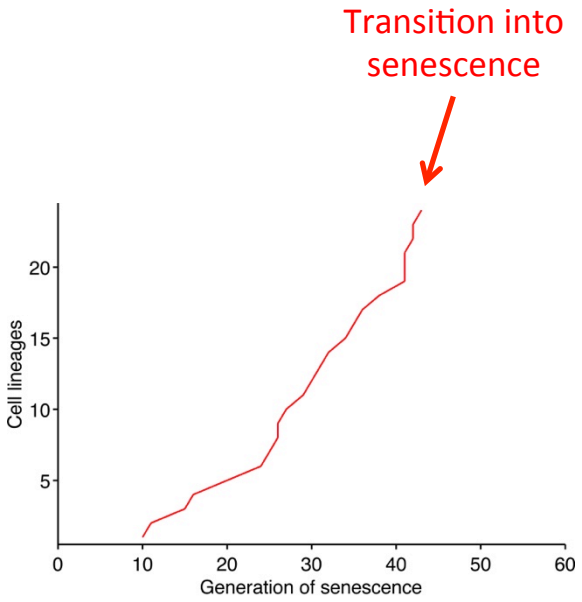
Approach

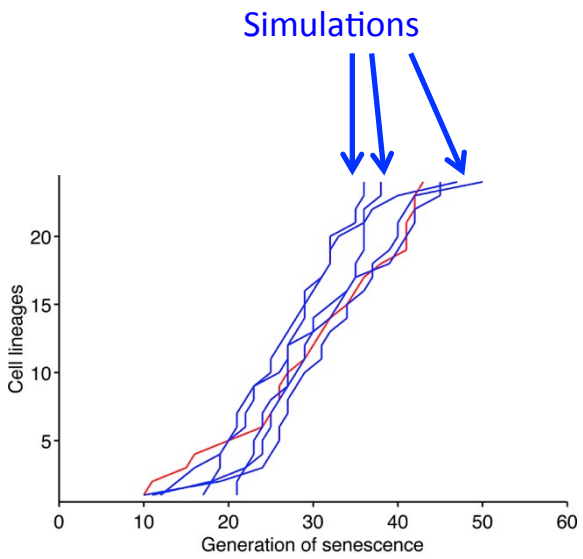
Experimental data:
Type A lineages

Extract transition data

Run the model and fit the transition profile





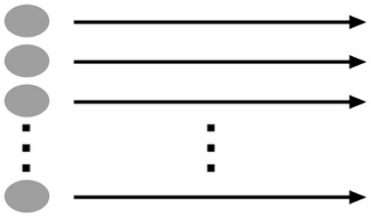


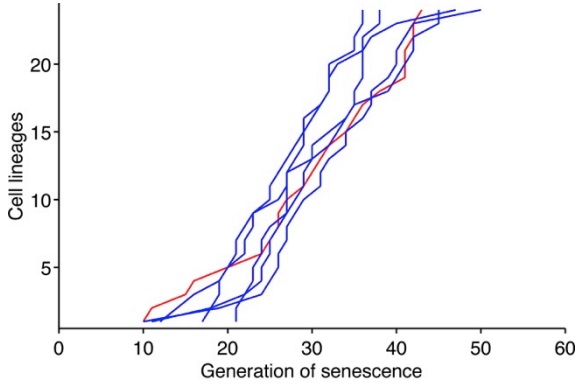
- The model was able to reproduce the experimental heterogeneity.

Sources of senescence heterogeneity

Interclonal variations in telomere distributions?

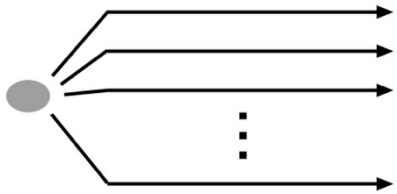
Different initial cells

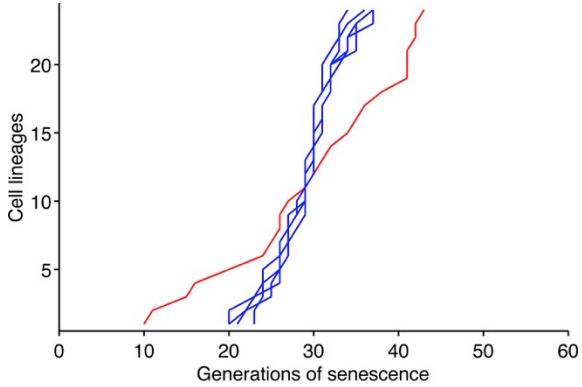




Coefficient of variations (CV) = 0.29

One initial cell





CV = 0.14

Sources of senescence heterogeneity

Interclonal variations in telomere distributions?

Asymmetry of telomere replication?

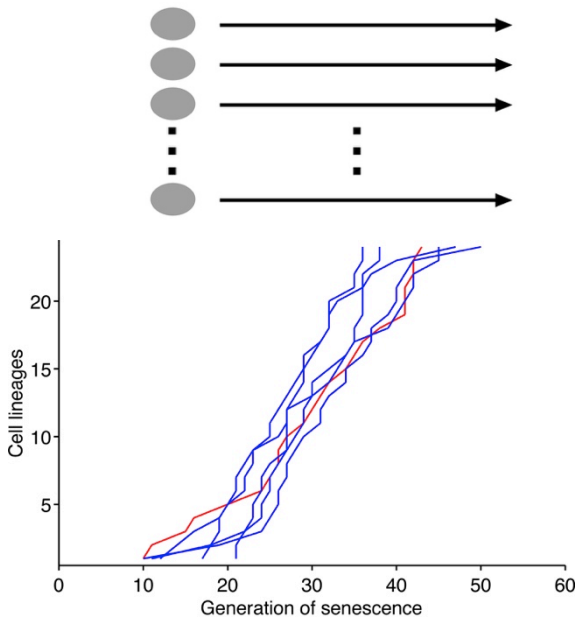
Asymmetric

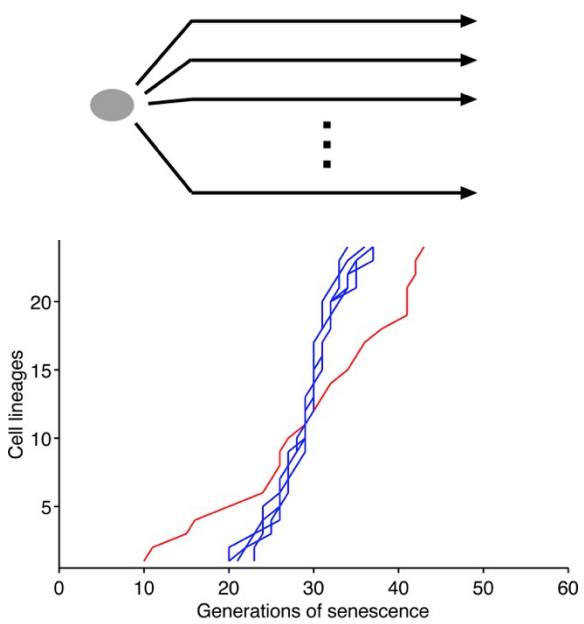
Symmetric

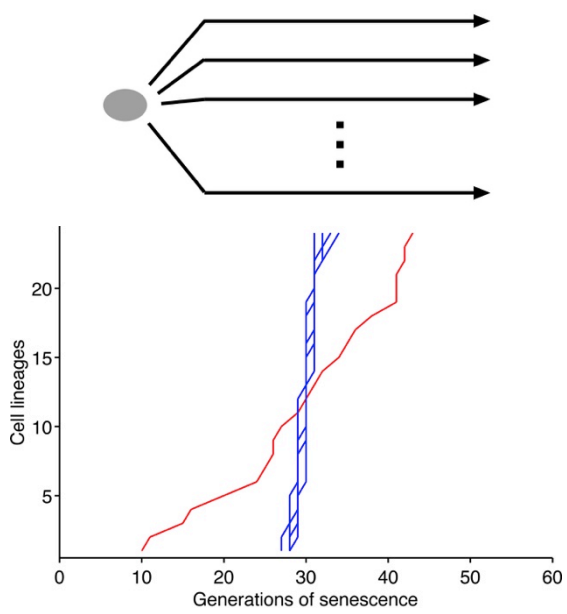
Different initial cells

One initial cell

One initial cell (symmetric replication)







Coefficient of variations (CV) = 0.29

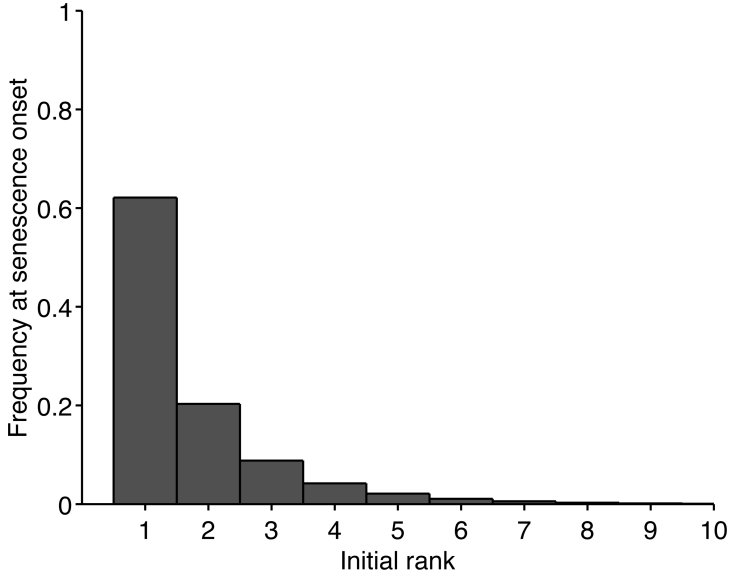
CV = 0.14

CV = 0.04

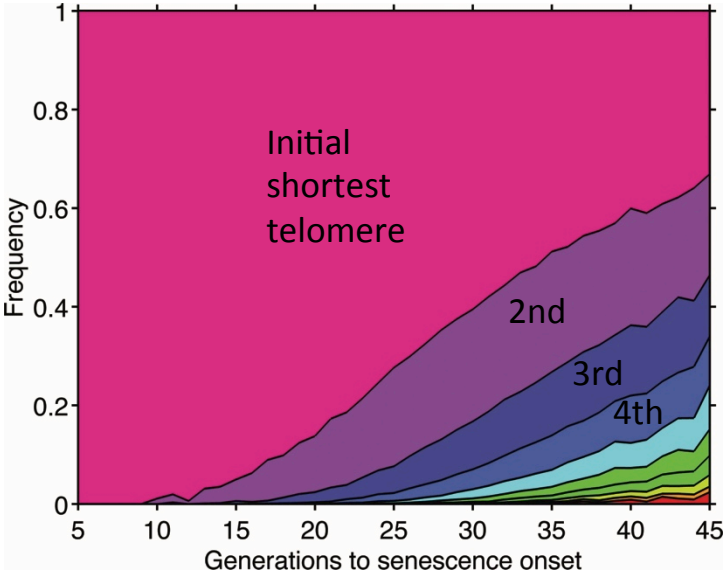
- Different sources of heterogeneity.
- The asymmetry of telomere replication is an unexpected source.

Consequences of the asymmetry of telomere replication

Initial rank of the final shortest telomere



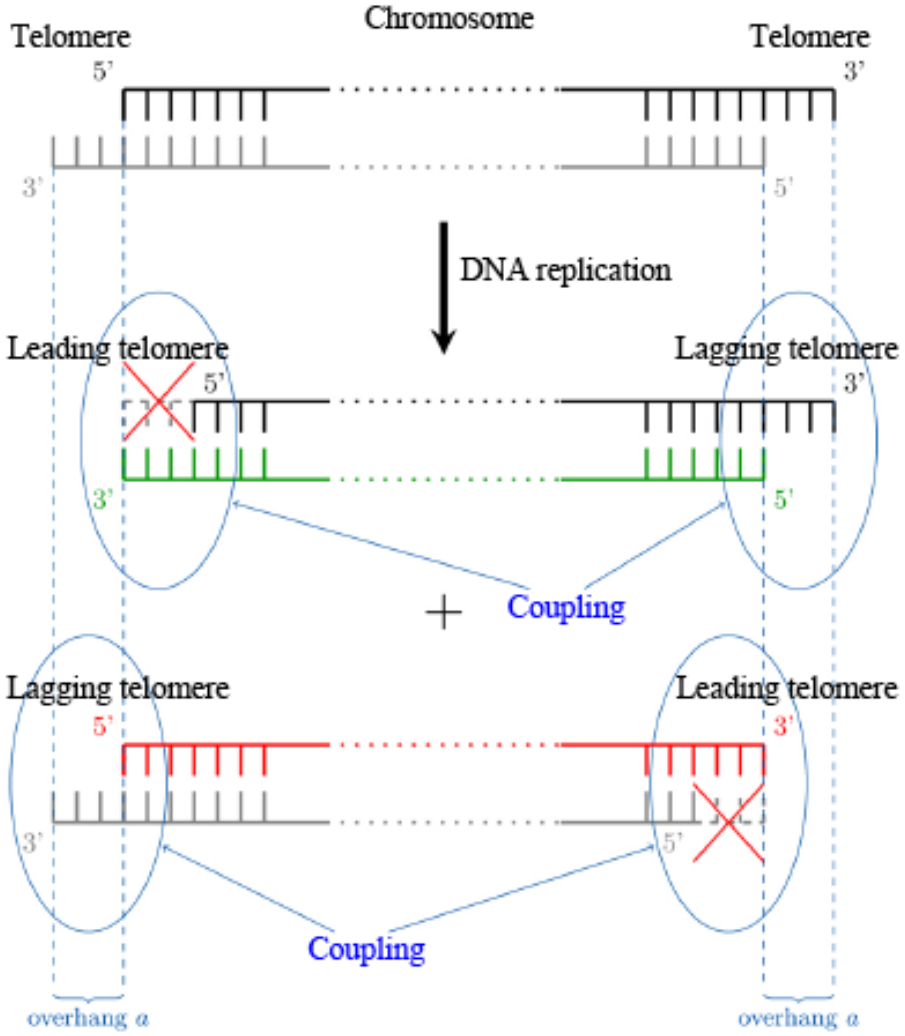
As a function of senescence onset timing



- Short-lived and long-lived lineages experience different telomere dynamics.

A new layer of complexity:
The coupling effect

Collaboration with Sarah Eugène (INRIA) and Thibault Bourgeron (ENS Lyon),
with help from Marie Doumic, Philippe Robert and Teresa Teixeira



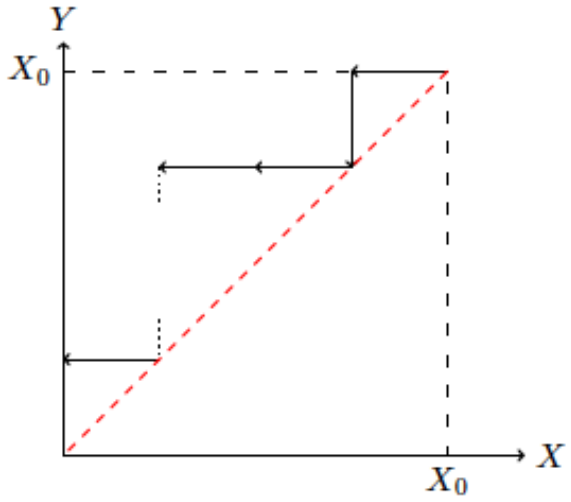
With or without telomerase

$$\begin{pmatrix} L^1_{n+1} \\ L^2_{n+1} \end{pmatrix} = \begin{pmatrix} (L^1_n - a.\mathrm{B}_n)^+ + \mathrm{C}^1_n.\mathcal{G}^1_n \\ (L^1_n - a.(1-\mathrm{B}_n))^+ + \mathrm{C}^1_n.\mathcal{G}^1_n \end{pmatrix}$$

- The coupling effect adds a new constraint.

Eugène *et al.* Submitted (see Arxiv)

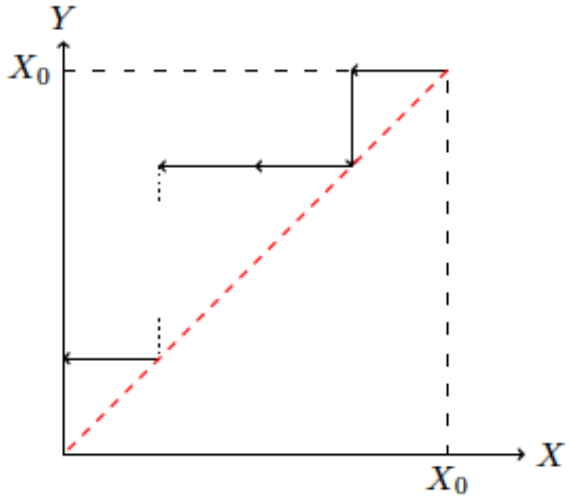
Case of a single chromosome with two equal telomeres (x_0,x_0)



Asymptotic expansion of the time of senescence:

$$\mathbb{E}(T^1_{x_0}) \sim 2x_0 \quad \text{and} \quad \frac{2x_0 - T^1_{x_0}}{\sqrt{x_0}} \underset{x_0 \to +\infty}{\overset{dist.}{\longrightarrow}} |N|, \qquad \text{with } N \overset{dist.}{\sim} \mathcal{N}(0,2).$$

Case of a single chromosome with two equal telomeres (x_0,x_0) and general case



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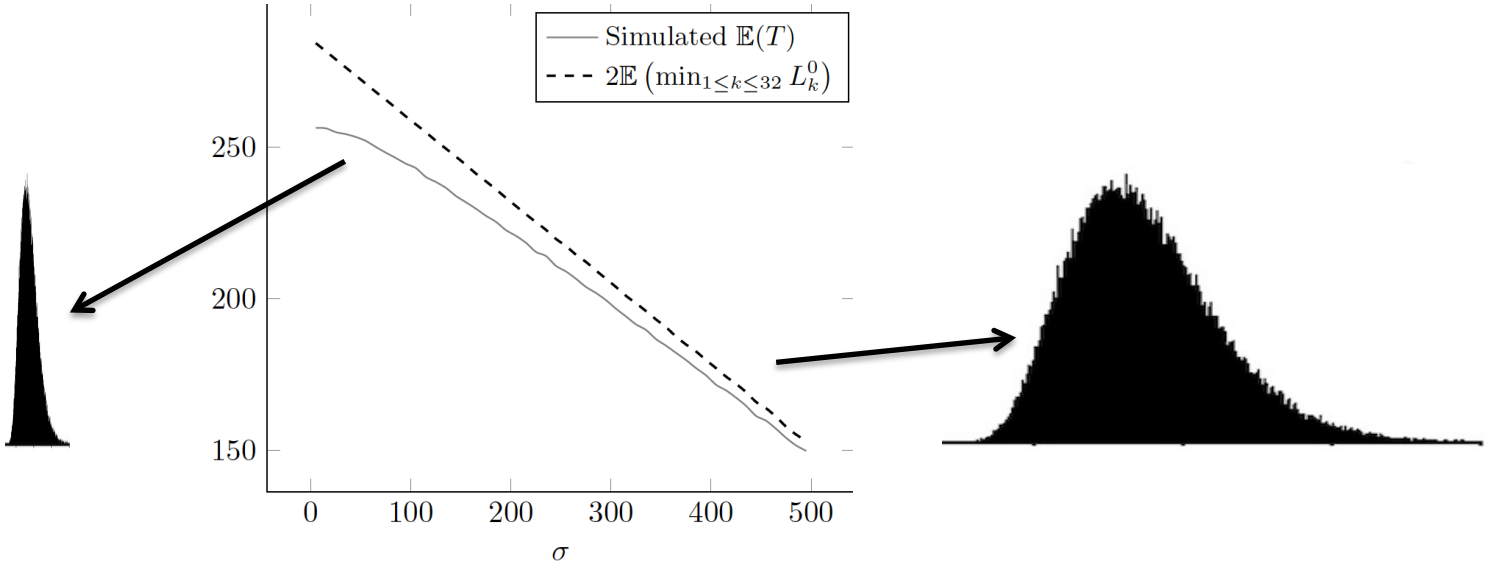
And for 16 pairs of telomeres:

$$\mathbb{E}(T_{x_0}) \approx x_0 + \sum_{n=x_0}^{2x_0-1} \left[\mathbb{P}(2x_0 - \sqrt{x_0}\,|N| > n)\right]^{16} = x_0 + \sum_{k=0}^{x_0-1} \left[\operatorname{erf}\left(\frac{k}{2\sqrt{x_0}}\right)\right]^{16}$$

Biological relevance of the expansion



Variance of the initial distribution

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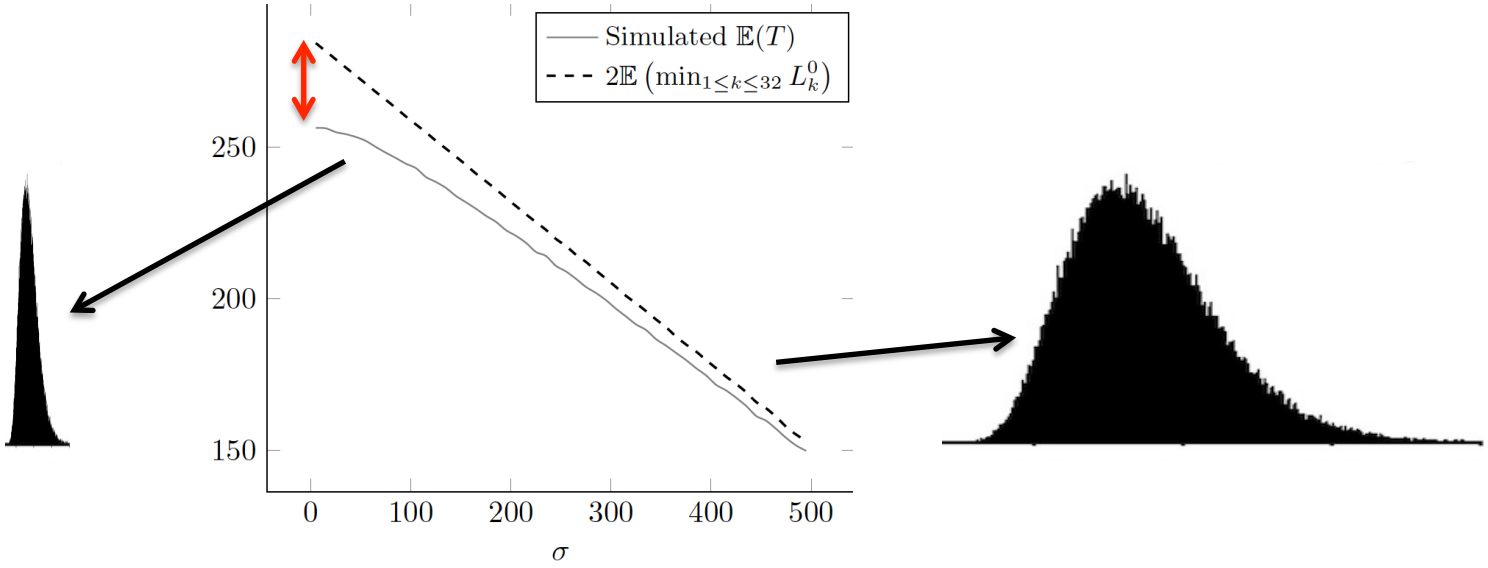

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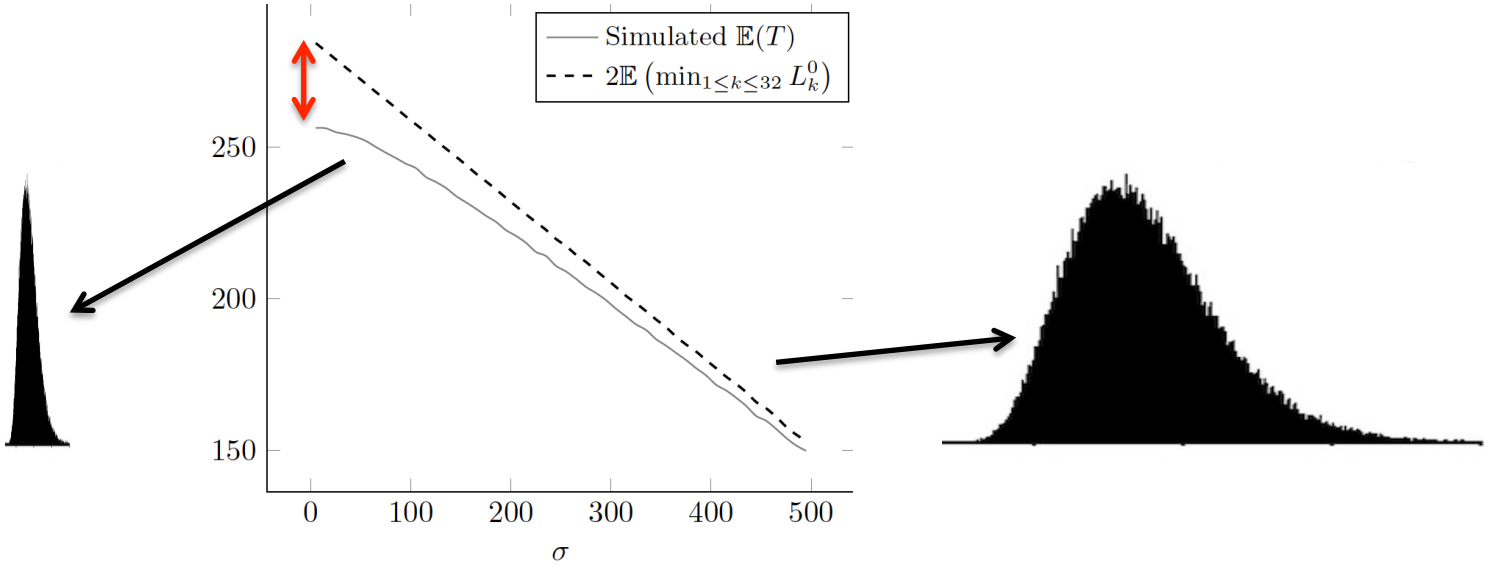
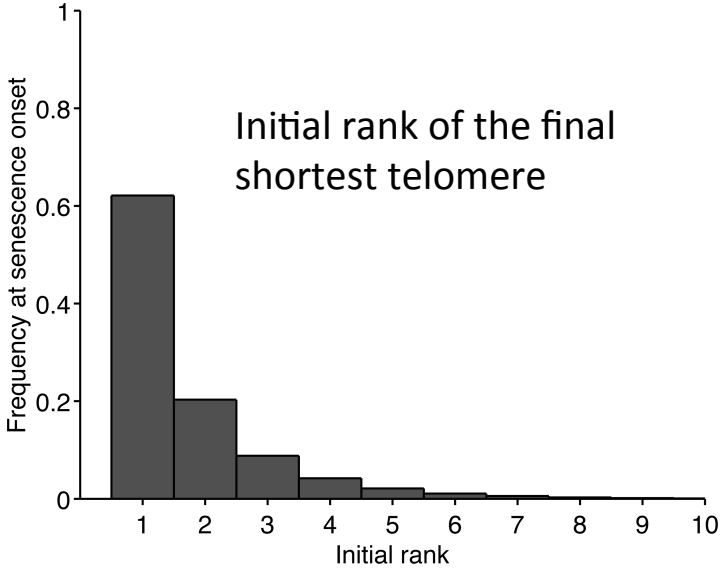
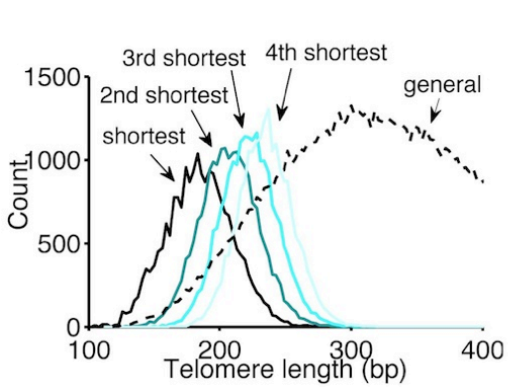
Shortening dynamics depends on the initial variance

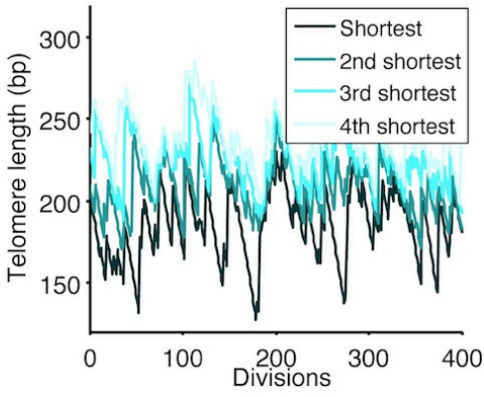


Initial rank of the final shortest telomere



Dynamics of the shortest telomeres



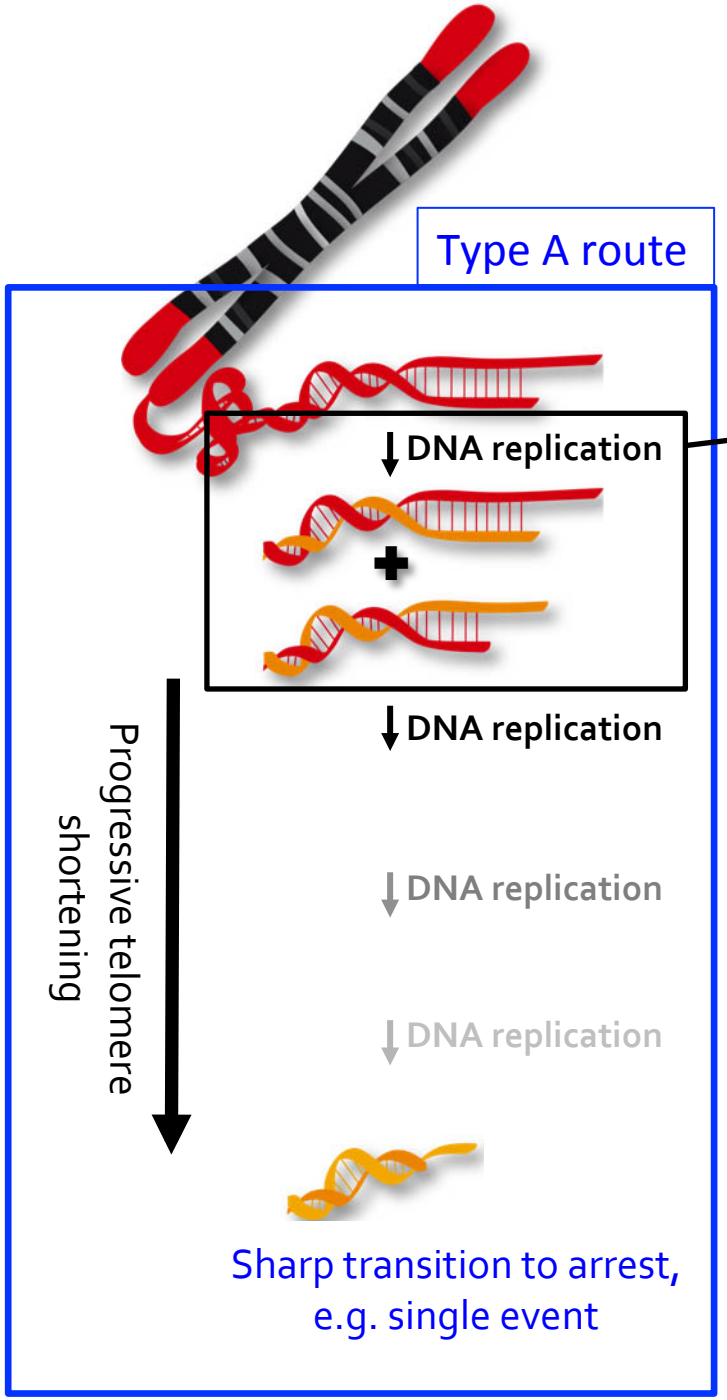


Summary of the mathematical models

Publication	Shortening without telomerase	Shortening and elongation with telomerase	Elongation probability	Comment
Xu *et al.* 2013 Genetics	N/A	$L_{n+1} = \begin{cases} L_n - a \\ L_n - a + b \end{cases}$	$P(L_n) = \begin{cases} (1+\beta(L_n - L_0))^{-1} \\ 1 \end{cases}$	Deterministic shortening. **Simulations.**
Bourgeron *et al.* 2015 Sci Rep	$L_k^{n+1} = \begin{cases} L_k^n \\ L_k^n - s_k \end{cases}$, if $k \in E$ / $k \in E^c$ where $E = \sigma(\{1, \dots, 16\})$	$L_k^{n+1} = \begin{cases} L_k^n - s \\ L_k^n - s + B \end{cases}$	$P(L_k^n) = \begin{cases} (1+\beta(L_k^n - L_0))^{-1} \\ 1 \end{cases}$	Stochastic shortening but not for steady-state telomere distribution. **Simulations.**
Eugène *et al.* 2016 Submitted (and arxiv)	$\begin{pmatrix} L_{n+1}^1 \\ L_{n+1}^2 \end{pmatrix} = \begin{pmatrix} (L_n^1 - a.\mathrm{B}_n)^+ + \mathrm{C}_n^1.\mathcal{G}_n^1 \\ (L_n^2 - a.(1-\mathrm{B}_n))^+ + \mathrm{C}_n^1.\mathcal{G}_n^1 \end{pmatrix}$		$P(L_n) = \begin{cases} (1+\beta(L_n - L_0))^{-1} \\ 1 \end{cases}$ OR $P(L_n) = 1_{\{L_n \le i_s\}}$	Stochastic shortening (also for steady-state telomere distribution) and coupling. **Theoretical.**

- Each model provided new insights
- Successive layers of refinements

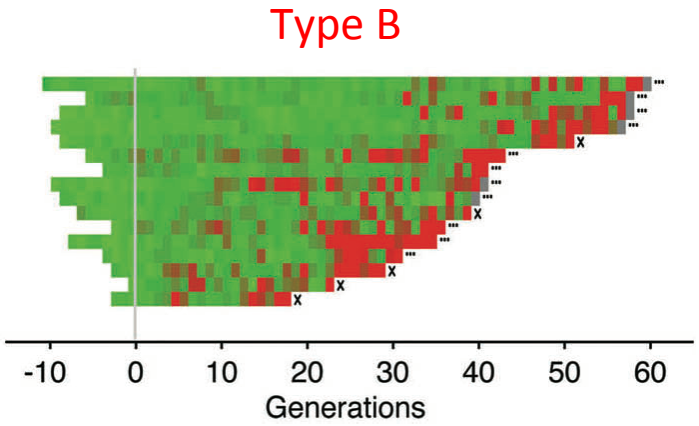
Replicative senescence



Intrinsic source of heterogeneity

(Xu *et al.*, 2015, *Nature Communications*)
(Bourgeron *et al.*, 2015, *Scientific Reports*)

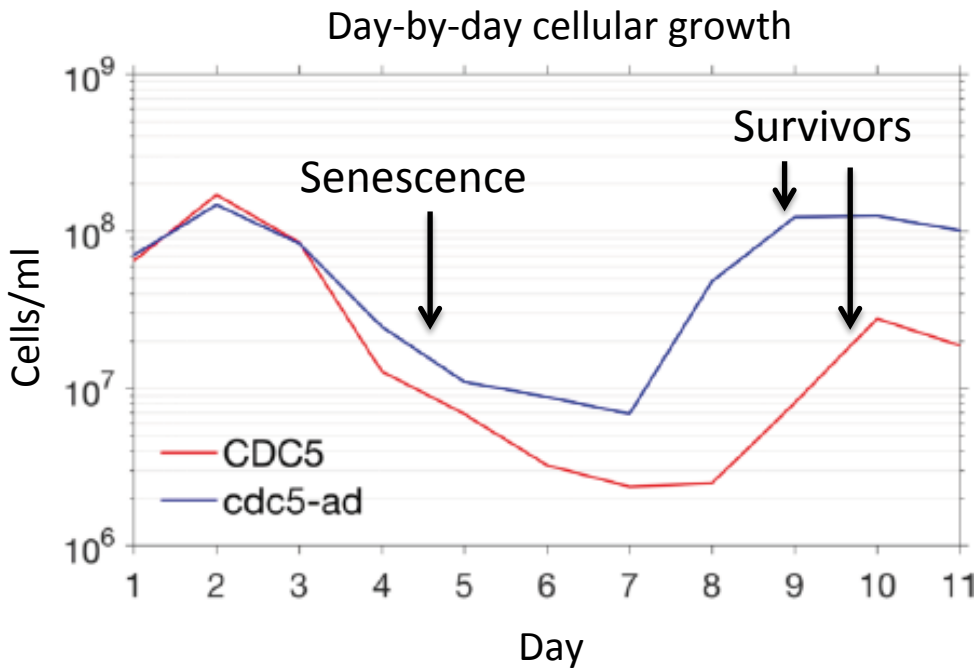
What are Type B lineages? Are they important?



Our hypothesis:

Type B lineages contributes to many aspects of senescence, for instance the emergence of **post-senescence survivors** (analogous to **cancer** cells).

What are Type B lineages? Are they important?

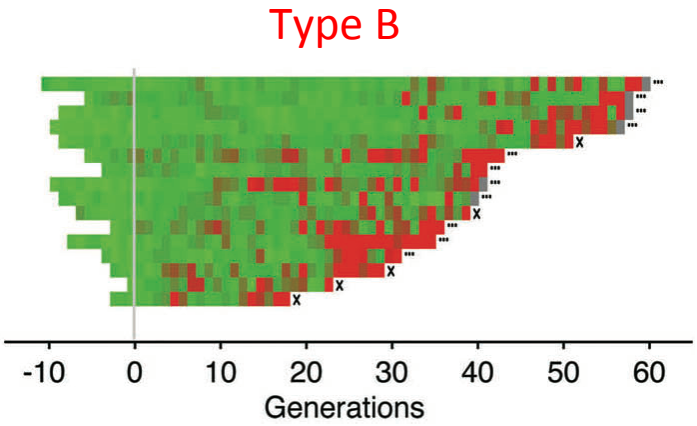


Our hypothesis:

Type B lineages contributes to many aspects of senescence, for instance the emergence of **post-senescence survivors** (analogous to **cancer** cells).

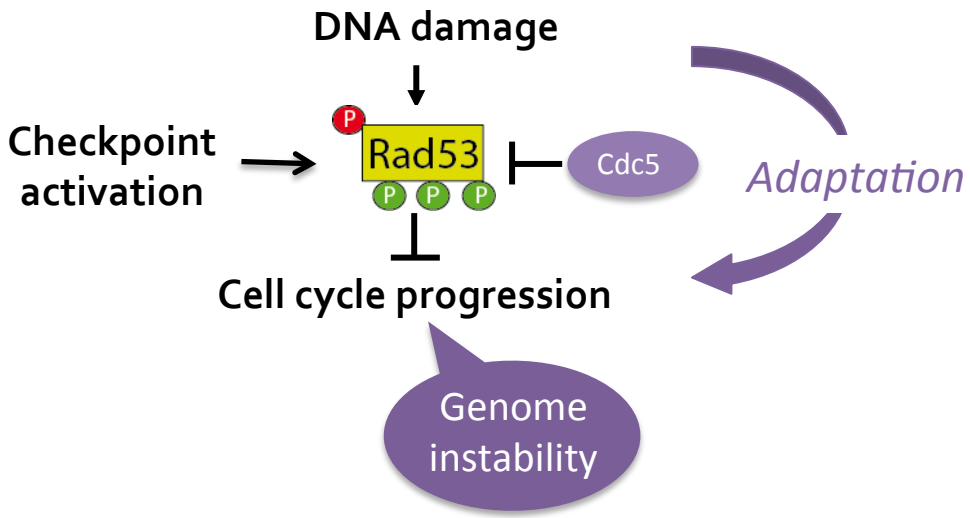
- Several lines of experimental evidence suggesting this is true.

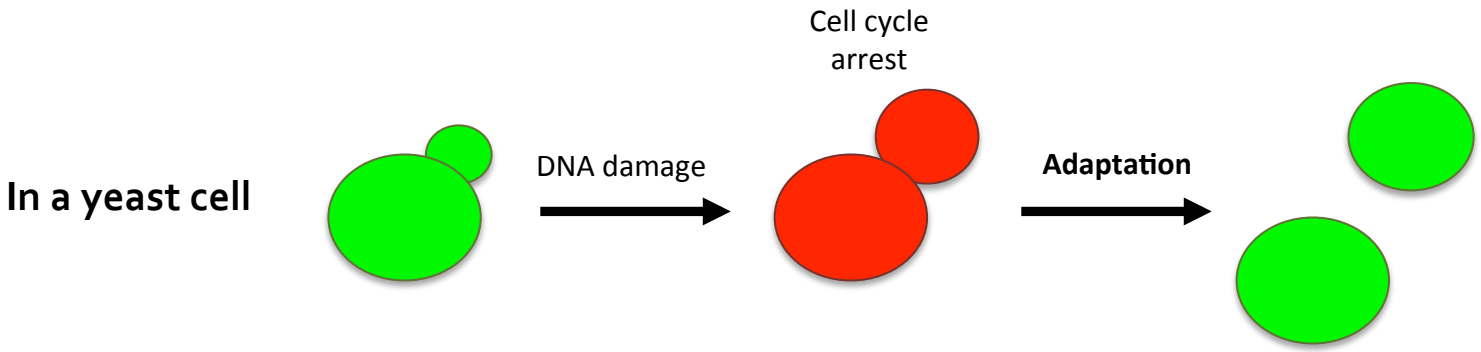
Most recent evidence



The **early delays** in type B lineages generate **genome instability**

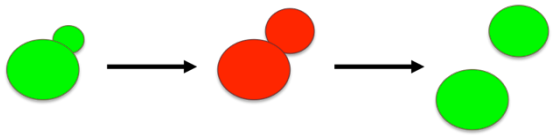
Adaptation
to DNA damage



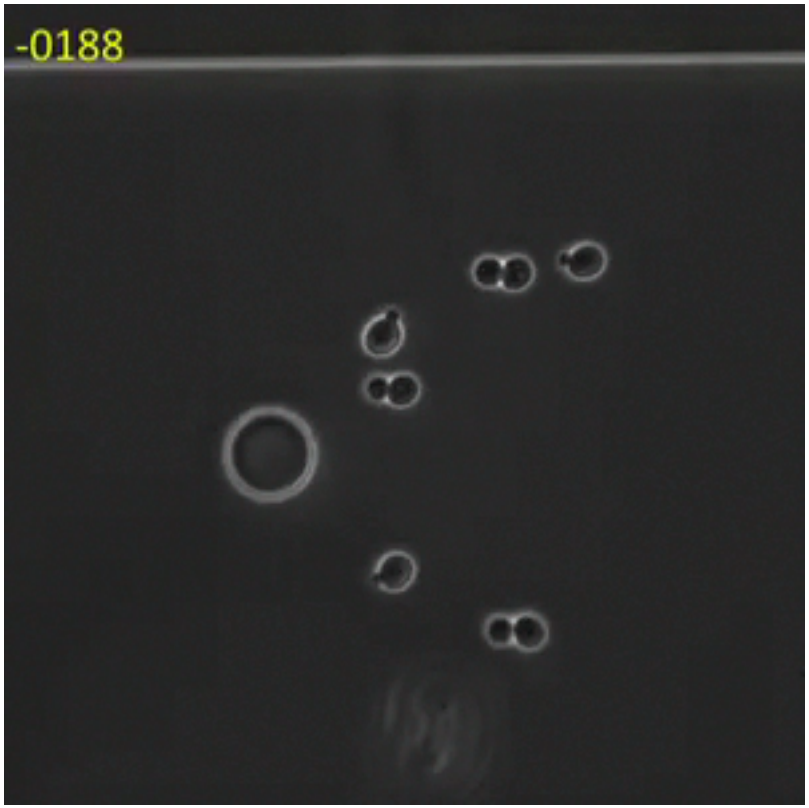


Live cell imaging of adaptation

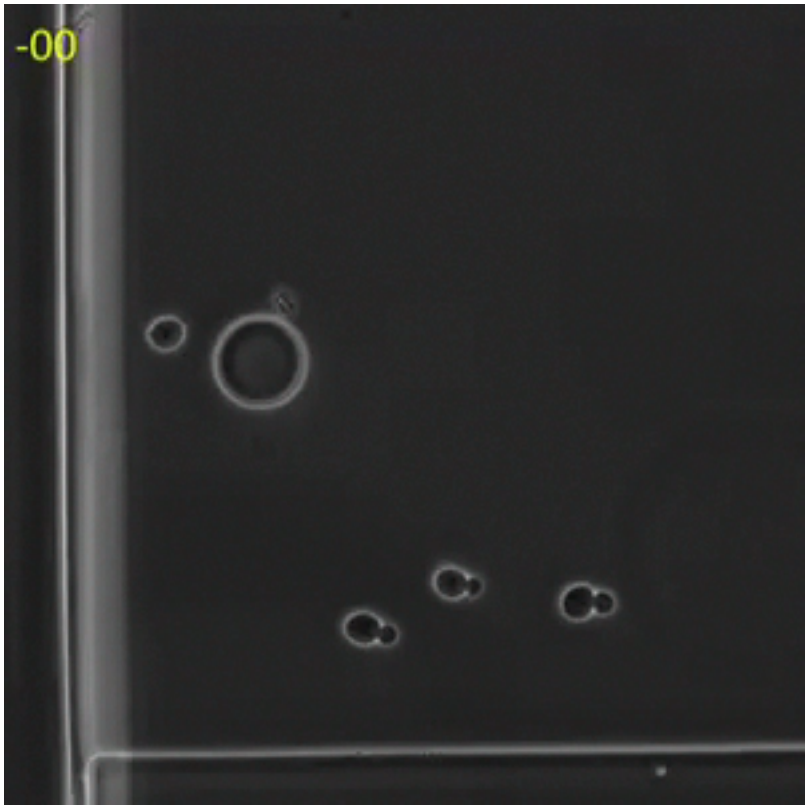
Central gene: *CDC5*



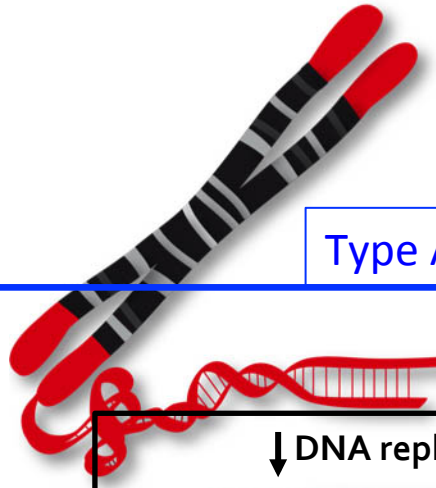
CDC5 cdc13-1 32°C



cdc5-ad cdc13-1 32°C



Replicative senescence



Type A route

DNA replication

DNA replication

DNA replication

DNA replication

Progressive telomere shortening

Sharp transition to arrest, e.g. single event

Intrinsic source of heterogeneity

Cancer

Type B route

Stochastic telomeric damage

x N

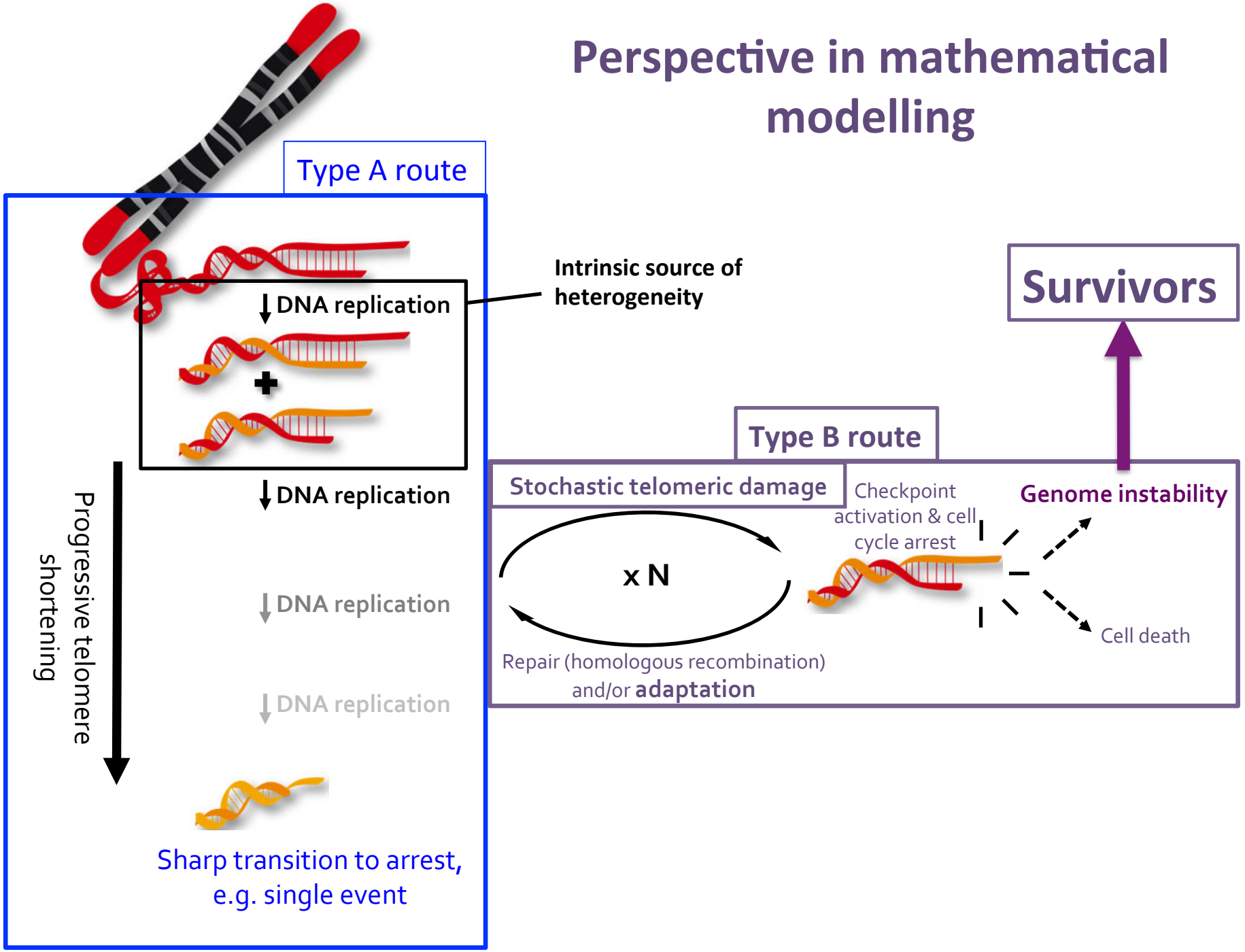
Repair (homologous recombination) and/or **adaptation**

Checkpoint activation & cell cycle arrest

Genome instability

Cell death

Perspective in mathematical modelling



70 years of progress in the study of the Luria-Delbrück distribution

Assumptions in the Luria-Delbrück model:

- The process starts with one wild-type cell and no mutant,
- Deterministic growth for wild-type and mutant cells,
- Exponential growth (to infinity),
- Mutations occur randomly at a rate proportional to the population size.

Luria and Delbrück	Mean and variance
Lea and Coulson	Approximate pgf, stochastic model
Haldane	Combinatorial approach
Armitage	Exact pgf
Kendall	Arbitrary distribution for cell cycle
Bartlett	Exact pgf, fully stochastic model
Crump and Hoel	Filtered Poisson process theory
Ma and Sarkar	Algorithm for probability function
Many others…	But no closed expression!

70 years of progress in the study of the Luria-Delbrück distribution

Assumptions in the Luria-Delbrück model:

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Anyone?	Distribution of **post-senescence survivors**!

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Assumptions in the Luria-Delbrück model:

- The process starts with one wild-type cell and no mutant,
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- ~~Exponential growth (to infinity)~~,
- ~~Mutations~~ occur randomly at a rate proportional to the population size.

Assumptions in the **SURVIVOR** model:

- Stochastic and heterogeneous growth,
- Growth slows down in wild-type cells (senescence),
- Recombination events occur stochastically as a function of telomere lengths,
- No easy way to identify survivors.

Goals:

- To understand how survivors (~cancer cells) emerge,
- To develop a method to estimate the rate of survivor emergence.

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