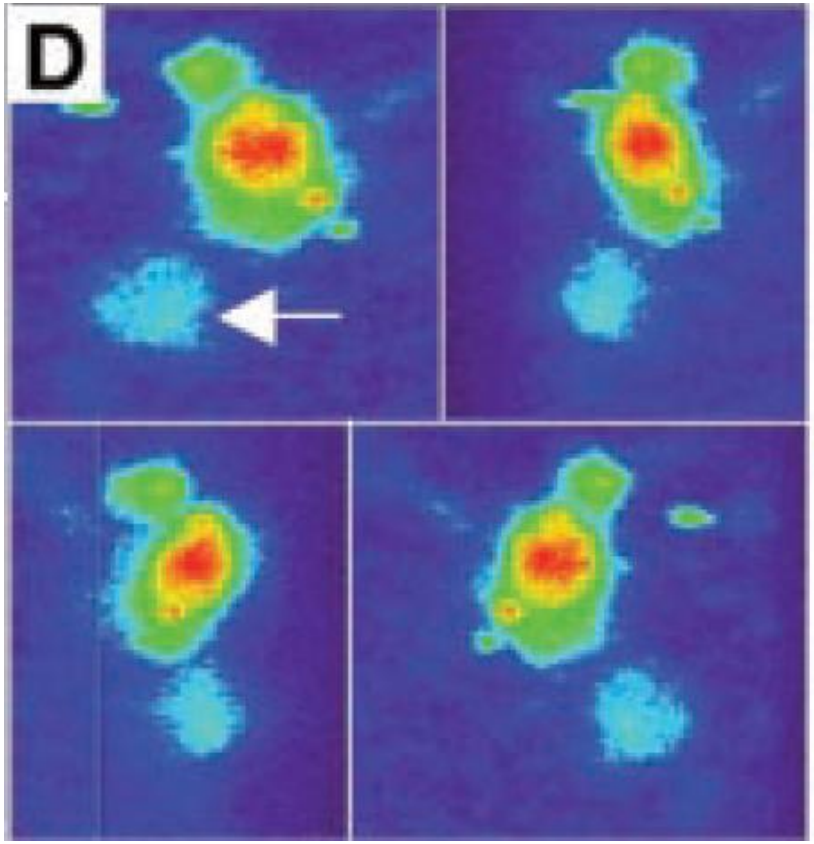
BMM5828/BTC5819 – Utilização de *Saccharomyces cerevisiae* como organismo-modelo em biologia molecular



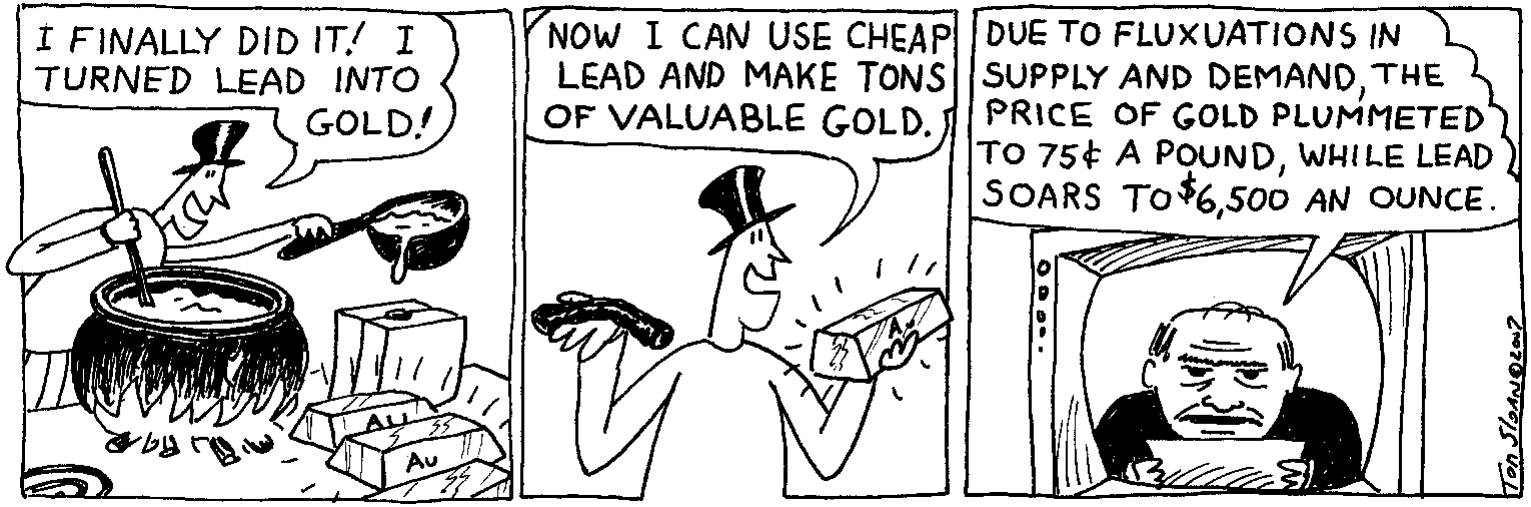
ICB, 10/11/2017

Estrutura da apresentação

1. A procura pelo elixir da imortalidade/eterna juventude (e cura de todas as doenças);
2. Teorias Evolucionárias do Envelhecimento
   A. Teoria do acúmulo de mutações
   B. Teoria da Pleiotropia antagonística
   C. Teoria do Soma descartável;
3. O que *Saccharomyces cerevisiae* nos ensinou sobre as teorias evolucionárias do envelhecimento;

O Laboratório dos Alquimistas

Pedra filosofal – transmutação de Pb em Au



Elixir da imortalidade

Paracelsus

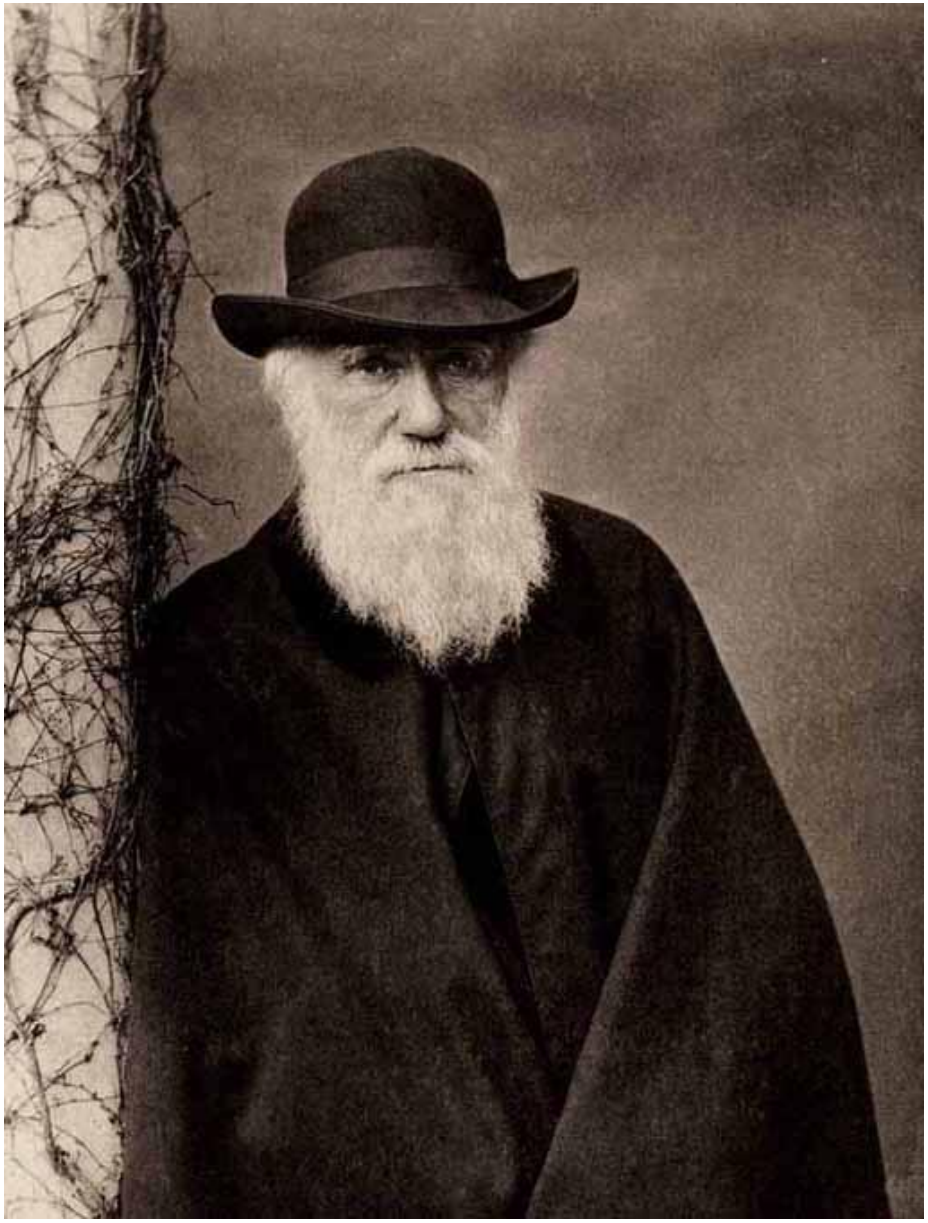
Philippus Aureolus Theophrastus Bombastus von Hohenheim

Paracelsus

Poison is in everything, and no thing is without poison. The dosage makes it either a poison or a remedy.

AZ QUOTES

Charles Robert Darwin



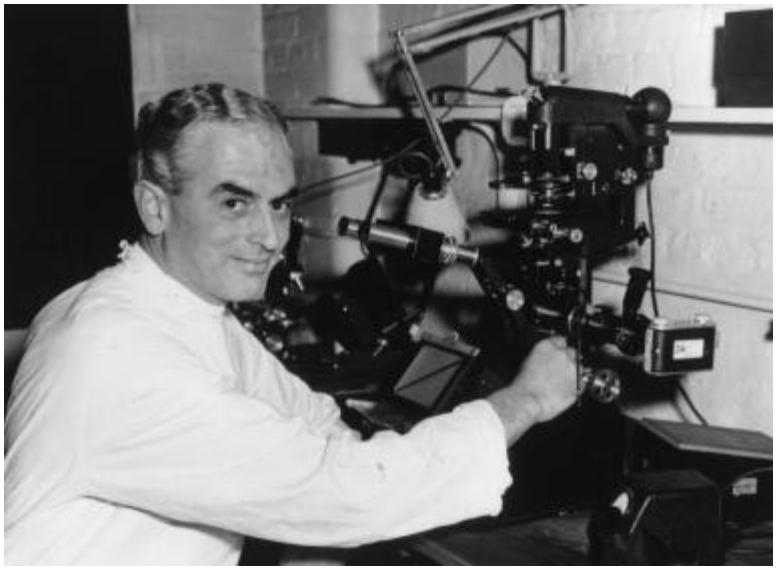
Teorias Evolucionárias do Envelhecimento

1. Teoria do acúmulo de mutações

2. Teoria da pleiotropia antagonística

3. Teoria do SOMA descartável

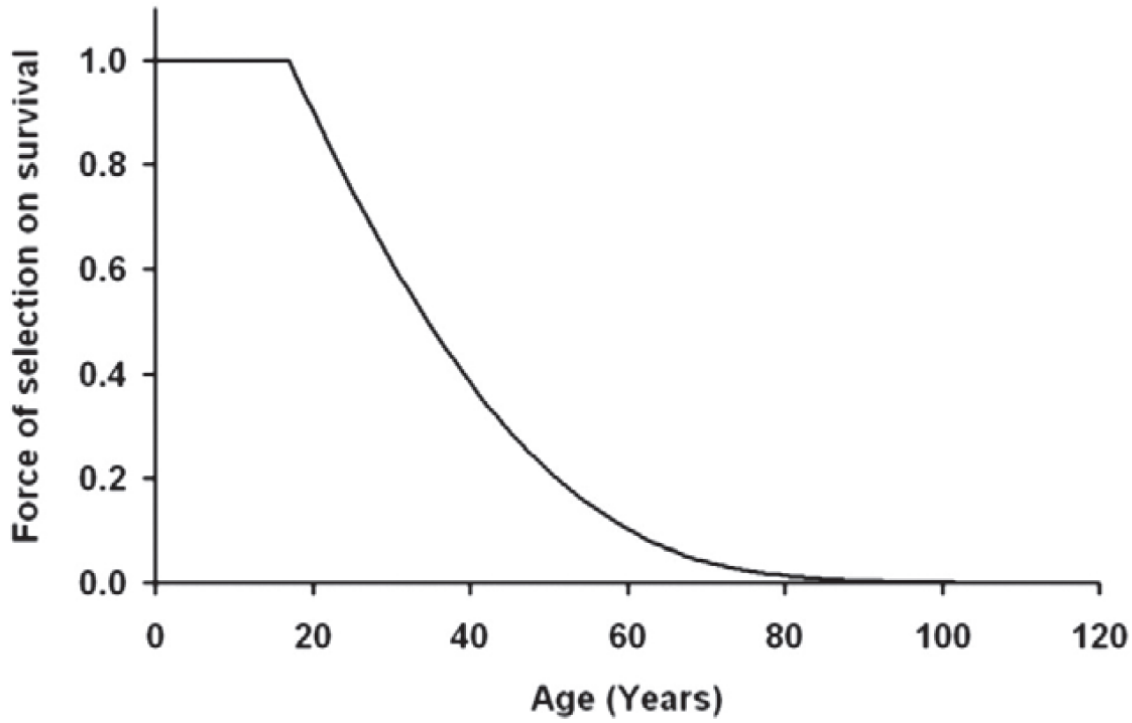
Teorias do acúmulo de mutações



Peter Medawar

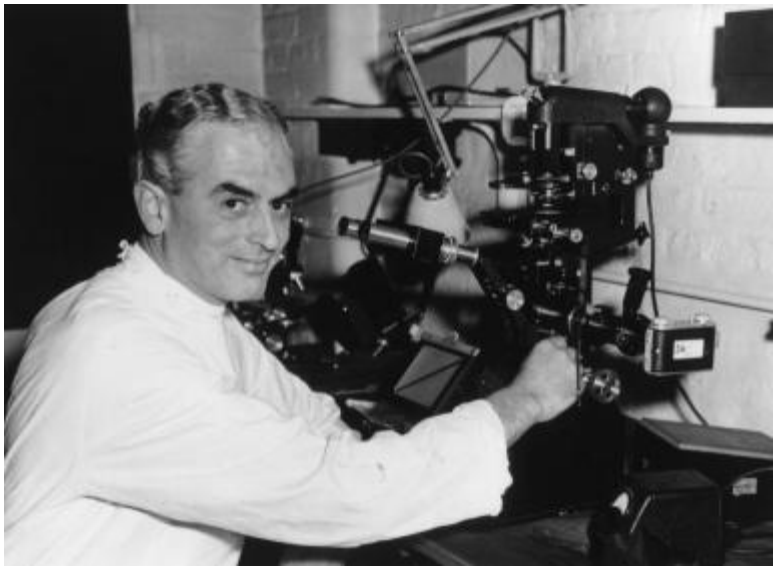
A força da seleção natural declina com o aumento da idade.

A força da seleção natural decai em função da idade



Biochimica et Biophysica Acta **1790** (2009) 951–962

Teorias do acúmulo de mutações



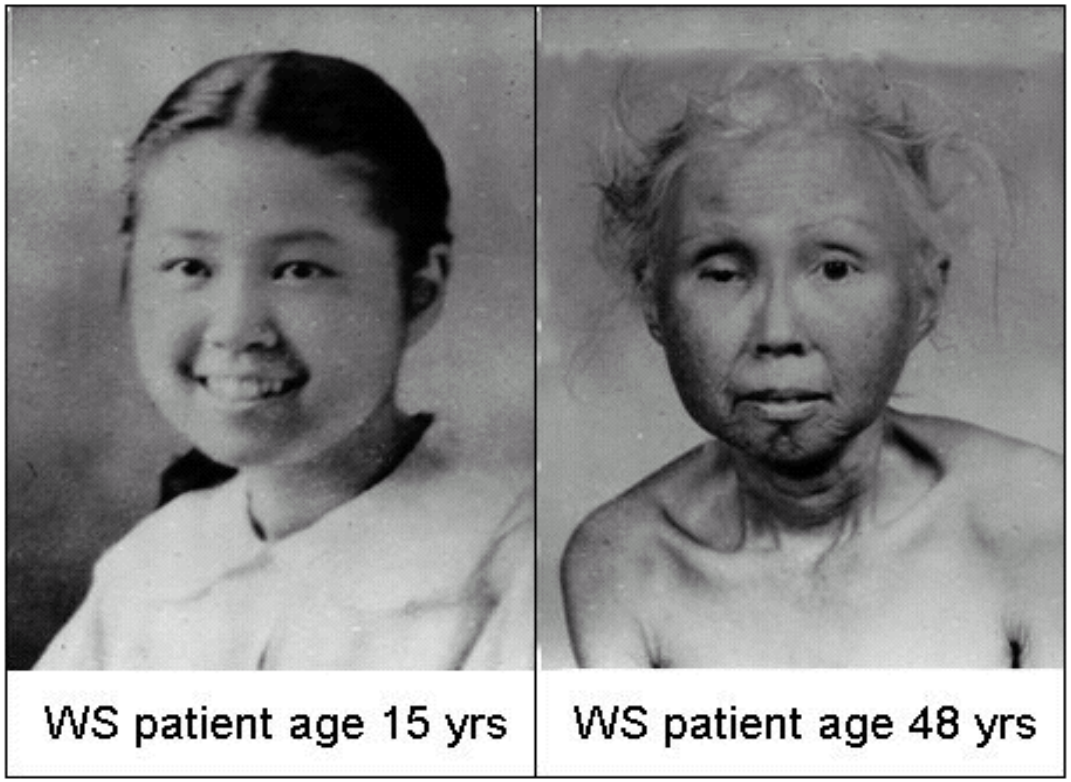
Peter Medawar

A força da seleção natural declina com o aumento da idade.

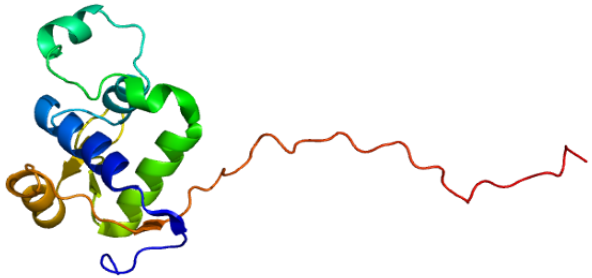
Genes benéficos na fase inicial da vida são favorecidos pela seleção natural, mas isso não ocorre com os genes benéficos na fase tardia.

Não há mecanismos para eliminar a população de mutações que causam efeitos deletérios em animais velhos.

Síndrome de Werner – herança autossômica, recessiva



RecQ helicase
(gene WRN)



Aging Cell (2015) **14**, pp366–371 Doi: 10.1111/acel.12290

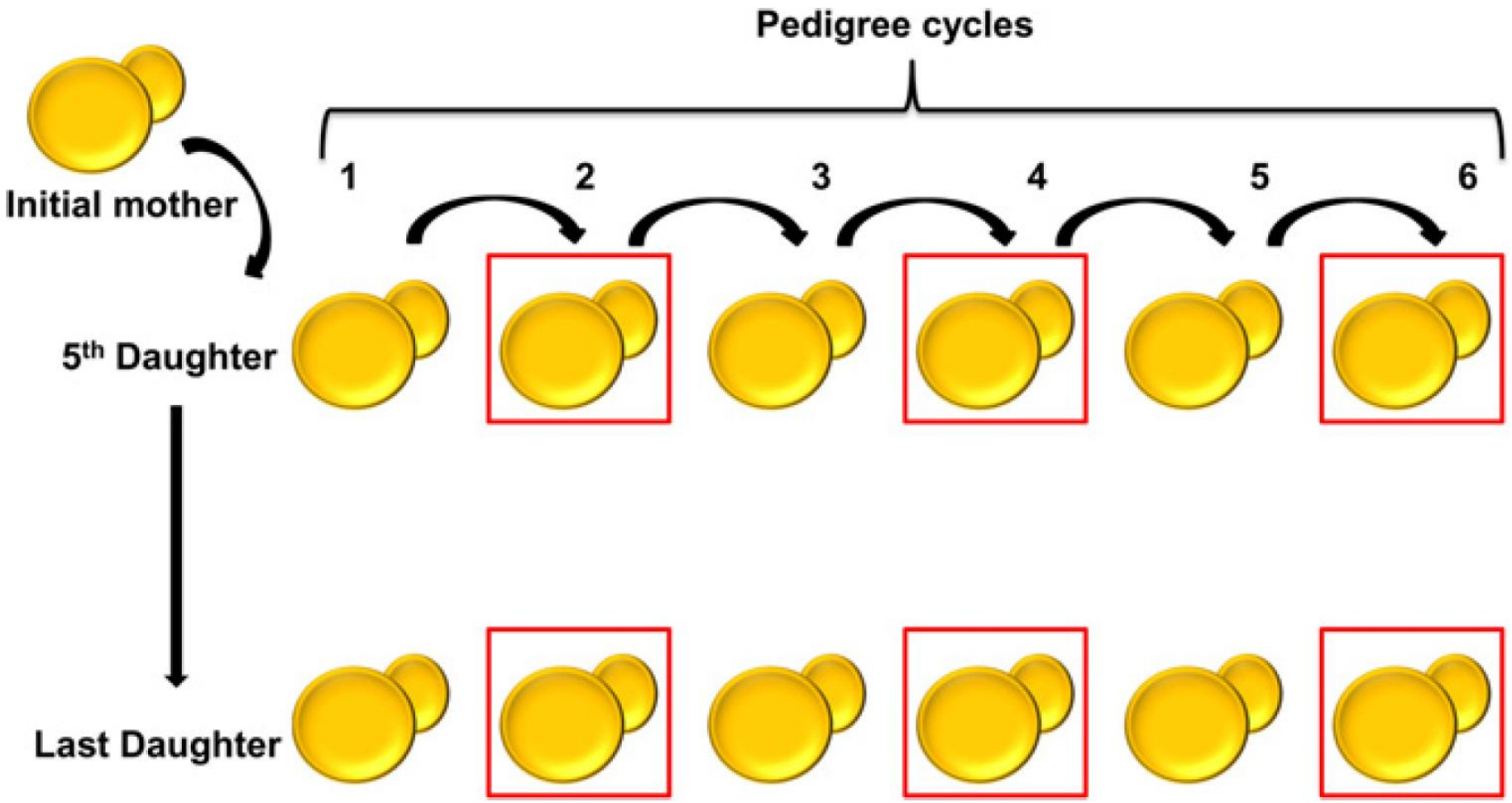
Evidence that mutation accumulation does not cause aging in *Saccharomyces cerevisiae*

Alaattin Kaya, Alexei V. Lobanov and Vadim N. Gladyshev

Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

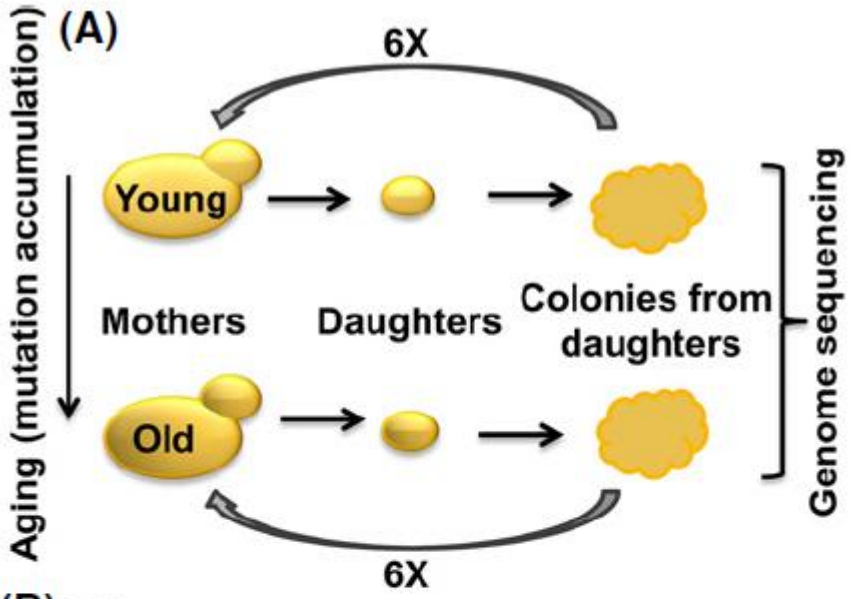
can lead to premature aging, but whether they limit lifespan under conditions of their sufficiency is unclear. Another possibility is the accumulation of oxidative damage, which decreases or abolishes

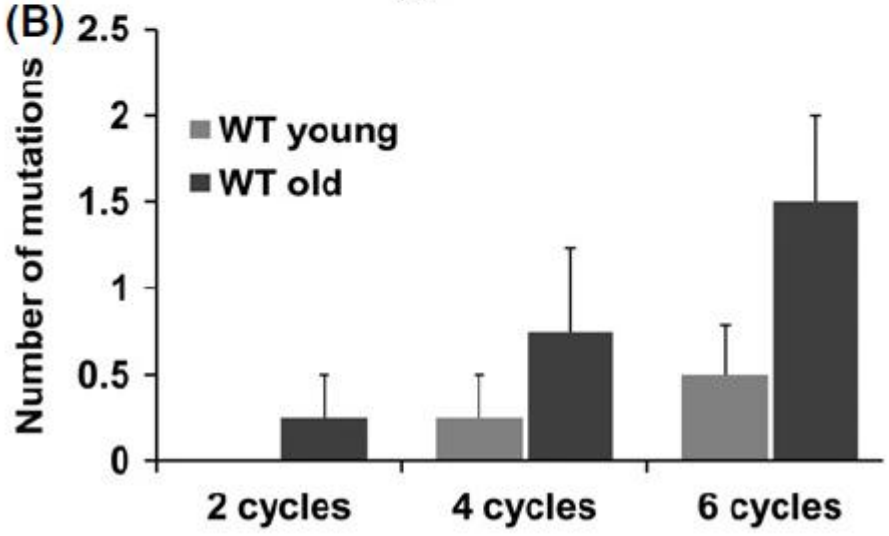
Experimental design of the pedigree assay

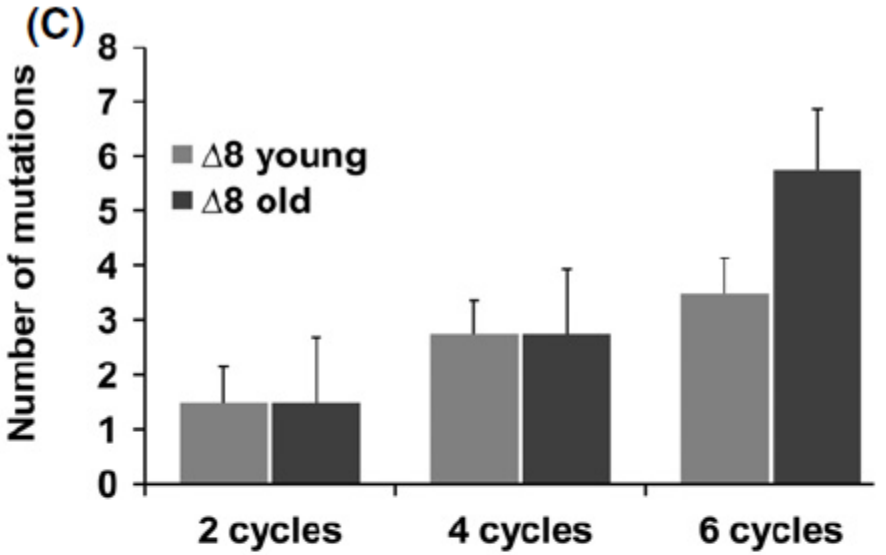


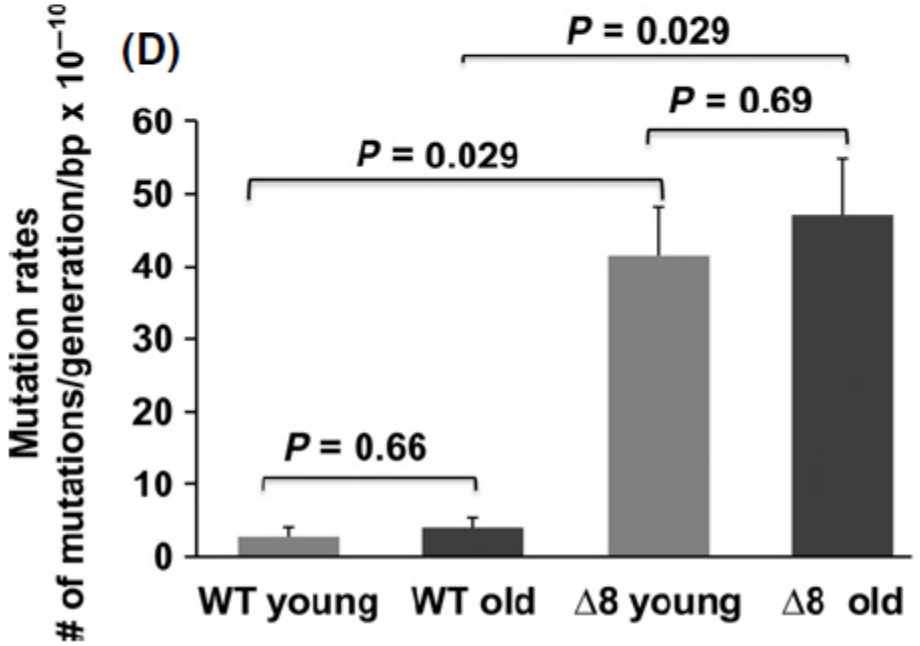
Aging Cell (2015) **14**, pp366–371

Mutation accumulation does not cause aging in yeast









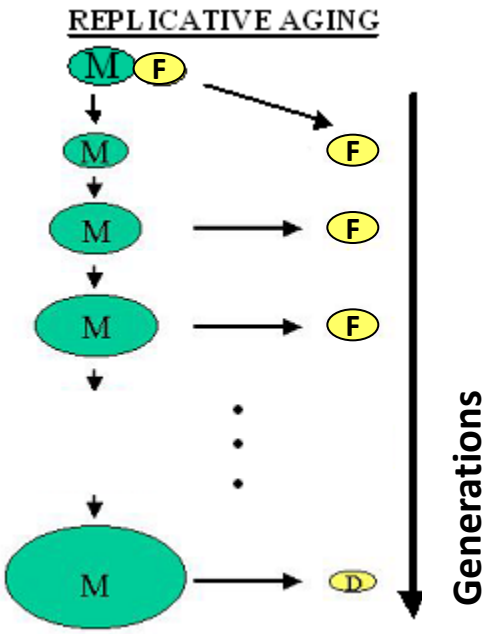
Aging Cell (2015) **14**, pp366–371

Our further analysis of the observed mutations revealed that many of them occurred on **chromosome XII**, where the **ribosomal DNA (rDNA) repeats** are located. rDNA has been identified as a factor leading to **ERCs**, which accumulate during **replicative aging** (**Sinclair** & **Guarente**, 1997).

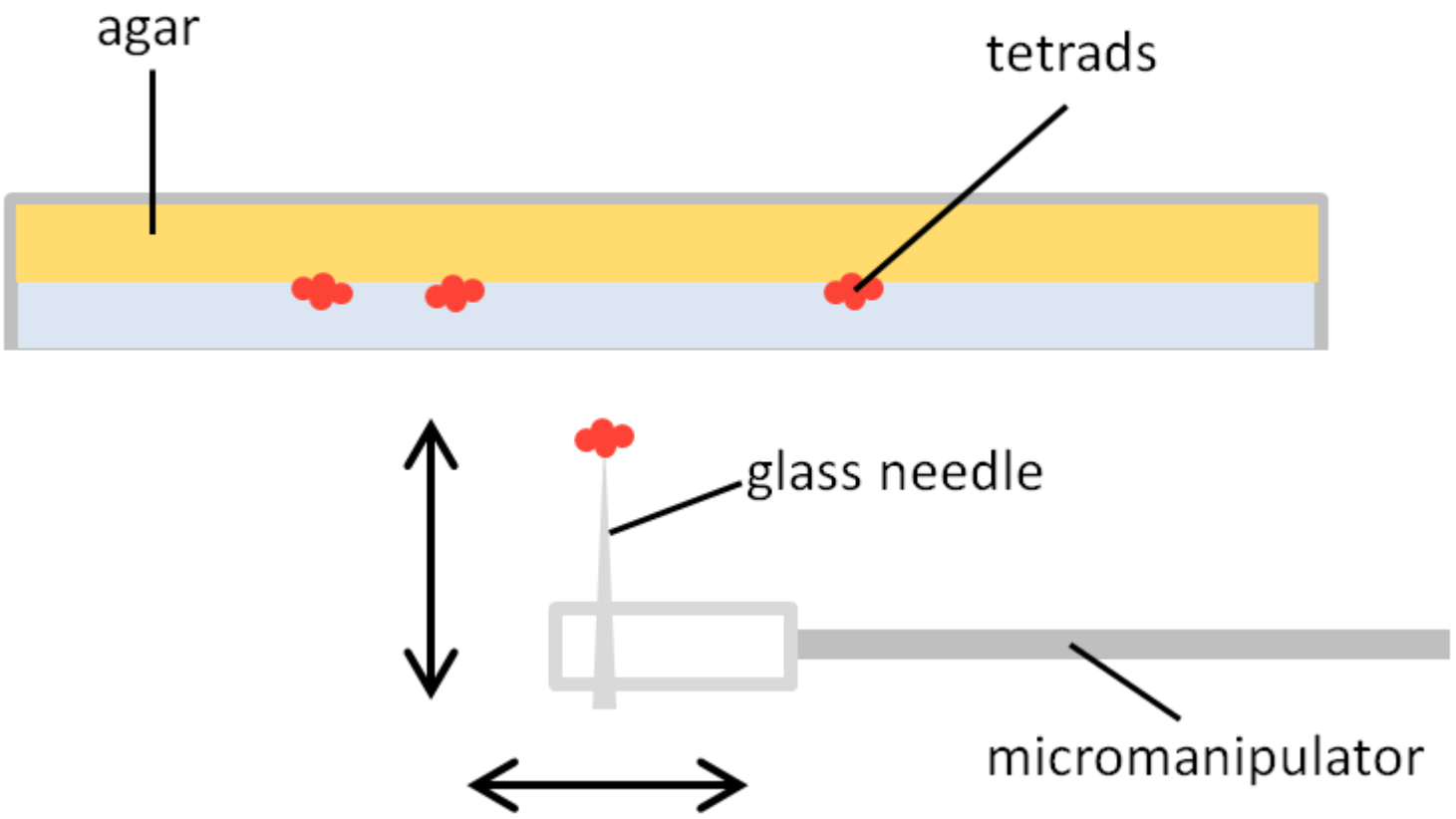
David Sinclair

Leonard Guarente

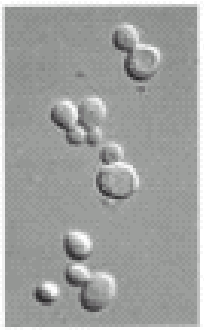
Yeast Replicative Life Span

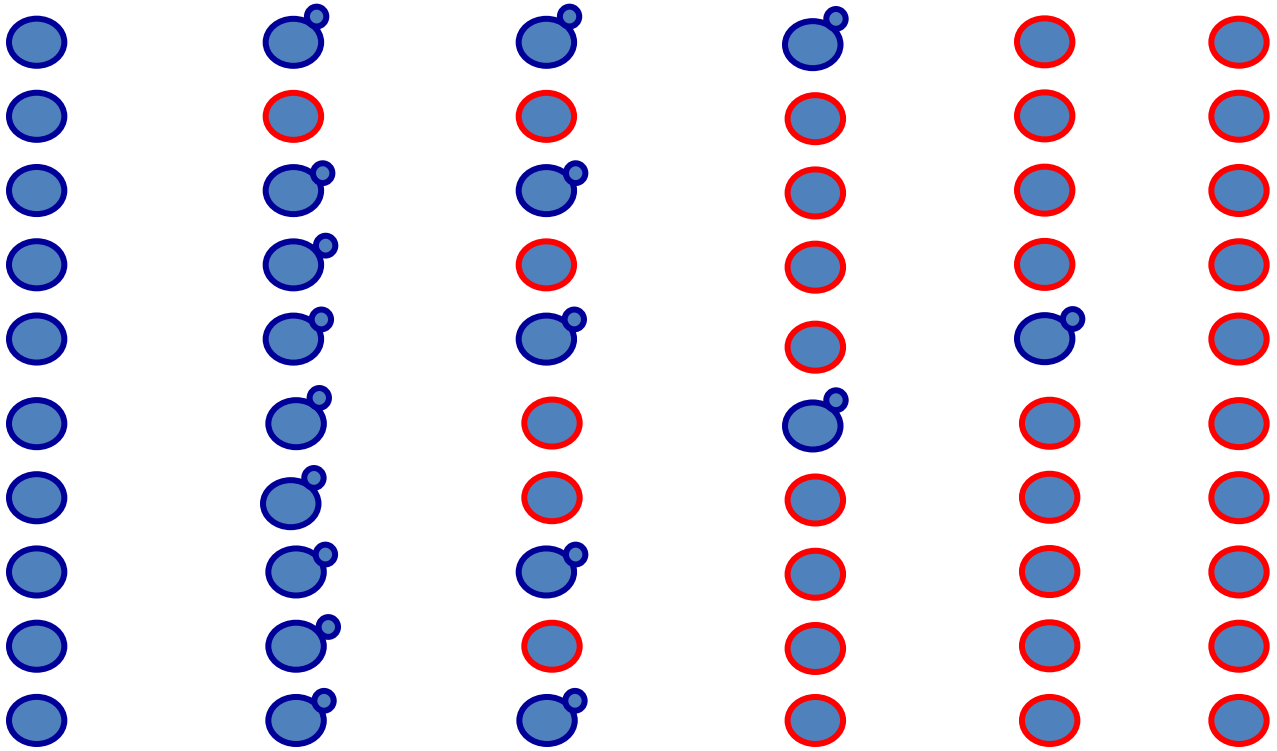


Yeast micromanipulator



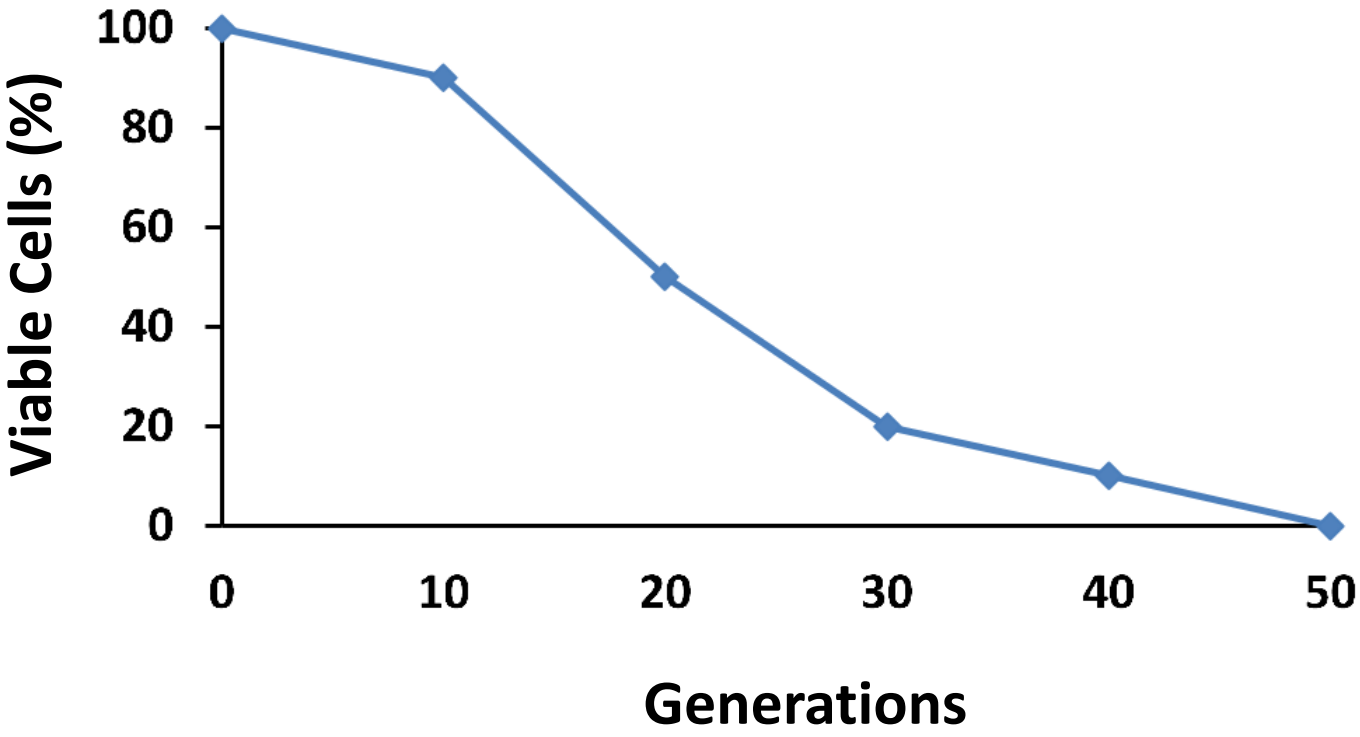
Replicative Life Span (RLS) Assay in *Saccharomyces cerevisiae*



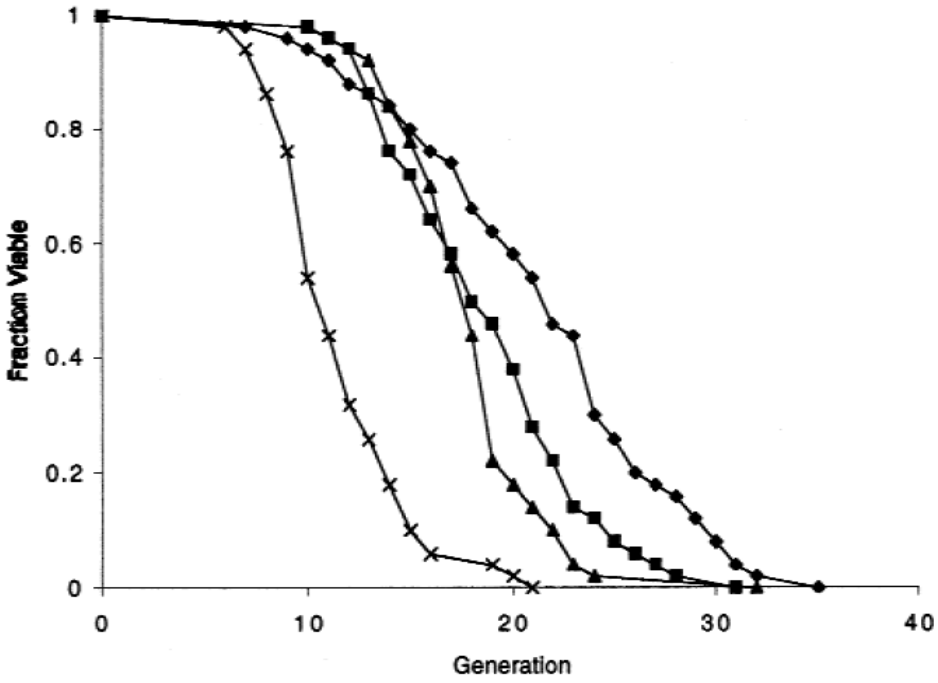


Gerations	0	10	20	30	40	50
% Survival (Viable Fraction)	100	90	50	20	10	0

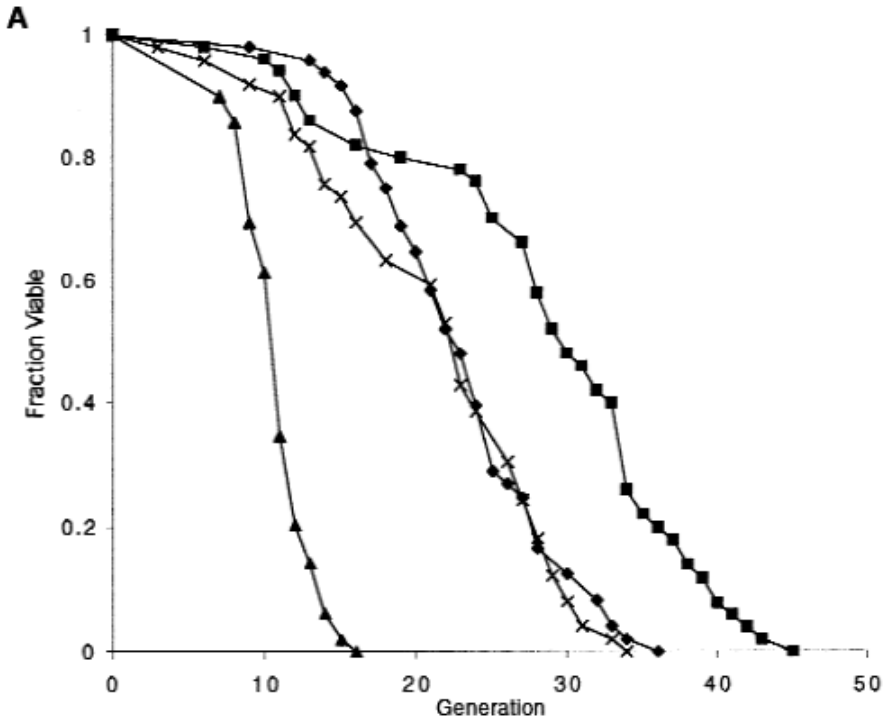
Yeast RLS Curve



SIR2 regula a longevidade replicativa em *Saccharomyces cerevisiae*



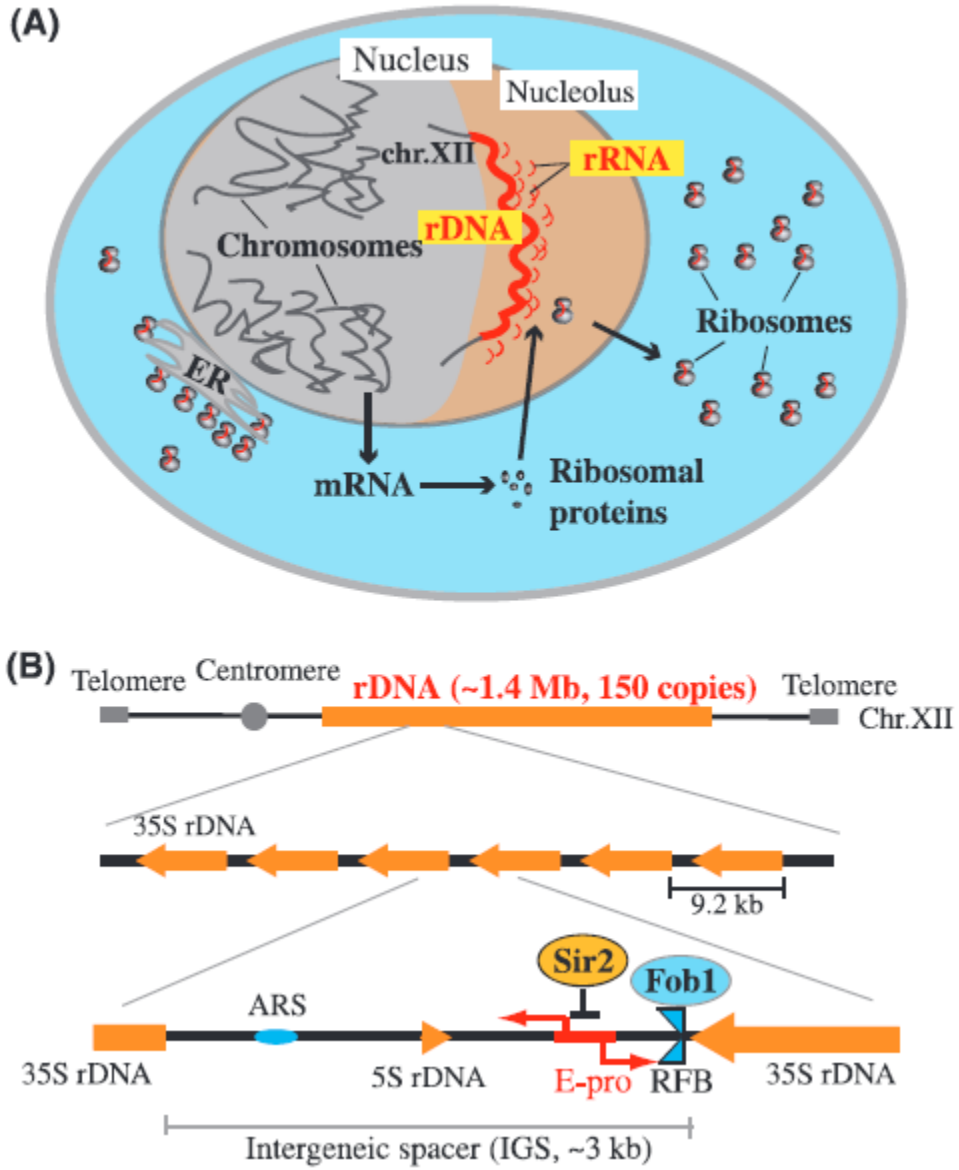
(◆) *W303R*; (■) *sir4*; (▲) *sir3*; (x) *sir2*.



(■) *W303R SIR2/URA3*; (▲) *sir2*; (×) *sir2 SIR2/URA3*.

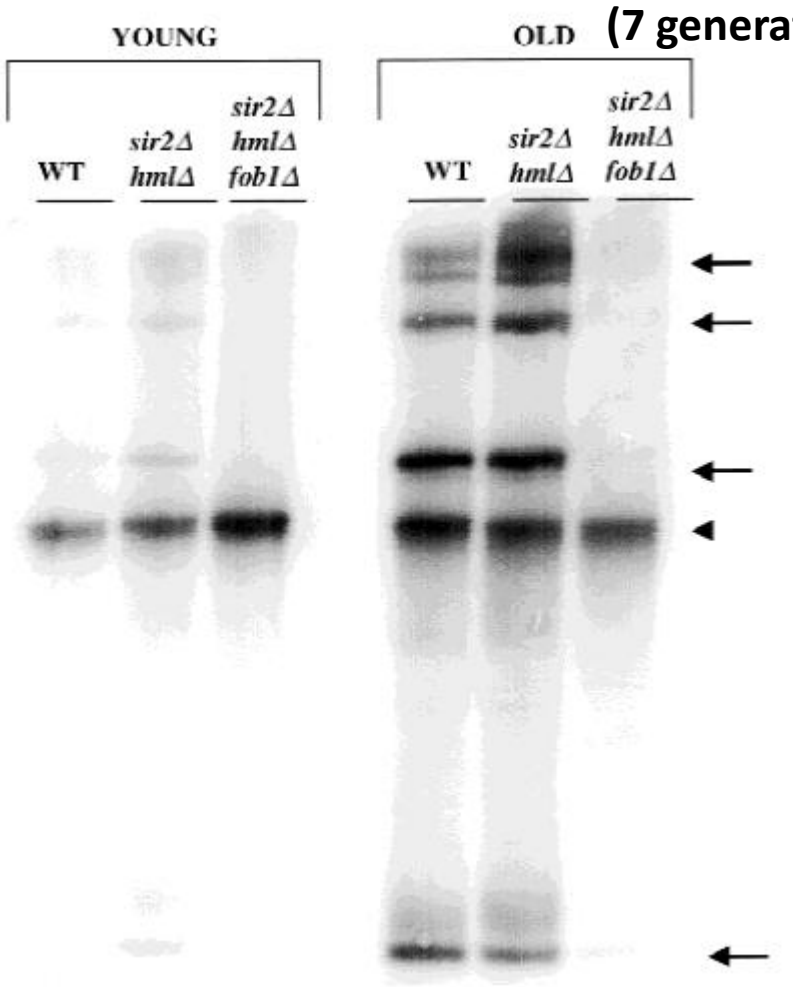
GENES & DEVELOPMENT 13:2570–2580

Organização do rDNA no cromossomo XII



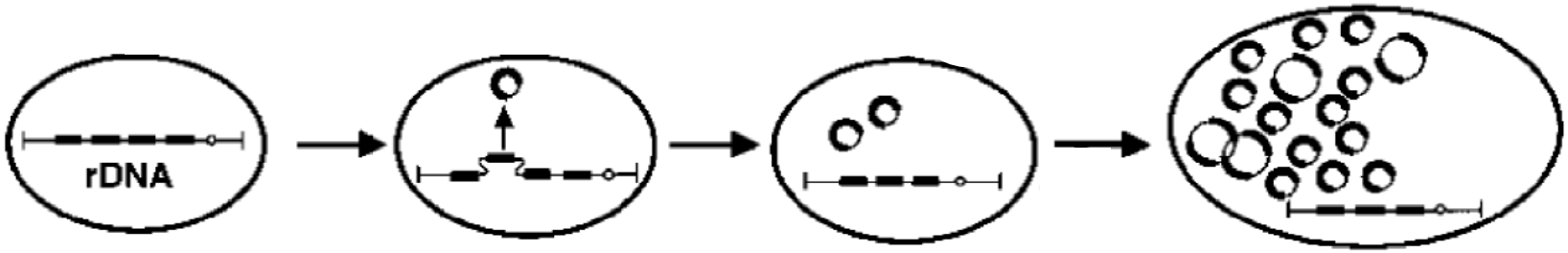
Genes to Cells (2011) **16**, 617–624

FOB1-dependent accumulation of ERCs occurs more rapidly in a sir2 mutant than in wild-type mother cells



GENES & DEVELOPMENT 13:2570–2580

Minicírculos de rDNA são formados ao longo do envelhecimento



a young cell

b excision/ inheritance of ERC

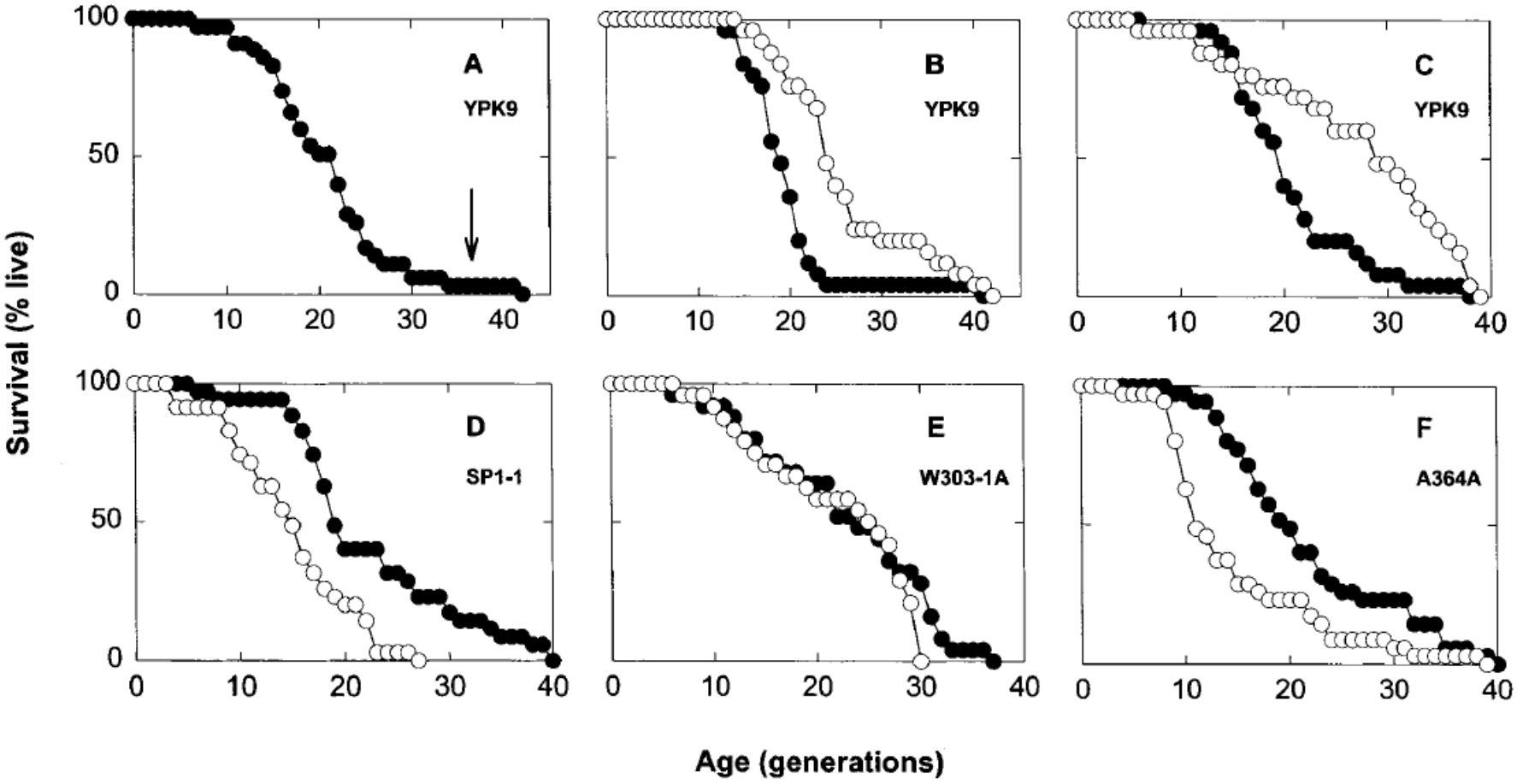
c replication recombination

d old cell nucleolar fragmentation death

Cell, Vol. 91, 1033–1042, December 26, 1997,

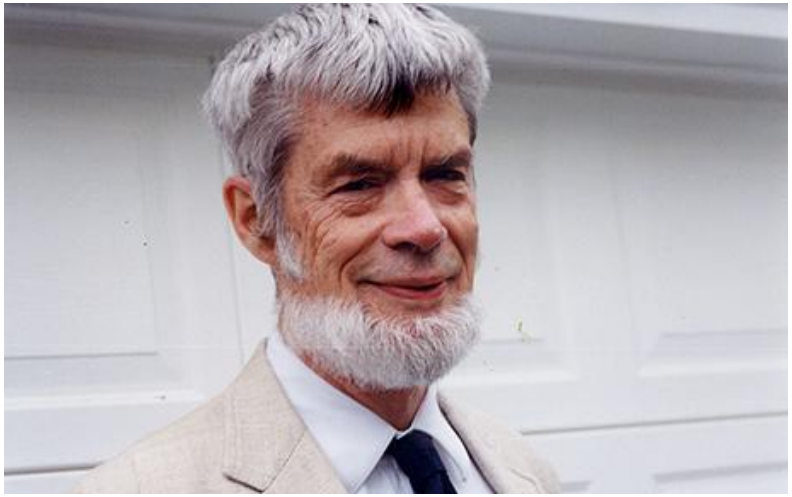
E o DNA mitocondrial?

Life spans of grande and petite strains with glucose as a carbon source



Genetics **152:** 179–190 (May 1999)

Teorias da pleiotropia antagonística



George Williams

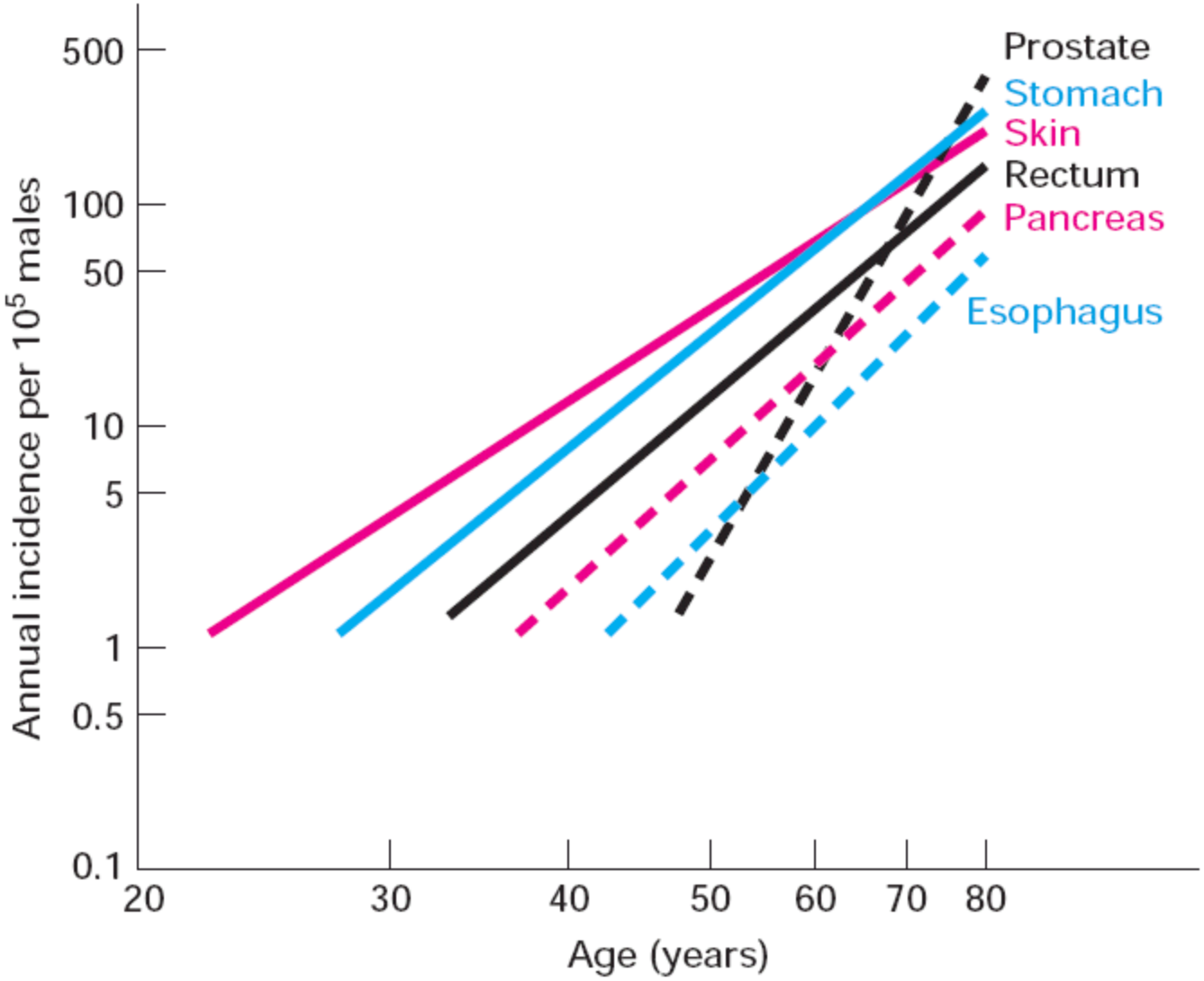
Teoria do “pague depois”.

Alguns genes são benéficos na fase inicial da vida, mas são deletérios na fase tardia (genes pleitrópicos).

A seleção natural frequentemente maximizará o vigor na juventude às custas do vigor na fase adulta, produzindo um declínio do vigor (envelhecimento) na fase tardia da vida.

Pleiotropia antagonística em pavões machos

Incidência de câncer em homens



Cell Reports
Article

Cell PRESS
Open ACCESS

The Genomic Landscape and Evolutionary Resolution of Antagonistic Pleiotropy in Yeast

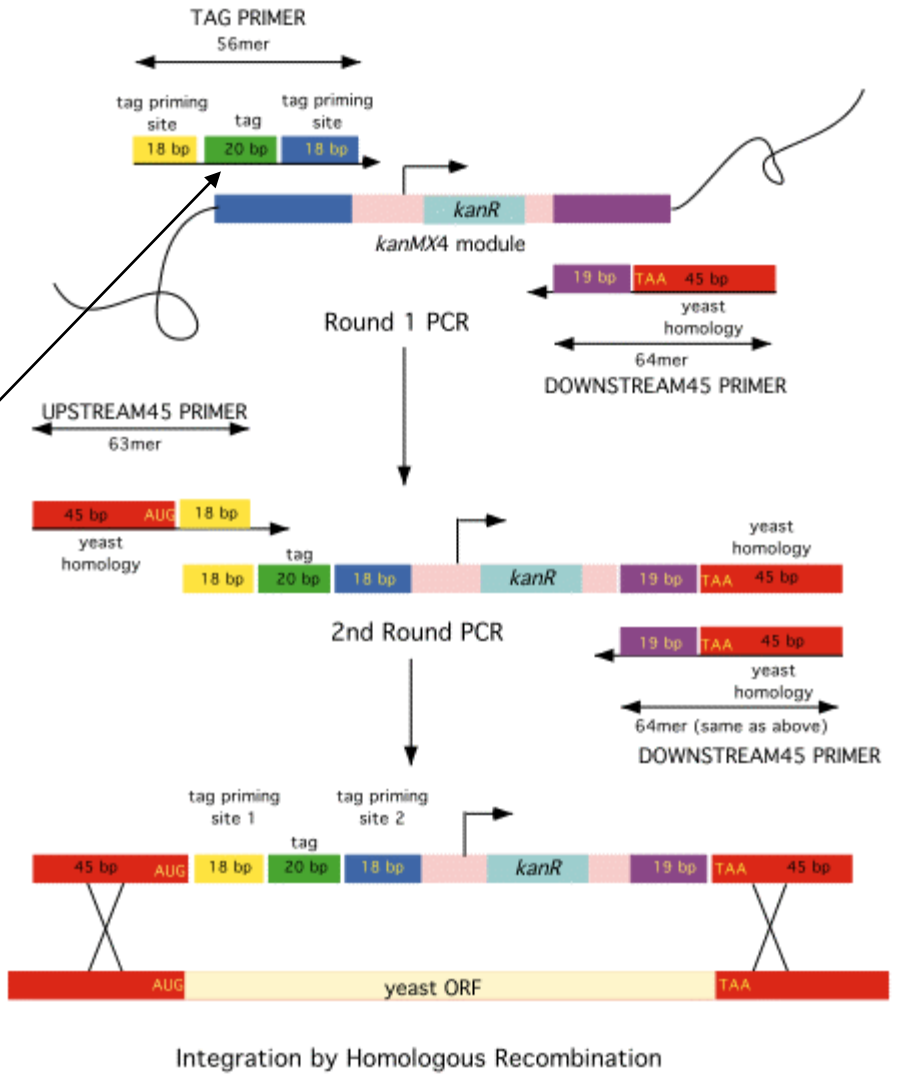
Wenfeng Qian,[1] Di Ma,[2,3] Che Xiao,[1,4,5] Zhi Wang,[1,6] and Jianzhi Zhang[1,*]
[1]Department of Ecology and Evolutionary Biology
[2]Department of Cell and Developmental Biology
[3]Life Sciences Institute
University of Michigan, Ann Arbor, MI 48109, USA
[4]School of Life Sciences, Peking University, Beijing 100871, China
[5]Present address: Graduate Program in Genetics, Stony Brook University, Stony Brook, NY 11794, USA
[6]Present address: The Biodesign Institute, Arizona State University, Tempe, AZ 85287, USA
*Correspondence: jianzhi@umich.edu
http://dx.doi.org/10.1016/j.celrep.2012.09.017

Construção da coleção de cepas de levedura com rompimento gênico único

≅6000 coleção de diploides heterozigotos

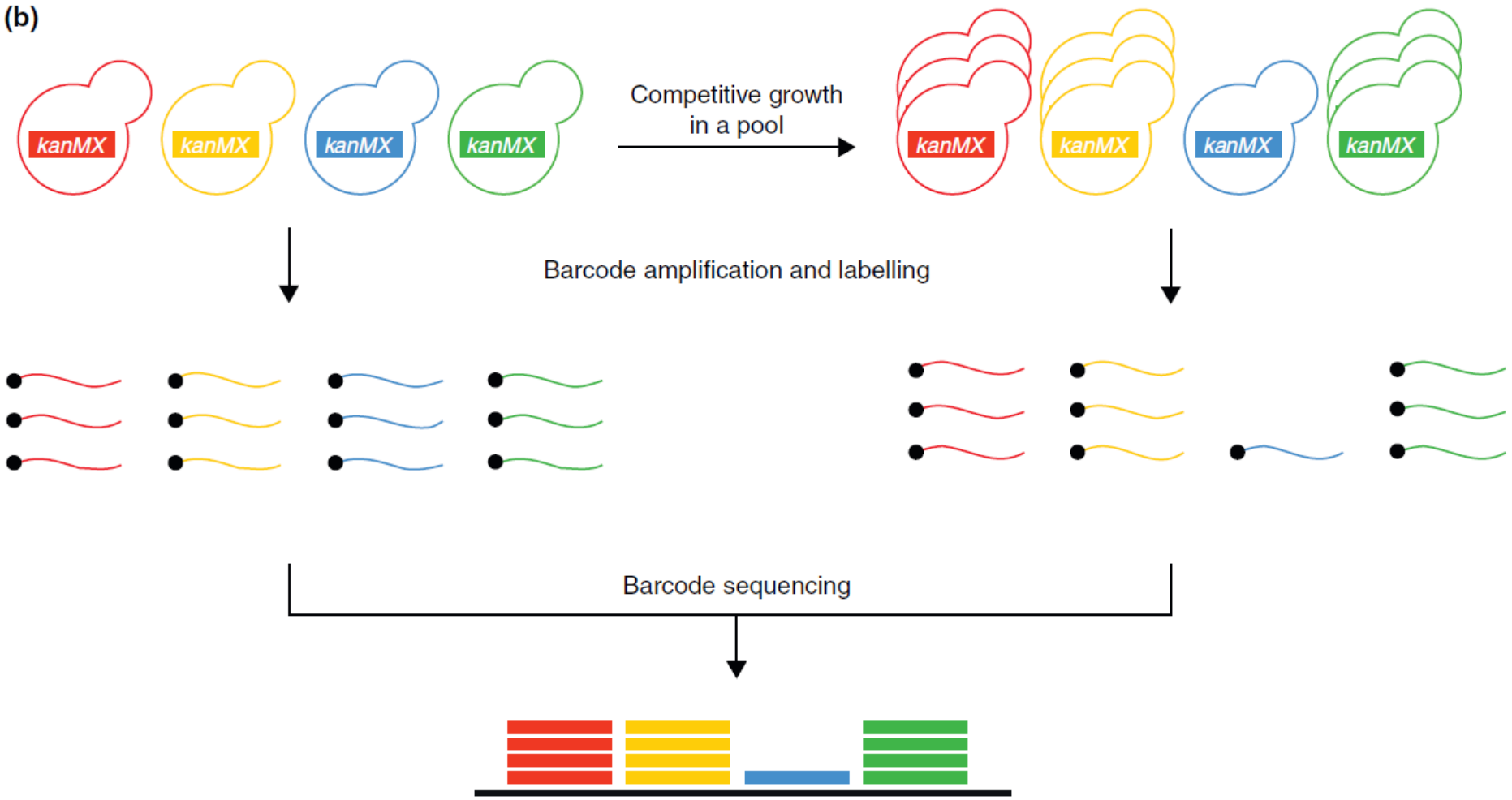
≅5000 haplóides viáveis

“código de barras” (20 pb)



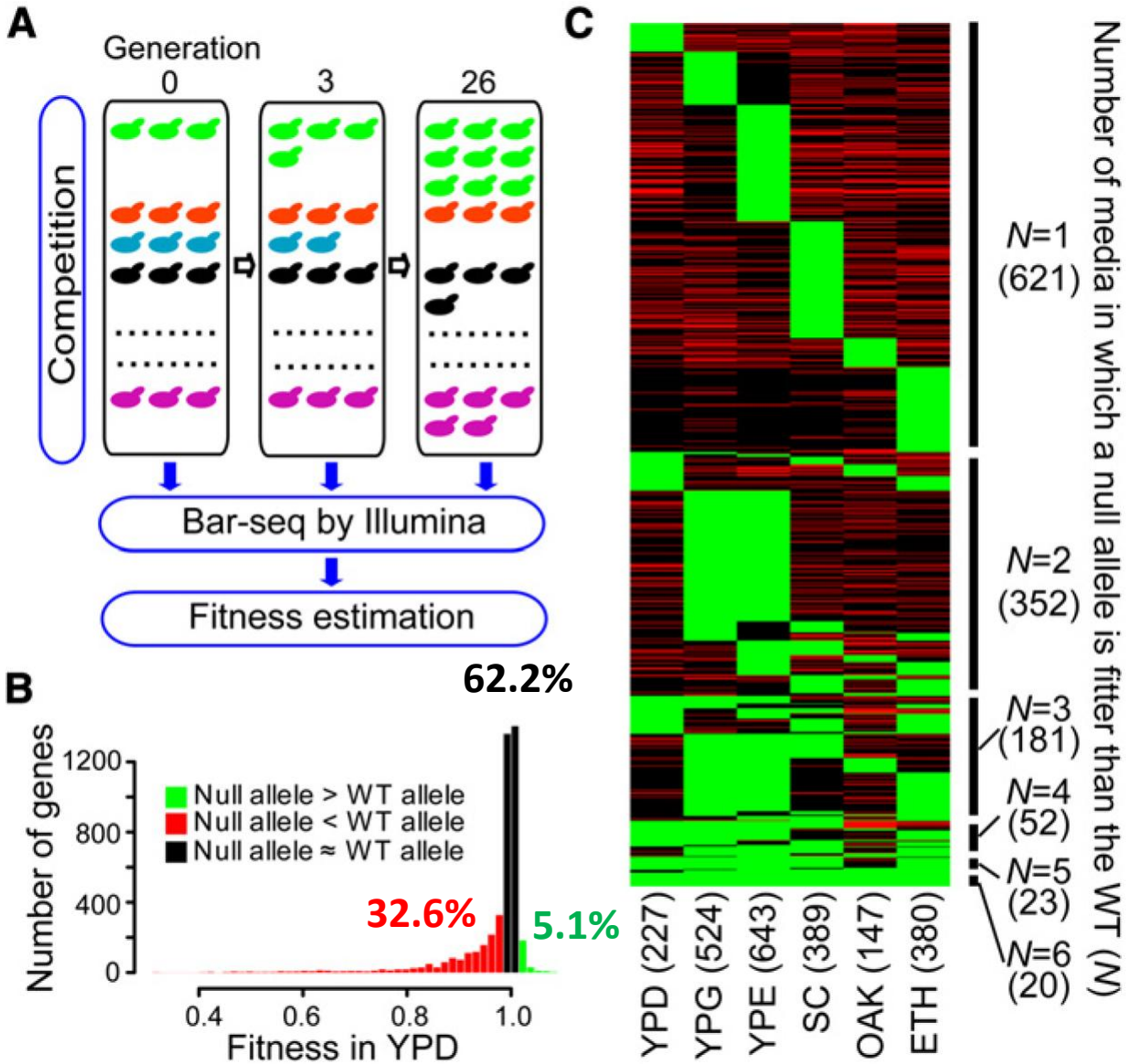
http://www-sequence.stanford.edu/group/yeast_deletion_project/first_round.html

A levedura como modelo de estudo da ação de pequenas moléculas

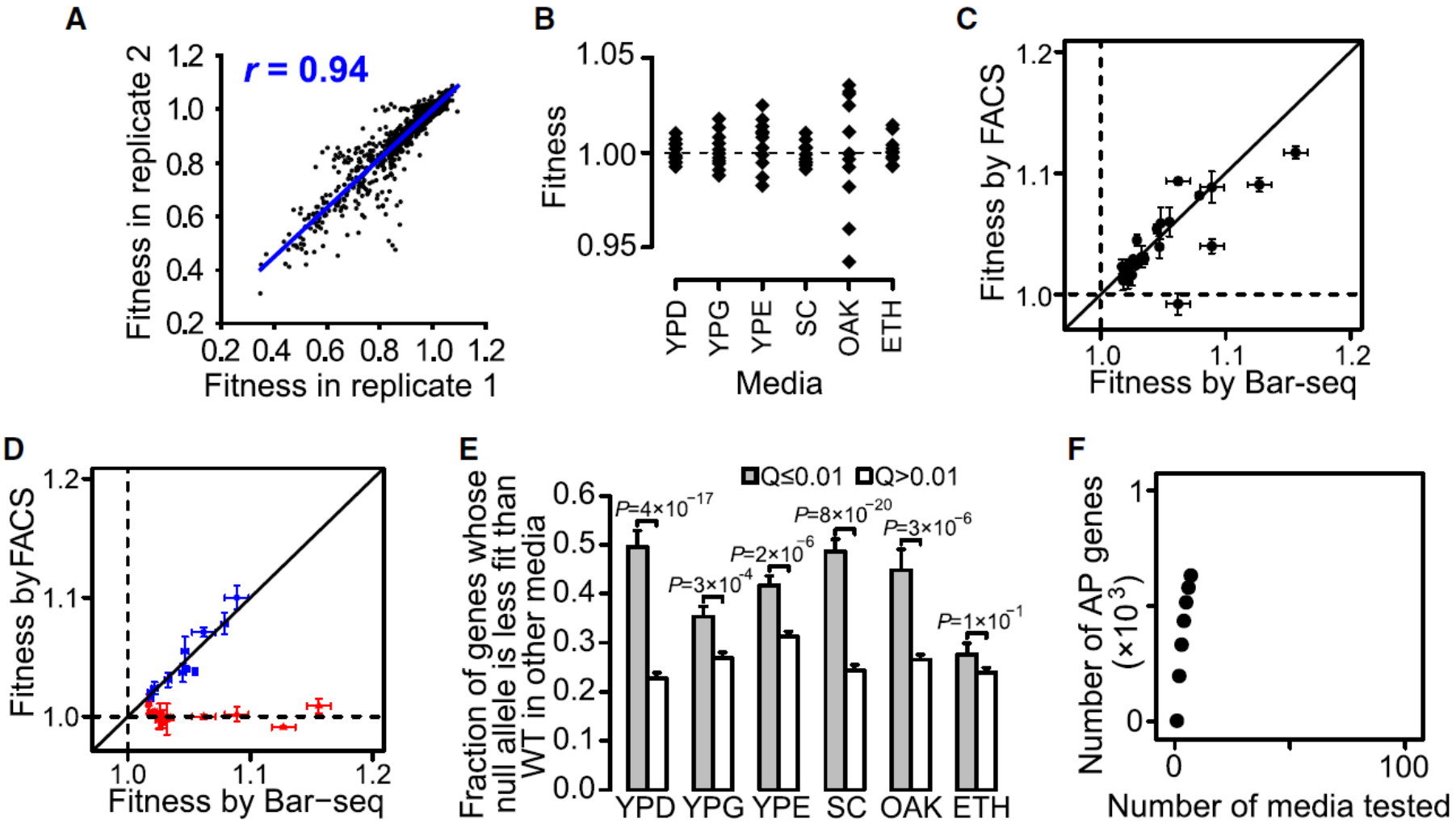


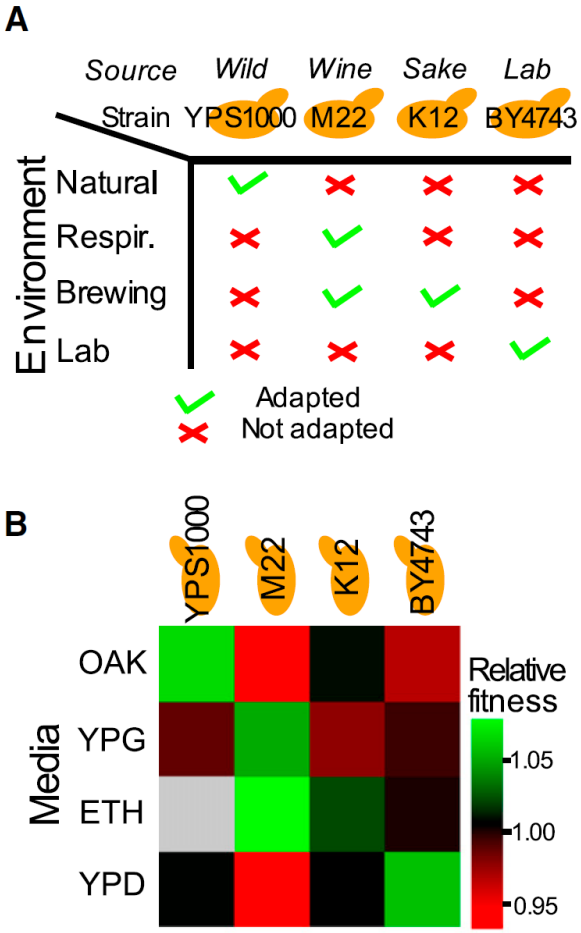
Current Opinion in Cell Biology

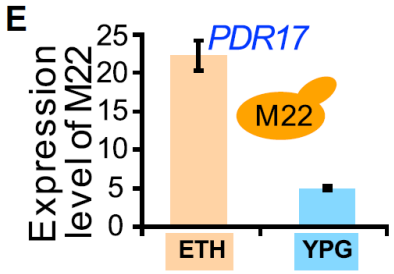
Genome-wide Identification of Yeast Genes Subject to AP in Six Environments



Validations of Bar-Seq Results







AP Is at Least Partially Resolved by Gene Regulation in the Presence of Sufficient Selection

If the expression of a gene is beneficial in environment A but harmful in environment B.

Predictions

1. the expression level of the gene in B should be lower for a strain more adapted to B than for a strain less adapted to B;

2. for a strain adapted to both environments, the expression of the gene should be lower in B than in A;

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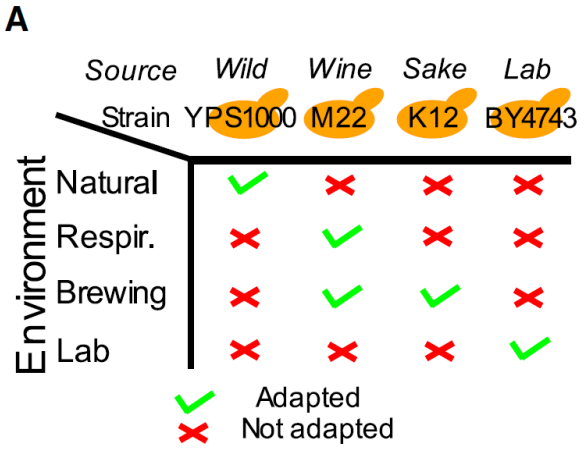

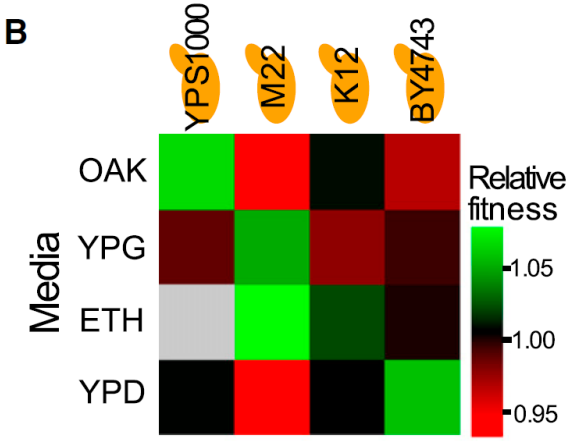

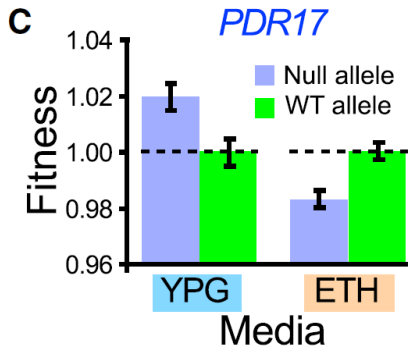

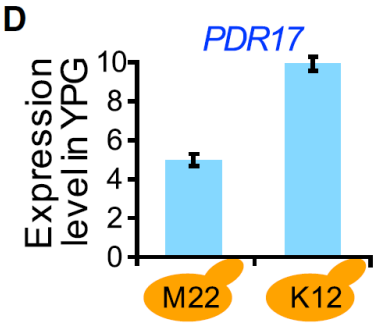

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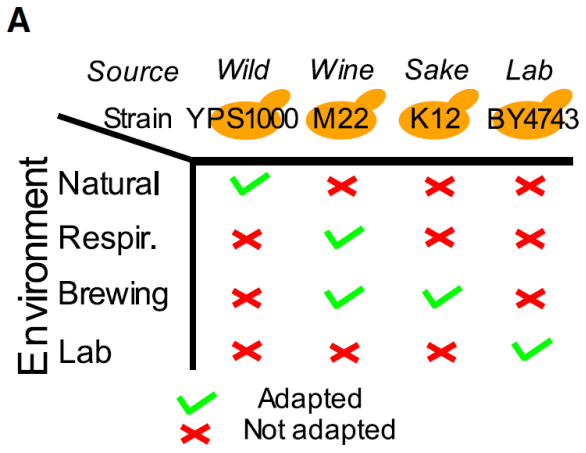

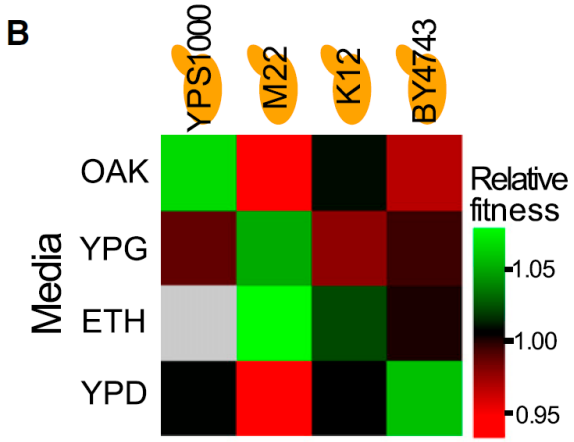

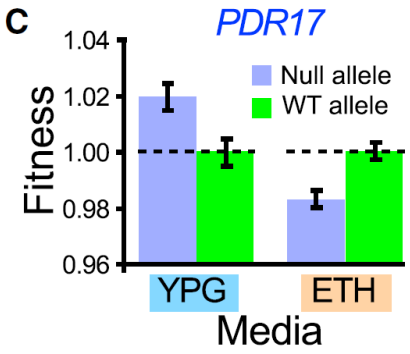

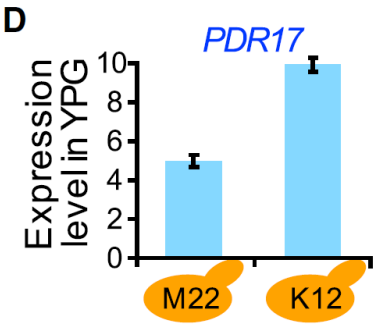

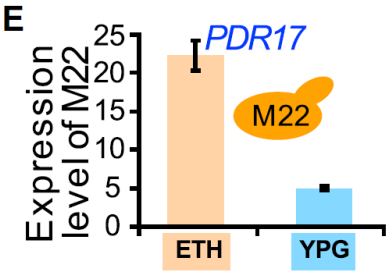

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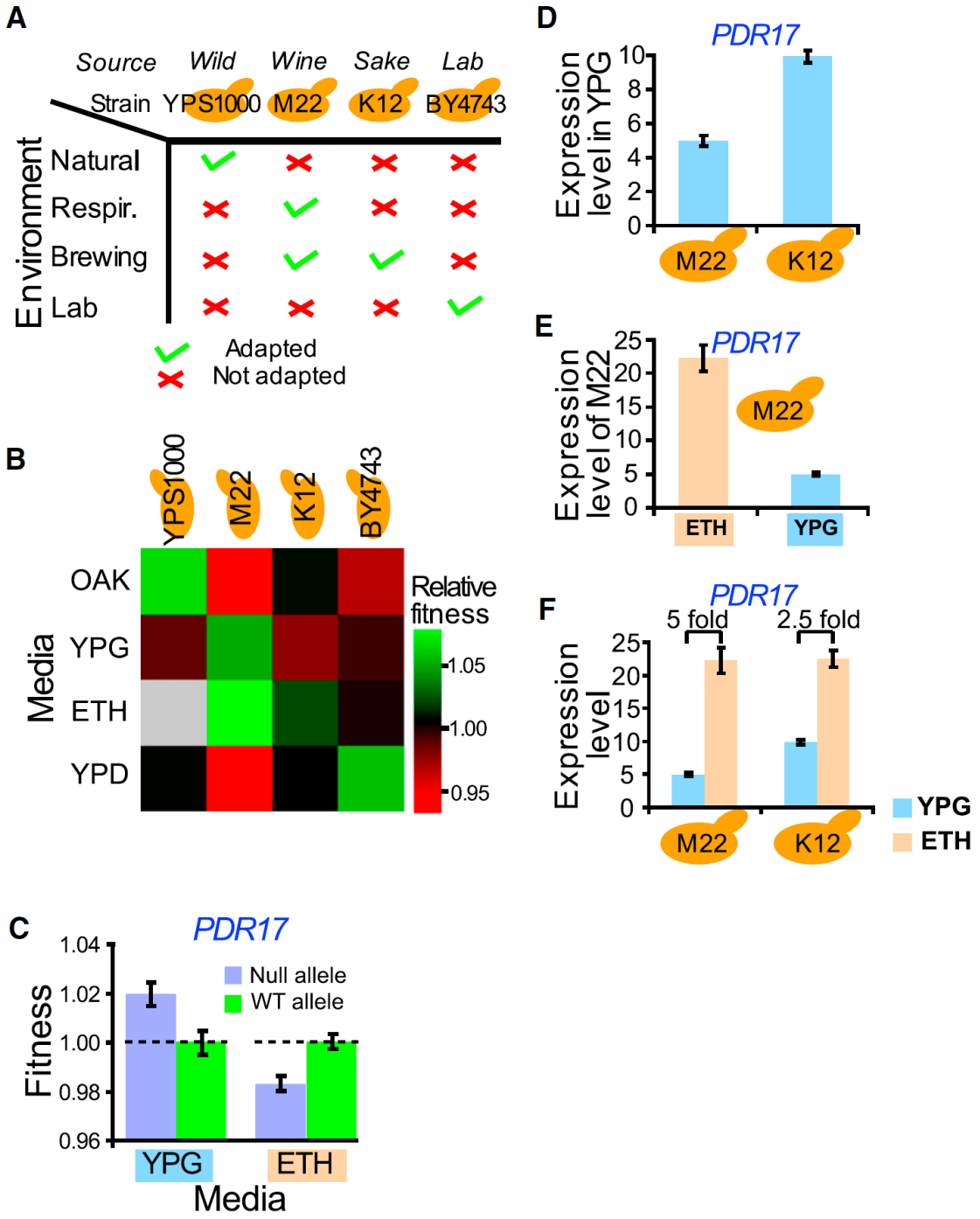

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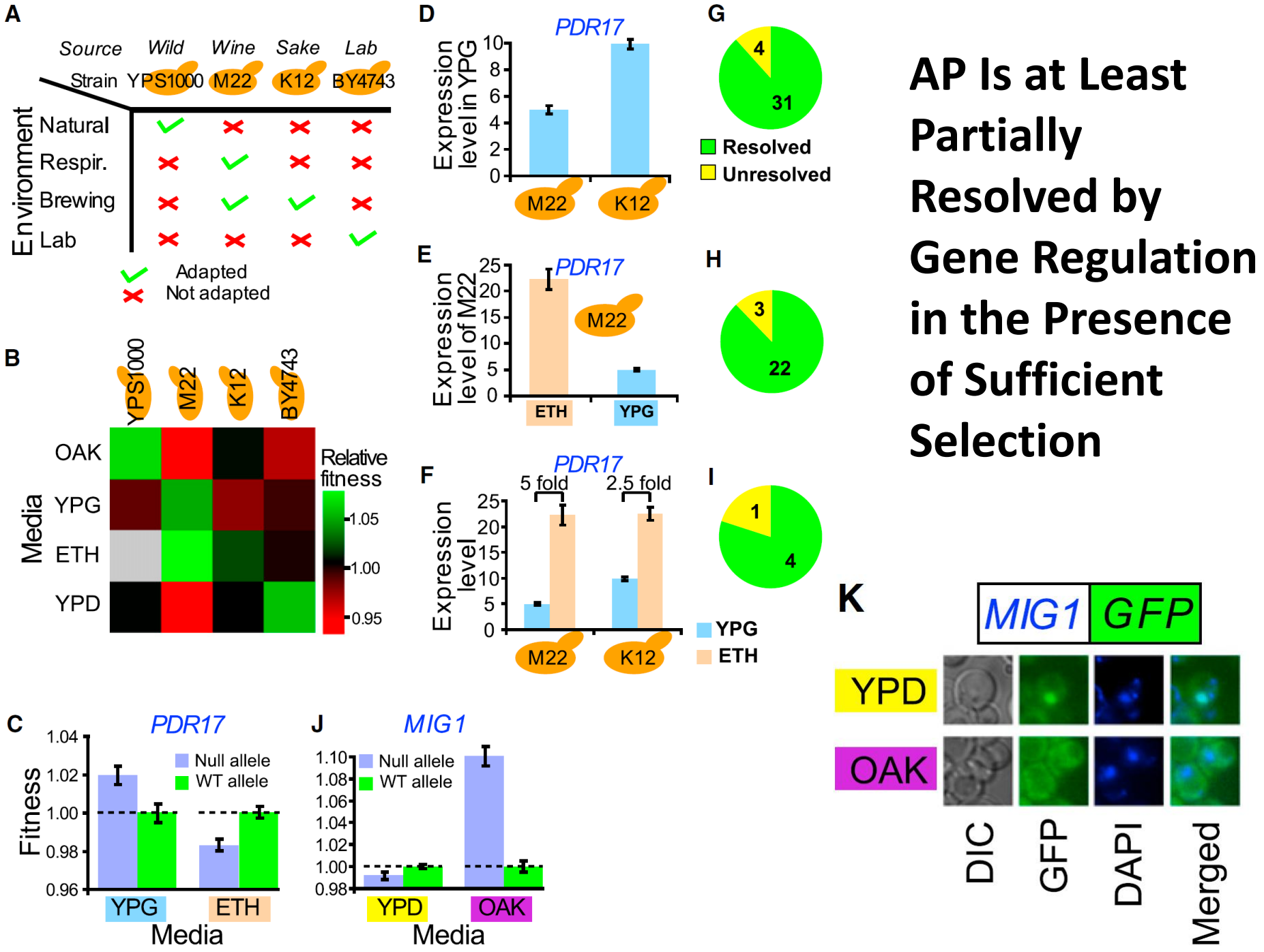


AP Is at Least Partially Resolved by Gene Regulation in the Presence of Sufficient Selection

Teoria do SOMA descartável

Thomas Kirkwood

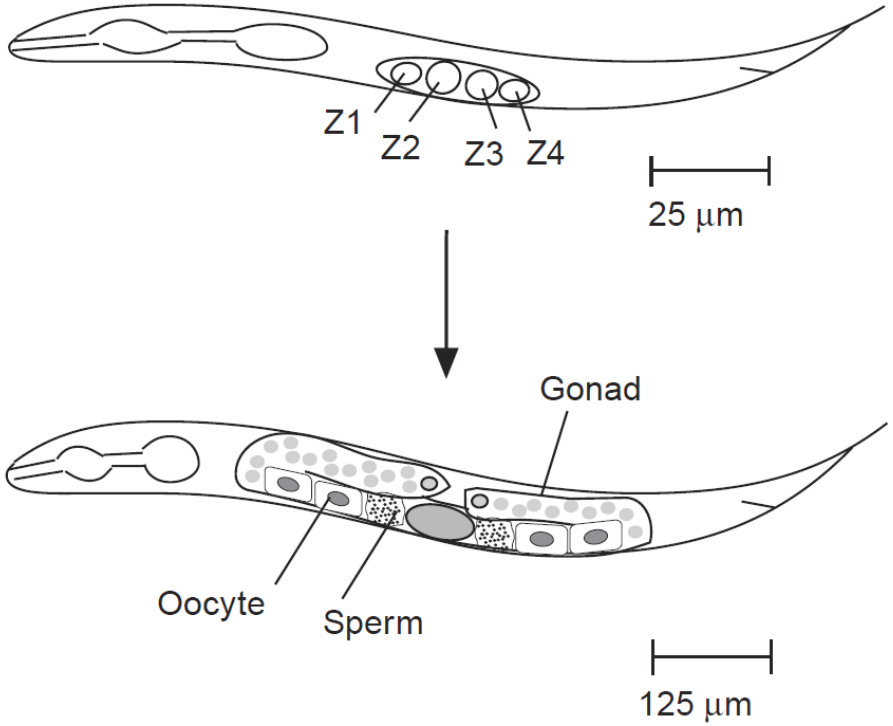
Extensão da Teoria da Pleiotropia Antagonística;

Compromisso entre a energia investida na reprodução versus manutenção somática, reparo e sobrevivência;

O envelhecimento surgiu porque a Seleção Natural favorece genes que aumentam investimento em reprodução às custas da energia necessária à sobrevivência.

Caenorhabditis elegans

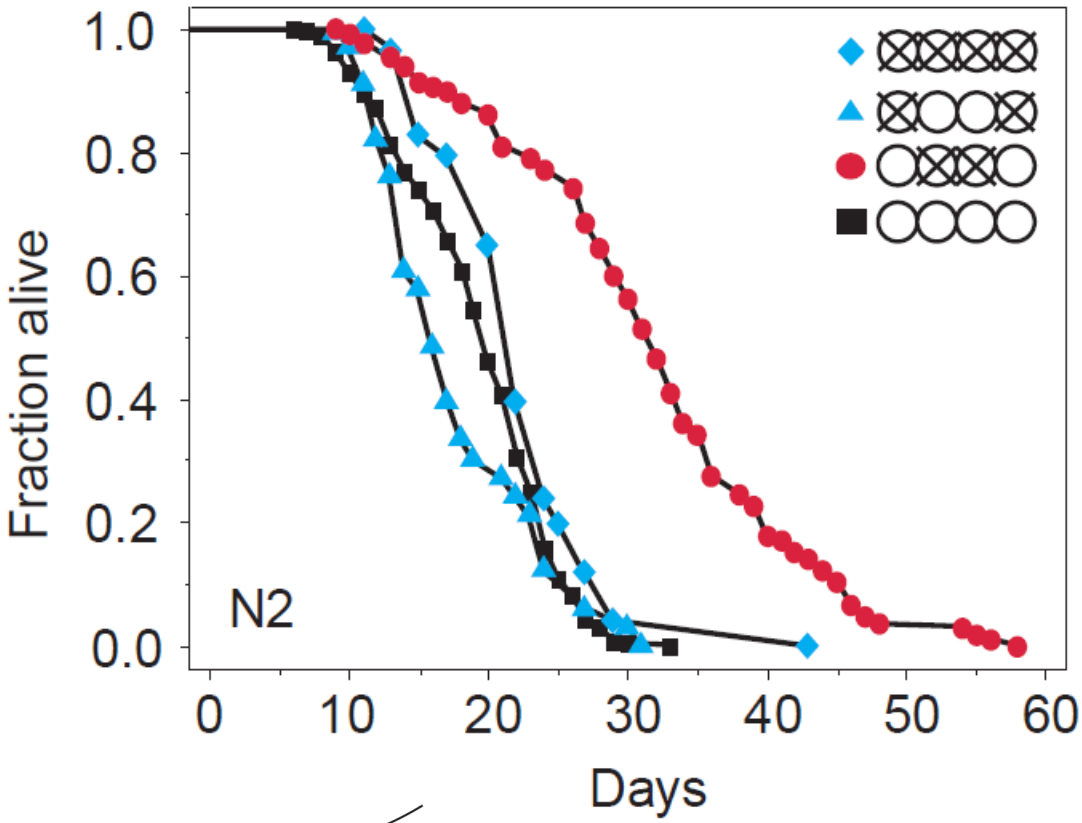
A gônada de *C. elegans*

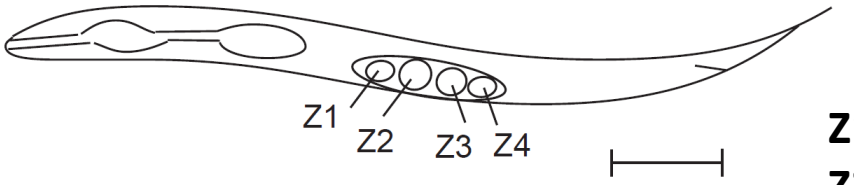


Z1, Z4 – somatic gonad
Z2, Z3 – germ line gonad

Nature (1999) **399**:362-6.

Comparação entre as longevidades de linhagens de *C. elegans* férteis e estéreis



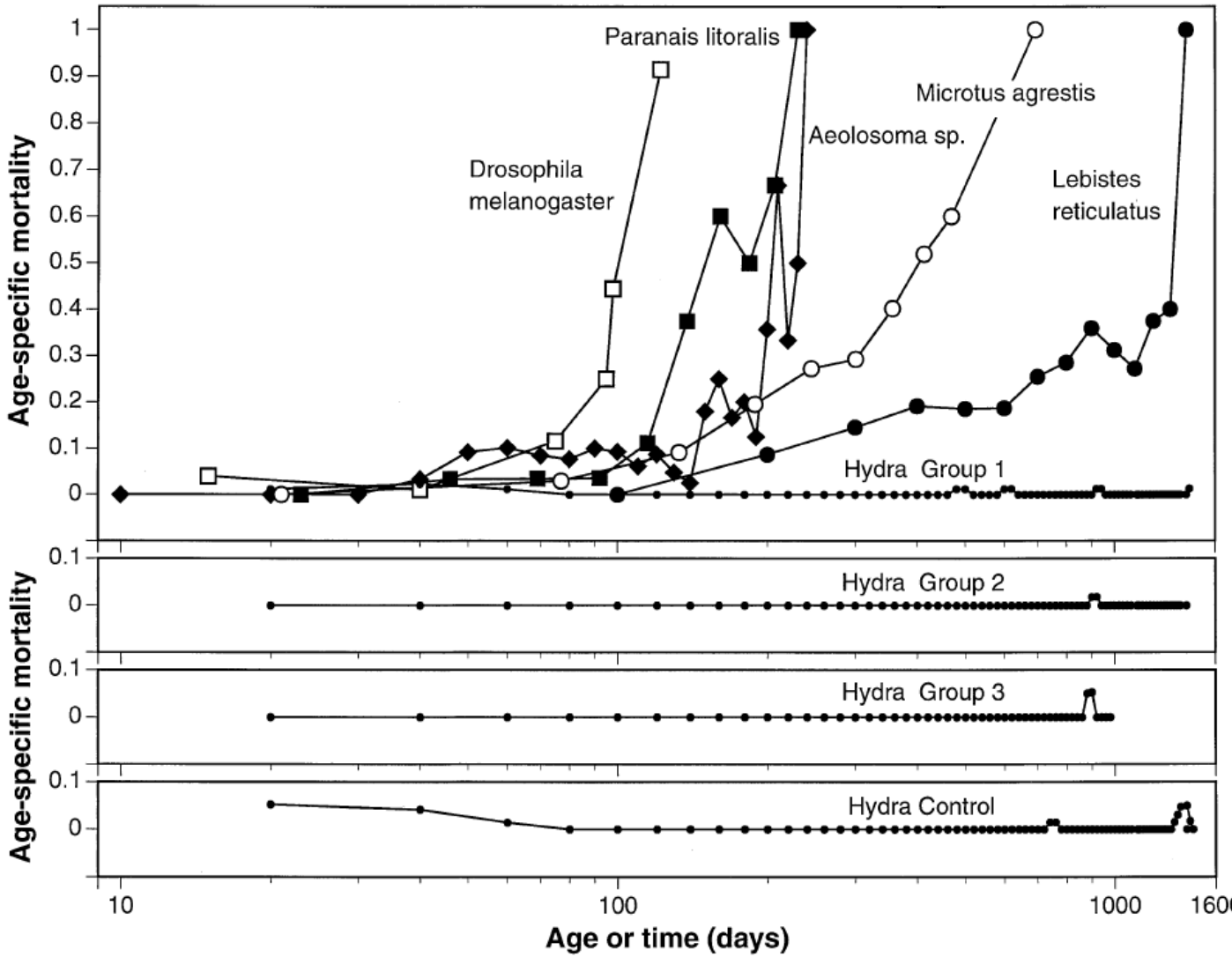


Z1, Z4 – somatic gonad
Z2, Z3 – germ line gonad

Nature (1999) **399**:362-6.

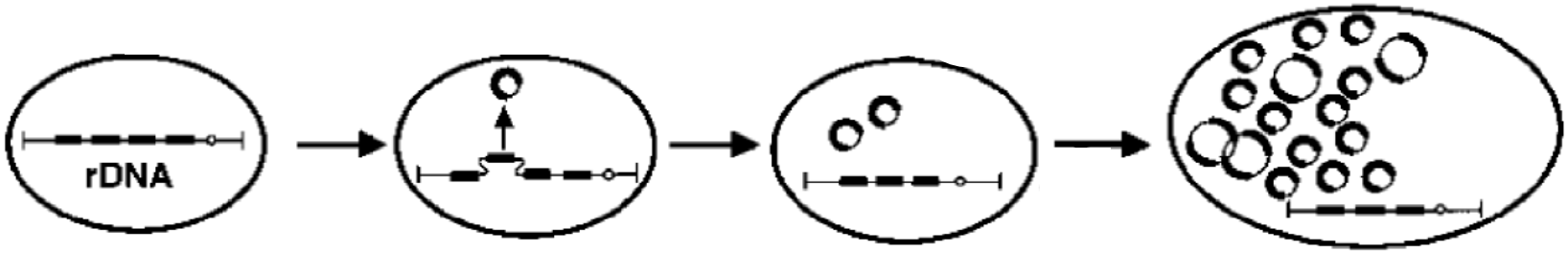
Hydra, um modelo para a teoria do SOMA descartável

Hydra viridissima



Experimental Gerontology, **33**: 217-225

Minicírculos de rDNA são formados ao longo do envelhecimento



a young cell

b excision/ inheritance of ERC

c replication recombination

d old cell nucleolar fragmentation death

Cell, Vol. 91, 1033–1042, December 26, 1997,

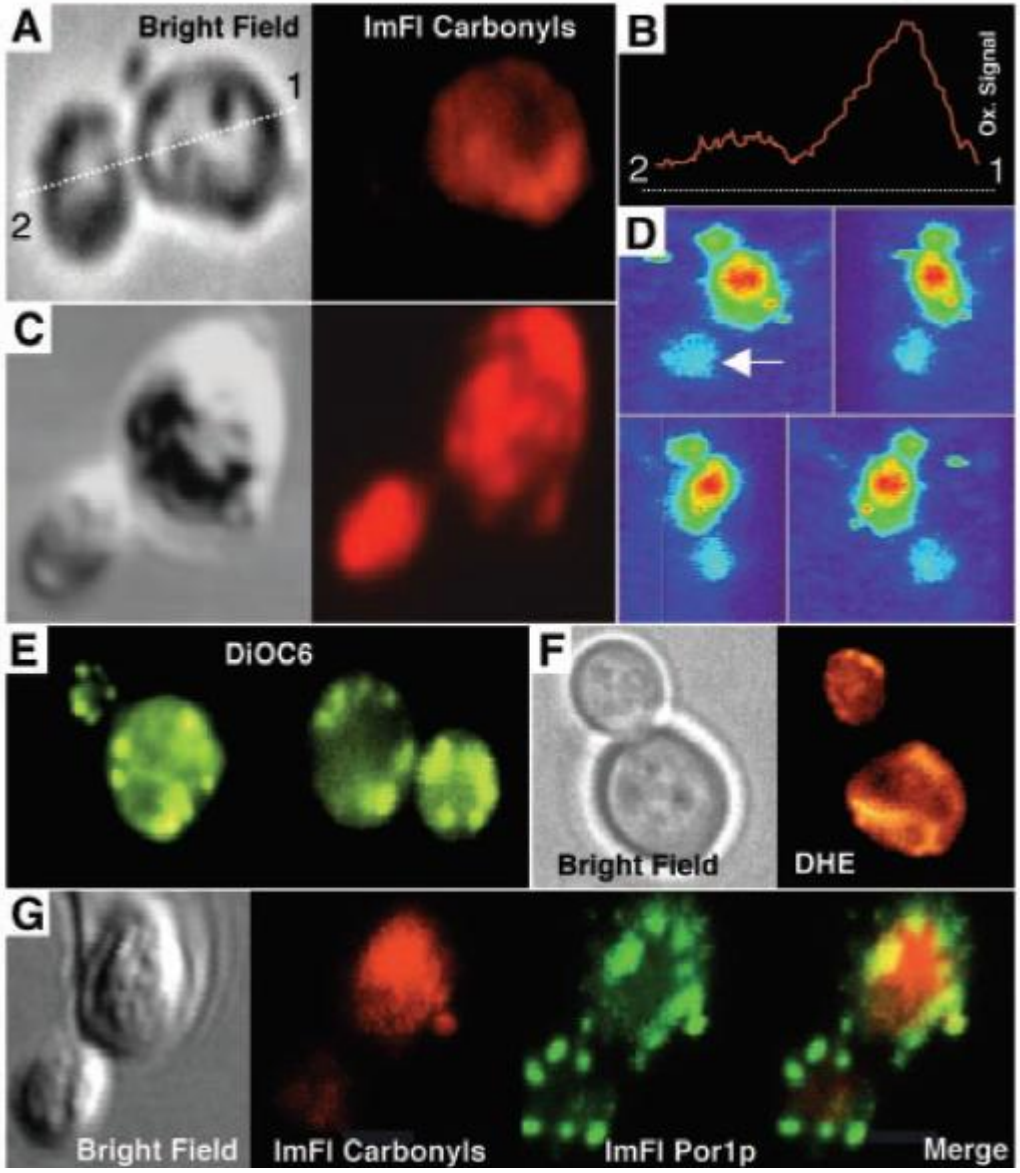
Asymmetric Inheritance of Oxidatively Damaged Proteins During Cytokinesis

Hugo Aguilaniu,[1,2,3] Lena Gustafsson,[2] Michel Rigoulet,[3] Thomas Nyström[1]

Carbonylated proteins were visualized in single cells of the budding yeast *Saccharomyces cerevisiae*, revealing that they accumulate with replicative age. Furthermore, carbonylated proteins were not inherited by daughter cells during cytokinesis. Mother cells of a yeast strain lacking the *sir2* gene, a life-span determinant, failed to retain oxidatively damaged proteins during cytokinesis. These findings suggest that a genetically determined, Sir2p-dependent asymmetric inheritance of oxidatively damaged proteins may contribute to free-radical defense and the fitness of newborn cells.

Science (2003) **299**:1751-3

Distribution of oxidatively damaged proteins, mitochondria, and ROS during yeast cytokinesis.



Scan of (A)

Paraquat

Confocal depth scan

Mito $\Delta\Psi$

$O_2^{\bullet-}$

Science (2003) **299**:1751-3

Sir2p-dependent protein segregation gives rise to a superior reactive oxygen species management in the progeny of *Saccharomyces cerevisiae*

Nika Erjavec and Thomas Nyström*

Department of Cell and Molecular Biology–Microbiology, Göteborg University, Medicinaregatan 9C, 413 90 Göteborg, Sweden

Edited by E. R. Stadtman, National Institutes of Health, Bethesda, MD, and approved May 11, 2007 (received for review February 21, 2007)

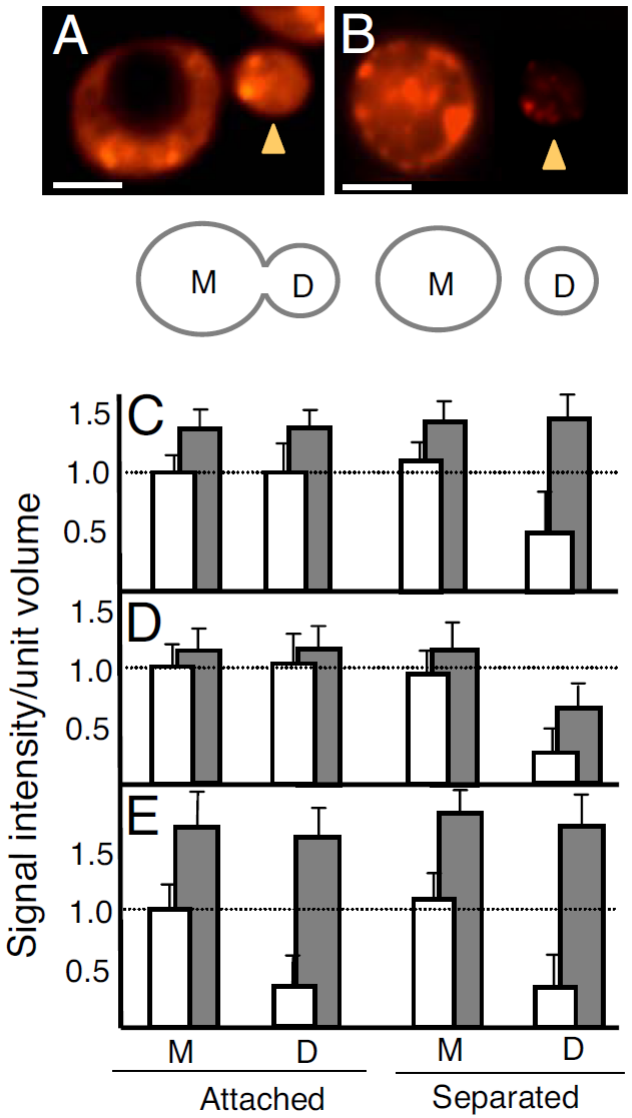
Yeast cytokinesis entails a rejuvenation process by which the aged mother cell generates daughter cells enjoying full replicative potential. Here we show that this process includes a precipitous reduction in the levels of reactive oxygen species in the progeny immediately after completion of cytokinesis. The reduction in hydrogen peroxide is the result of a Sir2p and actin cytoskeleton-dependent segregation of the cytosolic catalase Ctt1p such that the daughter cell receives a higher load of undamaged and active Ctt1p than the progenitor cell. Such spatial quality control provides the daughter cells with a superior capacity to combat external oxidative stress and delays self-inflicted oxidative damage to their

under the control of Sir2p, a conserved NAD-dependent histone deacetylase, acting as a key regulator of aging in a variety of organisms, including yeast, worms, and flies (18–21). The failure of the *sir2*Δ mutant mother cells to retain damage appears to act independently of ERC accumulation, because preventing the propagation of ERCs in a *sir2* mutant by deleting the replication fork block gene *FOB1* does not rescue the loss of damage asymmetry caused by deleting *SIR2* (unpublished data). In addition, the failure in damage segregation is not due to aging as such because the *sir2*Δ*fob1*Δ double mutant does not age prematurely

ROS levels are reduced in daughter cells in a Sir2p-dependent way after completion of cytokinesis

WT

sir2Δ



H_2O_2

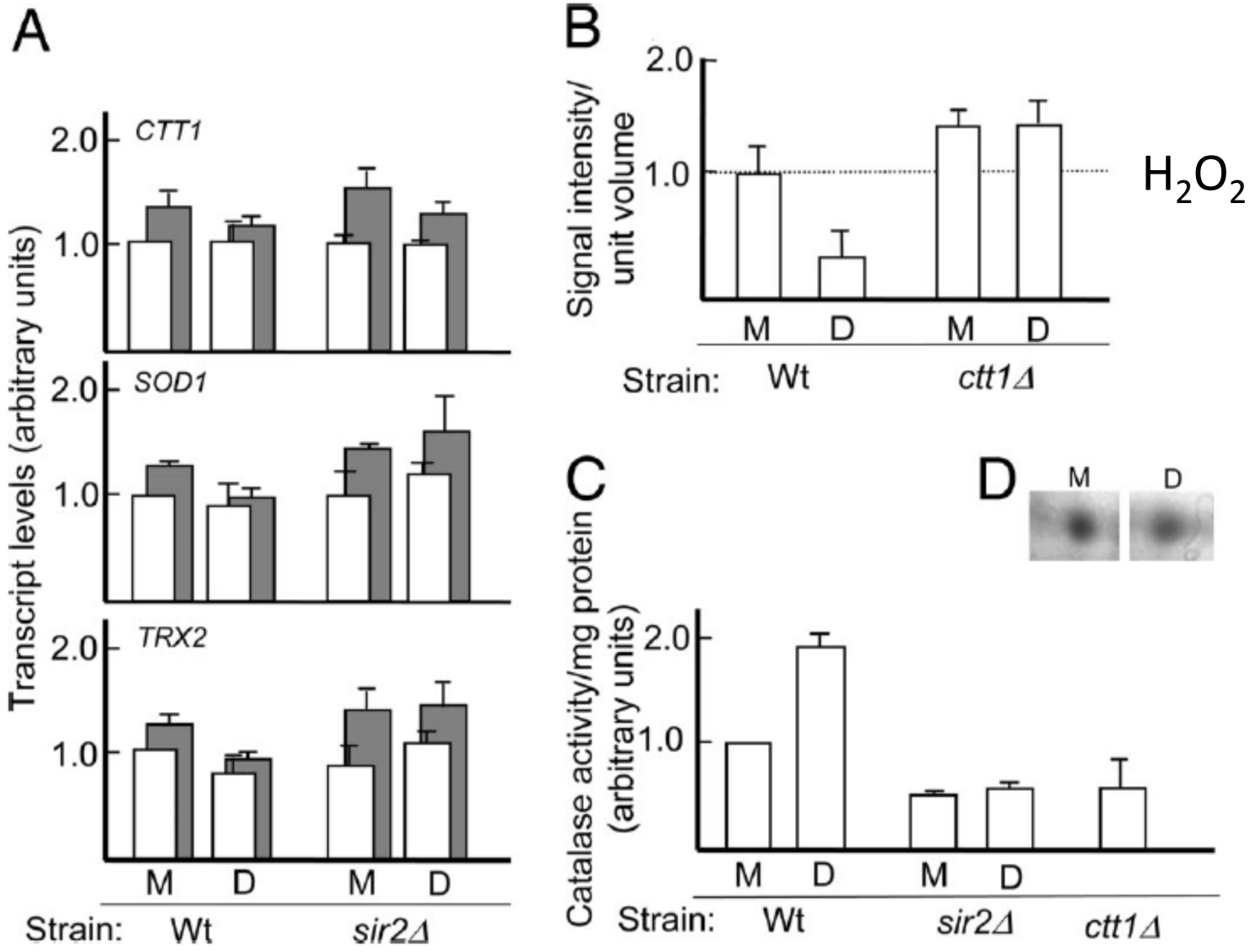
$O_2^{\bullet-}$

H_2O_2

Carbonyl levels

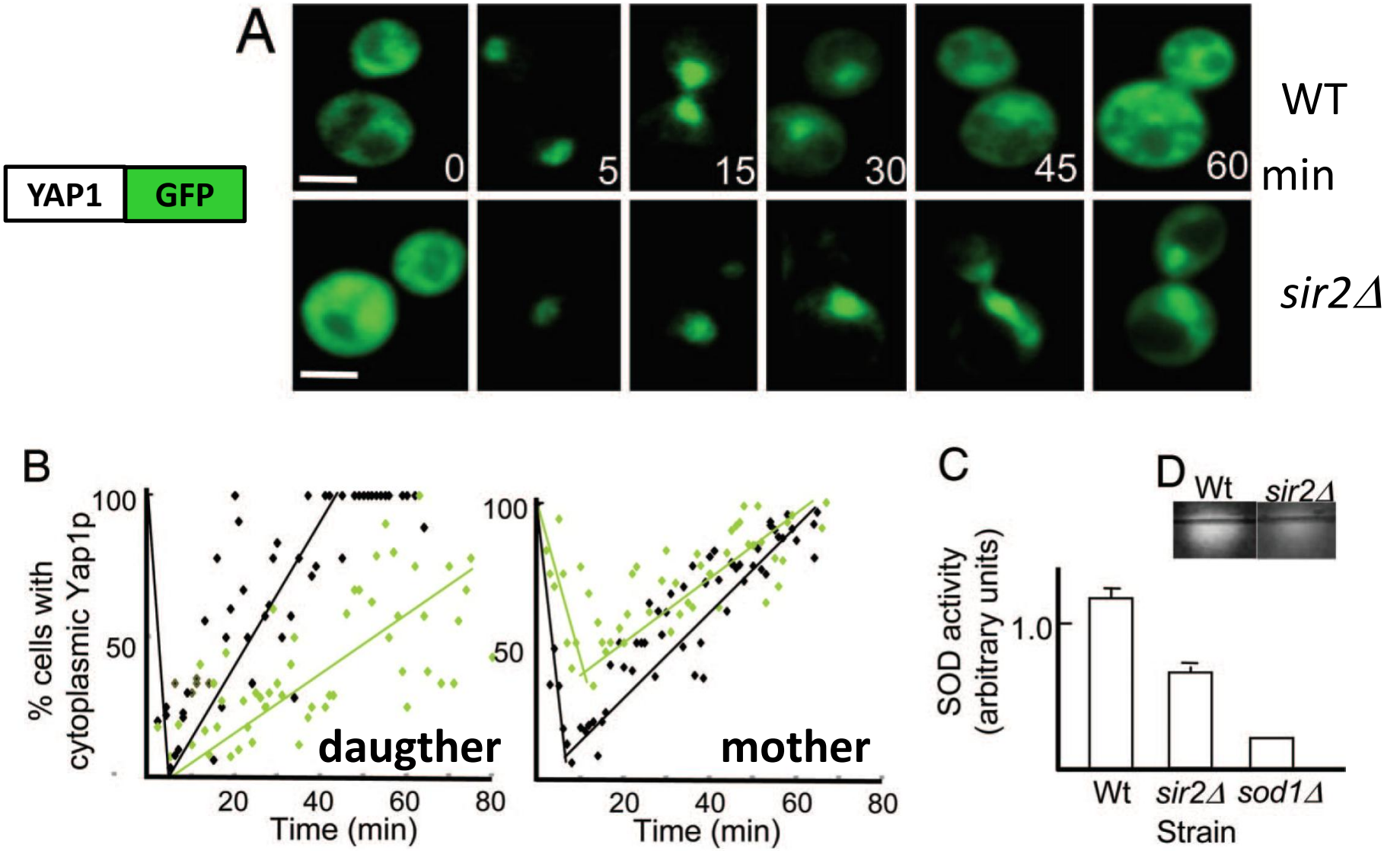
Proc Natl Acad Sci U S A. (2007) **104**:10877-81

A Sir2p- and Ctt1p-dependent process ensures asymmetrical hydrogen peroxide scavenging



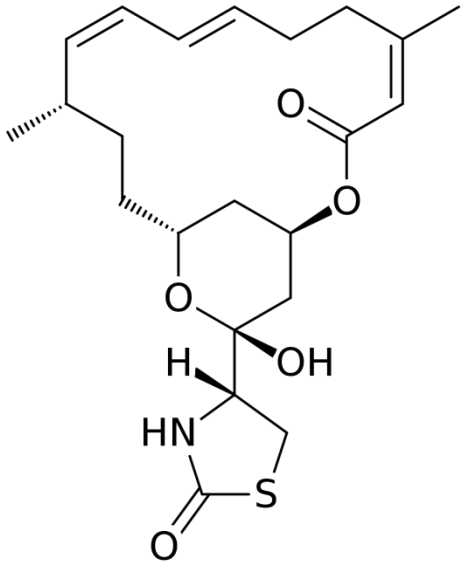
Proc Natl Acad Sci U S A. (2007) **104**:10877-81

Daughter cells display a Sir2p-dependent superior capacity to respond to external oxidants

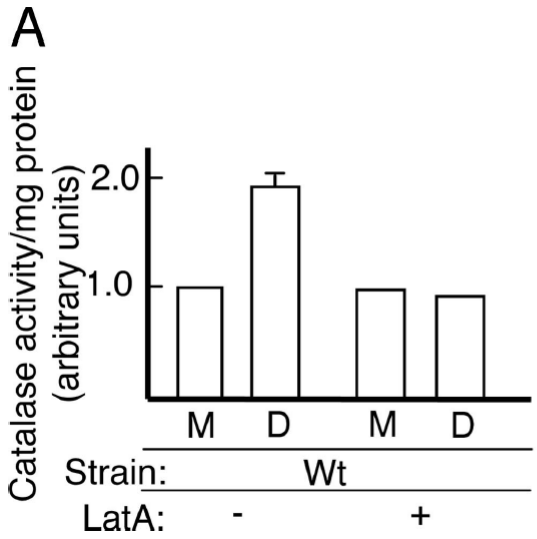


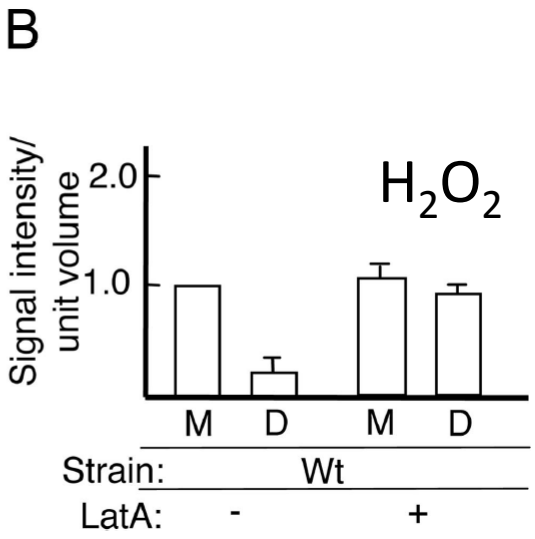
Proc Natl Acad Sci U S A. (2007) **104**:10877-81

The actin cytoskeleton is required for elevated catalase activity and the purging of peroxide in the yeast progeny



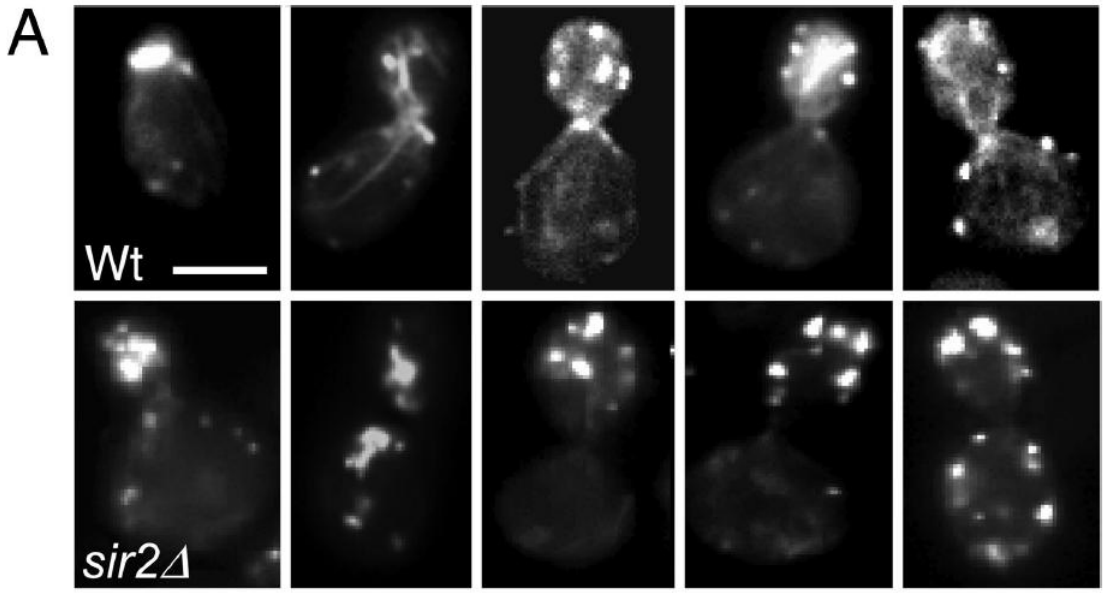
Latrunculin A

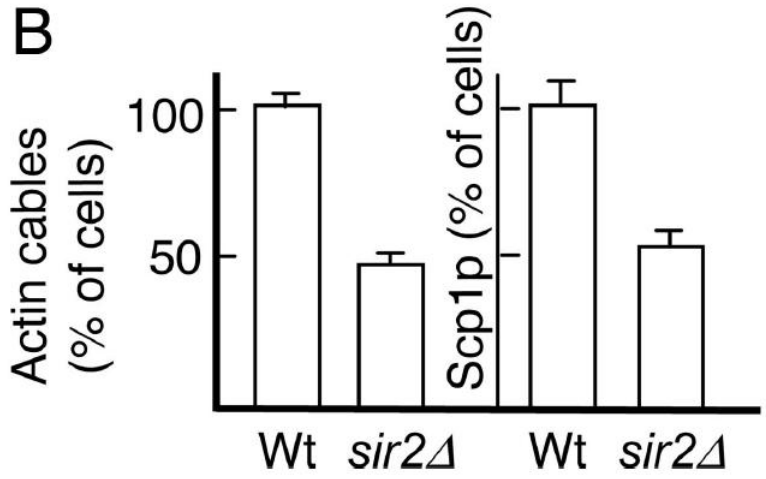




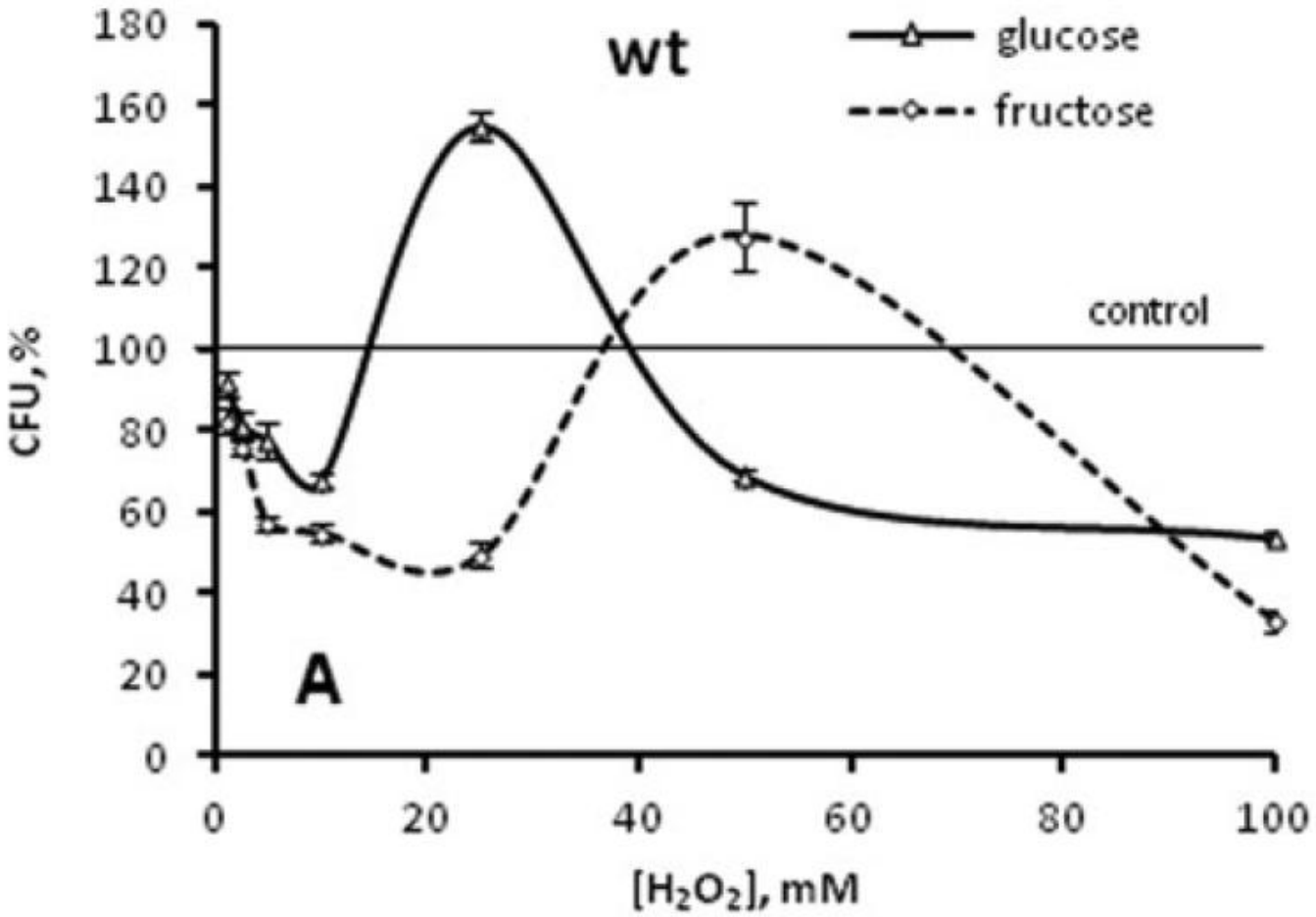
Proc Natl Acad Sci U S A. (2007) **104**:10877-81

Lack of Sir2p results in loss of actin cables and cable-dependent Scp1p localization.





Proc Natl Acad Sci U S A. (2007) **104**:10877-81



Dose Response. (2016) **Vol. 14**: Issue 2 doi: 10.1177/1559325816636130

Paracelsus

Poison is in everything, and no thing is without poison. The dosage makes it either a poison or a remedy.

AZ QUOTES

Síntese

1. A teoria do Acúmulo de Mutações não se aplica ao envelhecimento de *Saccharomyces cerevisiae* porque o número de mutações que acumulam não é suficiente para causar senescência;
2. *SIR2* é um gene envolvido na supressão da formação de círculos extracromossomais de rDNA (ERCs); sua deleção causa diminuição da longevidade;
3. ERCs acumulam ao longo do envelhecimento replicativo da levedura;
4. A Pleiotropia Antagonística é observada em *S. cerevisiae* e as células podem resolvê-la modulando expressão gênica ou modificações pós-traducionais das proteínas;
5. ERCs e proteínas carboniladas segregam com a célula-mãe na citocinese. A célula-filha, além de não ter esses fatores, possui forte defesa antioxidante, cuja segregação depende do citoesqueleto e de *SIR2*. Isso está relacionado à Teoria do Soma descartável.

David Sinclair

https://www.youtube.com/watch?v=YAIck1DUHHU&index=23&list=PL4p9eGkQRuo4eblxlQ1J90lk_PlXmWxH_

Leonard Guarente

https://www.youtube.com/watch?v=1DT6MUUdZDs

Links das imagens

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http://media.npr.org/assets/img/2016/02/01/joseph_wright_of_derby_the_alchemist-1-_slide-b2793277121ff321a1c245ee423cc49c3304c251-s900-c85.jpg

https://upload.wikimedia.org/wikipedia/commons/4/4b/Philippus_aureolus_theophrastus_paracelsus.jpg

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Vittoria de Lima Camandona forneceu gentilmente a foto que consta nesta apresentação

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