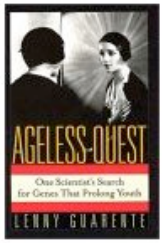
Sirulins



Ageless Quest: One Scientist's Search for Genes That Prolong Youth

Leonard Guarente

A&S300-002 Jim Lund

Functions of yeast *SIR2*

- The structure of the nucleolus.
- For silencing near chromosome telomeres.
  - Distinct from repression.
  - Does not allow any transcription machinery-activators or repressors-to bind.
- For silencing of special sequences involved in cell-type differentiation.

Silencing:

- The term given to a mechanism by which gene expression in regions of the genome is repressed
- Silencing can be modified by changes in chromatin structure

- In eukaryotes, altering chromatin states around a gene allows cells to achieve complex patterns of regulation.
- SIR2p is a component of chromatin
- SIR2p sets up silent chromatin states around the genes it regulates.

Transcriptional silencing

Occurs at three specific locations in the *S. cerevisiae* genome

1. Telomeres (including sub-telomeric repeats)
2. Mating loci: HML/HMR
3. Ribosomal DNA (rDNA)

SIR2 is a deacetylase

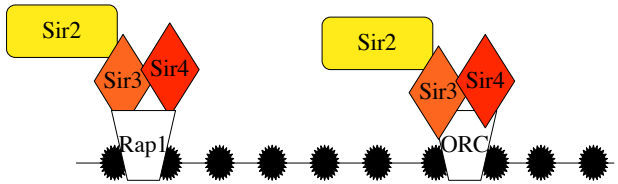
Yeast SIR2p and SIR2-like proteins have an NAD+-dependent deacetylase activity which is responsible for underacetylated histones within silent chromatin.

This deacetylation is coupled to NAD cleavage.

NAD is cleaved into : an ADP-ribose moiety and nicotinamide

A novel cellular metabolite:
O-acetyl-ADP-ribose is generated.

SIR2 chromatin repression



Sir3 and Sir4 are both capable of binding to the N-terminal tails of histones H3 and H4 with particular patterns of acetylation

Transcriptional silencing and aging
Sir2 and the formation of extrachromosomal rDNA circles (ERCs)

Ribosomal DNA repeats (rDNA)

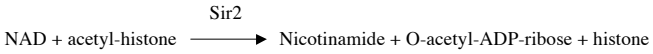
100-200 copies of a 9.1kB unit in tandem repeats

Specialized form of silencing - RNA pol I and III are still able to transcribe DNA, but RNA pol II is repressed by the structure formed here

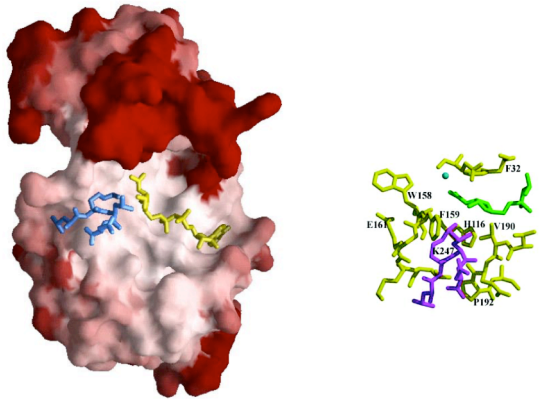
Sir2 is key player in maintaining this unique structure

Recent work has uncovered links between the rDNA locus, the silencing machinery, and aging

Sir2 is an NAD-dependent histone deacetylase (HDAC)

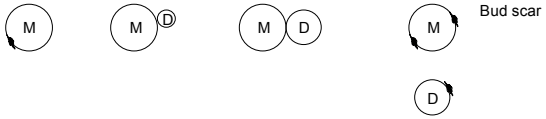


Potential substrate binding cleft of Sir2

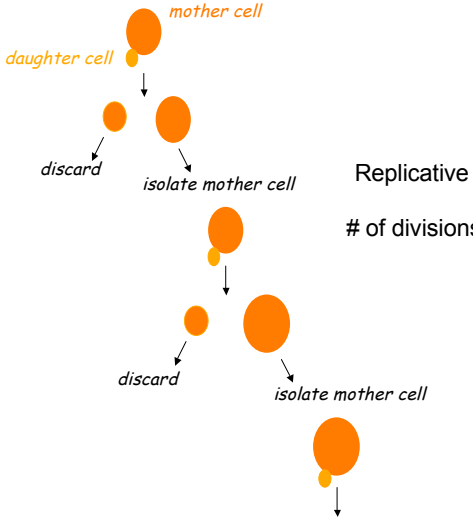


Yeast aging

Replicative aging in yeast is defined as the number of times a cell buds before senescence



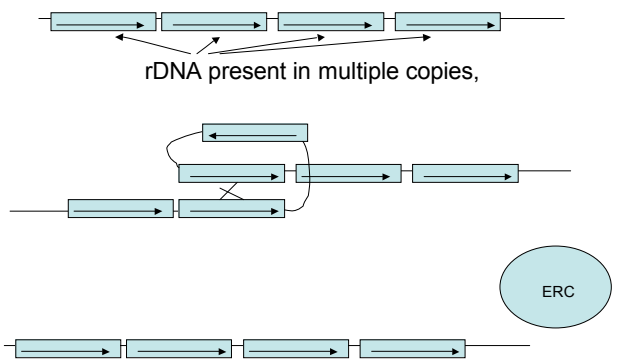
Average of about 25 daughters



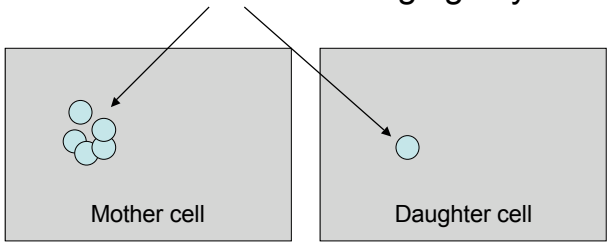
Replicative aging in yeast =

# of divisions of a mother cell

Transcriptional silencing and aging in yeast
Formation of extrachromosomal rDNA circles (ERCs)



How do ERCs affect aging in yeast?



- ERCs do not segregate equally during budding so that the 'old mother' cell retains more than the 'new daughter' cell (No centromeric sequences).
- They are amplified during each round of cell division because of the ARS (Autonomous Replicating Sequence)
- They accumulate over time
- ERC formation has been causatively linked to aging (artificially produced and senescence ensues)
- Mechanism not known, but it is proposed that the unsilenced excess of ERCs titrates away essential transcription and/or replication factors

What do silencing proteins have to do with ERC formation?

- Silencing proteins were observed to relocate from the telomeres to the nucleolus and this is associated with extension of life span
- Sir2 has been shown to suppress recombination of rDNA repeats
- Sir2 has been shown to suppress the formation of ERCs
- **Deletion of *SIR2* shortens life span, and an additional copy of *SIR2* increases life span (Sinclair and Guarent, 1997).**

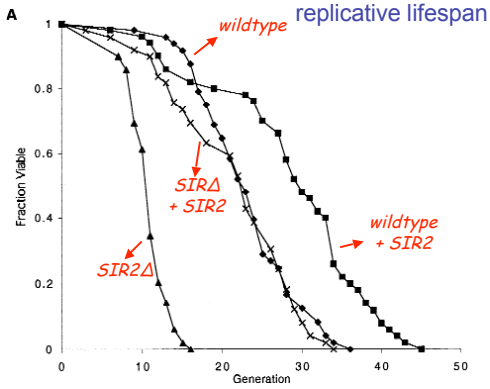
MODEL: Repressive chromatin formation at the rDNA locus is important for increased life span. It is likely that in yeast, the direct mechanism by which this repressive chromatin structure affects life span is via decreased levels of recombination. The secondary consequence of this may be inappropriate gene expression, but that is speculative.

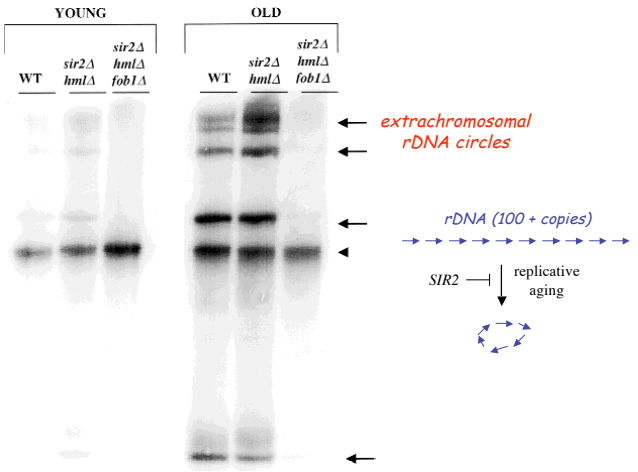
The *SIR2/3/4* complex and *SIR2* alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms

Matt Kaeberlein,[1] Mitch McVey,[1] and Leonard Guarente[2]
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 USA

GENES & DEVELOPMENT 13:2570-2580 © 1999

One extra copy of the *SIR2* gene increases replicative lifespan in yeast





SIR2 and the formation of extrachromosomal rDNA circles (ERCs): Links to metabolic rate

- Life span can be increased in many organisms, including yeast, by calorie restriction.
- NAD and *SIR2* are required for life-span extension by calorie restriction in yeast.

Decreased glycolysis

Proposed model

↓

Increased cellular NAD levels

↓

NAD + acetyl-histone —SIR2→ Nicotinamide + O-acetyl-ADP-ribose + histone

Increased silencing
Decreased rDNA recombination

Is there any relevance of this pathway to other organisms?

- *SIR2* is well conserved throughout evolution
- Family members are found in archaea (where the crystal structure and mechanism were determined), bacteria (more distantly related) and throughout eukaryotes.

- In *C. elegans*, there is evidence that a SIR2 homolog is also involved in aging.
- It has been shown that the human SIR2 homolog (SIRT1) is also an NAD-dependent HDAC.
- Notably, the human protein deacetylates p53 and FOXO proteins.

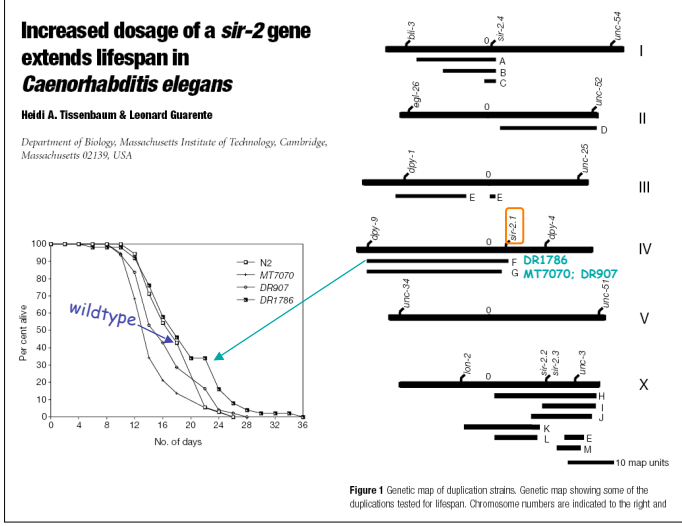
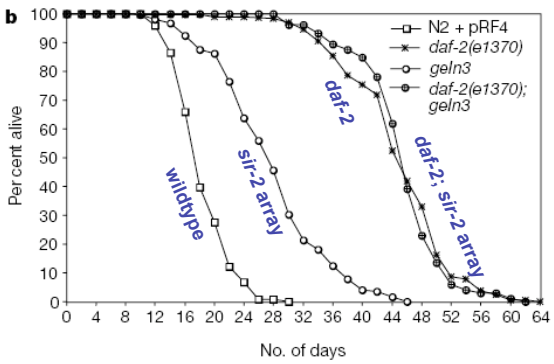
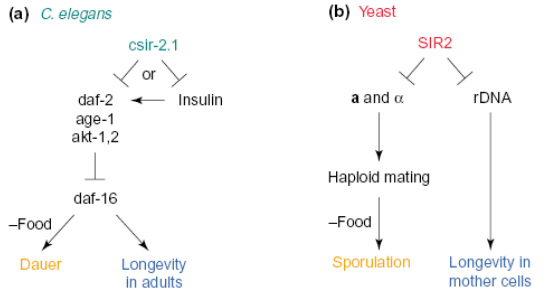


Figure 1 Genetic map of duplication strains. Genetic map showing some of the duplications tested for lifespan. Chromosome numbers are indicated to the right and



sir-2.1 overexpression does not further extend the lifespan of a long-lived *daf-2* insulin-signaling mutant.



MODEL:

sir-2.1 overexpression can extend post-replicative *C. elegans* lifespan and replicative yeast lifespan

Kenyon and Guarente, *Nature*, 2000
Hekimi and Guarente, *Science*, 2003

SIR2 is an NAD^+-dependent (histone) deacetylase

↓

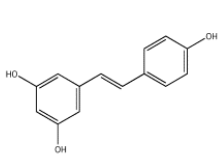
SIR2 activity can extend lifespan

↓

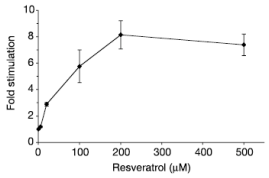
Screen small molecule library to define Sir2 agonists

↓

Identify resveratrol (and related compounds)



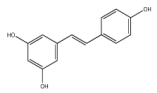
resveratrol



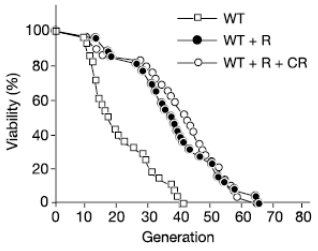
Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan

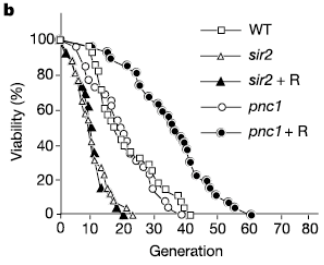
Konrad T. Howitz[1], Kevin J. Bitterman[2], Haim Y. Cohen[2], Dudley W. Lamming[1], Siva Lavu[1], Jason G. Wood[1], Robert E. Zipkin[1], Phuong Chung[1], Anne Kisielewski[1], Li-Li Zhang[1], Brandy Scherer[1] & David A. Sinclair[1]

NATURE | VOL 425 | 11 SEPTEMBER 2003



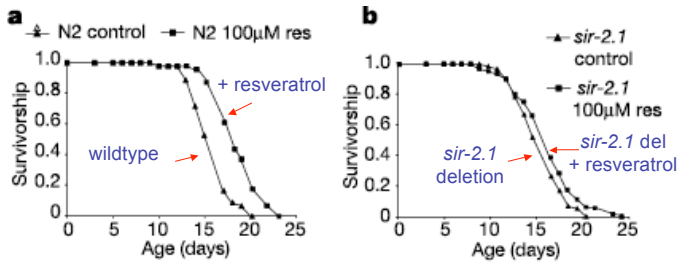
resveratrol



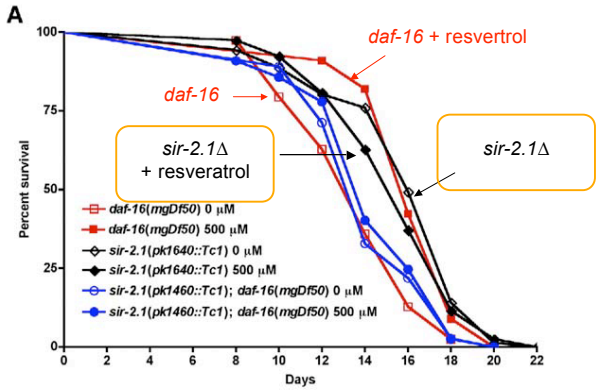


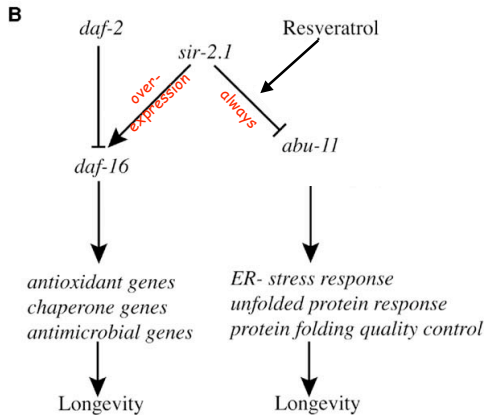
Resveratrol can extend lifespan in yeast

Resveratrol extends lifespan in *C. elegans*



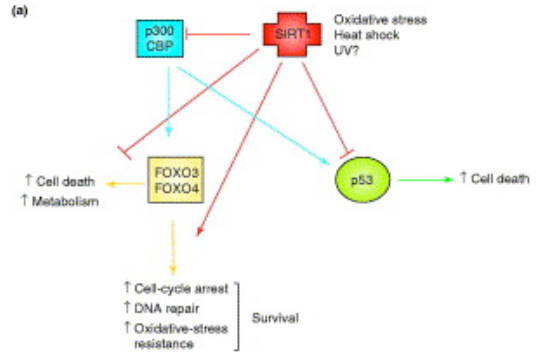
Resveratrol-mediated lifespan extension is independent of *daf-16*!





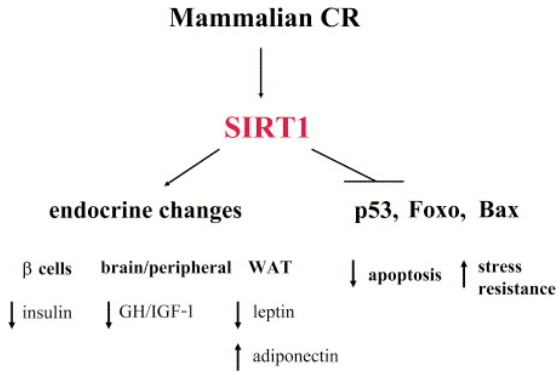
C. elegans SIR-2.1 has 2 activities that extend lifespan!

How SIRT1 Mediates Some of the Effects of CR in Mammals



Giannakou and Partridge, 2005

How SIRT1 Mediates Some of the Effects of CR in Mammals



Guarente and Picard, 2005

Resveratrol in humans

- lowers the Michaelis Constant of SIRT1 for both:
  - the acetylated substrate
  - and NAD+.
- Increases cell survival in tissue culture by stimulating SIRT1-dependent deacetylation of gene p53.
  - Protects cultured human cells from radiation.