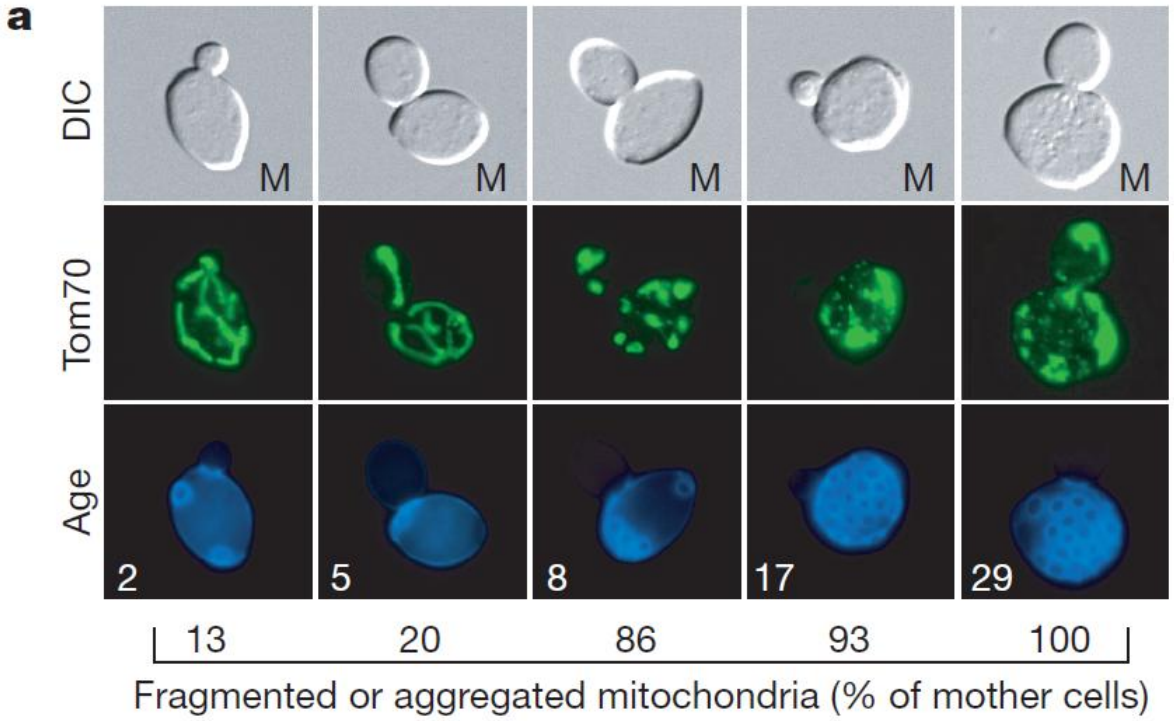
BMM5828/BTC5819 - Utilização de *Saccharomyces cerevisiae* como organismo modelo em biologia molecular

Controle do processo do envelhecimento celular



Objetivos

1. A Curva de Gompertz-Makeham;

2. Ensaio de Longevidade Replicativa;

3. O sacrifício da célula-mãe (segrega ROS, mitocôndria e ERCs);

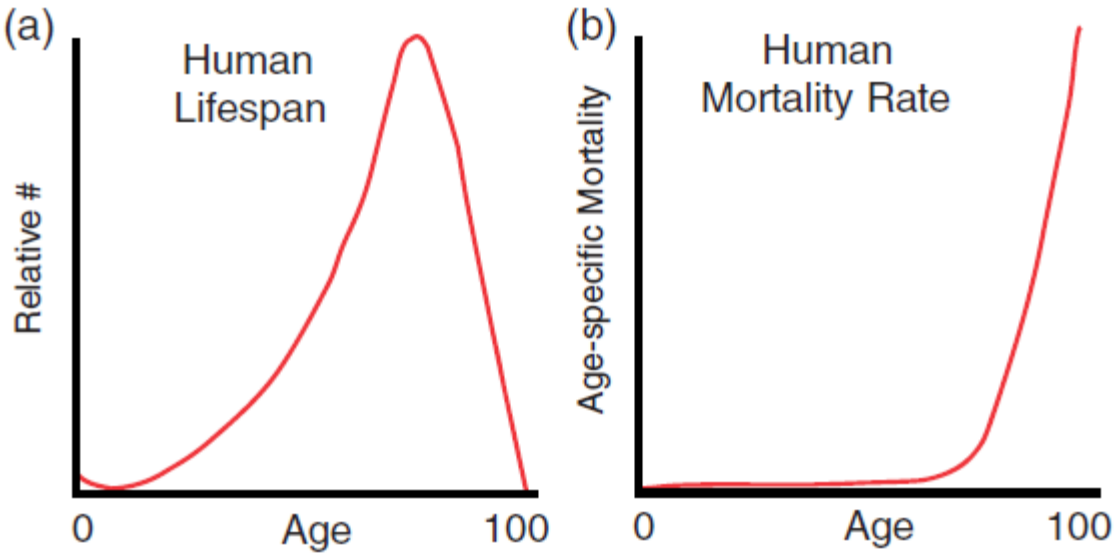
4. Participação das organelas nos mecanismos do envelhecimento: mitocôndria, retículo endoplasmático e vacúolo;

5. Via TOR e restrição calórica;

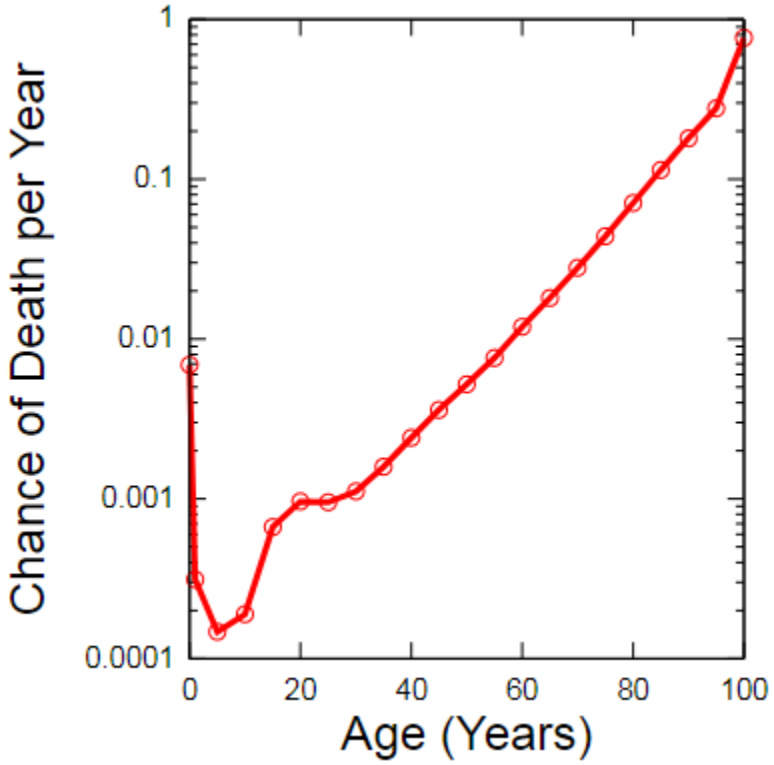
6. Ensaio de Longevidade Cronológica;

7. Mecanismos biológicos da longevidade cronológica.

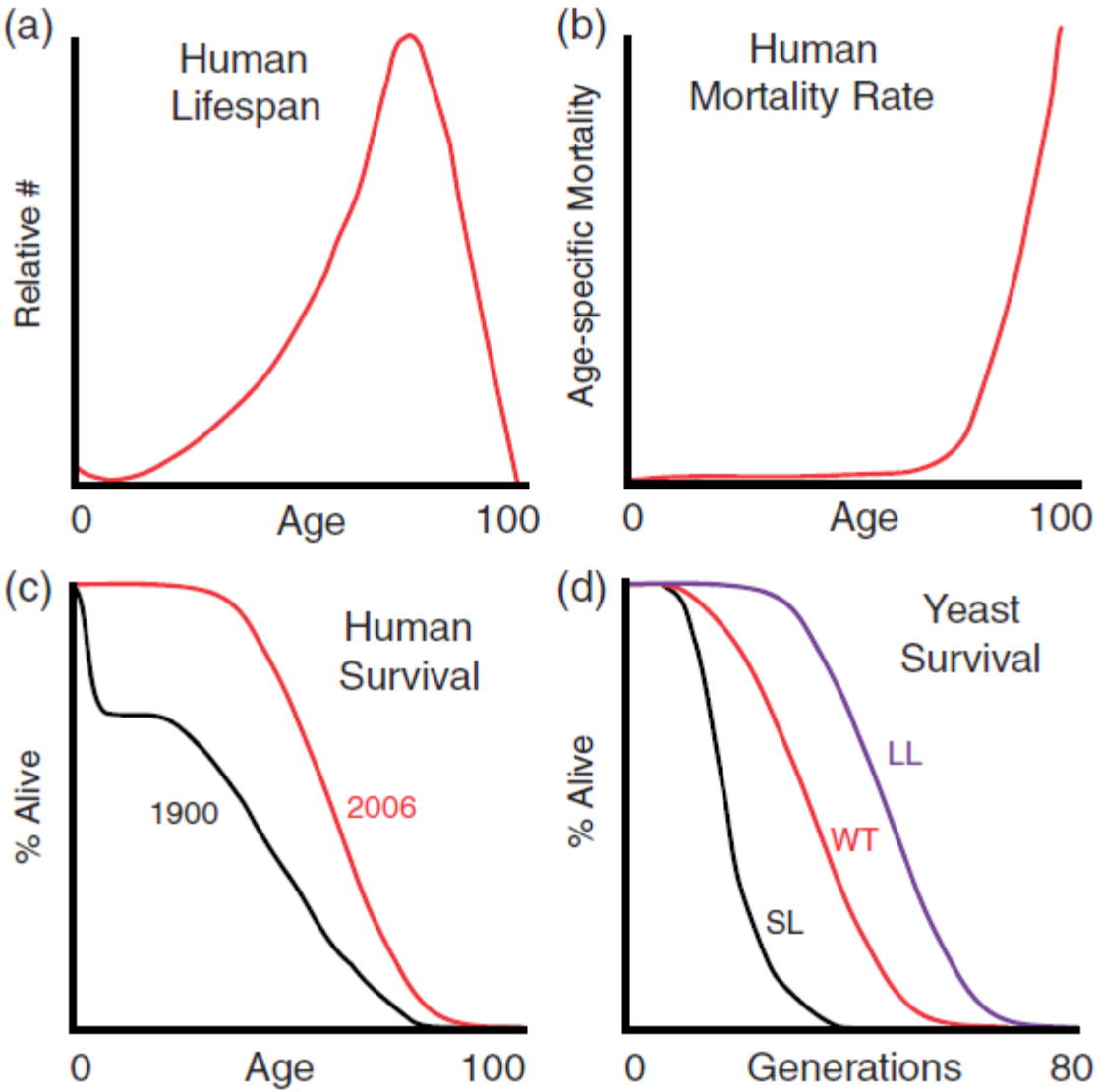
Graphical representation of lifespan & mortality rate



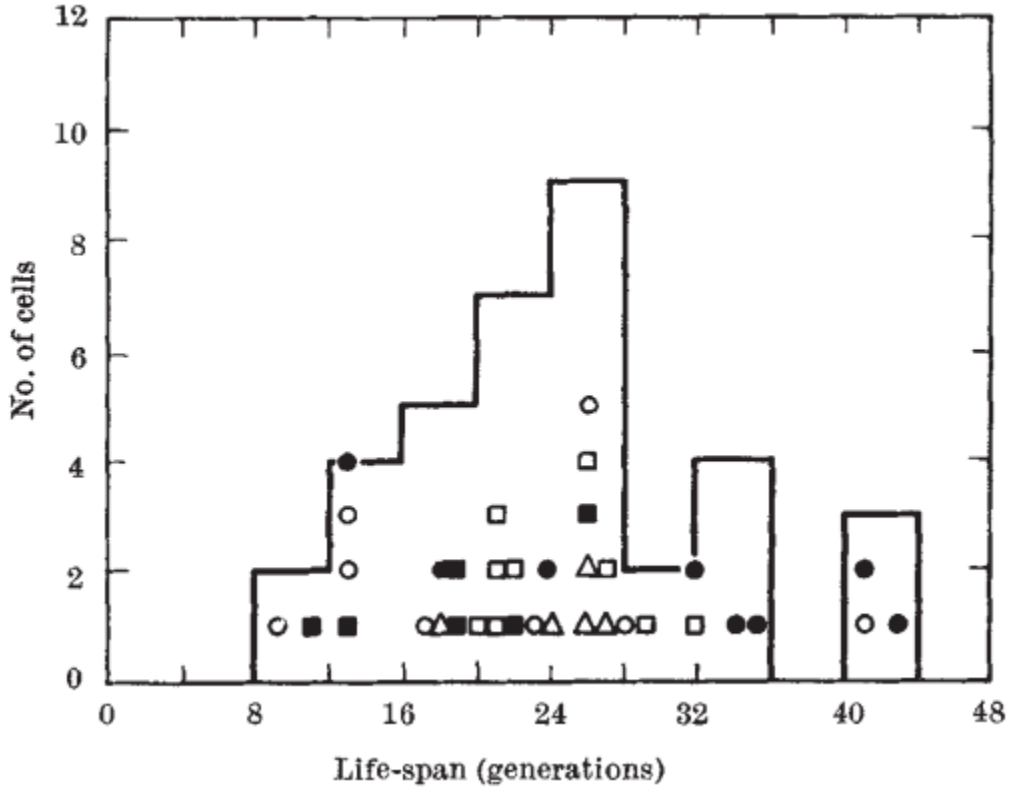
Gompertz–Makeham law of mortality



Graphical representation of lifespan & mortality rate

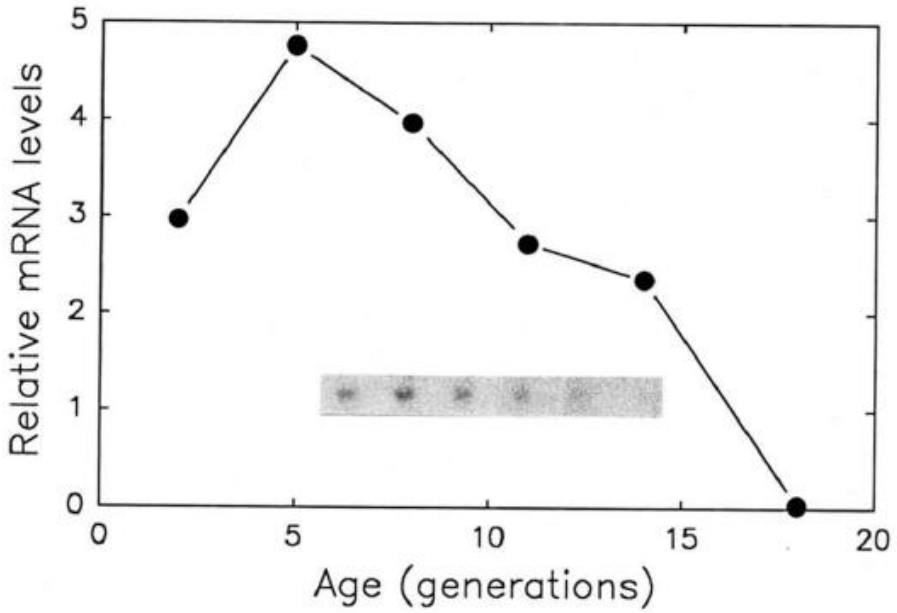


Lifespan distribution for single yeast cells



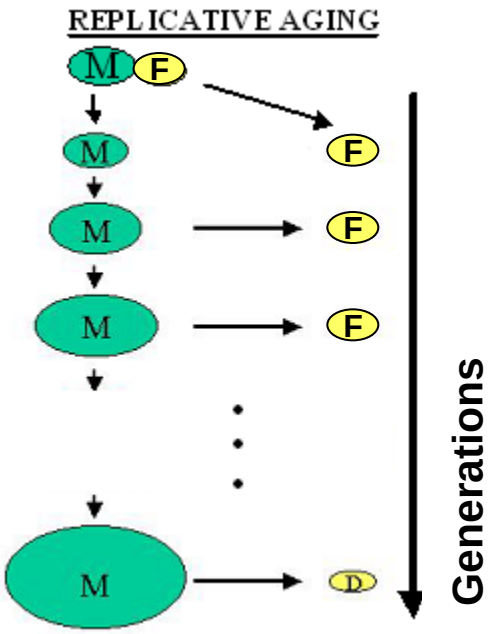
Mortimer and Johnson (1959) Nature**183**:1751–2

LAG1 deletion extends lifespan

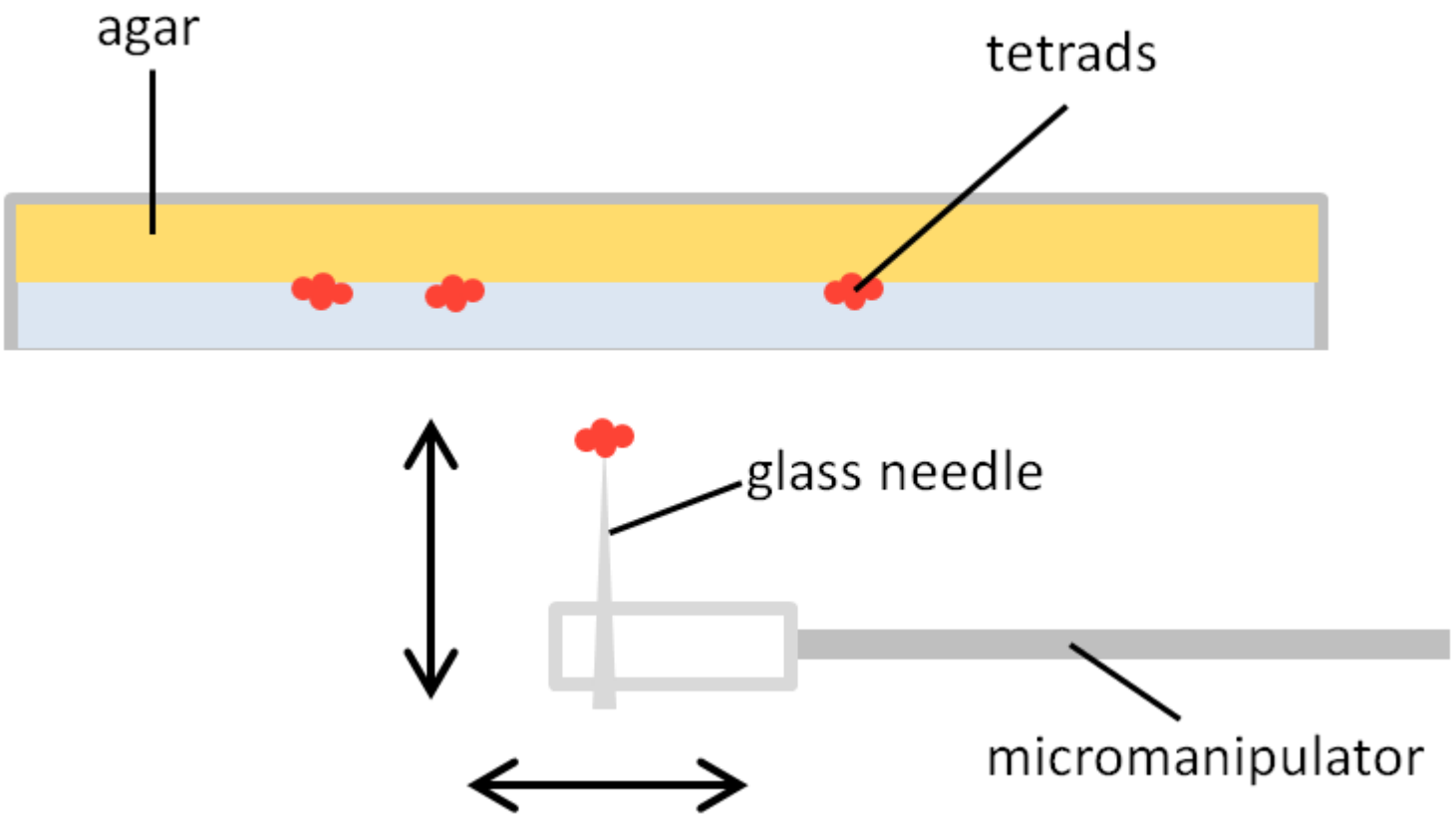


D´mello, N.P. et al. (1994) J Biol Chem **269**:15451-15459

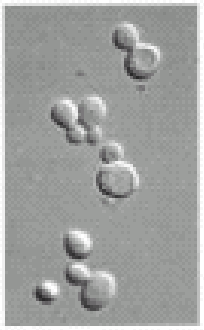
Yeast Replicative Life Span

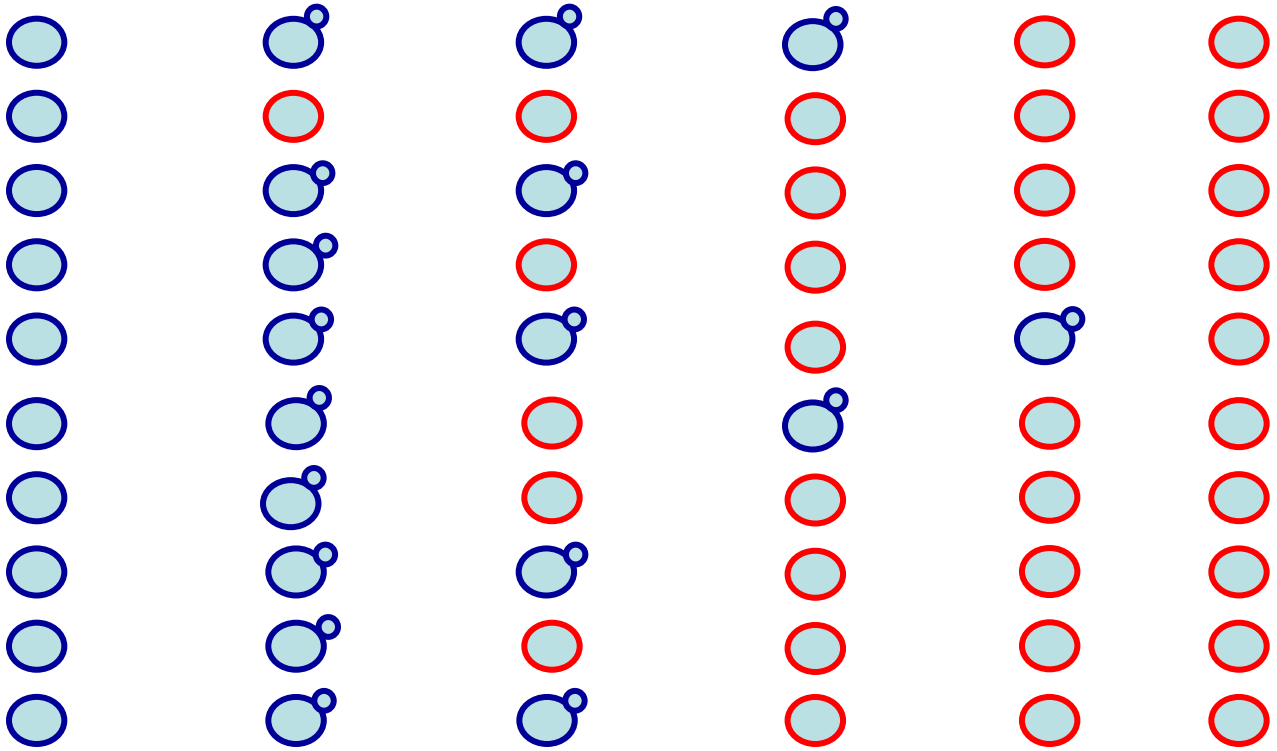


Yeast micromanipulator



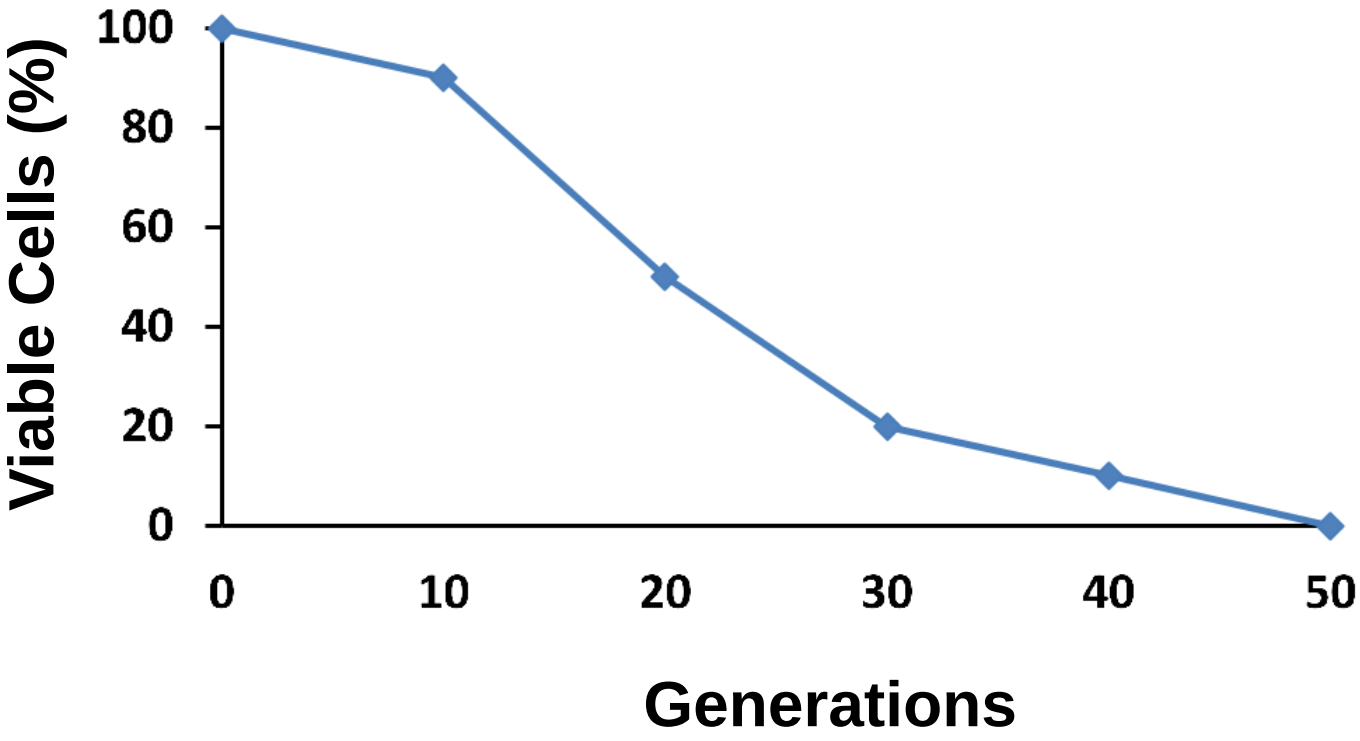
Replicative Life Span (RLS) Assay in *Saccharomyces cerevisiae*



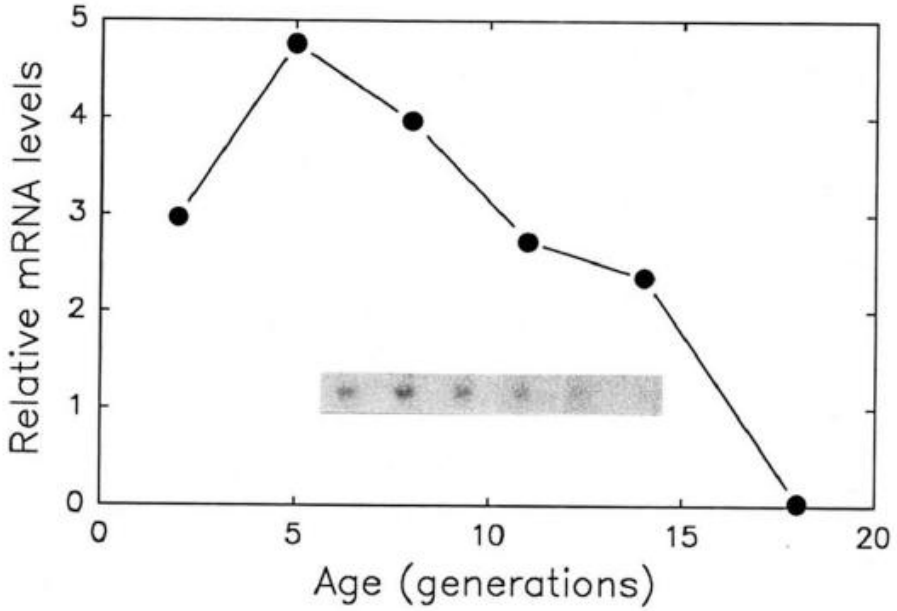


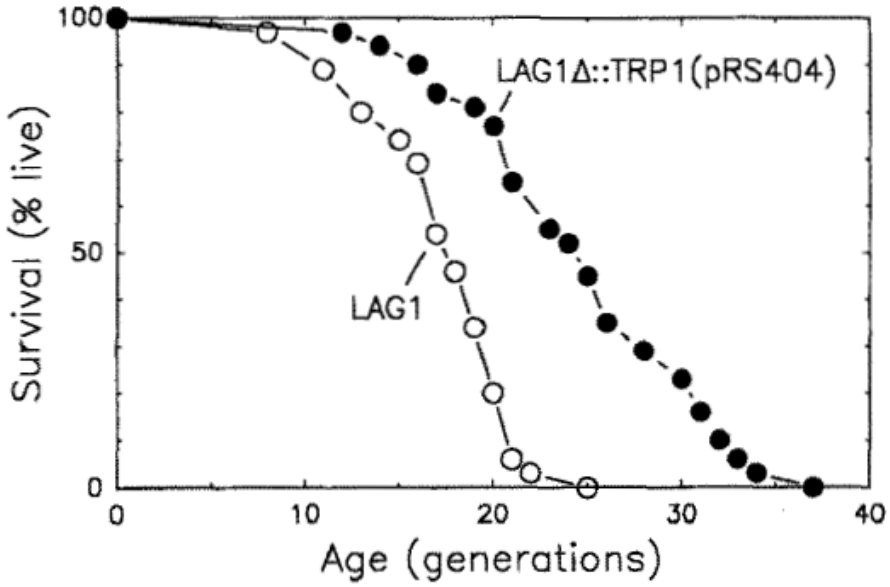
Gerations	0	10	20	30	40	50
% Survival (Viable Fraction)	100	90	50	20	10	0

Yeast RLS Curve



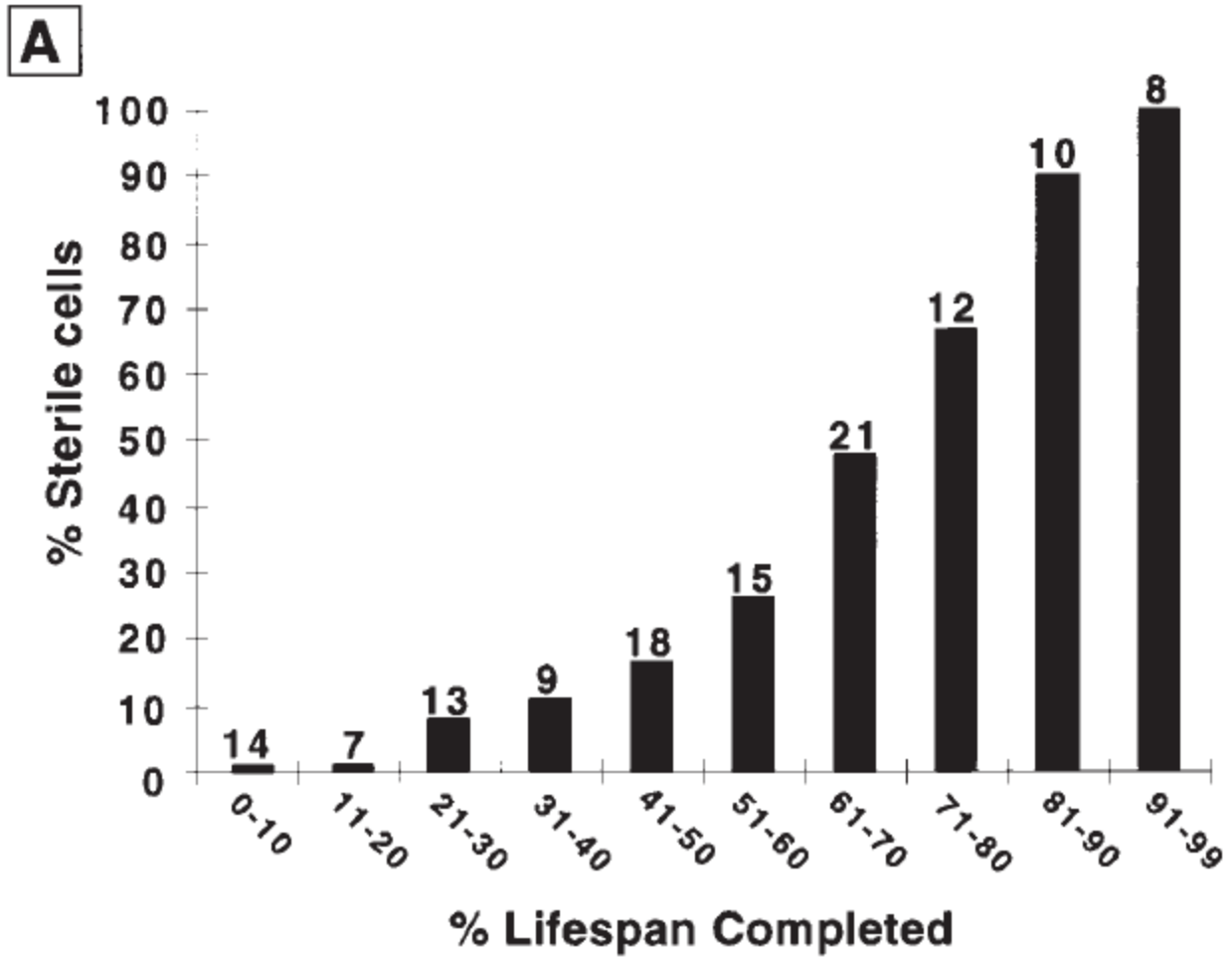
LAG1 deletion extends lifespan





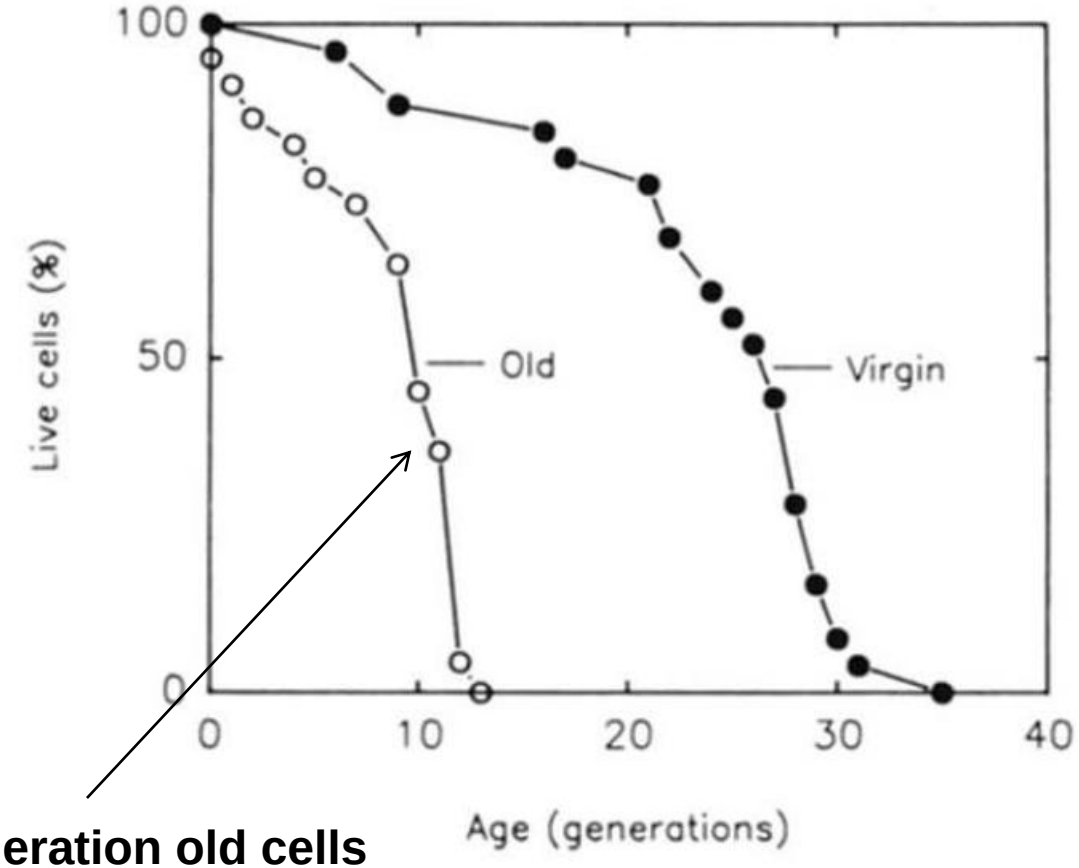
D´mello, N.P. et al. (1994) J Biol Chem **269**:15451-15459

Reduced lifespan of cells with prior stationary phase experience is accompanied by sterility



Ashrafi, K. et al. (1999) PNAS **96:**9100-9105

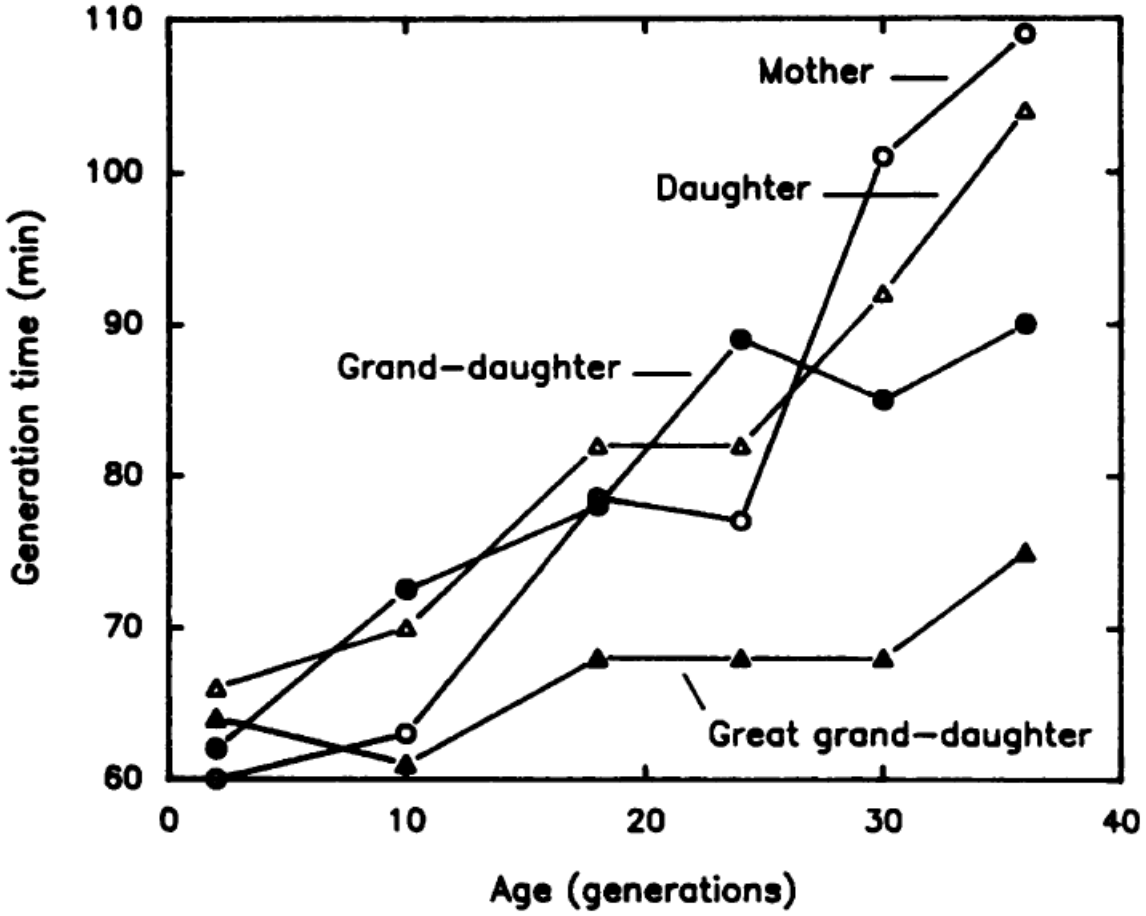
Life spans of cells from old yeast cell preparations



18-generation old cells

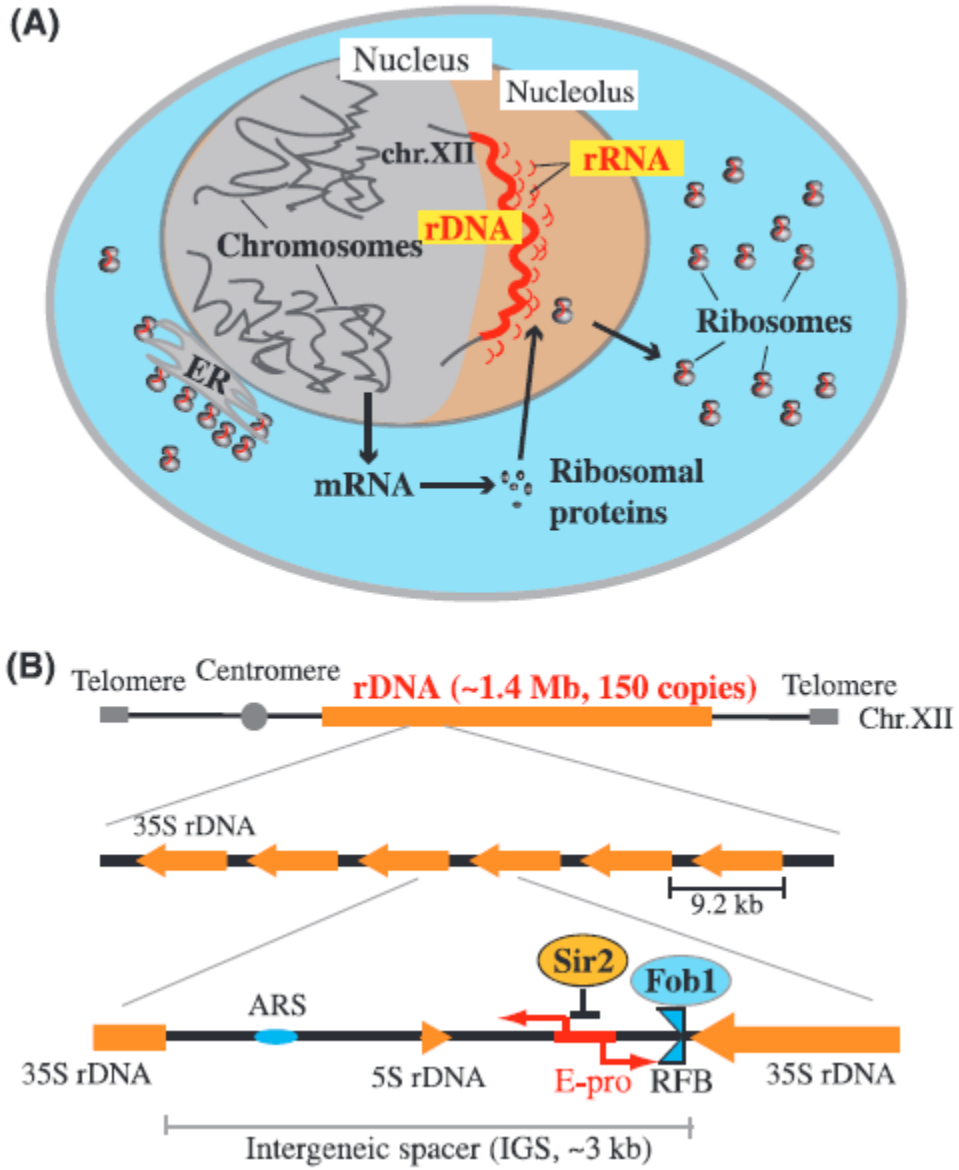
Egilmez, N.K. (1989) J Biol Chem **264**:14312-14317

Generation time of yeast mother, daughter, grand-daughter and great grand-daughter



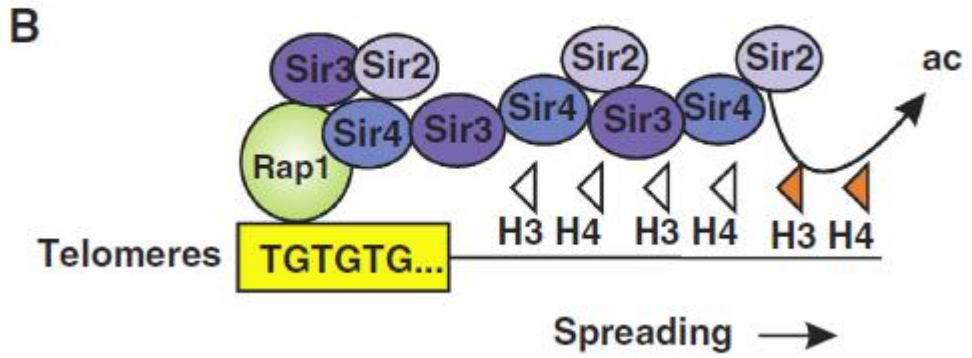
Egilmez, N.K. (1989) J Bacteriol **171**:37-42

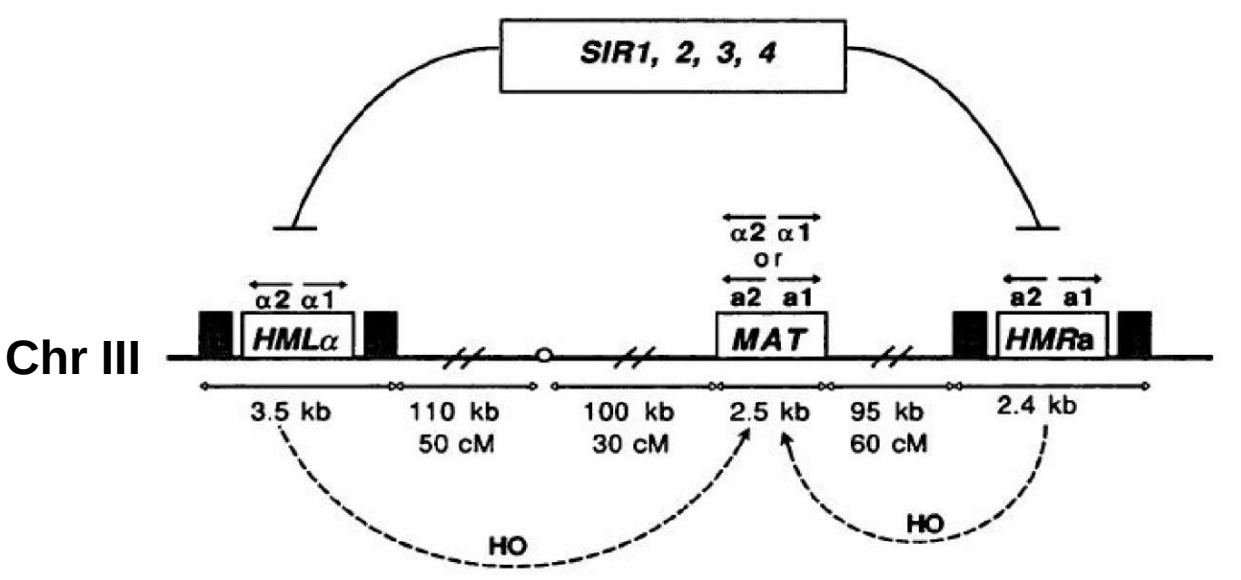
Organização do rDNA no cromossomo XII



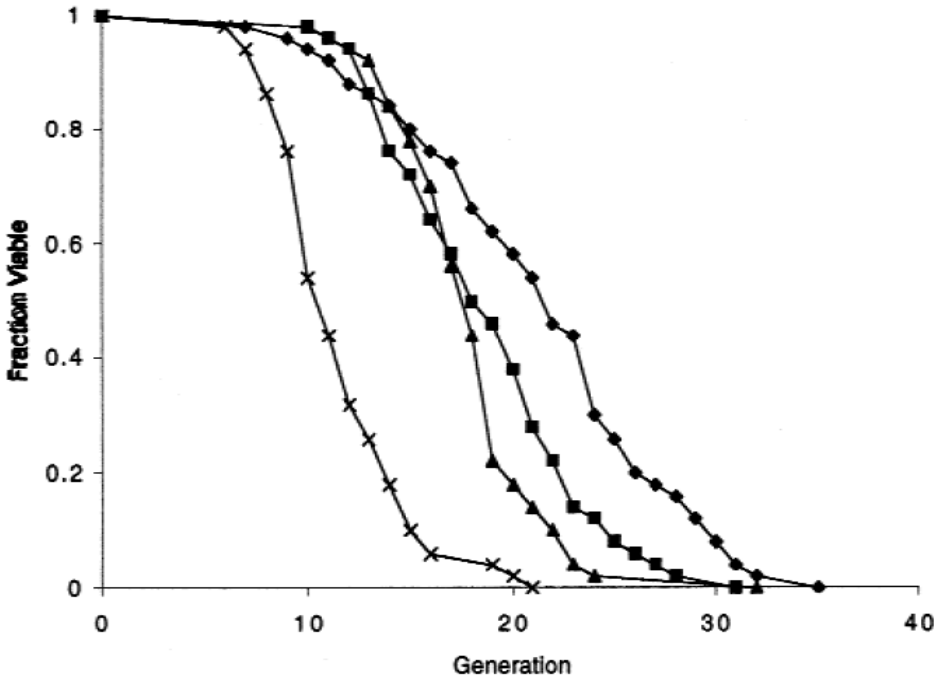
Genes to Cells (2011) **16**, 617–624

Complexo SIR controla extensão da heterocromatina

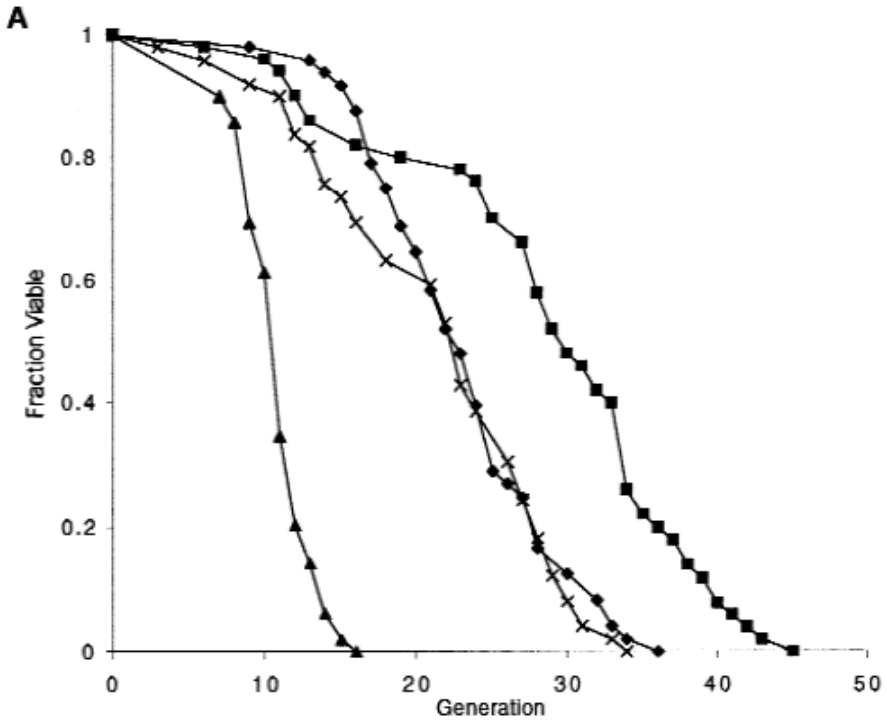




SIR2, *SIR3*, and *SIR4* are required for longevity of haploid mother cells



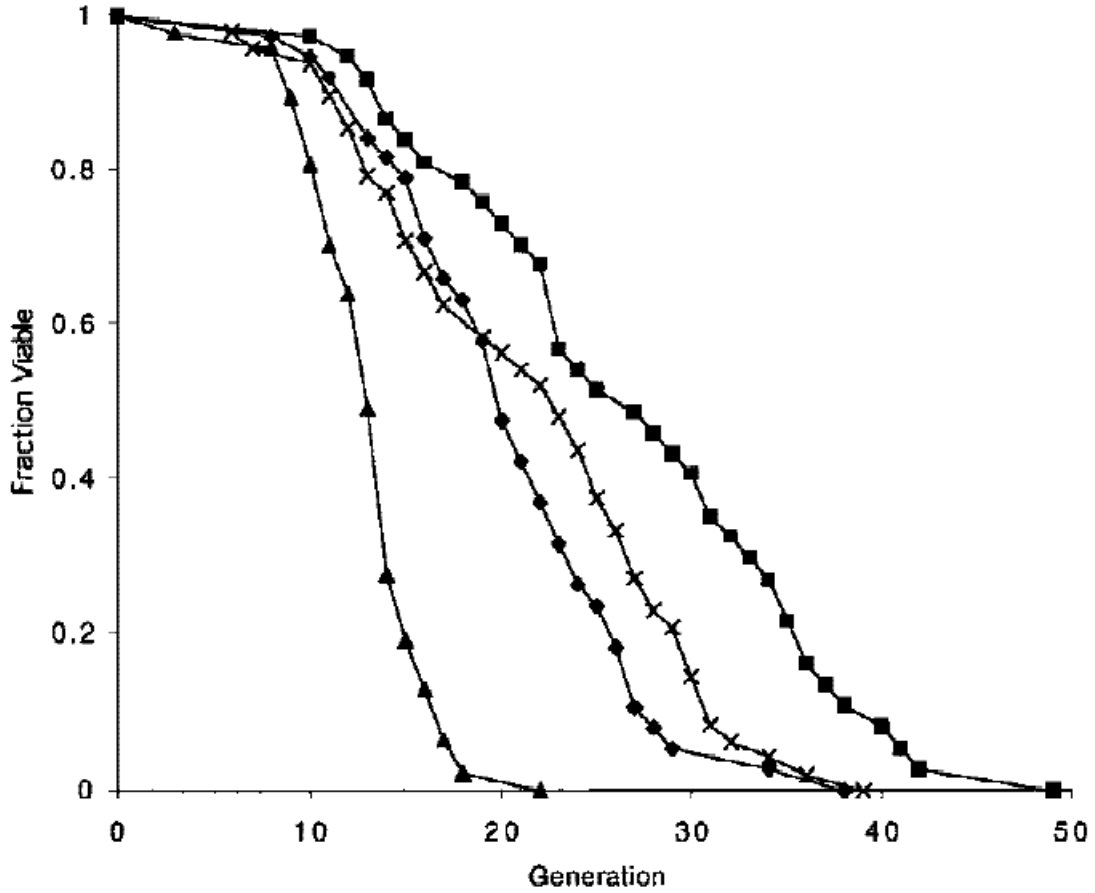
(◆) *W303R*; (■) *sir4*; (▲) *sir3*; (x) *sir2*.



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

GENES & DEVELOPMENT 13:2570–2580

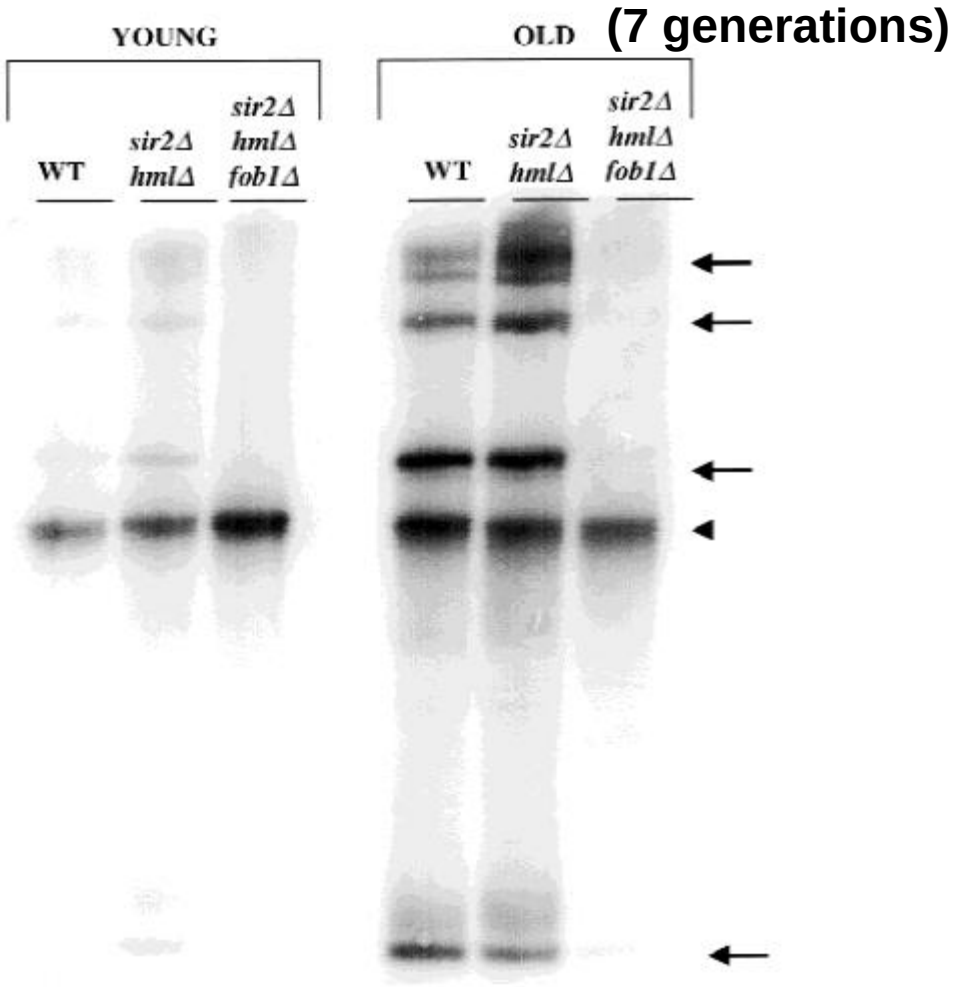
Mutation of *fob1* suppresses the life span defect of a *sir2* mutant



(◆) *W303R*; (■) *fob1*; (▲) *sir2 hml*; (×) *sir2 hml fob1*

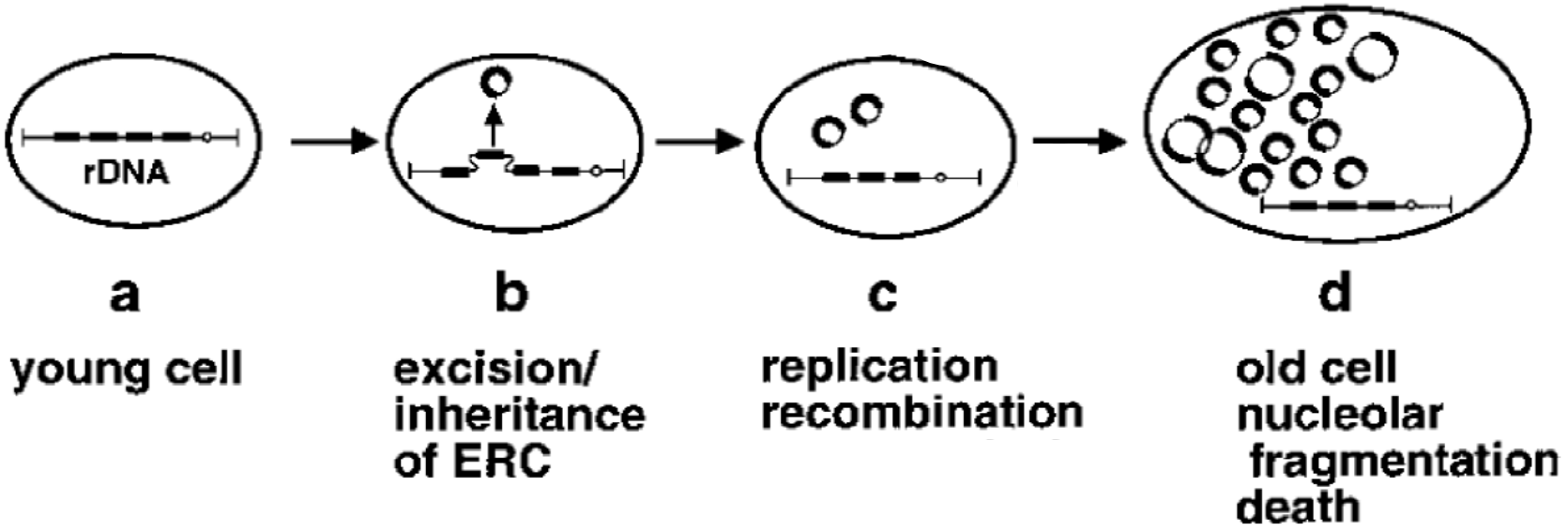
GENES & DEVELOPMENT 13:2570–2580

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



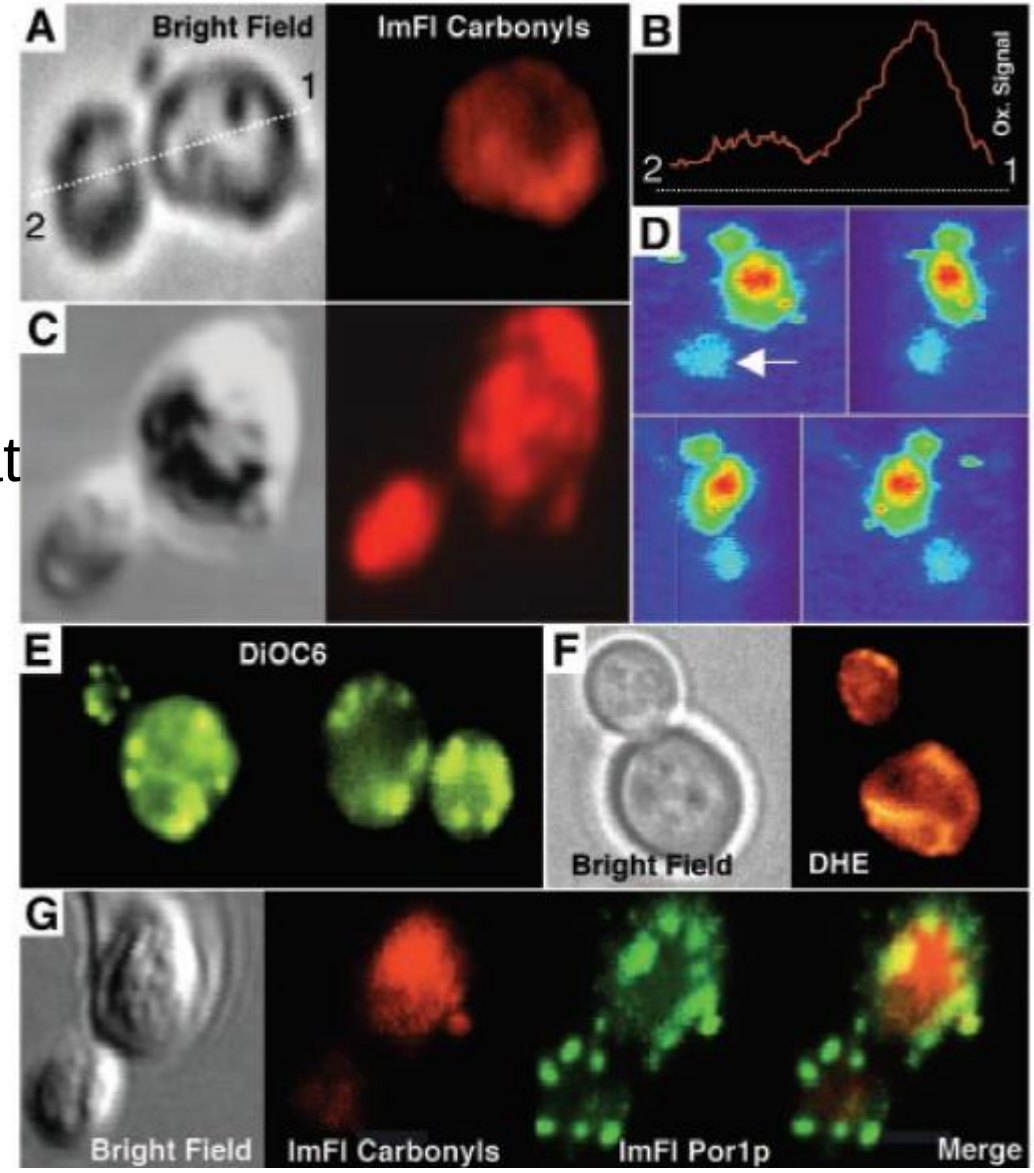
GENES & DEVELOPMENT 13:2570–2580

Minicírculos de rDNA são formados ao longo do envelhecimento



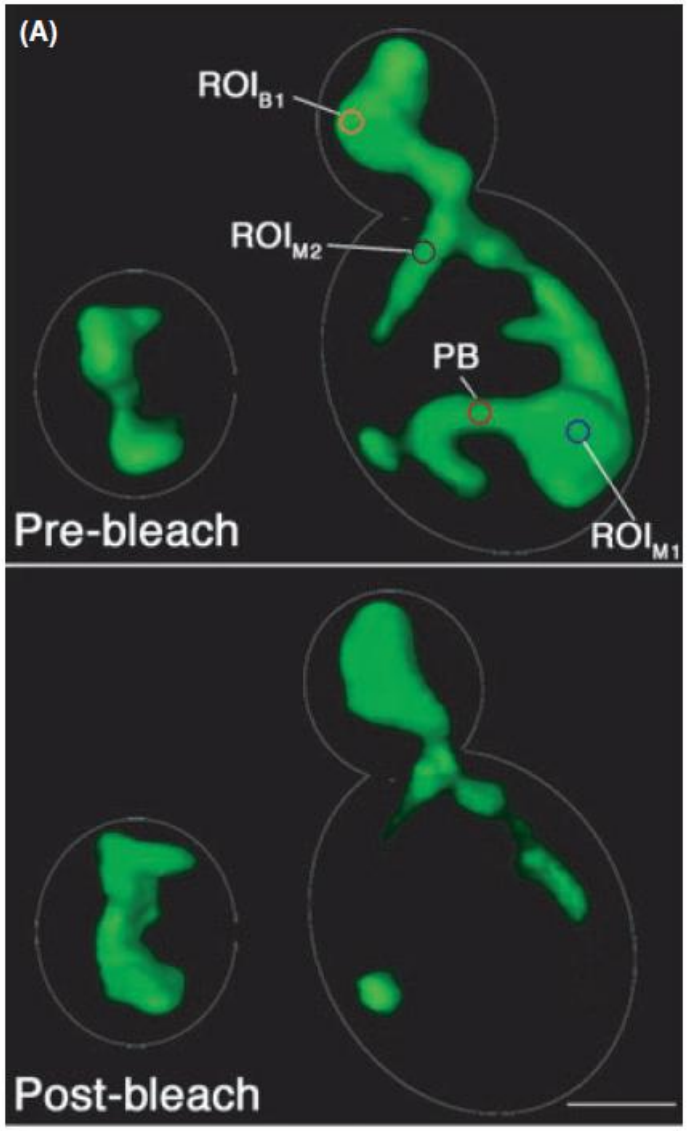
Cell, Vol. 91, 1033–1042, December 26, 1997,

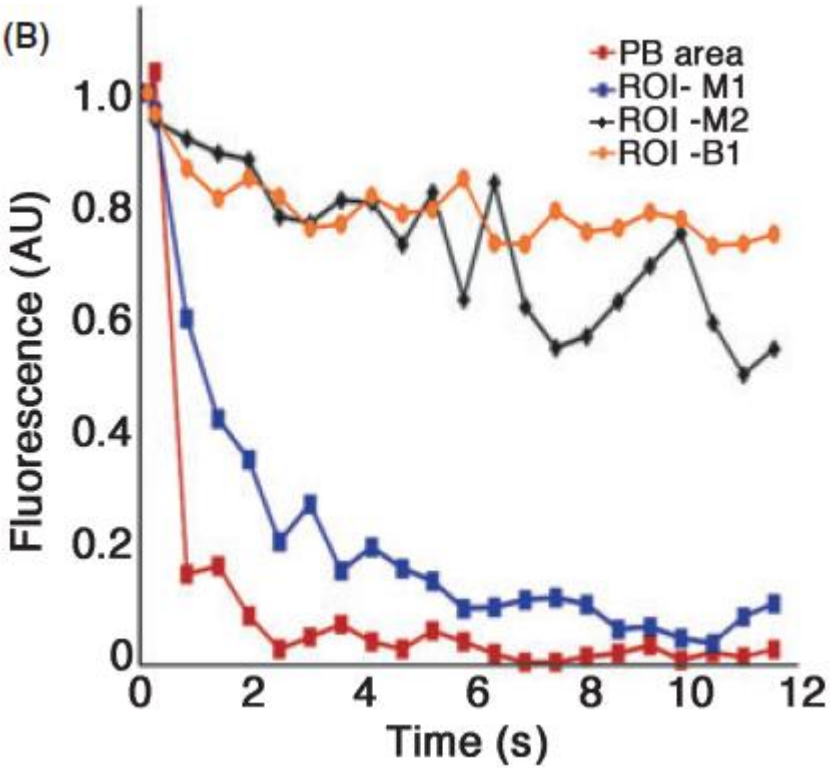
Distribution of oxidatively damaged proteins, mitochondria, and ROS during yeast cytokinesis.

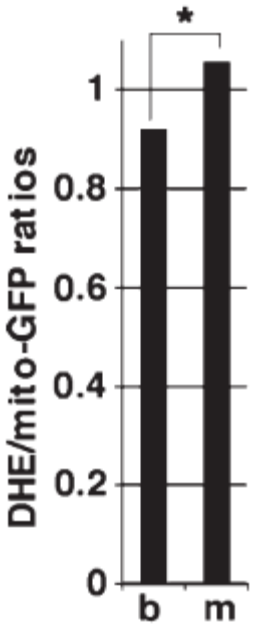


Science (2003) **299**:1751-3

Mitochondria in the mother cell are physically distinct

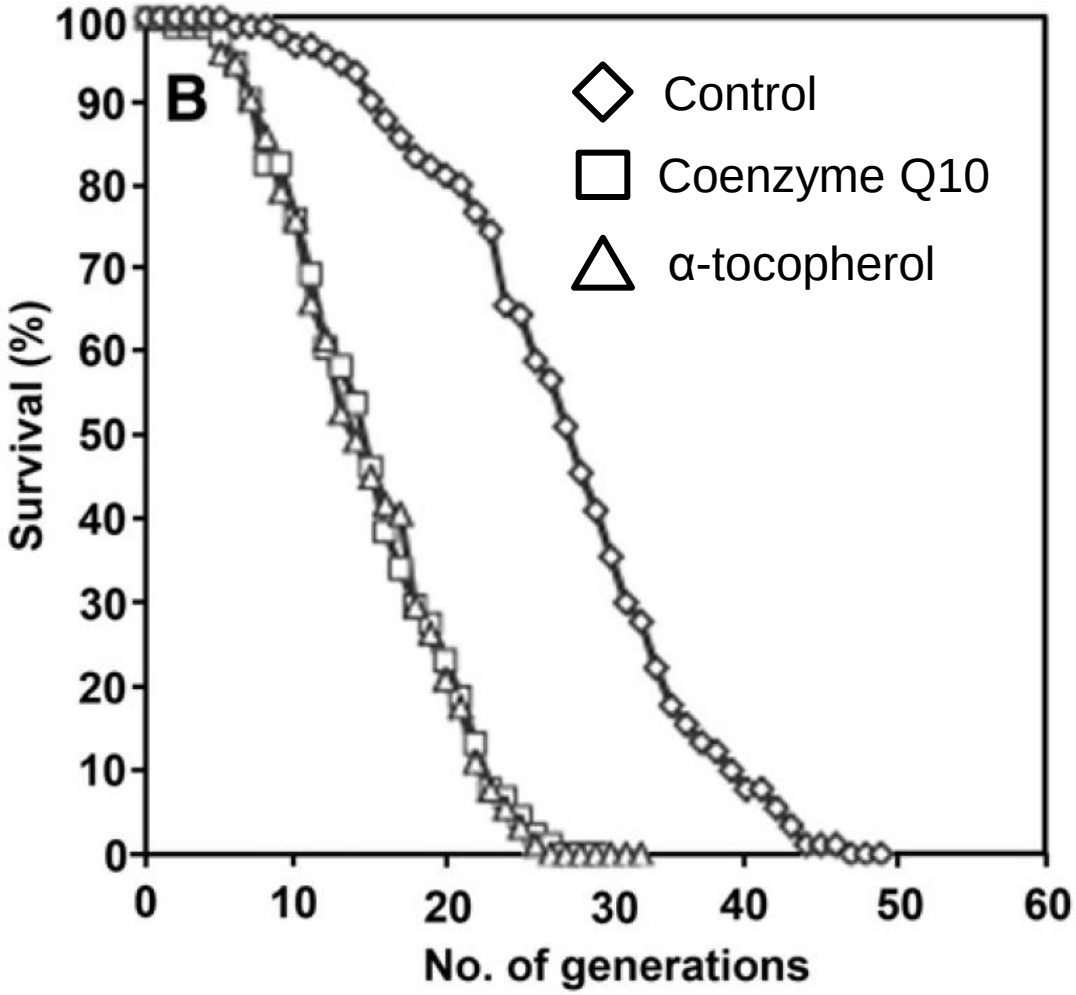






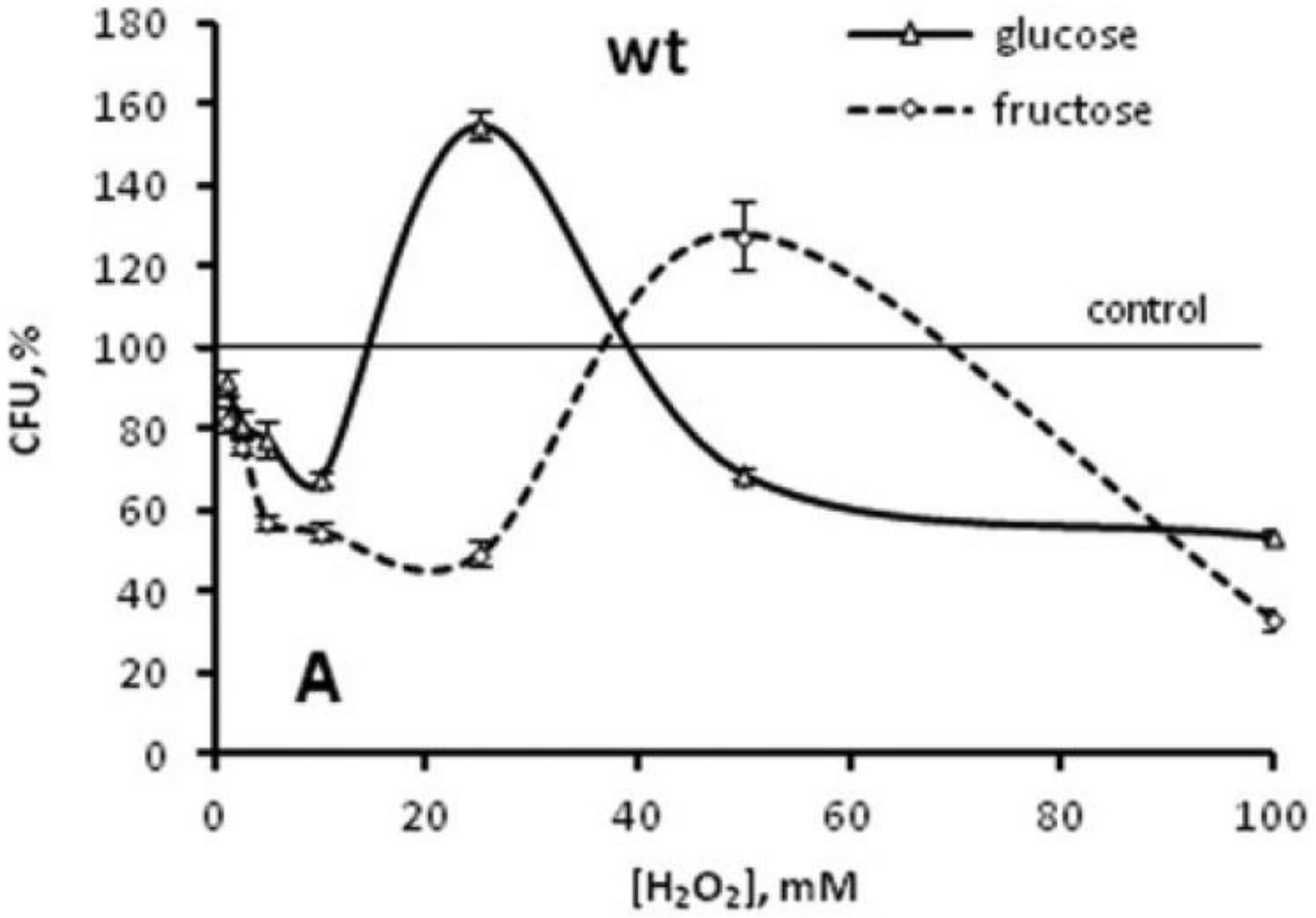
McFaline-Figueroa, J.R. et al. (2011) Aging Cell **10**: 885–895

Antioxidants do not increase longevity



Lam et al. (2010) Free Radical Biology & Medicine **49**: 237–244

ROS activates a stress response mechanism called hormesis that promotes longevity



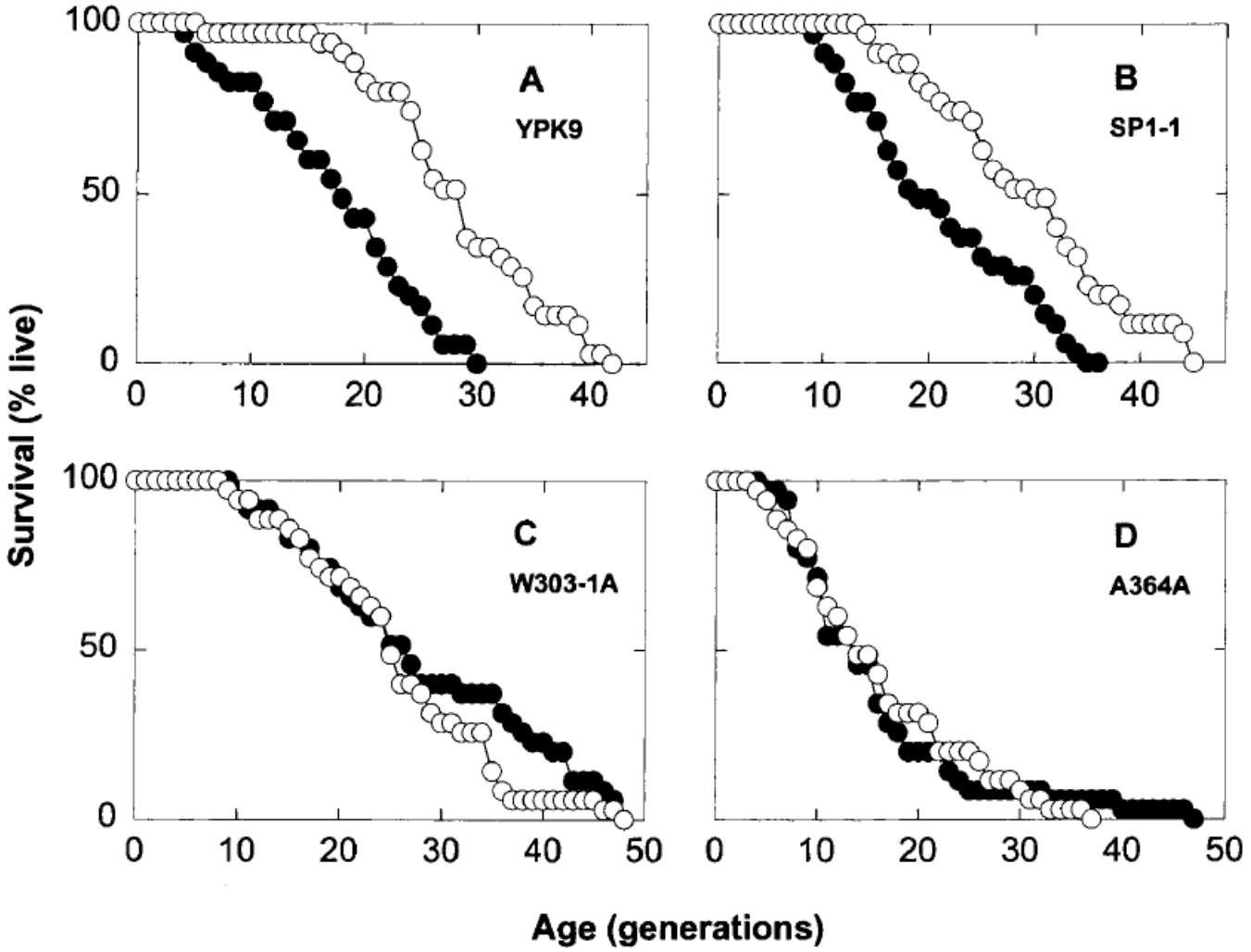
Dose Response. (2016) **Vol. 14**: Issue 2 doi: 10.1177/1559325816636130

Paracelsus

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

AZ QUOTES

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

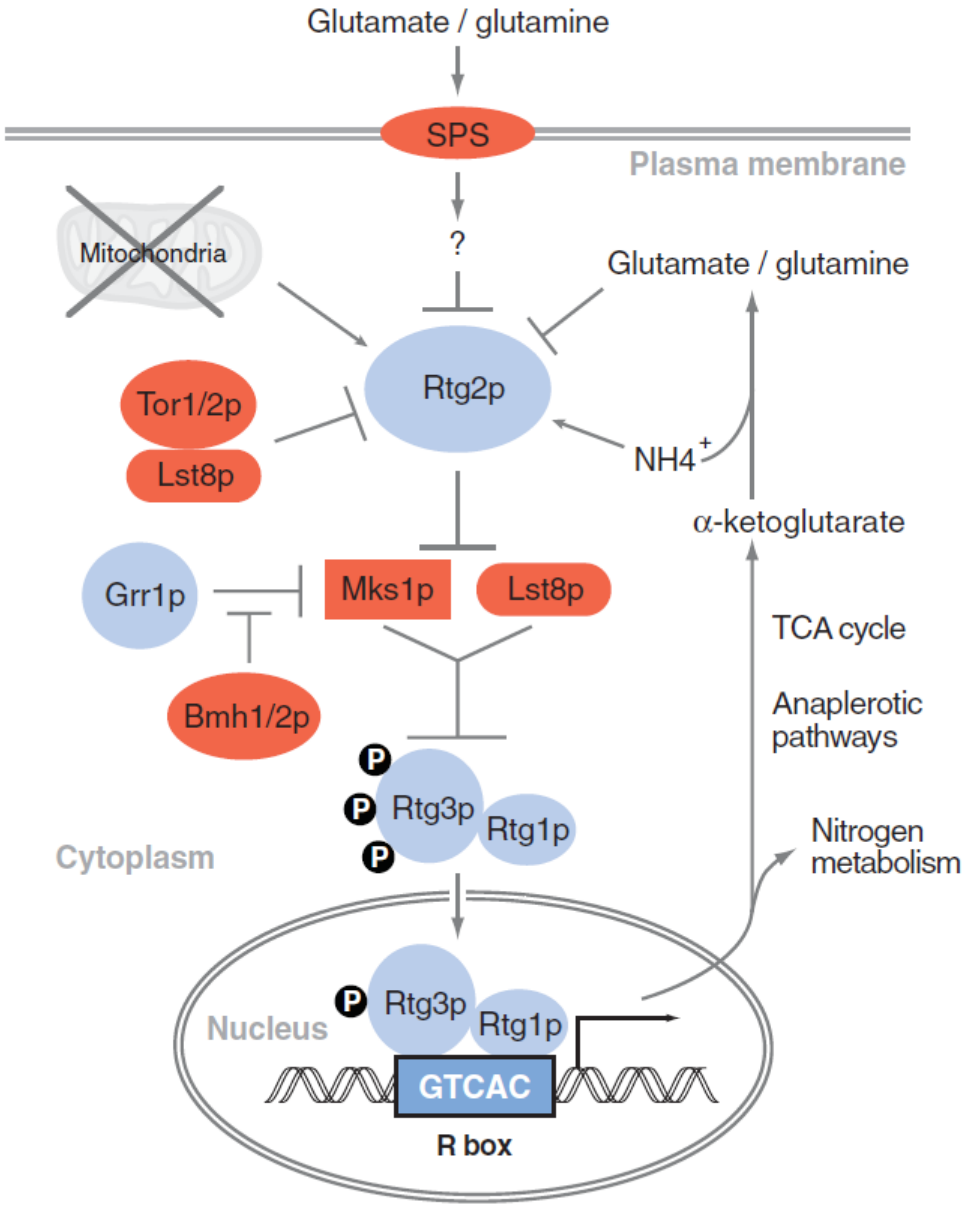


● grande

○ petite

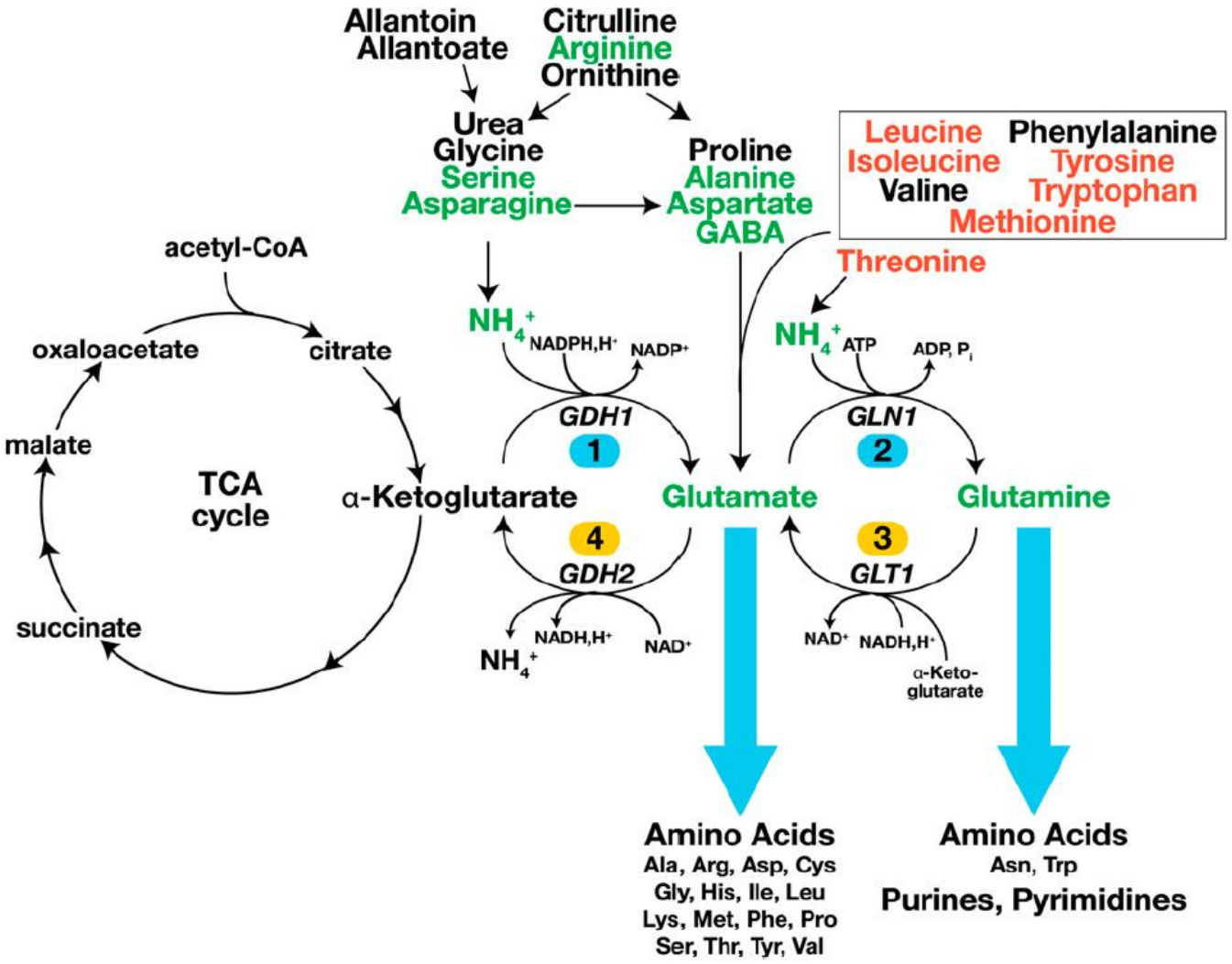
Kirchman, P.A. et al. (1999) Genetics **152**: 179–190

Retrograde (RTG) signaling pathway



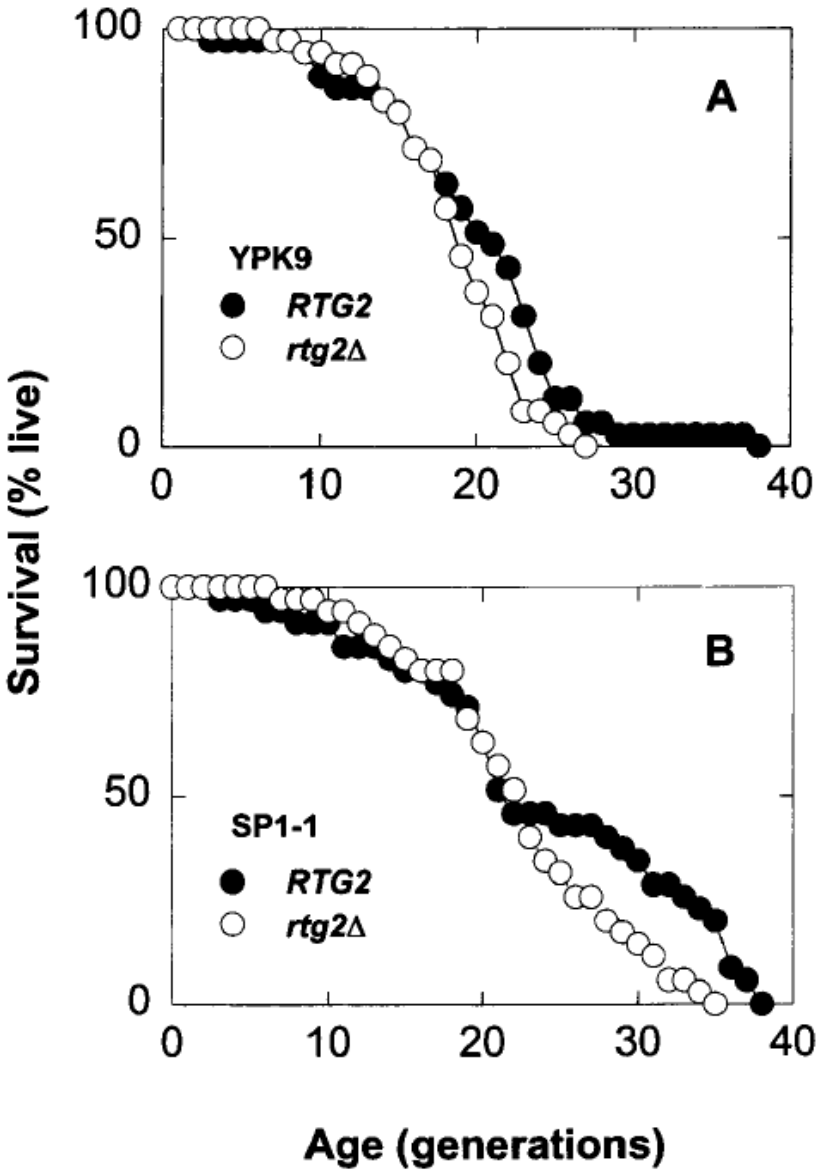
Liu and Butow (2006), Ann Rev Genet, **40**: 159-185

Glutamate and glutamine provide all nitrogen for biosynthetic reactions



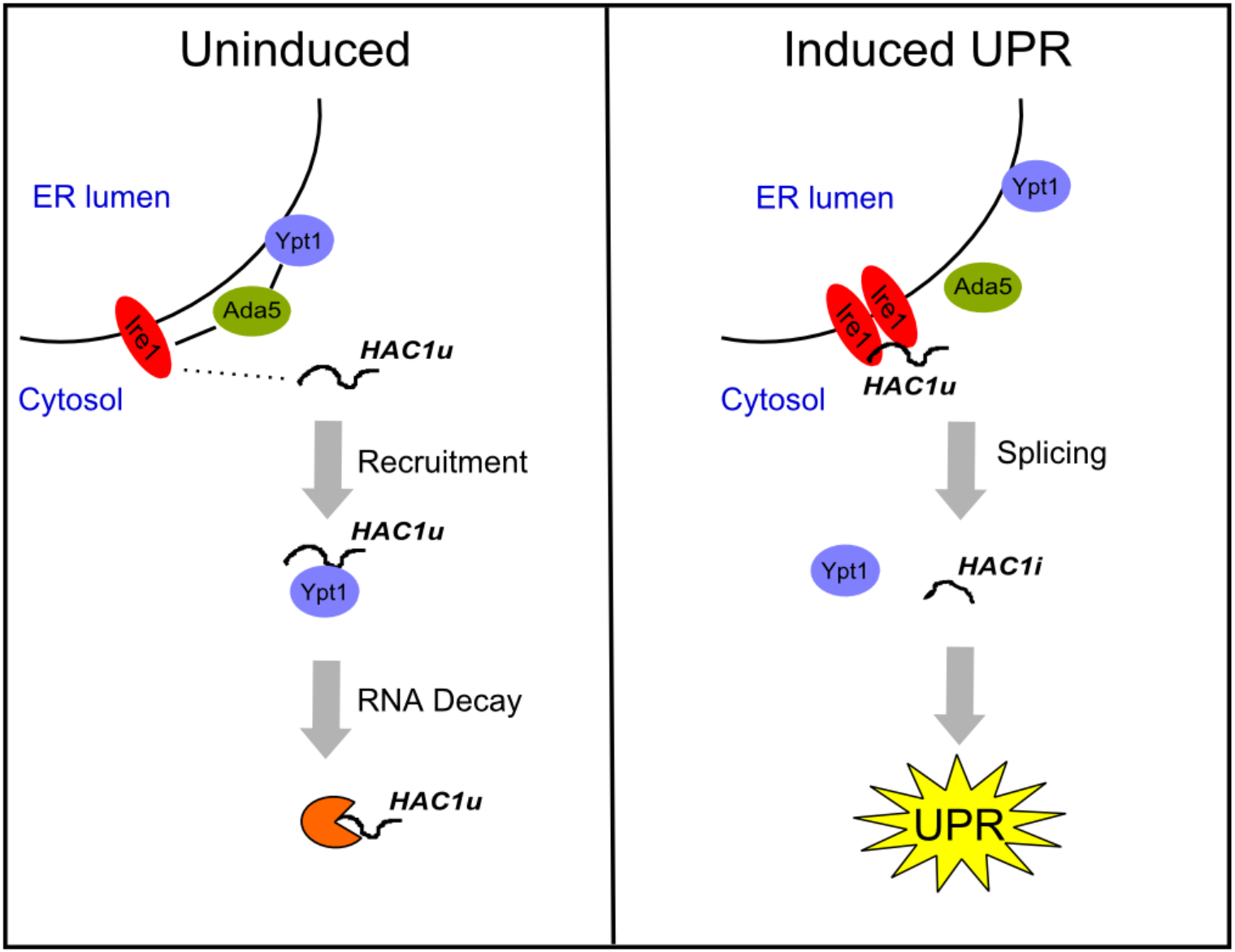
Ljungdahl and Daignan-Fornier (2002), Genetics, **190:** 885-929

RTG2 is required for extension of petite lifespan

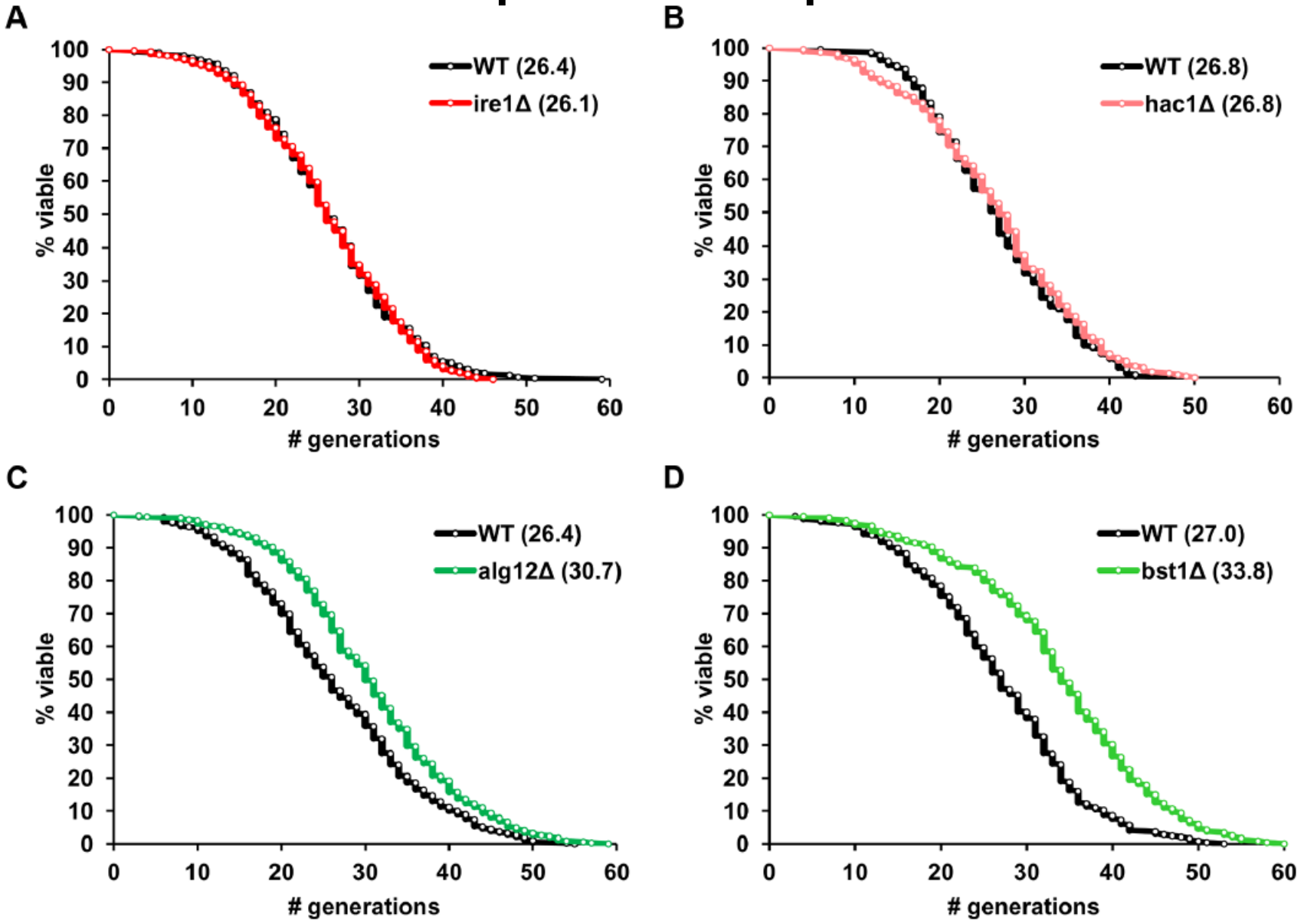


Kirchman, P.A. et al. (1999) Genetics **152**: 179–190

Yeast ER unfolded protein response

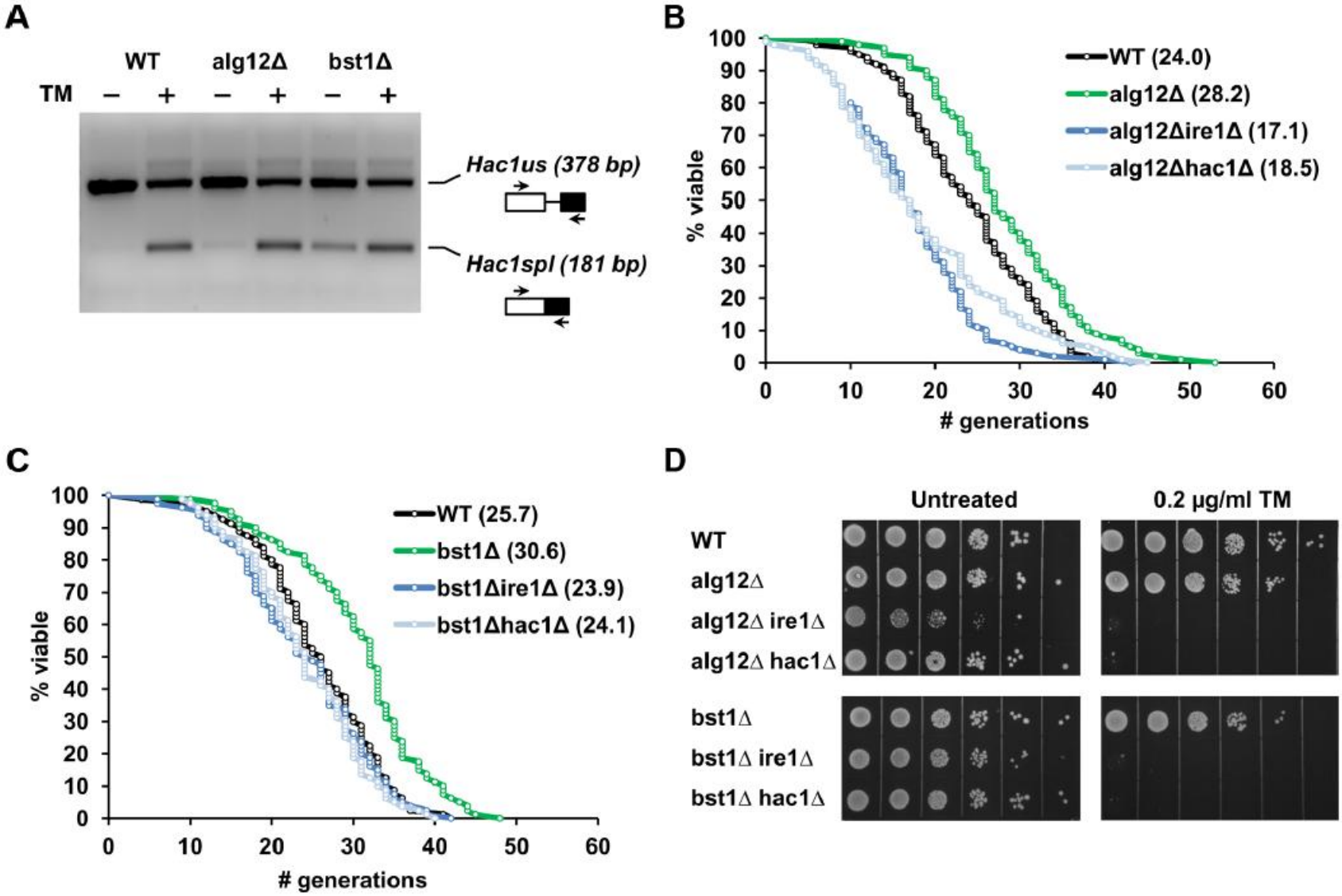


ER stress response genes differentially modulate yeast replicative lifespan



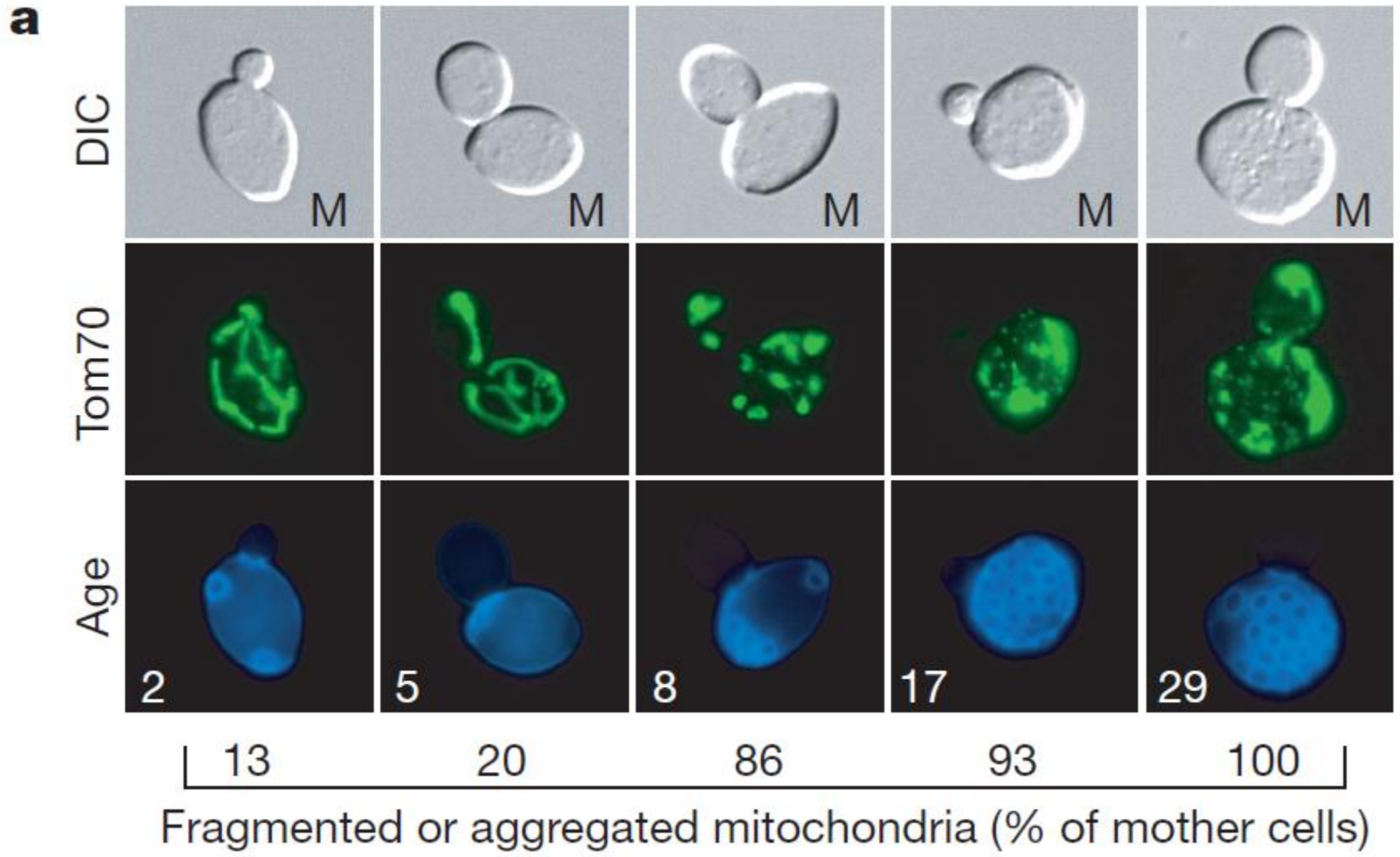
Labunskyy, V.M. et al. (2014) PLoS Genetics **10**:e1004019

Extended lifespan in *alg12Δ* and *bst1Δ* mutants is dependent on functional Ire1p and Hac1p and is associated with increased basal UPR activity



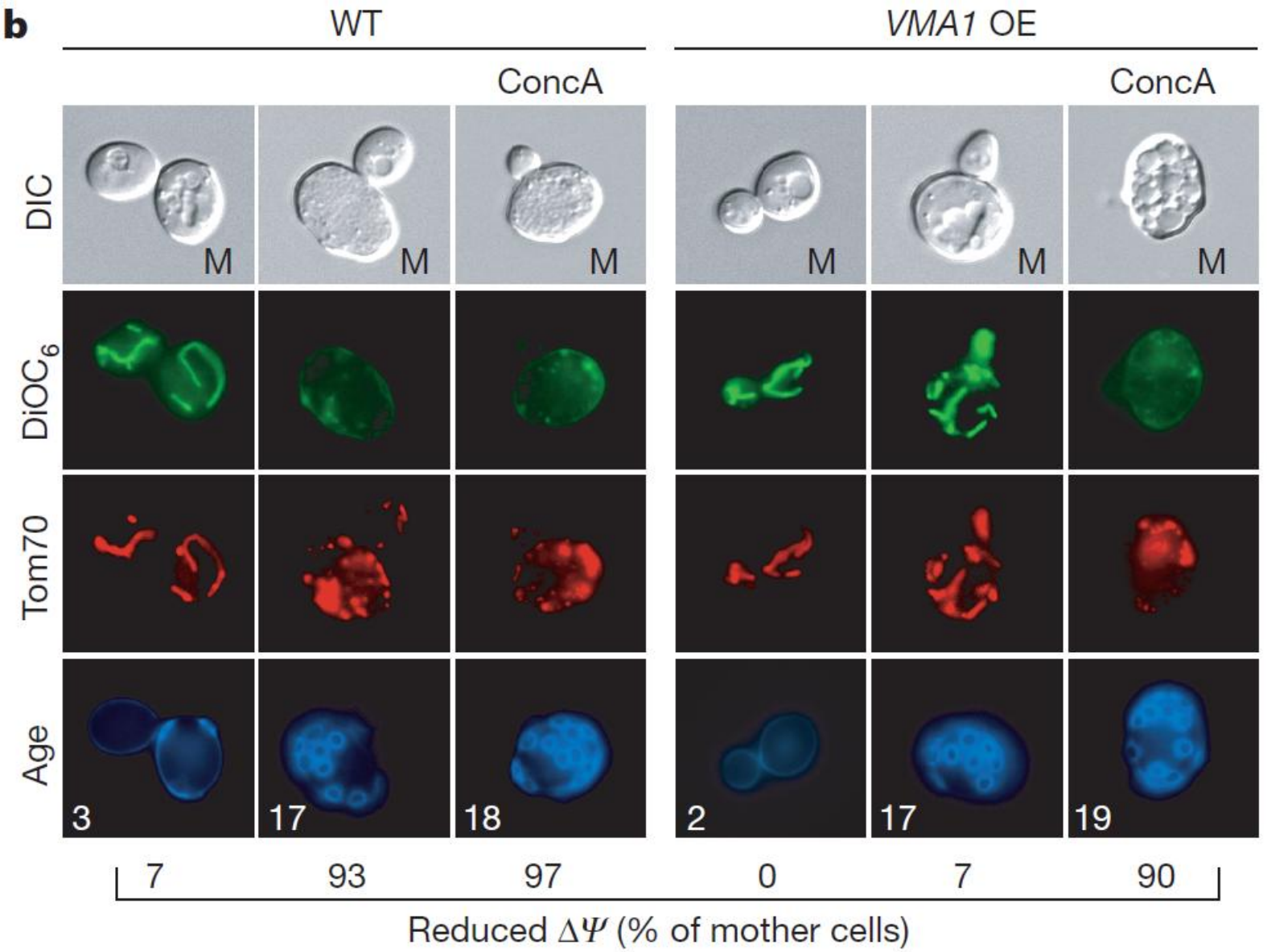
Labunskyy, V.M. et al. (2014) PLoS Genetics **10**:e1004019

Age-induced mitochondrial dysfunction



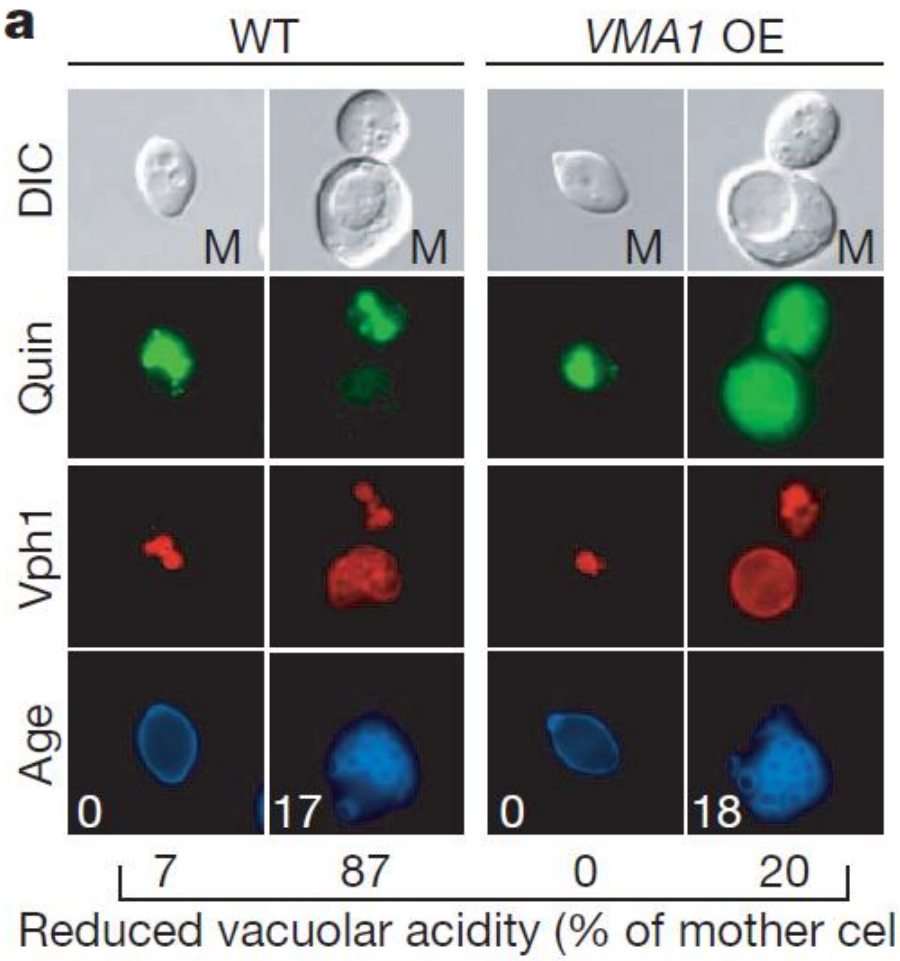
Hughes, A.L. (2012) Nature **492:**261-267

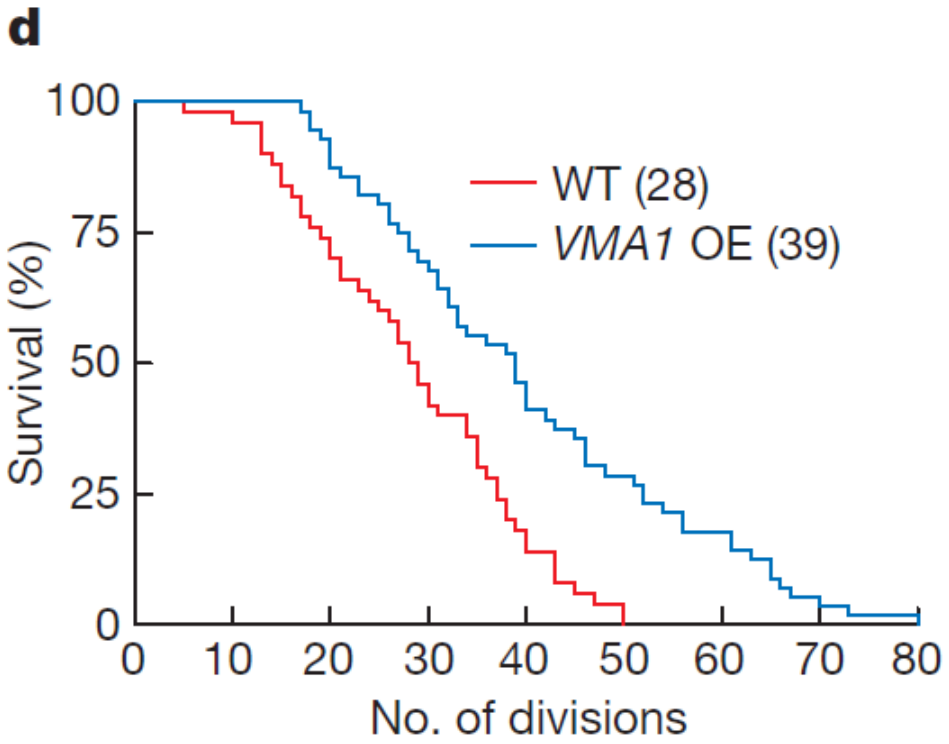
Age-induced mitochondrial dysfunction is suppressed by VMA1 overexpression



Hughes, A.L. (2012) Nature **492:**261-267

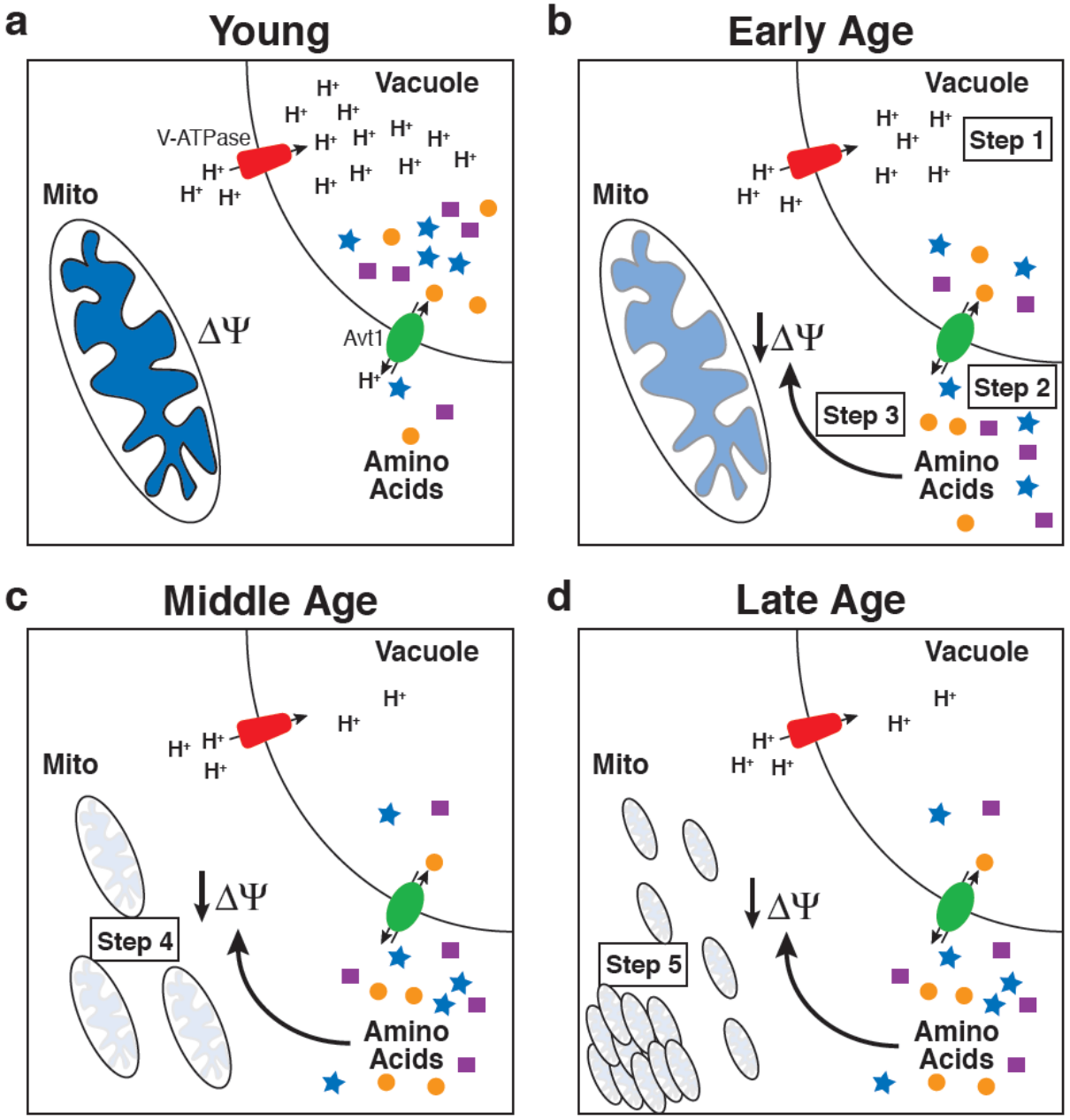
Vacuolar acidity is reduced in ageing cells and regulates mitochondrial function and lifespan





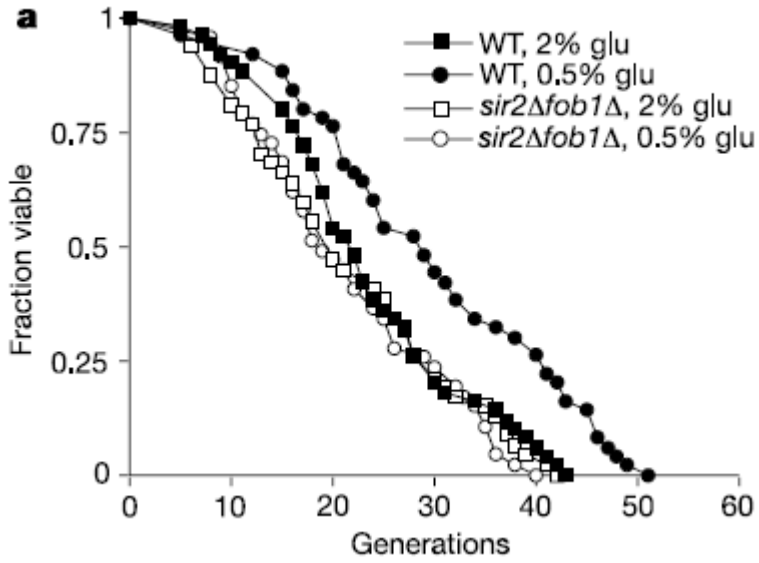
Hughes, A.L. (2012) Nature **492:**261-267

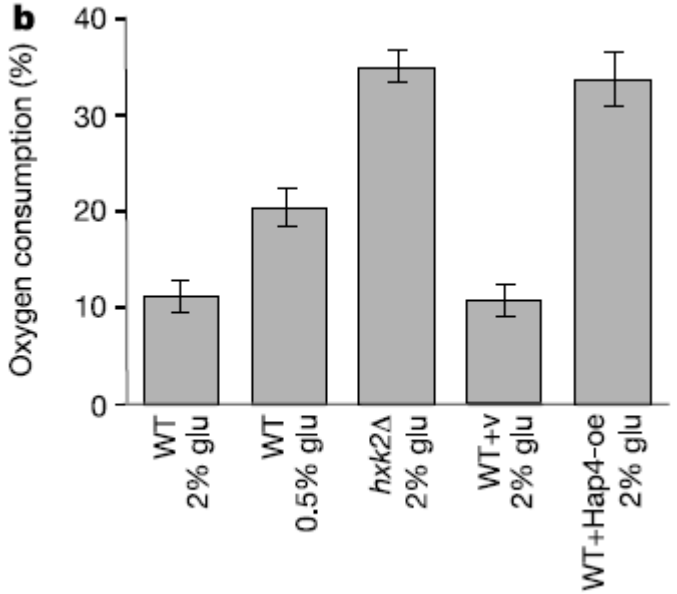
Model of the yeast ageing process

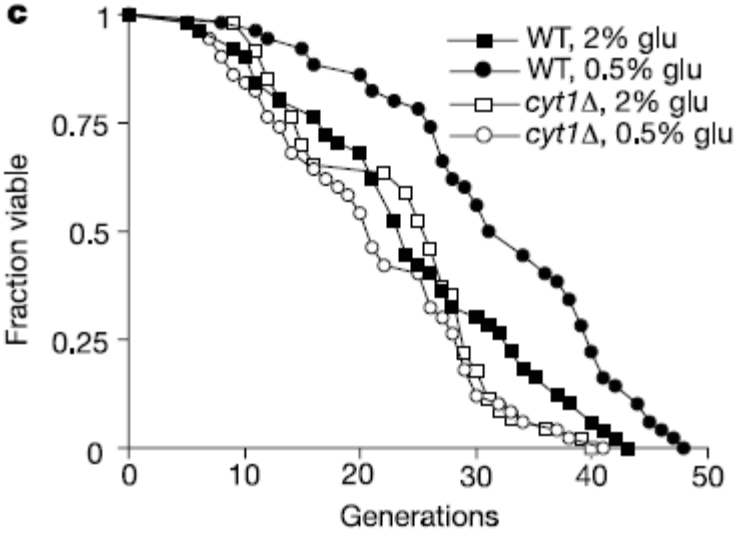


Hughes, A.L. (2012) Nature **492:**261-267

Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration

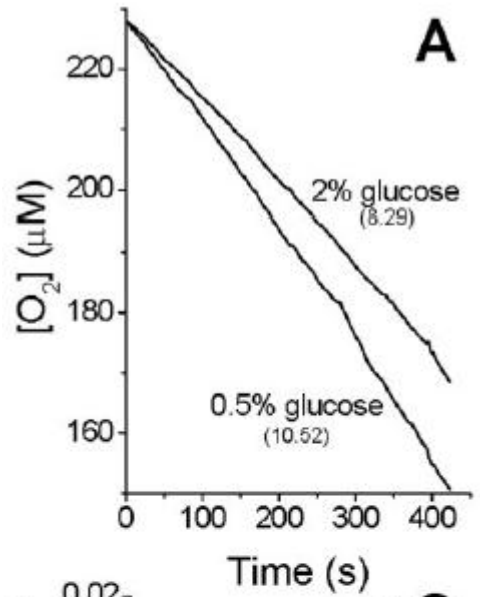


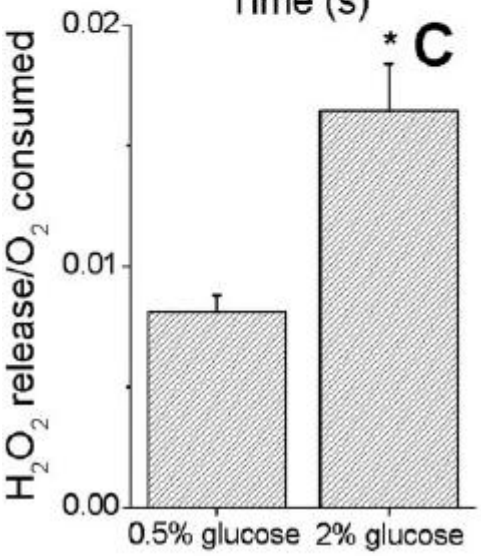


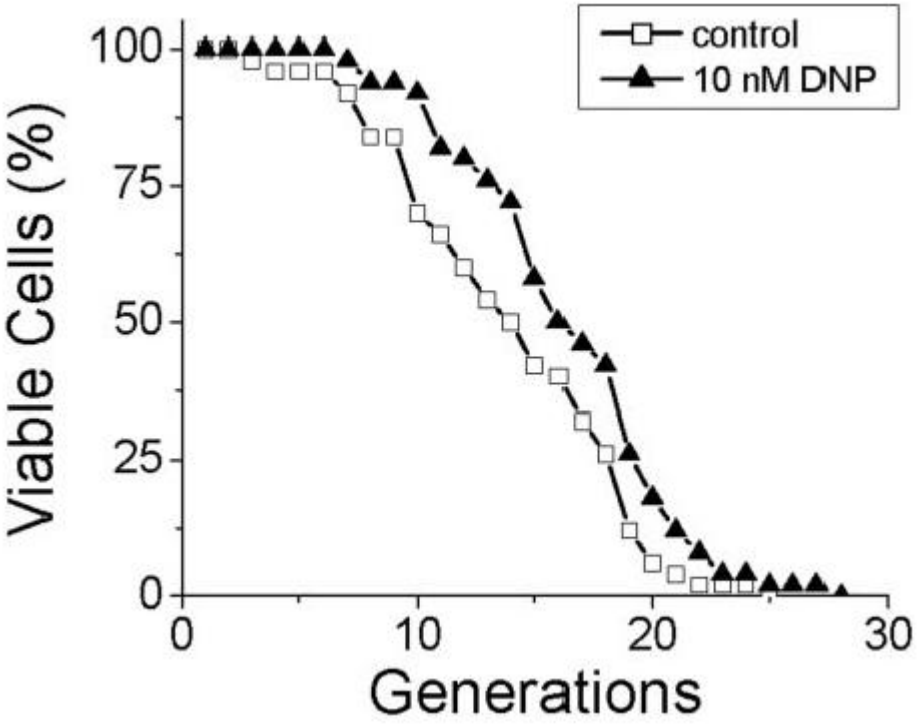


Lin, S-J et al. (2002) **418**: 344-348

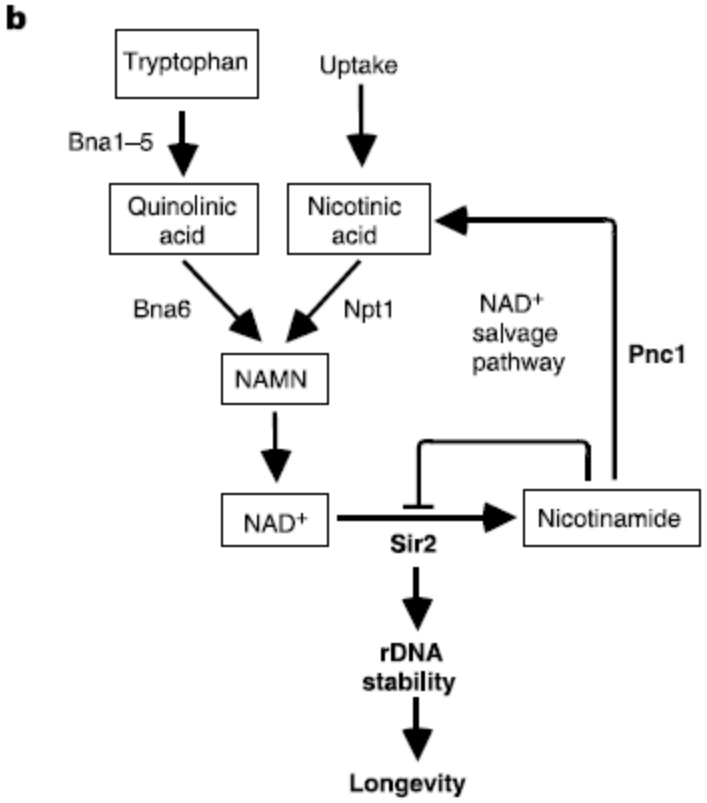
Higher Respiratory Activity Decreases Mitochondrial Reactive Oxygen Release and Increases Life Span in *Saccharomyces cerevisiae*

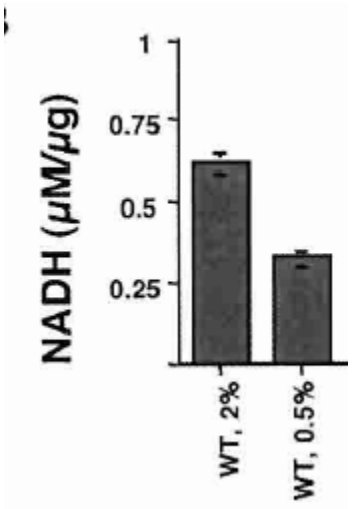


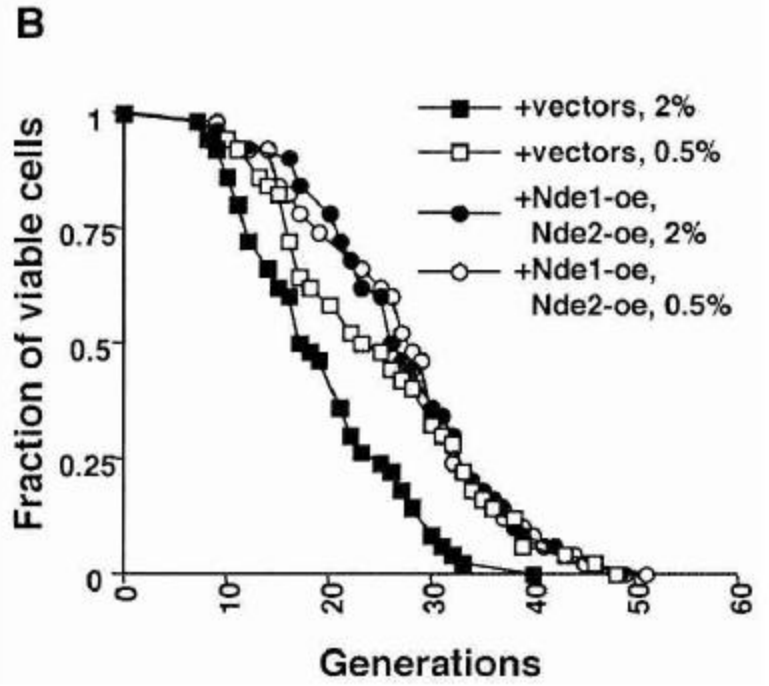


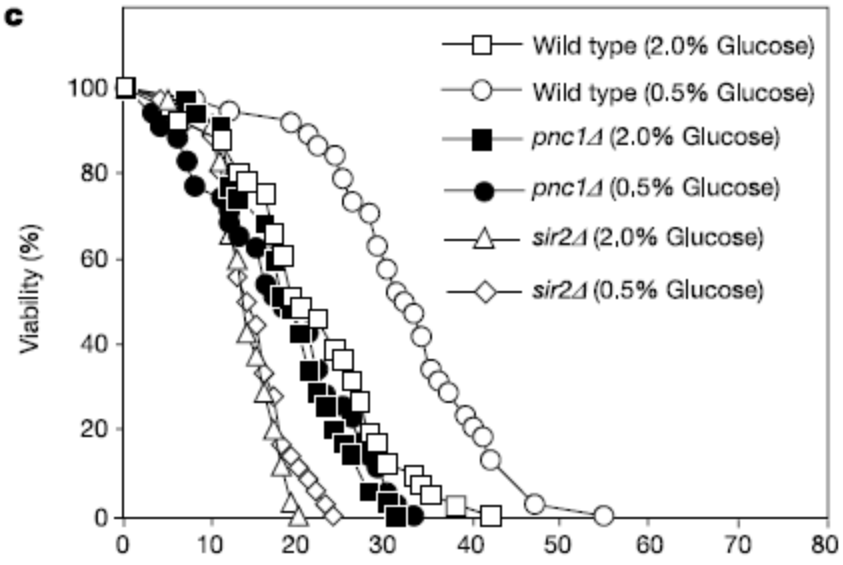


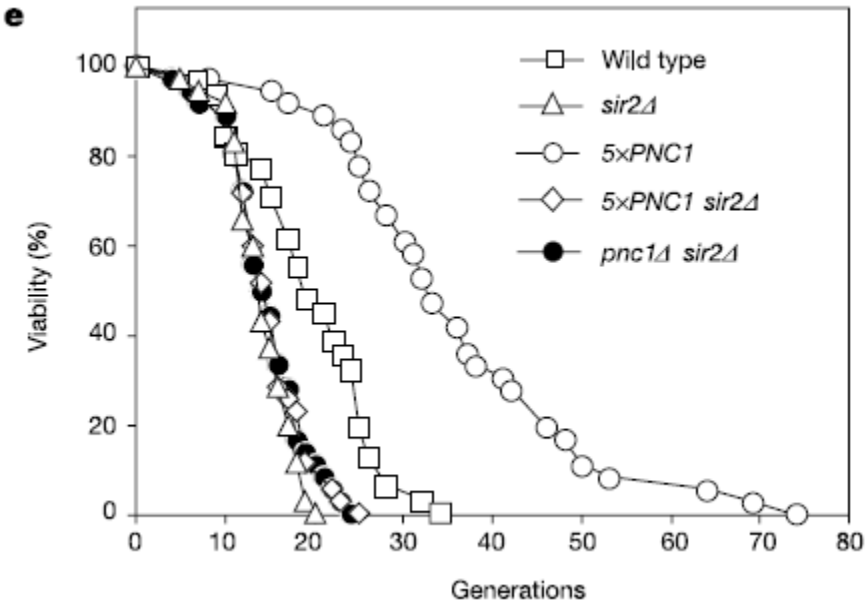
Barros. M.H. et al. (2004) **279:**49883-49888





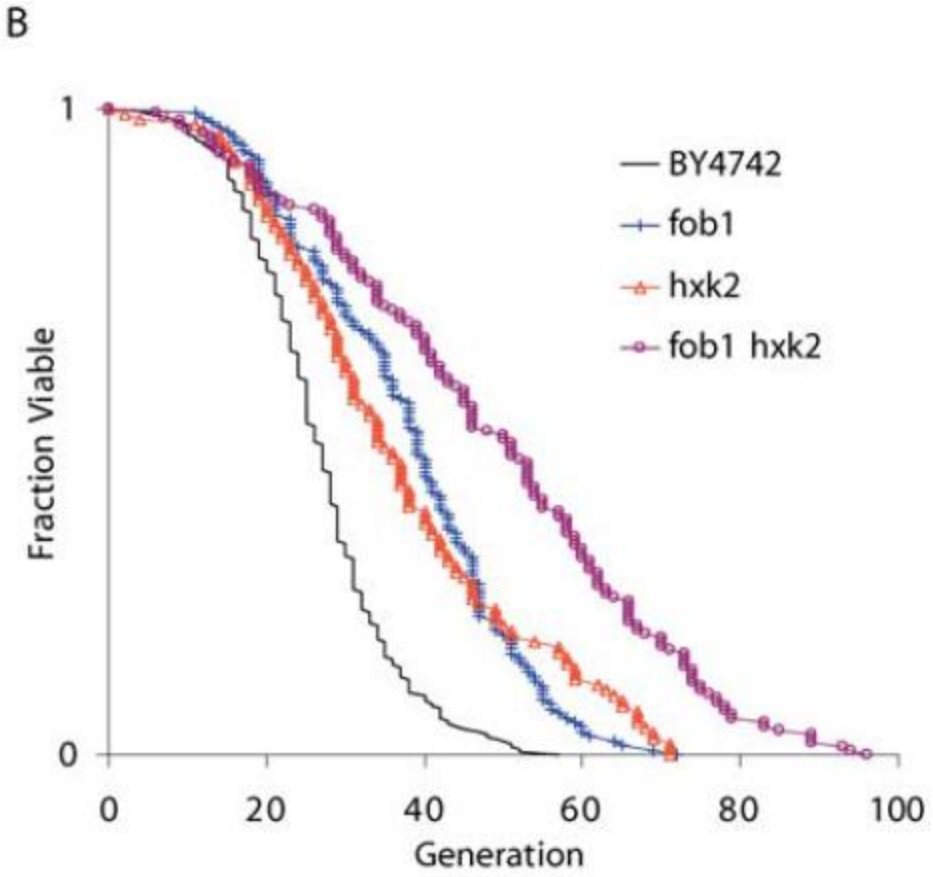






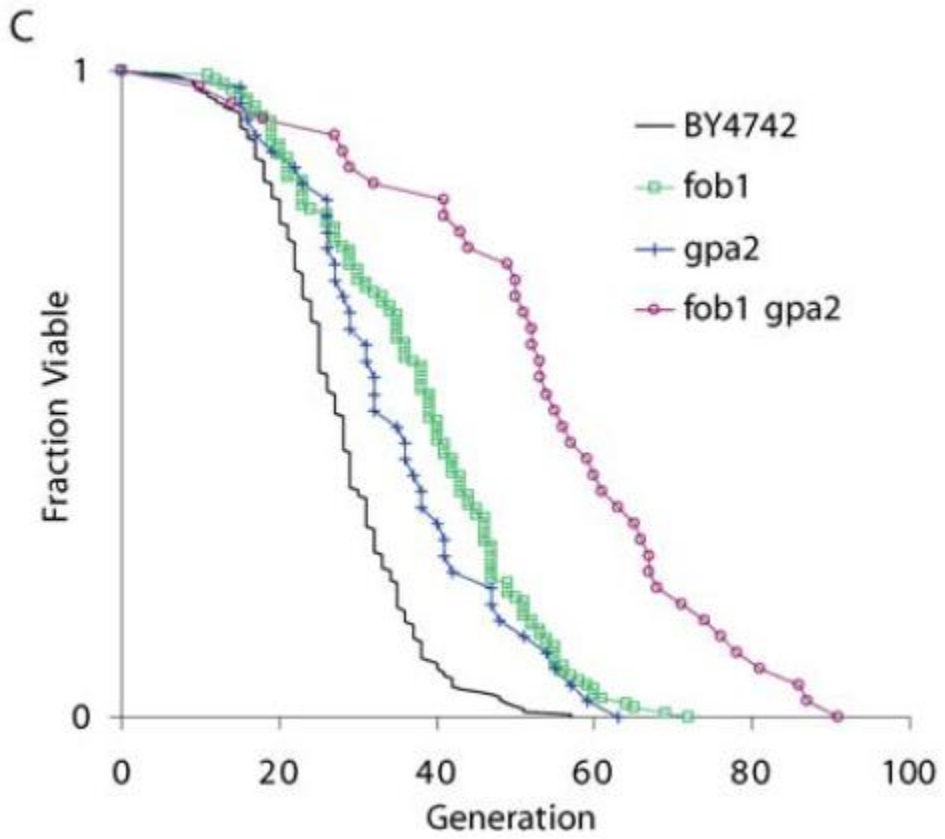
Anderson, R.M. et al. (2003) Nature **423:**181-185
Lin, S-J. et al. (2004) Genes Dev **18:**12-16

Regulation of Longevity by CR and Fob1



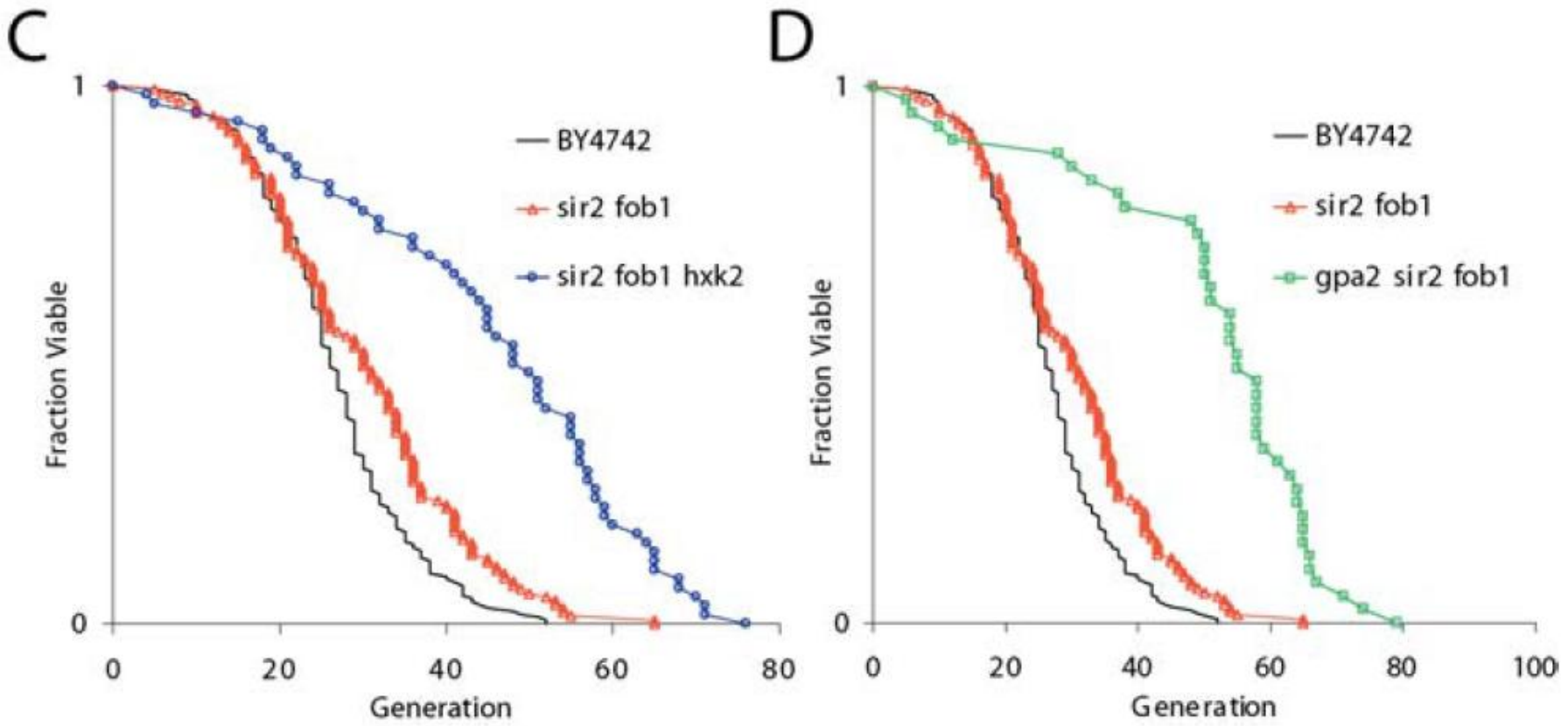
Kaeberlein et al. (2004) PLoS Biol 2(9): e296

Regulation of Longevity by CR and Fob1



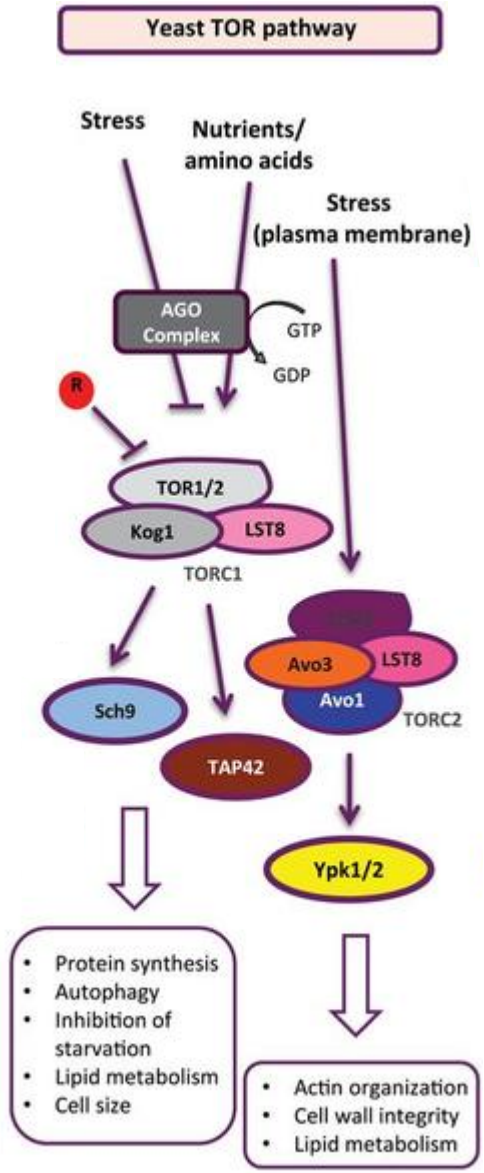
Kaeberlein et al. (2004) PLoS Biol 2(9): e296

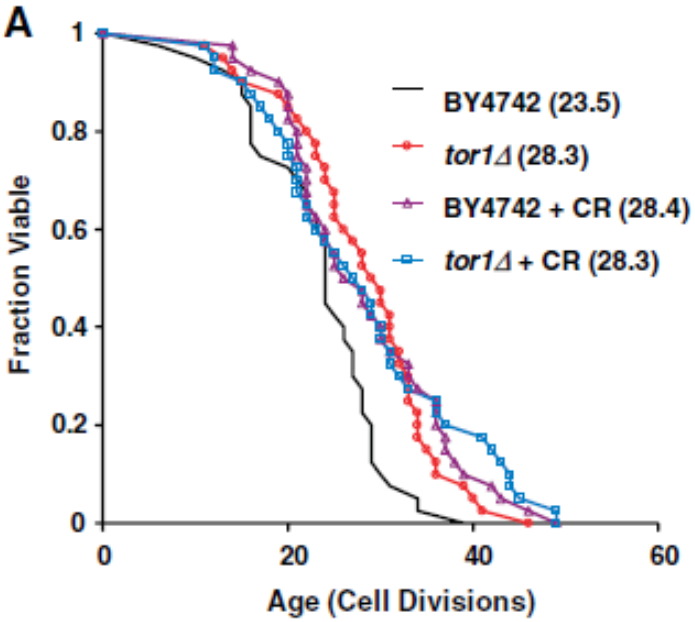
Life Span Extension by CR Does Not Require Sir2

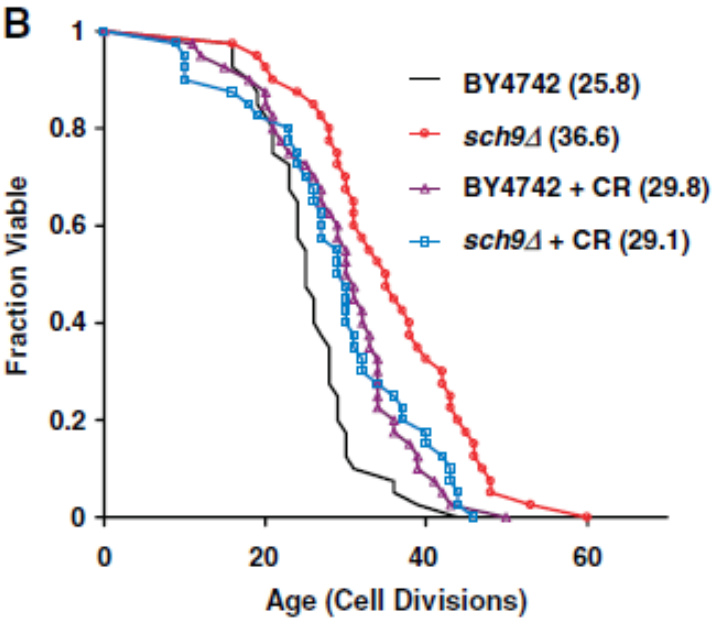


Kaeberlein et al. (2004) PLoS Biol 2(9): e296

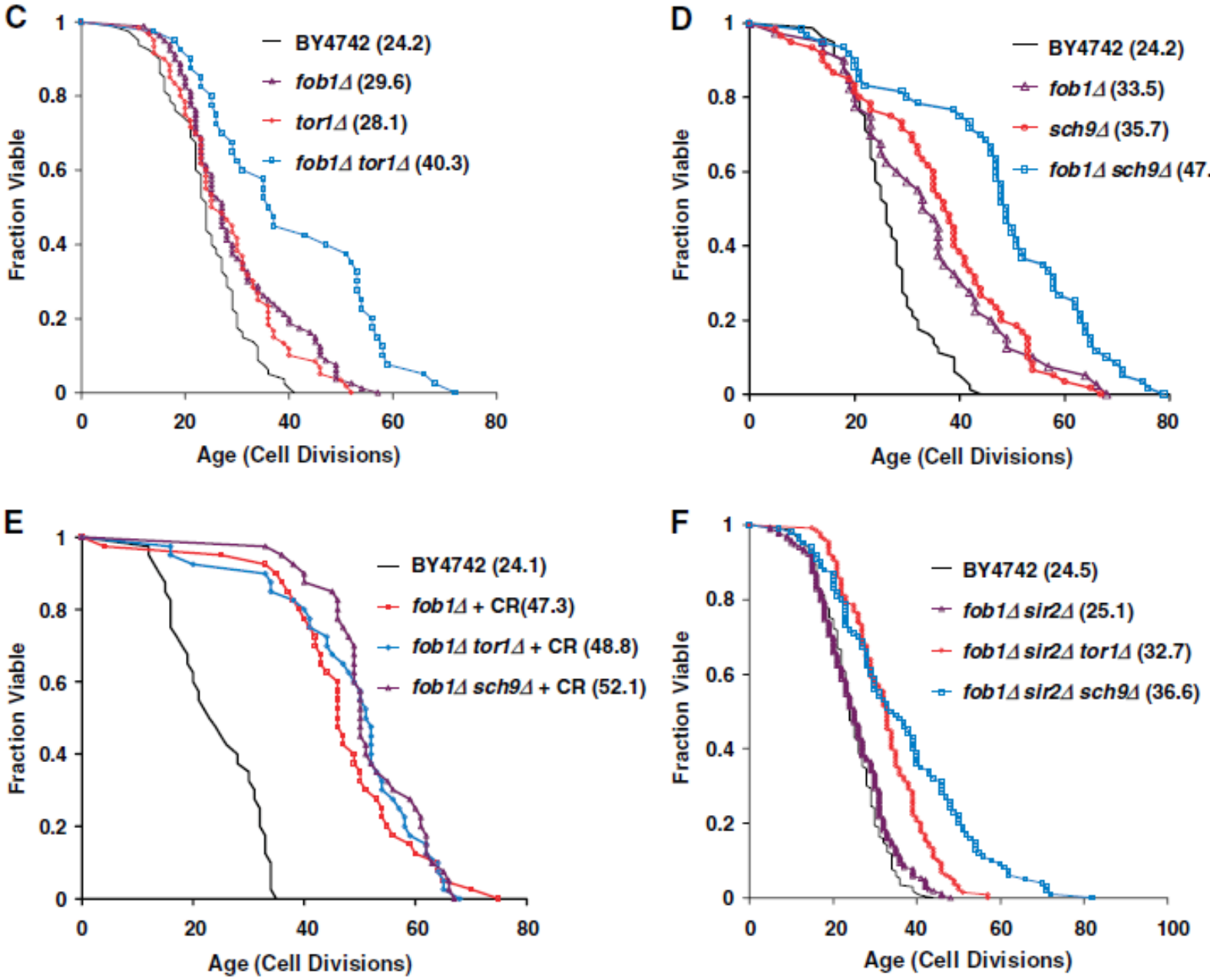
TOR1 or SCH9 deletion mutants are genetic mimics of CR.





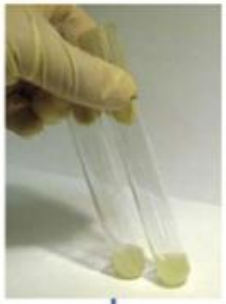


Kaeberlein et al. (2005) Science 310:1193-1196



Kaeberlein et al. (2005) Science 310:1193-1196

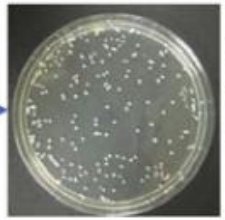
Yeast Chronological Lifespan Assay

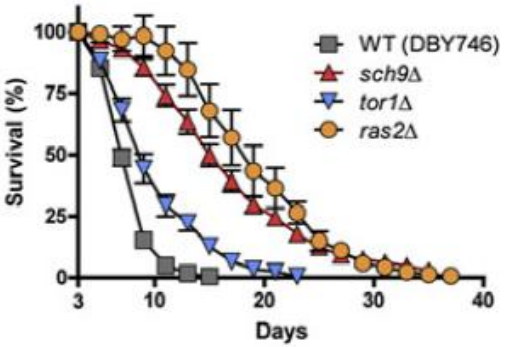


Dilute in fresh SDC using aluminum foil caps

A Standard CLS method

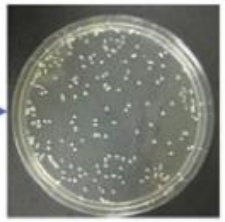
Age cells in liquid medium.
Plate cells onto YPD plates for CFU.





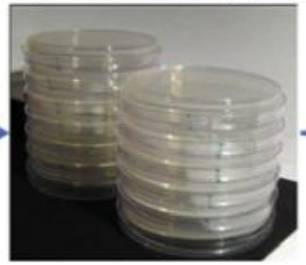
B Extreme CR/Starvation

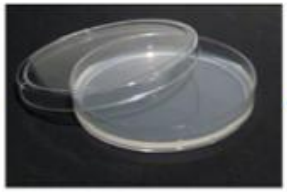
Switch cells to water on day 3. Plate cells onto YPD plates for viability.

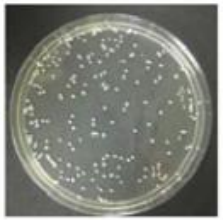


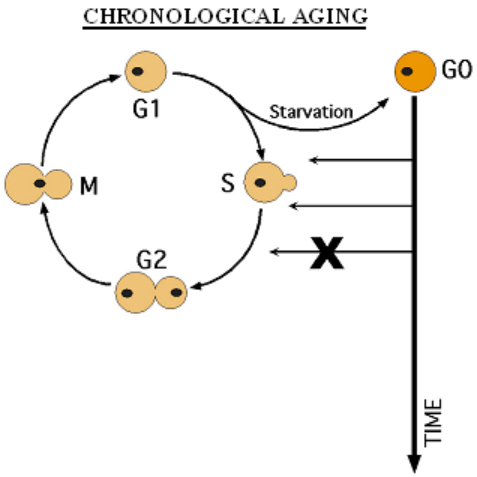
C *in situ* (plate) viability

Age cells on a set of SDC–trp or other nutrient deficient plates. Every two days, take one plate, add nutrient(s) required for growth to determine viability.



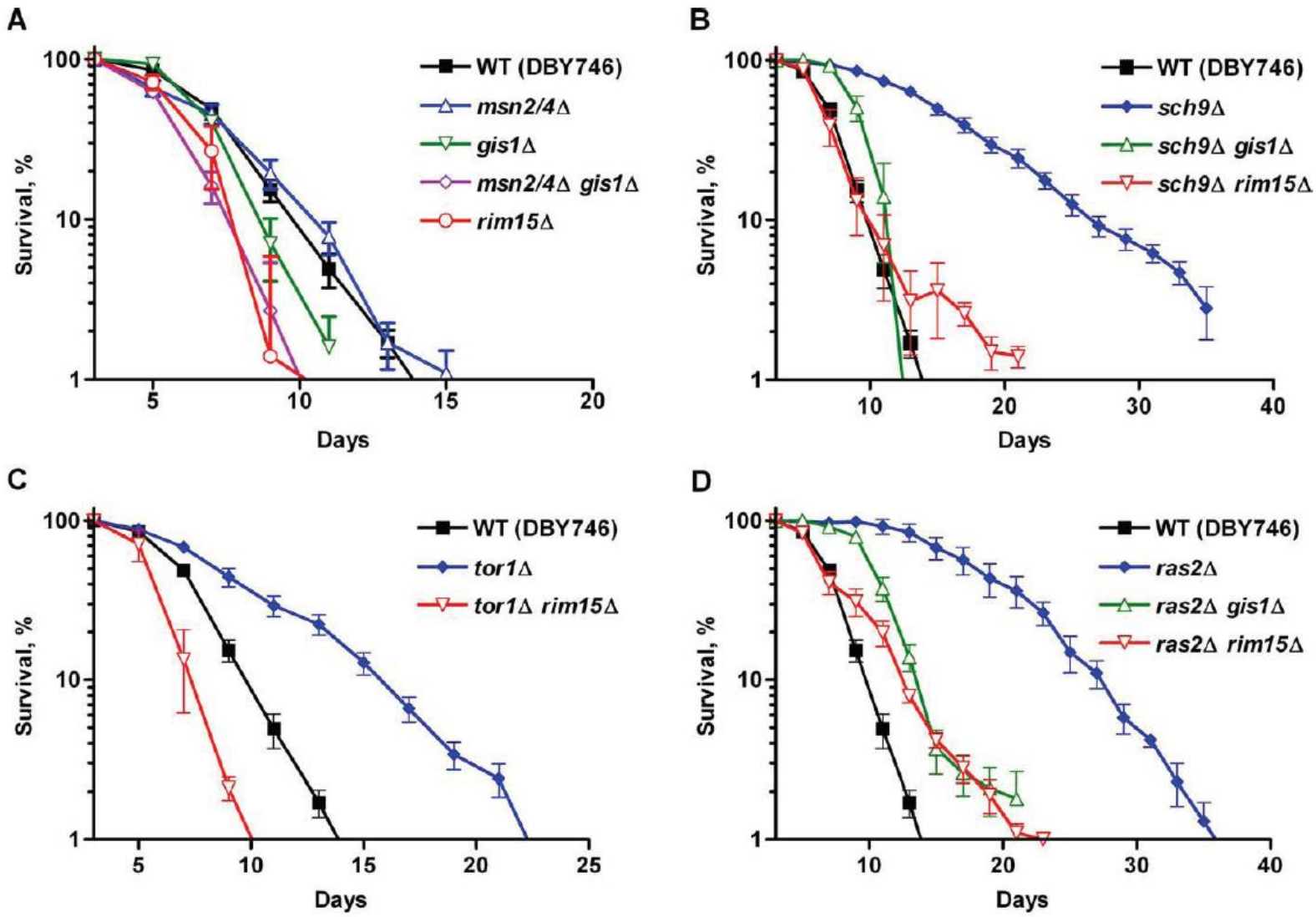






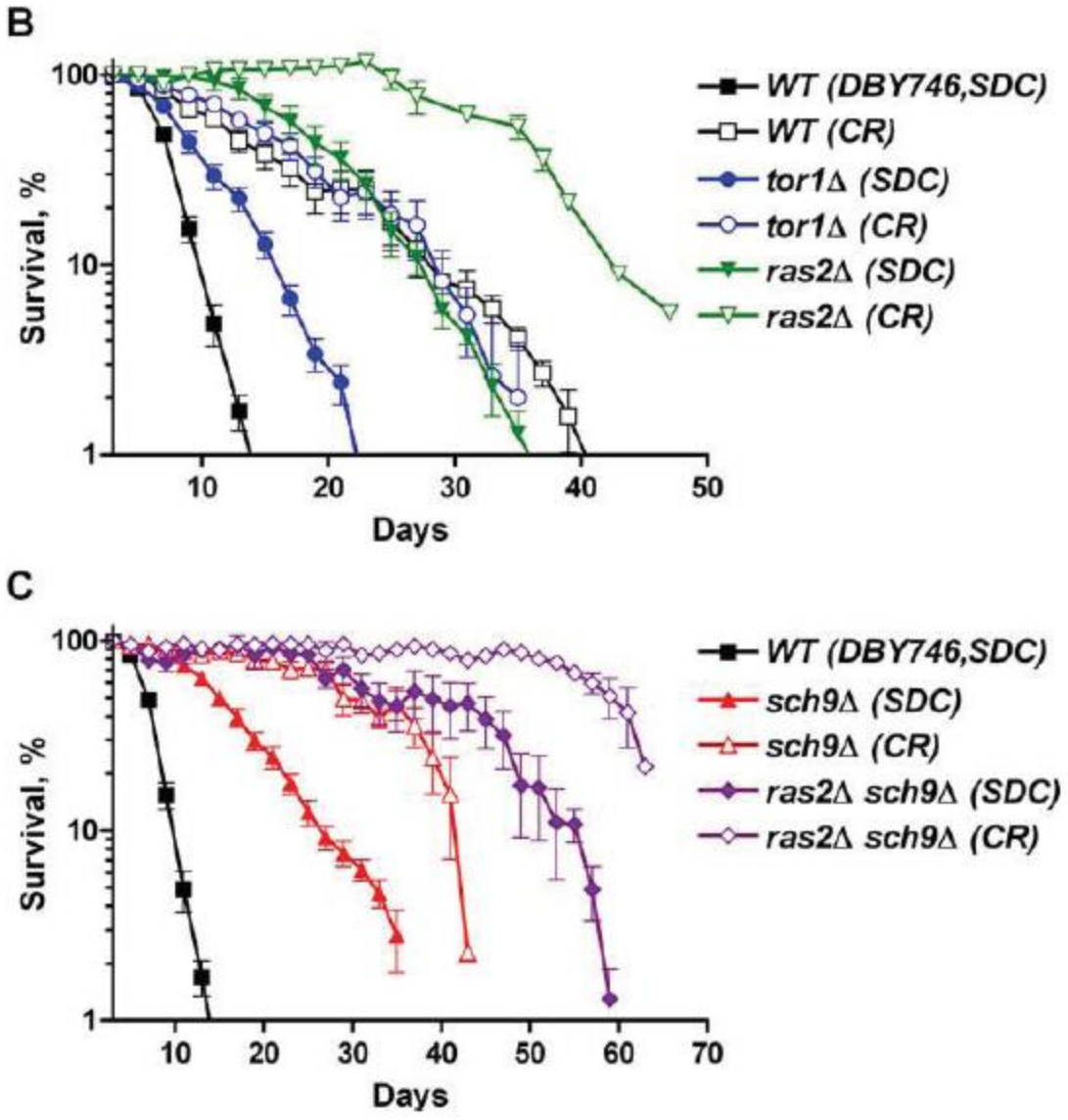
Longo, V.D. (2012) Cell Metabol **16:**18-31

Rim15 Is Required for Chronological Lifespan Extension



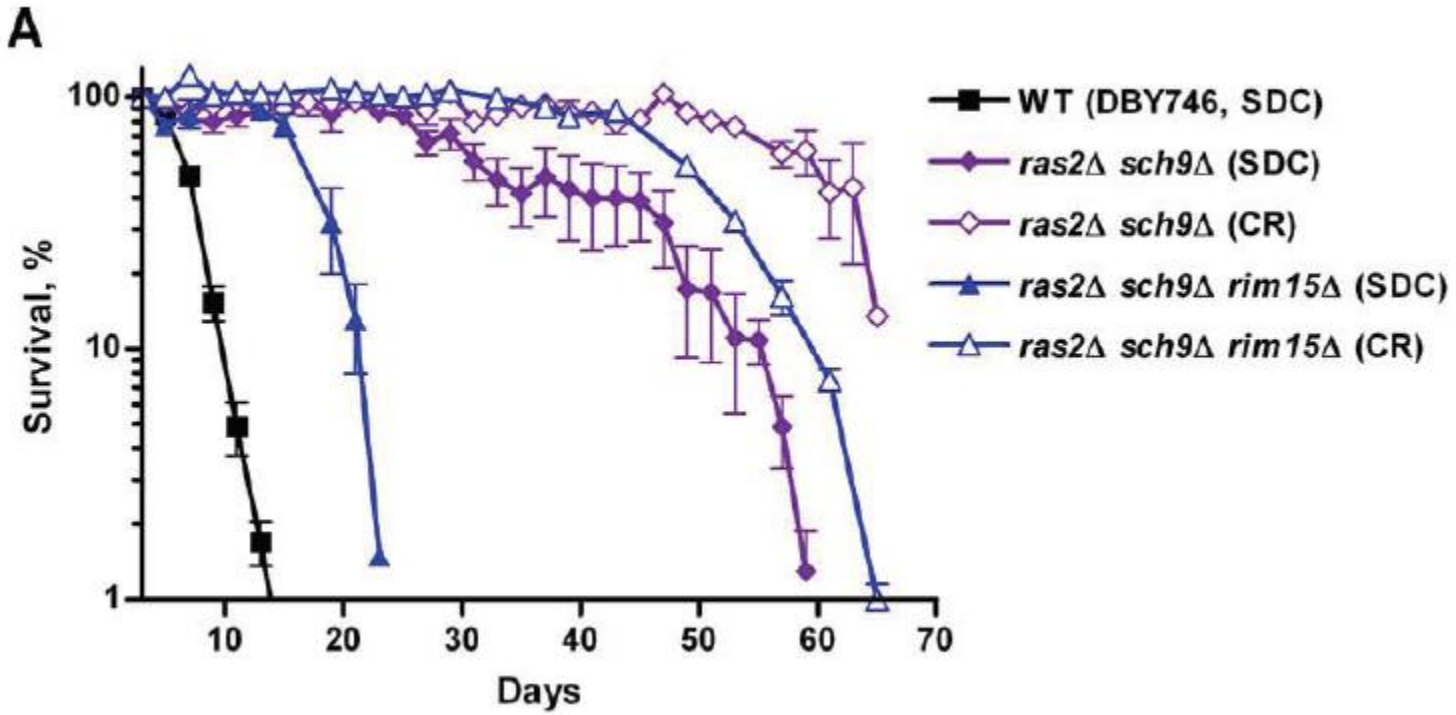
Wei et al. (2008) PLoS Genet 4(1): e13. doi:10.1371/journal.pgen.0040013

CLS under Extreme CR



Wei et al. (2008) PLoS Genet 4(1): e13. doi:10.1371/journal.pgen.0040013

Rim15-independent Mechanism(s) Is Required for Maximal Life Span Extension



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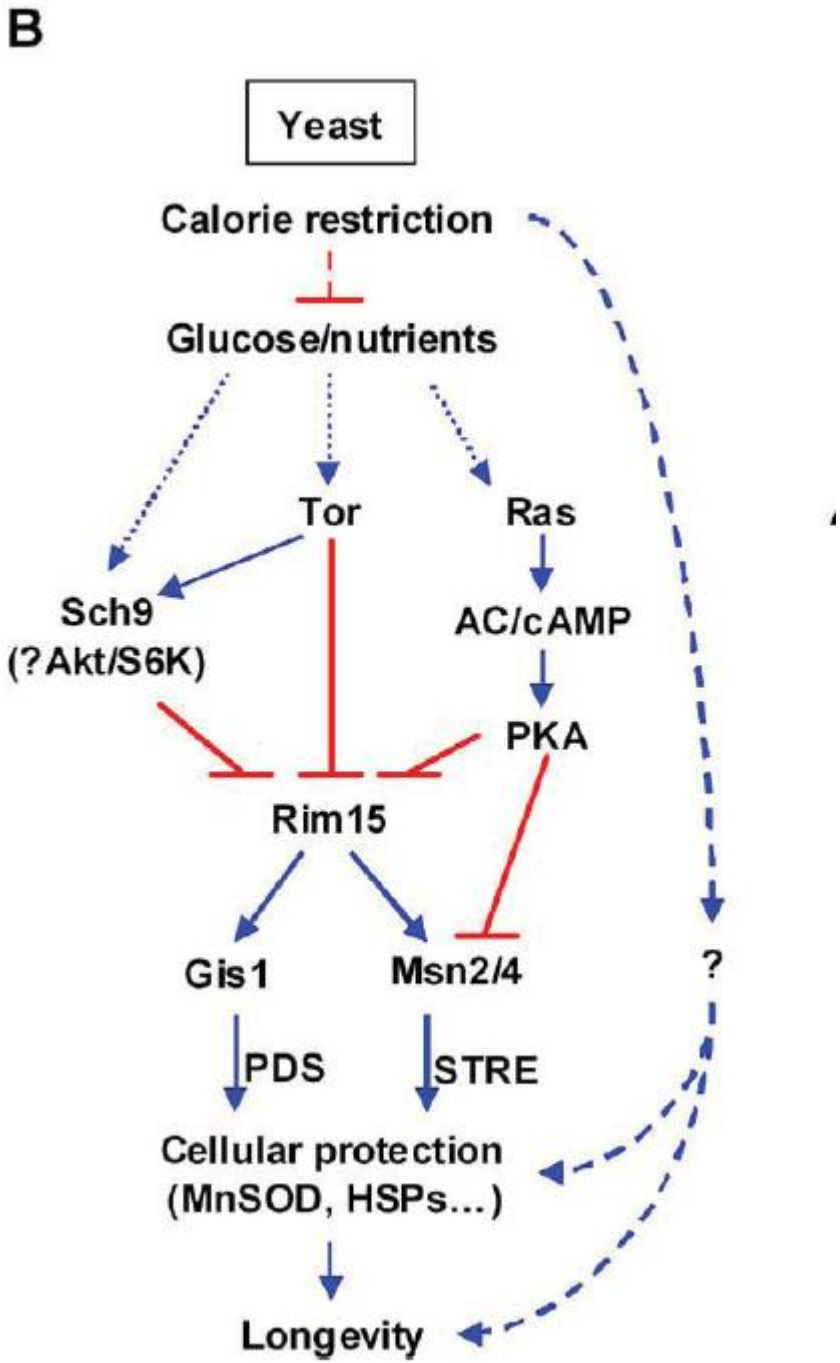

Wei et al. (2008) PLoS Genet 4(1): e13. doi:10.1371/journal.pgen.0040013

Resumo

1. A Curva de Gompertz-Makeham mostra como evolui a mortalidade de uma população ao longo do tempo;

2. Ensaio de Longevidade Replicativa mede o número de brotos que uma célula-mãe é capaz de produzir até a senescência;

3. A célula-mãe segrega ROS, mitocôndria funcional e ERCs em seu citoplasma para gerar uma célula-filha “jovem”;

4. A sinalização retrógrada estende a RLS aumentando a síntese de precursores biossintéticos da célula. A resposta a proteínas mal-enoveladas no RE e a acidificação do vacúolo são processos que também estendem a RLS;

5. A via TOR está associada ao mecanismo de restrição calórica (intervenção que estende a RLS);

6. O Ensaio de Longevidade Cronológica mede quanto tempo uma célula pode permanecer em estado quiescente;

7. A restrição calórica ativa o fator Rim15 cuja atividade é modulada por TOR e Ras2/Via da PKA.