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INSTITUT LADY DAVIS DE RECHERCHES MÉDICALES / LADY DAVIS INSTITUTE FOR MEDICAL RESEARCH

Centre Bloomfield de recherche sur le vieillissement

The Bloomfield Centre for Research in Aging

Cancer and Aging: Two Faces of the Same Coin

(1) Theories, Mechanisms and Models of Aging

Cancer and Aging: Two Faces of the Same Coin

(1) Theories, Mechanisms and Models of Aging
(2) Telomere Biology and Aging
(3) Telomere Biology and Cancer-Part 1 and Part 2
(4) Telomerase and Telomere Regulation
(5) Telomeres, Telomerase and The Premature Aging Syndrome Dyskeratosis congenita
(6) Telomeres in Premature Aging and Degenerative Diseases

References:

Background reading and reference material of interest

Ljubuncic, P. and Reznick, A.Z. 2009. The evolutionary theories of aging revisited-A mini-review. Gerontology 55, 205-216.

Smith, D.L. et al. 2010. Calorie restriction: what recent results suggest for the future of ageing research. European Journal of Clinical Investigation 40, 440-450.

Lapointe, J. and Hekimi, S. 2010. When a theory of aging ages badly. Cellular and Molecular Life Science 67, 1-8.

Hekimi, S., Lapointe, J. and Wen, Y. 2011. Taking a "good" look at free radicals in the aging process. Cell 21, 569-576

Imai, S. and Guarente, L. 2010. Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases. Trends in Parmacological Sciences 31, 212-220.

Sahin, E. and DePinho, R.A. 2010. Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature 464 520-528.

Rando, T.A. and Chang, H.Y. 2012. Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. Cell 148, 46-57.

Lecture outline:

A. Introduction

•Lifespan and life expectancy
•Characteristics of aging
•Approaches to studying aging, use of model organisms
•Caloric restriction

B. Mechanisms/Causes of Aging

Evolutionary theory of aging
Free Radical (Oxidative Stress)/ Mitochondrial DNA theory of aging
Gene regulation theory of aging (Sir proteins)
Telomere theory of aging

- •Replicative senescence and the Hayflick limit
- •Characteristics of senescent cells
- •Cellular senescence versus aging
- •What are telomeres?
- •Telomeres and aging
- •End replication problem and telomere shortening

A. INTRODUCTION

What comes to mind when I say aging?

A. INTRODUCTION

What comes to mind when I say aging?

hair graying
wrinkled skin
wisdom
knowledge
natural process
death

Loss of function:

hearing loss
decreased reproduction
cataracts
fragility
muscle atrophy
anemia
feeble immune response
impaired wound healing
cell death
osteoporosis
Alzheimer's
cancer

Why do we study aging?

How do we study aging?

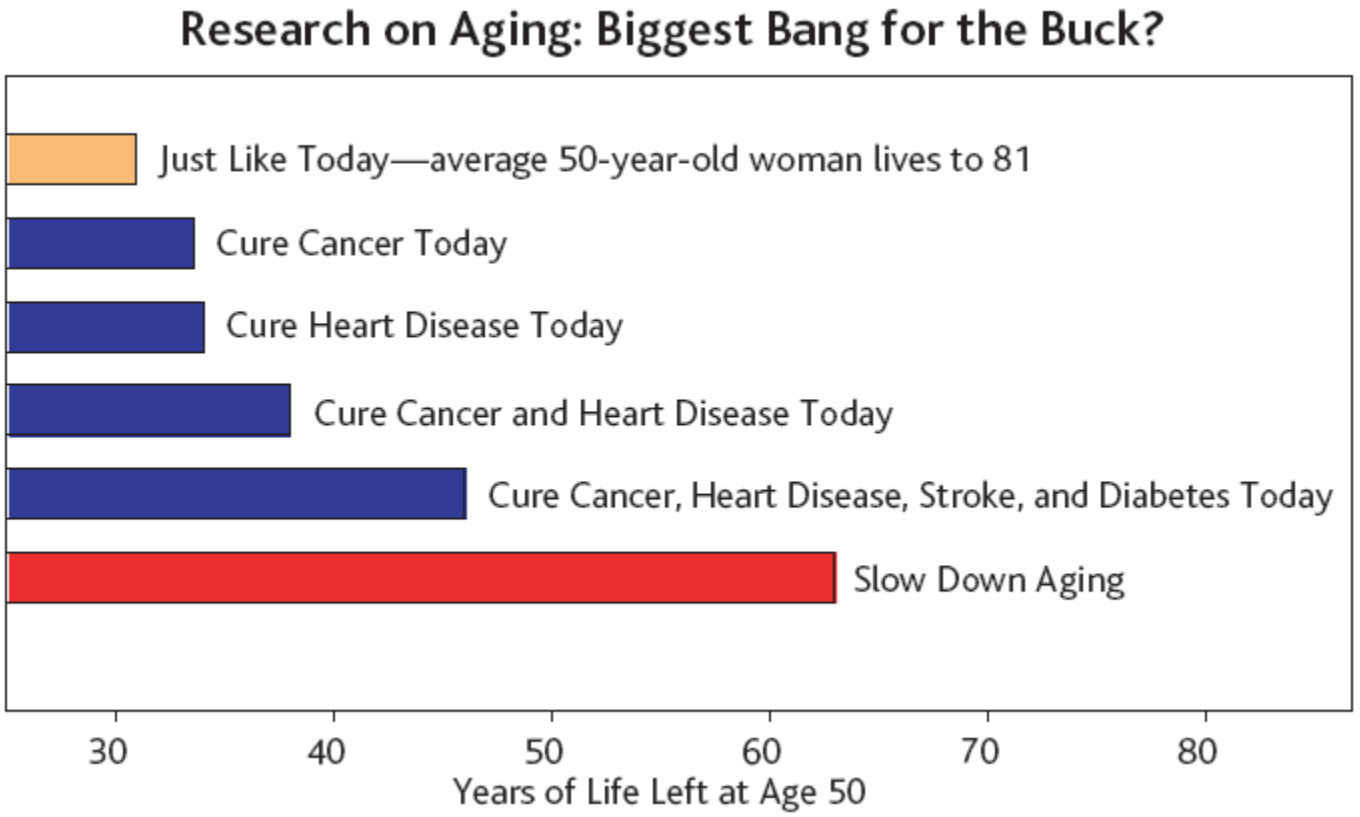
Why do we study aging?

-gain understanding of diseases of aging
-prevent/cure diseases of aging
-prolong life span
-stay young
-gain understanding of mechanisms of normal and abnormal aging
-improve quality of life in aging

How do we study aging?

Premature aging syndromes?
Age-related diseases-cancer, Alzheimer's
Model systems -mouse models (transgenic, knockout)
-C.elegans/cell culture (human/mouse)
Human population studies

Resolution of cardiovascular disease, diabetes and cancer would increase human life expectancy by 15 years



Martin, G.M. et al. 2003. Research on aging: the end of the beginning. Science 299: 1339-1341.
Hayflick, L. 2000. The future of ageing. Nature 408, 267-269.

Lifespan and Life Expectancy

- **Lifespan is the maximum number of years that a human can live (~125 years-unchanged)**

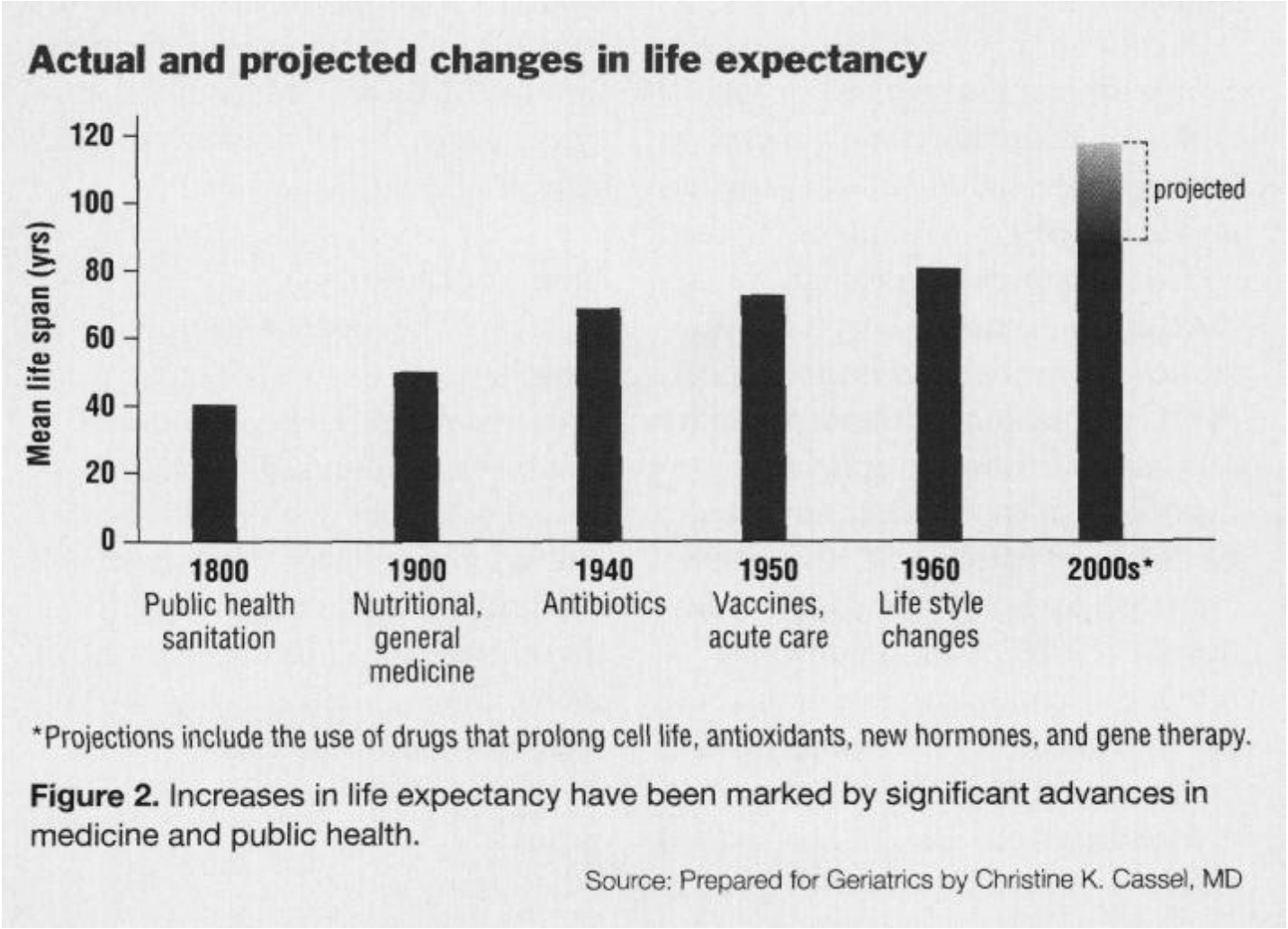
- **Life expectancy is defined as the average total number of years that a human expects to live**

In the last century there has been a significant gain in human longevity with the life expectancy increasing by 27 years, to approximately 80 years, in Western Countries (The life expectancy continues to rise, and based on 2008 statistics has now reached 76.4 years for men, and 82.4 years for women in the European Union)

Why?

Tosato, M. et al. 2007. The aging process and potential interventions to extend life expectancy. Clin. Inter. in Aging. 2, 401-412.

Ageing as an artefact of civilization



Cassel, C.K. 2001. Successful aging. Geriatrics. Vol. 56 35-39.
Hayflick, L. 2000. The future of ageing. Nature 408, 267-269.

Characteristics of Aging

TABLE 1

Characteristics of Aging

1.	Increased mortality with age after maturation.
2.	Changes in biochemical composition in tissues with age.
3.	Progressive decrease in physiological capacity with age.
4.	Reduced ability to respond adaptively to environmental stimuli with age.
5.	Increased susceptibility and vulnerability to disease.

1900: 4% >65 years of age
1992: 12%
2030: 22%

Troen, B.R. 2003. The Biology of Aging. The Mount Sinai Journal of Medicine Vol 70: 3-22.

Some North American statistics

Individuals over the age of 65

50% develop cardiovascular disease
35% develop arthropathies
15% develop type 2 diabetes
10% develop pulmonarydisease

Stroke and dementia, the most common cause of institutionalization cost 21 billion dollars per year.

Between the ages of 40 and 80, increased cancer incidence producing a lifetime cancer risk of nearly 1 in 2 in industrialized nations.

Sahin, E. and DePinho, R.A. 2010. nature 464, 520-528.

Model organisms

Table 1 | Conserved ageing phenotypes

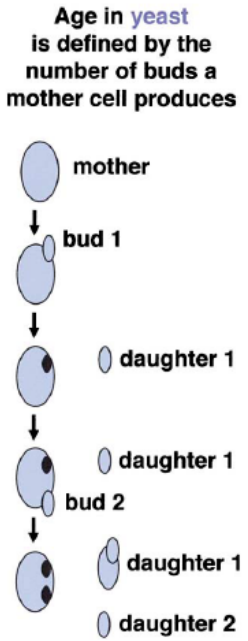
Phenotype	*H. sapiens*	*M. musculus*	*D. melanogaster*	*C. elegans*
Decreased cardiac function	Yes	Yes	Yes	NA
Apoptosis, senescence (somatic cells)	Yes	Yes	Yes	?
Cancer, hyperplasia	Yes	Yes	No	No
Genome instability	Yes	Yes	Yes	Yes
Macromolecular aggregates	Yes	Yes	Yes	Yes
Reduced memory and learning	Yes	Yes	Yes	NA
Decline in GH, DHEA, testosterone, IGF	Yes	Yes	?	?
Increase in gonadotropins, insulin	Yes	Yes	?	?
Decreased thyroid function	Yes	Yes	NA	NA
Decrease in innate immunity	Yes	Yes	Yes	Yes
Increase in inflammation	Yes	Yes	No	No
Skin/cuticle morphology changes	Yes	Yes	?	Yes
Decreased mitochondrial function	Yes	Yes	Yes	Yes
Sarcopenia	Yes	Yes	Yes	Yes
Osteoporosis	Yes	Yes	NA	NA
Abnormal sleep/rest patterns	Yes	Yes	Yes	?
Decrease in vision	Yes	Yes	?	NA
Demyelination	Yes	Yes	?	No
Decreased fitness	Yes	Yes	Yes	Yes
Arteriosclerosis	Yes	No	NA	NA
Changes in fat*	Yes	Yes	?	?

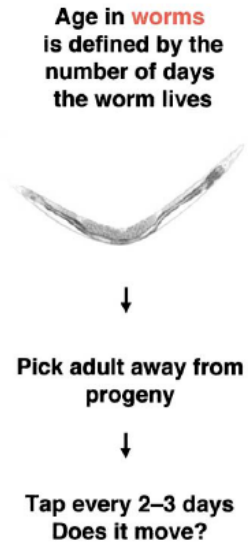
* Although changes in fat content and distribution have been reported for long-lived invertebrate mutants, at present there are no data on fat-related changes during normal ageing in these organisms. GH, growth hormone; DHEA, dehydroandrosterone; NA, not applicable.

Vijg, J. and Campisi, J. 2008. Puzzles, promises and a cure for ageing. Nature 454, 1065-1071.

Model organisms

Replicative lifespan:





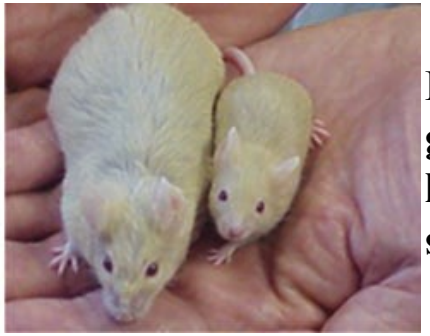
Chronological lifespan

Tissenbaum, H.A. and Guarante, L. 2003. Model Organisms as a Guide to Mammalian Aging. Dev. Cell 1, 9-19

Sch9 (a serine threonine kinase)

chico

GHR/BP (growth hormone receptor/binding protein)



Mutations that decrease glucose or insulin/IGF-1-like signaling/long-lived/ smaller

Longo, V.D. and Finch, C.E. 2003. Evolutionary Medicine: From Dwarf Model Systems to Healthy Centenarians? Science 299, 1342-1345. Liang et al. 2003. Genetic mouse models of extended lifespan. Experimental Gerontology 38, 1353-1364.

Caloric restriction

- **Typically refers to a diet in which calories are limited by 30-40% compared with animals fed *ad libitum***

- **Caloric restriction extends life span in rodents, worms, yeast (and nonhuman primates), and postpones or prevents a number of diseases (diabetes, cardiovascular disease, cancer) and age-dependent deterioration**

- **Selective value since reproduction can be postponed until food is available; when food is restored progeny are produced. Well fed controls become post-reproductive and die in the interim**

Longo, VD and Finch, CE. 2003. Evolutionary Medicine: From Dwarf Model Systems to Healthy Centenarians? Science 299, 1342-1345.

Piper, MDW and Bartke, A. 2008. Diet and aging. Cell Metabolism 8, 99-104.

Shanley DP and Kirkwood TBL. Caloric restriction does not enhance longevity in all species and is unlikely to do so in humans. Biogerontology (2006) 7, 165-168.

Smith, D.L., Nagy, T.R., Allison, D.B. 2010. Calorie restriction: what recent results suggest for the future of ageing research. Eur. J. Clin. Invest. 40, 440-450.

B. MECHANISMS/CAUSES OF AGING

***Evolutionary theory of aging**

***Mitochondrial free radical theory of aging (MFRTA)**

***Gene regulation theory of aging (Sir proteins)**

***Telomere theory of aging**

***Epigenetics and aging**

Tosato, M. et al. 2007. The aging process and potential interventions to extend life expectancy. Clin. Inter. in Aging. 2, 401-412.

Kirkwood, T.B.L. 2005. Understanding the odd science of aging. Cell 120, 437-447.

Blagonsklonny, M.V. et al. Impact papers on aging in 2009. Aging 2, 11-121.

Evolutionary Basis of Aging

Proposes two models for how aging can evolve:

1. **The theory of mutation accumulation**
2. **The antagonistic pleiotropy hypothesis**

Diminishing selection leads to the accumulation of late-acting harmful genes; old age is not under selective pressure per se, and there is no evolutionary mechanism to rid a population of mutations that cause detrimental effects only in old animals

A harmful late-acting gene remains in a population if it has a beneficial effect early in life (high testosterone in gorilla leads to artherosclerosis; large attractive feathers of male peacocks limits their ability to escape predators)

Lubuncic, P. and Reznick, A.Z. 2009. The evolutionary theories of aging revisited-A mini-review. Gerontology 55, 205-216.

Mitochondrial free radical theory of aging (MFRTA)

Initially proposed by Hartman in 1956 and refined in 1972

Damage to vital molecules, proteins, lipids and nucleic acids, can be caused by free radicals, byproducts of oxidative phosphorylation that occurs during aerobic metabolism.

Oxygen is reduced by the addition of electrons and converted into reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, hydroxyl radicals

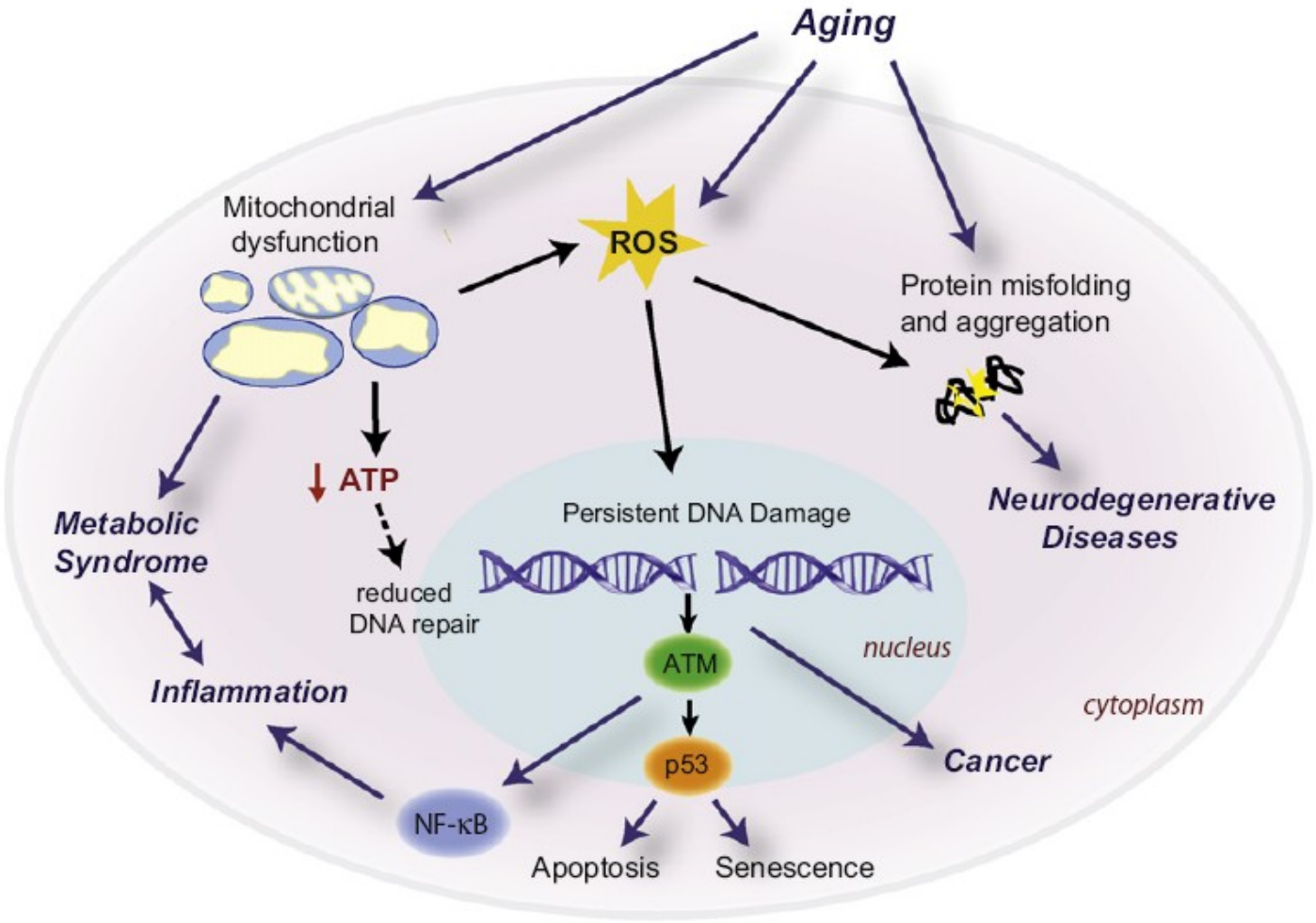
<u>Basis for theory:</u>

(i) Strong correlation between chronological age and the level of ROS generation and oxidative damage
(ii) Mitochondrial function is gradually lost during aging
(iii) Inhibition of mitochondrial function can enhance ROS production
(iv) Several age-dependent diseases are associated with severe increase in oxidative stress

But what if increased ROS generation is a consequence rather than a cause of aging?

Hekimi, S. et al. 2011. Taking a 'good' look at free radicals in the aging process. Trends in Cell Biology. 21, 569-576.

Age-related stress and disease



But what if increased ROS generation is a consequence rather than a cause of aging?

Haigis, M.C. and Yankner, B.A. 2010. The aging stress response. Mol. Cell 40, 333-344; Hekimi, S. et al. 2011. Taking a 'good' look at free radicals in the aging process. Trends in Cell Biology. 21, 569-576.

Data in support of the MFRTA

Orr, W.C., and Sohal, R.S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science *263*, 1128–1130.

Sod-1-/- mice have shortened lifespan and have high levels of oxidative damage (but they die from hepatocellular carcinoma)
(Elchuri et al., 2005 Oncogene 24, 367-380)

Extension of *Drosophila* lifespan by overexpression of human *SOD1* in motorneurons

Tony L. Parkes[1], Andrew J. Elia[2], Dale Dickinson[1], Arthur J. Hilliker[1], John P. Phillips[1] & Gabrielle L. Boulianne[2]

Extension of Life-Span with Superoxide Dismutase/Catalase Mimetics

Simon Melov,[1] Joanne Ravenscroft,[2*] Sarwatt Malik,[2] Matt S. Gill,[2] David W. Walker,[2†] Peter E. Clayton,[2] Douglas C. Wallace,[3] Bernard Malfroy,[4] Susan R. Doctrow,[4] Gordon J. Lithgow[2‡]

Age-1 mutant of C. elegans has increased lifespan, increased SOD and catalase, and increased resistance to oxidative stress, heat shock and UV radiation (reviewed In Johnson, F.B. et al. 1999. Cell 96, 291-302)

Lifespan Extension and Rescue of Spongiform Encephalopathy in Superoxide Dismutase 2 Nullizygous Mice Treated with Superoxide Dismutase–Catalase Mimetics

Simon Melov,[1] Susan R. Doctrow,[2] Julie A. Schneider,[3] Joanna Haberson,[1] Manisha Patel,[4] Pinar E. Coskun,[5] Karl Huffman,[2] Douglas C. Wallace,[5] and Bernard Malfroy[2]

Extension of Murine Life Span by Overexpression of Catalase Targeted to Mitochondria

Samuel E. Schriner,[1,5] Nancy J. Linford,[2] George M. Martin,[1,2] Piper Treuting,[3] Charles E. Ogburn,[2] Mary Emond,[4] Pinar E. Coskun,[5] Warren Ladiges,[3] Norman Wolf,[2] Holly Van Remmen,[6] Douglas C. Wallace,[5] Peter S. Rabinovitch[2*]

A *Sod2* Null Mutation Confers Severely Reduced Adult Life Span in Drosophila

Atanu Duttaroy,[1] Anirban Paul, Mukta Kundu and Amy Belton

Biology Department, Howard University, Washington, DC 20059

Manuscript received April 14, 2003
Accepted for publication August 26, 2003

FLP Recombinase-Mediated Induction of Cu/Zn-Superoxide Dismutase Transgene Expression Can Extend the Life Span of Adult *Drosophila melanogaster* Flies

Sun et al 1999 Mol. Cell. Biol. 19, 216-218

The lifespan extension becomes less evident after backcrossing,
Thus effect is likely the result of interaction with specific alleles at other loci

Evidence incompatible with the MFRTA

(i) A lack of correlation between the level of ROS production and longevity in various species

(ii) Deleterious rather than beneficial effects on lifespan from the administration of antioxidants in various species from invertebrates to humans

(iii) The inactivation or over-expression of antioxidants fails to produce outcomes that support the MFRTA

(iv) The existence of long-lived mutants and species with high ROS production and high levels of oxidative damage

Hekimi, S. et al. 2011. Taking a 'good' look at free radicals in the aging process. Trends in Cell Biology. 21, 569-576.

Data which do not support the MFRTA and which may contradict it

Increasing levels of a mitochondrial antioxidant, Coenzyme Q10, in mice, has no effects on lifesapn (Sohal et al. 2006 Free Radic Biol Med 40, 480-487)

Recent studies have also linked high oxidative stress to extended lifespan (Andziak et al 2006 Aging Cell 5, 463-471; Andziak et al. 2006 Aging Cell 5, 525-532; Csiszar et al. 2008 Am J Physiol Heart Circ Physiol 295, H1882-H1894; Ran et al 2007 J Gerontol A Biol Sci Med Sci 62, 932-942)

The overexpression of major antioxidant enzymes does not extend the lifespan of mice

(Perez et al. 2009 Aging Cell 8, 73-75)

Partial inactivation of Mclk1, a mitochondrial enzyme necessary for coenzyme Q biosynthesis, prolong average and maximum mouse lifespan despite high oxidative stress (Lapointe et al.2009 JBC 284, 20364-20374)

Expression of multiple copies of mitochondrially targeted catalase or genomic Mn superoxide dismutase transgenes does not extend the life span of *Drosophila melanogaster*

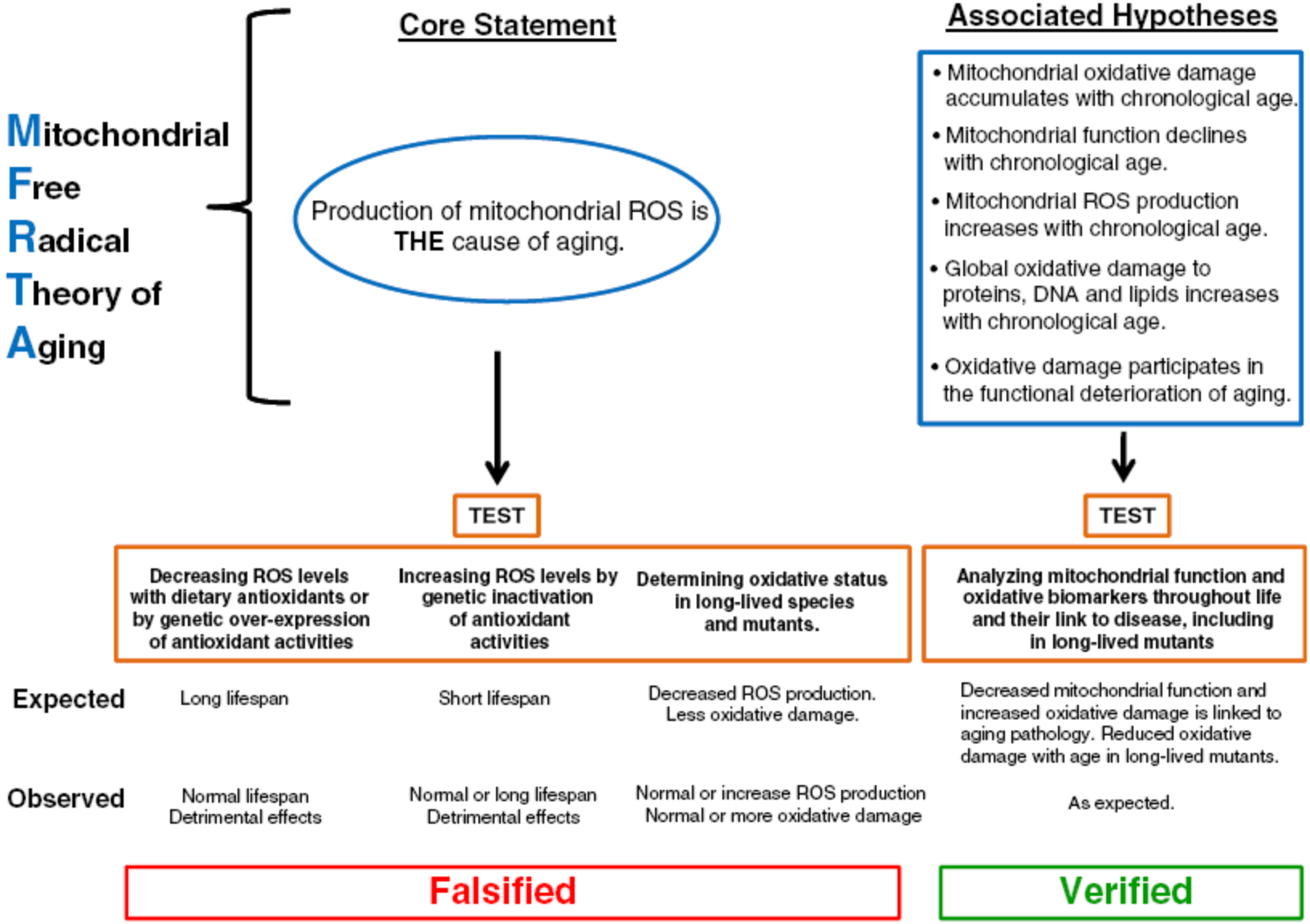
Robin J. Mockett [a], Barbara H. Sohal [b], Rajindar S. Sohal [b,*]

**Deletion of Sod-2 in C. elegans fails to shorten lifespan and actually prolongs it, despite increased oxidative stress (Van Raamsdonk and Hekimi 2009 PLoS Genet. 5, e1000361.

Overexpression of antioxidant enzymes such as SOD1, SOD2 and catalase does not increase the lifespan of mice (Muller et al. 2007 Free Radic Biol Med 43, 477-503; Huang et al. 2000 J Gerontol A Biol Sci Med Sci 55, B5-B9.

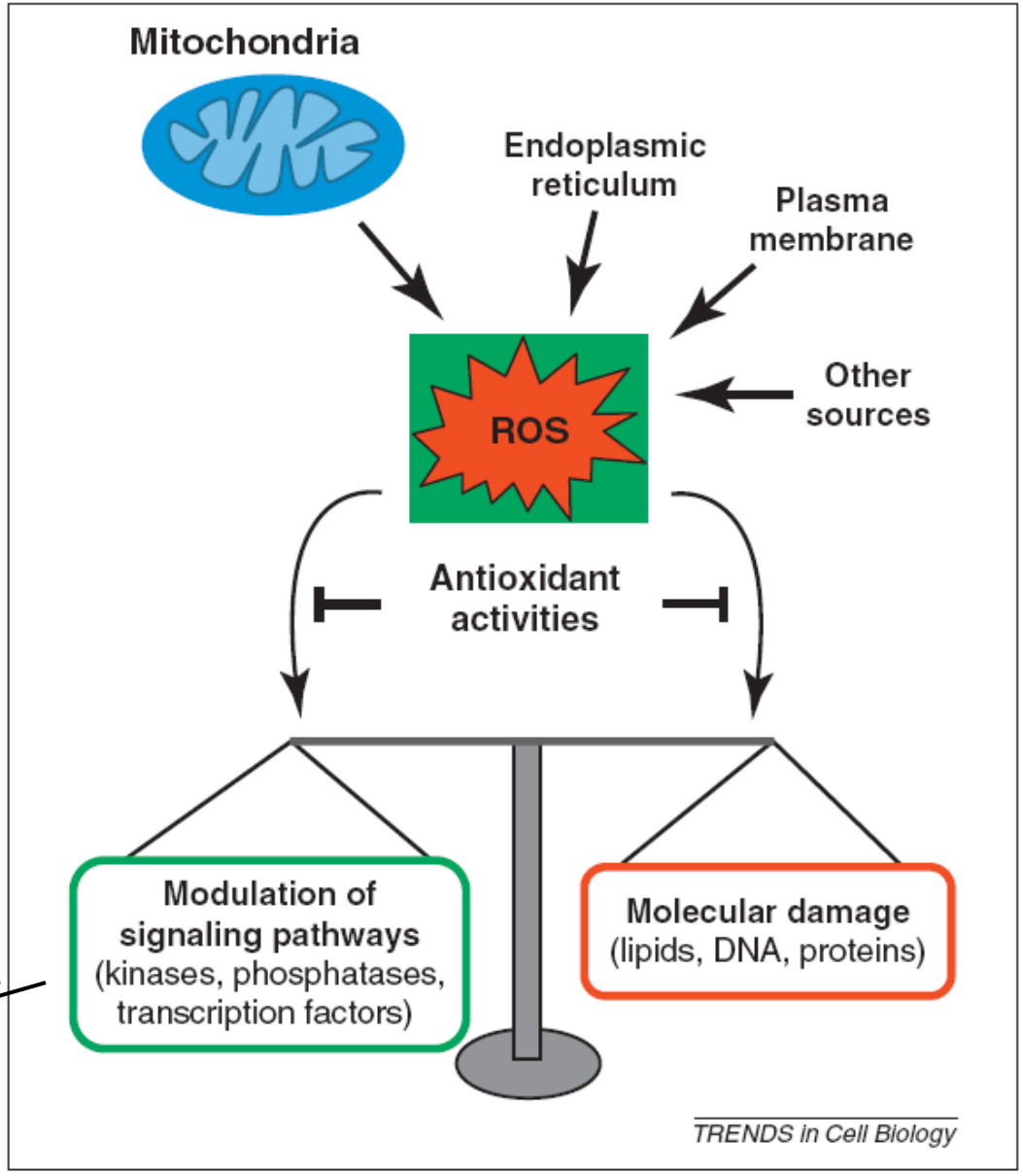
Mice knockouts of GPX1 (glutathione peroxidase, SOD1, 2 or 3 do not have decreased lifespan, despite increased oxidative stress (Van Remmen et al. 2003 Physiol Genomics 16, 29-37; Williams et al. 1998 JBC 273, 28510-28515)

Mice which overexpress a proofreading-deficient version of the mitochondrial DNA polymerase γ accumulate mtDNA mutations and display features of accelerated aging, which correlated with the induction of apoptotic markers, but not with increased markers of oxidative stress (Kujoth et al. 2005 Science 309, 481-484).



Lapointe J. and Hekimi, S. 2009. When a theory of aging ages badly. Cell. Mol. Life. Sci. Sep 3. [Epub ahead of print]

Sources and targets of ROS



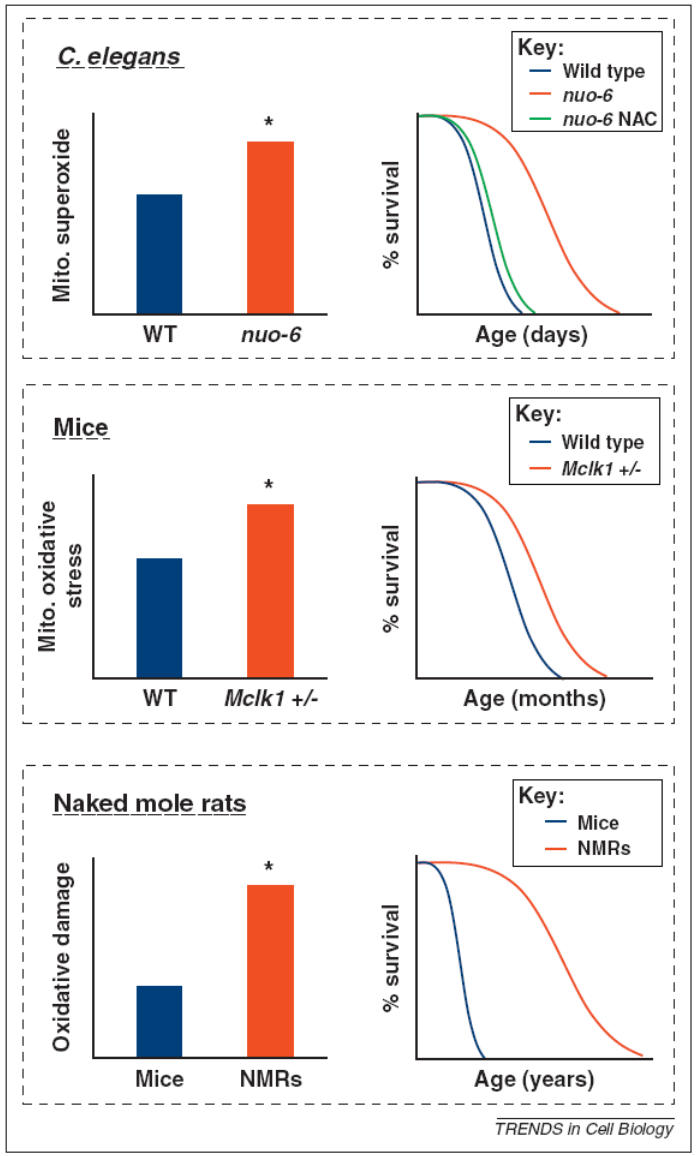
Enzymes modulating the oxidative status and stress response are regulated by kinases and phosphatases

Hekimi, S. et al. 2011. Taking a 'good' look at free radicals in the aging process. Trends in Cell Biology. 21, 569-576.

ROS as signaling molecules funtioning as stress signals in response to age-dependent damage

ROS can stimulate beneficial responses to cellular stresses produced by aging:

-autophagy
-DNA base excision repair
-protective transcription factor HIF-1a
-changes in gene expression?



Nuo-6 is a subunit of complex I of mitochondrial respiratory chain;
Mclk1+/ mice lack one copy of an enzyme
that is necessary for the synthesis of the antioxidant and
redox co-factor ubiquinone

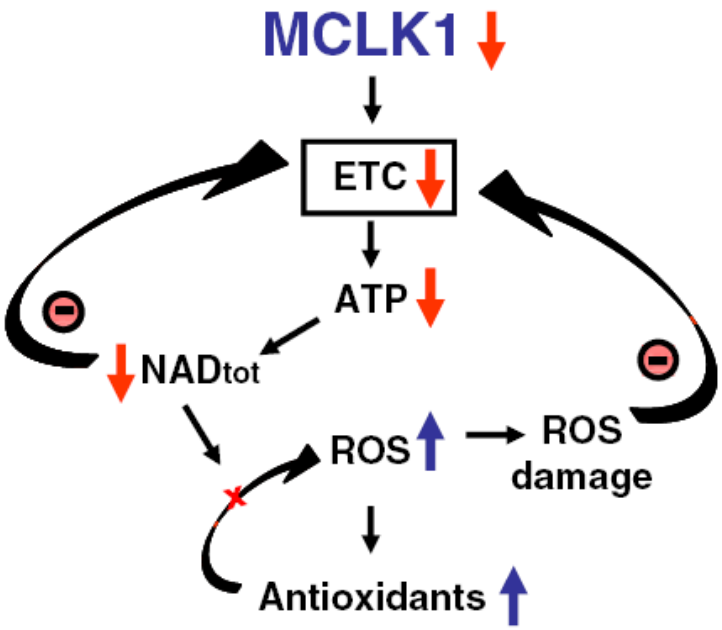
Hekimi, S. et al. 2011. Taking a 'good' look at free radicals in the aging process. Trends in Cell Biology. 21, 569-576.

Stress-response hormesis and aging?

Hormesis: a set of phenomena in which exposure to transient and/or repeated doses of a potentially harmful factor induces an adaptive beneficial effect on the cell or organism

MITOCHONDRIAL phenotype of young *Mclk1*$^{+/-}$ mutants

Changes resulting from the *Mclk1*$^{+/-}$ phenotype during chronological aging



Stabilization and **improvement** of mitochondrial function.

Gradual **reduction** of mitochondrial oxidative stress.

Slower accumulation of global oxidative damage to:

- DNA (8-OHdG)
- Membrane lipids (isoprostanes)

Observations with *Mclk1*$^{+/-}$ mutants that are irreconcilable with the MFRTA

Lapointe J. and Hekimi, S. 2010. When a theory of aging ages badly. Cell. Mol. Life. Sci. 67, 1-8.

Gradual ROS response hypothesis

Unlike hormesis, the gradual ROS response hypothesis proposes a process that is gradual, endogenous and occurs continuously as part of normal aging in wild type animals

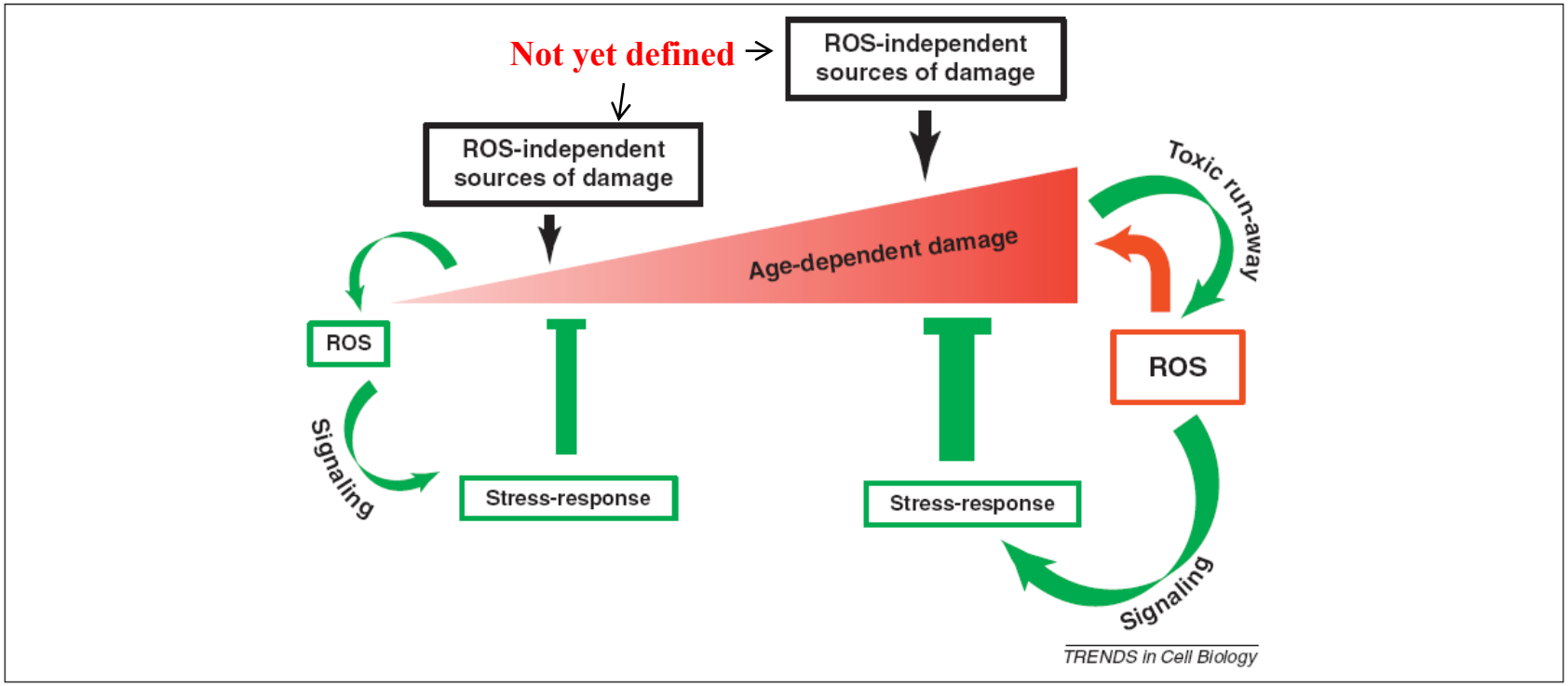
(i) As ROS are not the initial cause of aging in at least some species they cannot be a universal cause of aging, although high levels of ROS damage can contribute to the aged phenotype, in particular in disease states that develop later in life

(ii) ROS are signaling molecules that can modulate stress response pathways

(iii) Increased ROS levels can result in positive effects, including on cellular processes that limit lifespan

Hekimi, S. et al. 2011. Taking a 'good' look at free radicals in the aging process. Trends in Cell Biology. 21, 569-576.

Gradual ROS response hypothesis



(i) **Proposes that cellular constituents sustain age-dependent damages that trigger protective stress responses that use ROS as second messengers**

(ii) **Protective mechanisms are not completely effective leading to a gradual increase in age-related damage**

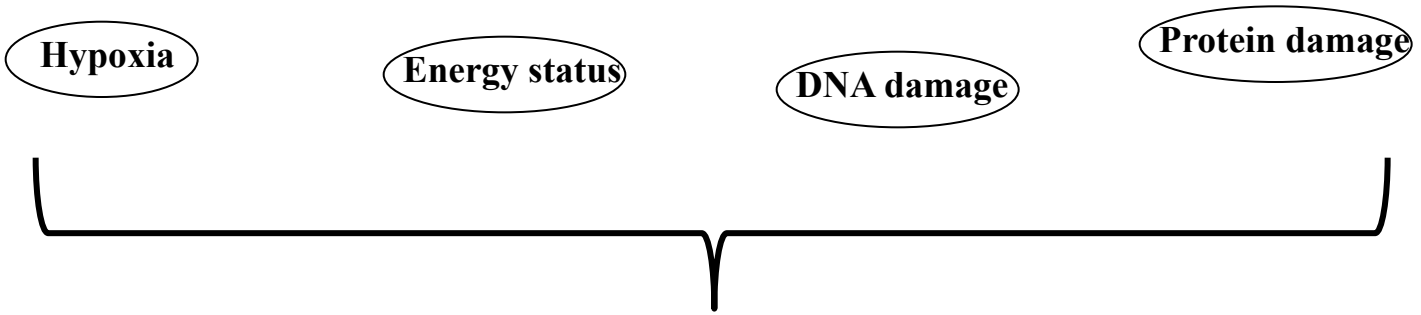
(iii) **The gradual increase in damage leads to a gradually intensifying stimulation of stress responses, and thus, a gradual and sustained generation of ROS**

(iv) **With aging, a threshold is reached where levels of ROS become maladaptive and ROS toxicity starts to contribute to the damage production which ROS dependent stress pathways were meant to combat**

(v) **The induced ROS-dependent damage could explain the involvement of ROS in age-dependent disease**

Hekimi, S. et al. 2011. Taking a 'good' look at free radicals in the aging process. Trends in Cell Biology. 21, 569-576.

Cellular stress response pathways



Insulin/Insulin-like growth factor (IGF) signaling
Sirtuins
Target of rapamycin (TOR)
AMP-activated protein kinase (AMPK) pathways

<u>Adaptive cellular responses</u>

Nutrient sensing
Redox metabolism
DNA damage response
Coordinated regulation of protein synthesis and turnover
Autophagy
Mitochondrial function

Based on Haigis, M.C. and Yankner, B.A. 2010. The aging stress response. Mol. Cell 40, 333-344;

Gene regulation theory of aging

- **Genes in yeast, worms, flies, and mice have been identified that affect lifespan**

- **Many of these genes regulate/promote growth (glucose or Insulin-like growth factor (IGF-1-like) signaling) and/or resistance against oxidative damage and other forms of stress**

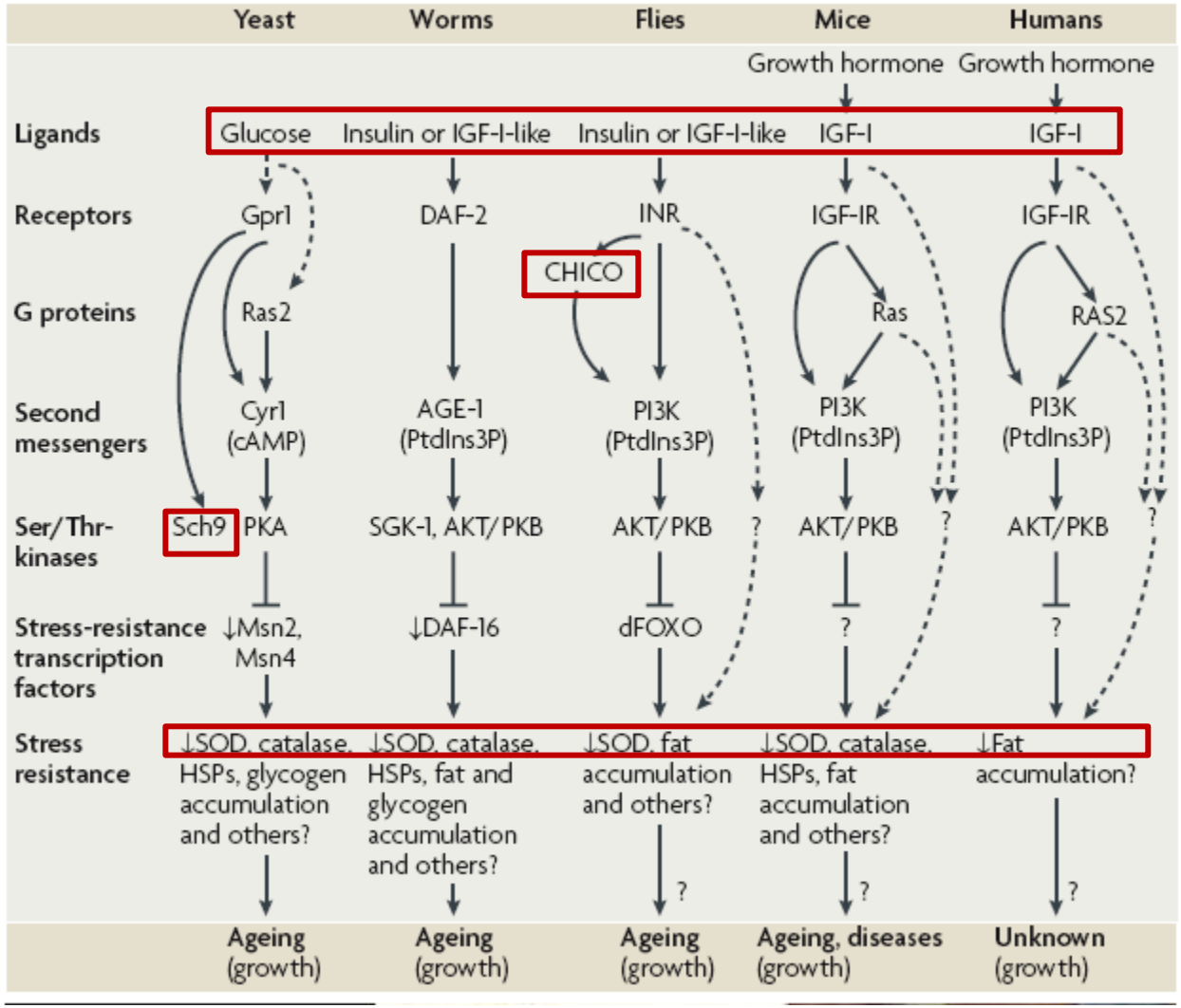
Table 1 Selected examples of genes identified to influence lifespan in model organisms

Organism*	Gene name/description	Function	Reference
Saccharomyces cerevisiae			
Sir2	NAD(+)-dependent deacetylase	Regulation of metabolism, stress resistance	Kaeberlein *et al.*, 1999
Caenorhabditis elegans			
age-1	Phosphatidylinositol kinase	Insulin signalling	Morris *et al.*, 1996
daf-2	Insulin receptor-like gene	Insulin signalling	Kimura *et al.*, 1997
daf-12	Nuclear hormone receptor	Regulation of metabolic and developmental pathways	Larsen *et al.*, 1995
daf-16	Forkhead transcription factor	Regulation of metabolic and developmental pathways	Ogg *et al.*, 1997
Drosophila melanogaster			
Cat	Catalase	Antioxidant activity	Orr & Sohal, 1994
Chico	Insulin receptor substrate	Insulin signalling	Clancy *et al.*, 2001
Sod1	Superoxide dismutase	Antioxidant activity	Parkes *et al.*, 1998
Sod2	Superoxide dismutase	Antioxidant activity	Sohal *et al.*, 1995
Mei-41	Phosphatidylinositol kinase, ATR kinase othologue	DNA repair	Symphorien & Woodruff, 2003
Pcmt	Protein carboxyl methyltransferase	Protein repair	Chavous *et al.*, 2001
Mus musculus			
Gh	Growth hormone	Insulin signalling, tissue proliferation	Bartke, 2005
Klotho	Beta-glucuronidase	Inhibits IIS signalling	Kuro-o *et al.*, 1997
p53	Tumour protein p53	Tumour suppression	Tyner *et al.*, 2002

*Organism in which the gene was first shown to influence lifespan.

Kuningas, M. et al. 2008. Genes encoding longevity: from model organisms to humans.

Conserved regulation of longevity



***Inherited SNPs in genes of insulin signaling pathway correlate with longevity**

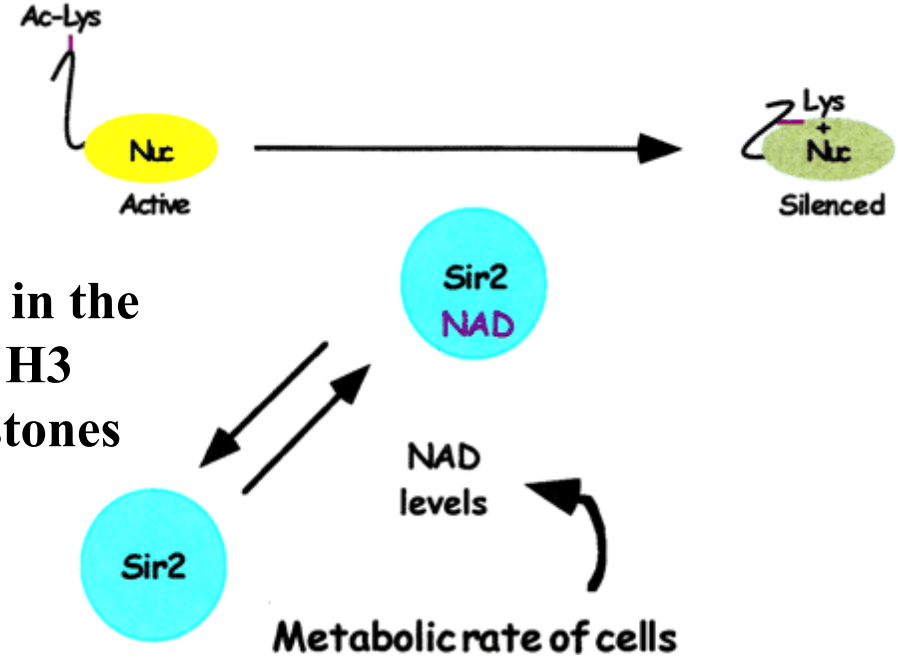
***SNPs in AKT1, FOXO1, FOXO3a found in multiple centenarian cohorts**

Longo, VD, Lieber, MR, Vijg, J. 2008 Turning anti-ageing genes against cancer. Nature Reviews Molecular Cell Biology, 9, 903-910; Haigis, M.C. and Yankner, B.A. 2010. The aging stress response. Mol. Cell 40, 333-344

SIR proteins

The yeast sirtuin 2 (Sir2-silent information regulator 2) is a nicotinamide adenine dinucleotide (NAD) histone deacetylase that modulates yeast replicative life span by suppressing genome instability through chromatin modification.

SIR2 is important for chromatin structure: it functions to silence several loci in yeast, including telomeres, ribosomal DNA (rDNA) and the mating loci.



Silencing requires that particular lysines in the extended amino-terminal tail of histones H3 and H4 be deacetylated (deacetylated histones can fold into a more compact, closed nucleosomal structure)

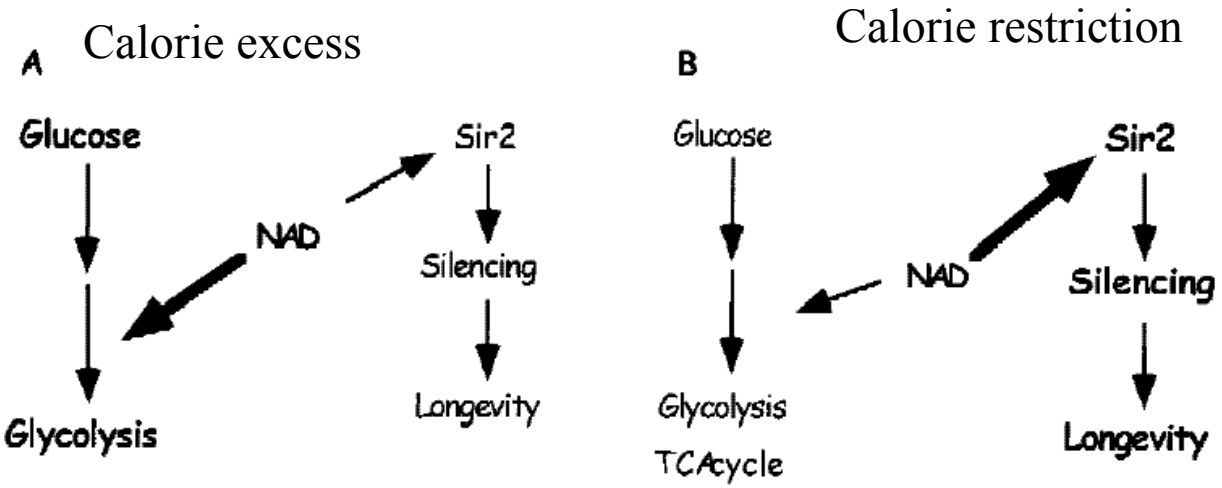
Guarante, L. 2000. Sir2 links chromatin silencing, metabolism, and aging. Genes & Development 14, 1021-1026.

Is SIR2 the link between caloric restriction and longevity?

Lifespan is not extended by caloric restriction of a yeast strain that lacks SIR2

Sirtuin activating compounds (STACs, e.g. resveratrol) can promote the survival of human cells, extend the replicative lifespan of yeast and delay aging in C.elegans and D. melanogaster likely by mimicing caloric restriction

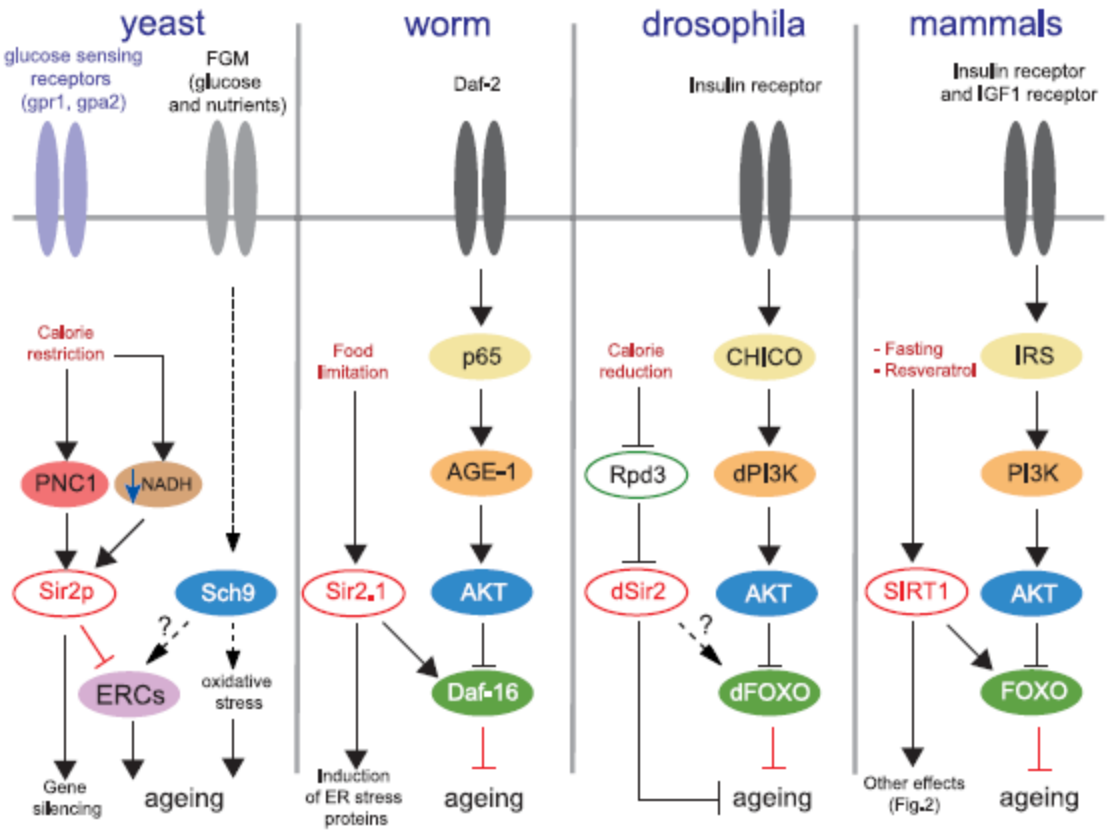
Model: More NAD becomes available when the physiological rate is slowed (caloric restriction), increasing SIR2 activity followed by increased silencing and lifespan



Guarente, L. 2000. Sir2 links chromatin silencing, metabolism, and aging. Genes & Development 14, 1021-1026. Howitz et al. 2003. Nature 425, 191-196. Guarente and Picard. 2005. Calorie Restriction—the SIR2 connection. Cell 120, 473-482.

SIR2 and signaling pathways in different species

Intersects the insulin/IGF-signaling pathway



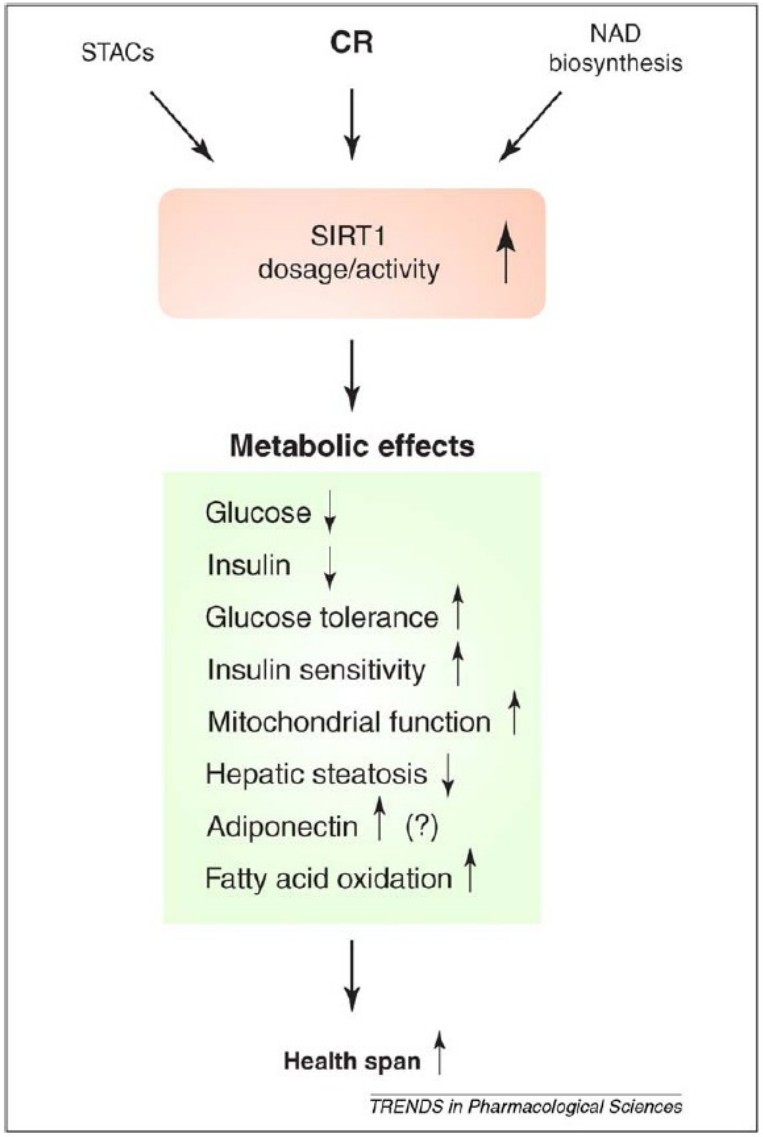
Dali-Youcef, N. et al. 2007. Sirtuins: The ‘magnificent seven’, function, metabolism and longevity. Annals of Medicine 39, 335-345.

SIRT1 and CR

•Unlike wild-type mice, SIRT1-deficient mice do not exhibit increased physical activity upon CR

•SIRT1 transgenic mice display phenotypes that mimic some of the physiological changes in response to CR

- oDecreased insulin and glucose levels in blood
- oImproved glucose tolerance
- oReduced fat mass and circulating levels of free fatty acid
- oReduced level of total cholesterol in blood
- oEnhanced oxygen consumption
- oImproved activity in rotarod tests
- oDelayed reproductive timing



Imai, S. and Guarente, L. 2010. Ten years of NAD-dependent SIR2 family deacetylases: implications For metabolic diseases. Trends in Pharmacological Sciences. 31, 212-220.

SIRT targets in mammals

Table 1. Mammalian sirtuins

	Enzymatic activity	Homologs	Subcellular localization	Function
SIRT1	Deacetylase	Sir2p (*S. cerevisiae*) Hst1p (*S. cerevisiae*) SIR-2.1(*C. elegans*) dSIR2 (*D. melanogaster*)	Nuclear, cytoplasmic	Glucose production (liver) Fatty-acid oxidation (liver) Cholesterol regulation (liver) Fatty-acid mobilization (WAT) Adipokine regulation (WAT) Fatty-acid oxidation (skeletal muscle) Insulin secretion (pancreatic β-cells) Neuroprotection (brain) Regulation of cellular differentiation Stress resistance and apoptosis control Mediator for caloric restriction
SIRT2	Deacetylase	Hst2p (*S. cerevisiae*) SIRT2 (*D. melanogaster*)	Cytoplasmic, nuclear	Tublin deacetylation Cell cycle control
SIRT3	Deacetylase		Mitochondrial	Mitochondrial protein deacetylation Acetate metabolism regulation ATP production Regulation of mitochondrial fatty-acid oxidation
SIRT4	ADP- ribosyltransferase	SIR-2.2 (*C. elegans*) SIR-2.3 (*C. elegans*) SIRT4 (*D. melanogaster*)	Mitochondrial	Amino acid-stimulated insulin secretion (pancreatic β-cells)
SIRT5	Deacetylase		Mitochondrial	Urea cycle regulation (liver)
SIRT6	ADP-ribosyltransferase Deacetylase	SIR-2.4 (*C. elegans*) SIRT6 (*D. melanogaster*)	Nuclear	Base excision repair Telomeric chromatin structure NF-βB regulation
SIRT7	Deacetylase	SIRT7 (*D. melanogaster*)	Nucleolar	Pol I transcription

WAT, white adipose tissue; BAT, brown adipose tissue.
References [22–26,121].

Imai, S. and Guarente, L. 2010. Ten years of NAD-dependent SIR2 family deacetylases: implications For metabolic diseases. Trends in Pharmacological Sciences. 31, 212-220.

SIRT targets in mammals

Table 1 | **Protein substrates and interactors of mammalian sirtuins***

Sirtuin	Disease area	Therapeutic strategy	Substrates/ interactors	Overexpression/knockout model summary
SIRT1	Metabolic, neurological, cardiovascular, renal, cancer, mitochondrial	Activation	p53, FOXO1, FOXO4, COUP-TF, CTIP2, NF-κB-p65, NCOR, histone H1, histone H4, KU70, p300, BCL11A, Tat, PGC1α, MEF2, eNOS, ACS1, E2F1, AR, p73, SMAD7, NBS1, RB, TLE1, IRS2, LXR, AROS, SUV39H1, WRN, DBC1, TORC2	• Efficacy observed in preclinical models of diabetes with small-molecule SIRT1 activators[24] • Transgenic overexpression of SIRT1 is cardioprotective against oxidative stress and heart ageing[57] • *Sirt1*-overexpressing mice show some phenotypes of calorie-restricted mice[7] • *SIRT1* overexpression shows beneficial effects in Alzheimer's disease and Huntington's disease models[59,63] • Knockout mice have genomic instability and developmental defects[58,61] • SIRT1 activates PGC1α by deacetylation and is involved in mitochondrial biogenesis[10]
SIRT2	Neurological, metabolic, cancer	Inhibition/ activation?	Tubulin, HOXA10, FOXO, histone H4, 14-3-3 protein	• Efficacy observed in a cellular and *Drosophila melanogaster* model of Parkinson's disease with small-molecule SIRT2 inhibitors[62]
SIRT3	Metabolic, mitochondrial	Activation	ACS2	• *Sirt3*-knockout mice have hyperacetylated proteins in mitochondria[60]
SIRT4	Metabolic, mitochondrial	Inhibition?	GDH, IDE, ANT2, ANT3	• *Sirt4*-knockout mice are viable and fertile; pancreatic mitochondrial lysates from knockout animals show higher GDH activity[31]
SIRT5	Neurological	Unknown	Unknown	• Increased expression of *Sirt5* observed in frontal cortex of brains from serotonin receptor knockout mice[64]
SIRT6	Cancer	Activation	Histone H3	• Knockout mice have genomic instability, premature ageing phenotype and predisposition to developing cancer[52]
SIRT7	Cardiovascular	Activation	RNA polymerase I, p53	• Knockout mice have decreased lifespan with inflammatory cardiac hypertrophy[56]

SIRT6 depletion leads to telomere dysfunction with end-to-end chromosomal fusions and premature cellular senescence

Lavu, S. et al. 2008. Sirtuins—novel therapeutic targets to treat age-associated diseases. Nature Reviews Drug Discovery 7, 841-853.

SIRT1 as a potent protector from age-associated pathologies, such as diabetes, liver steatosis, cardiovascular disease, neuro-degeneration, and cancer

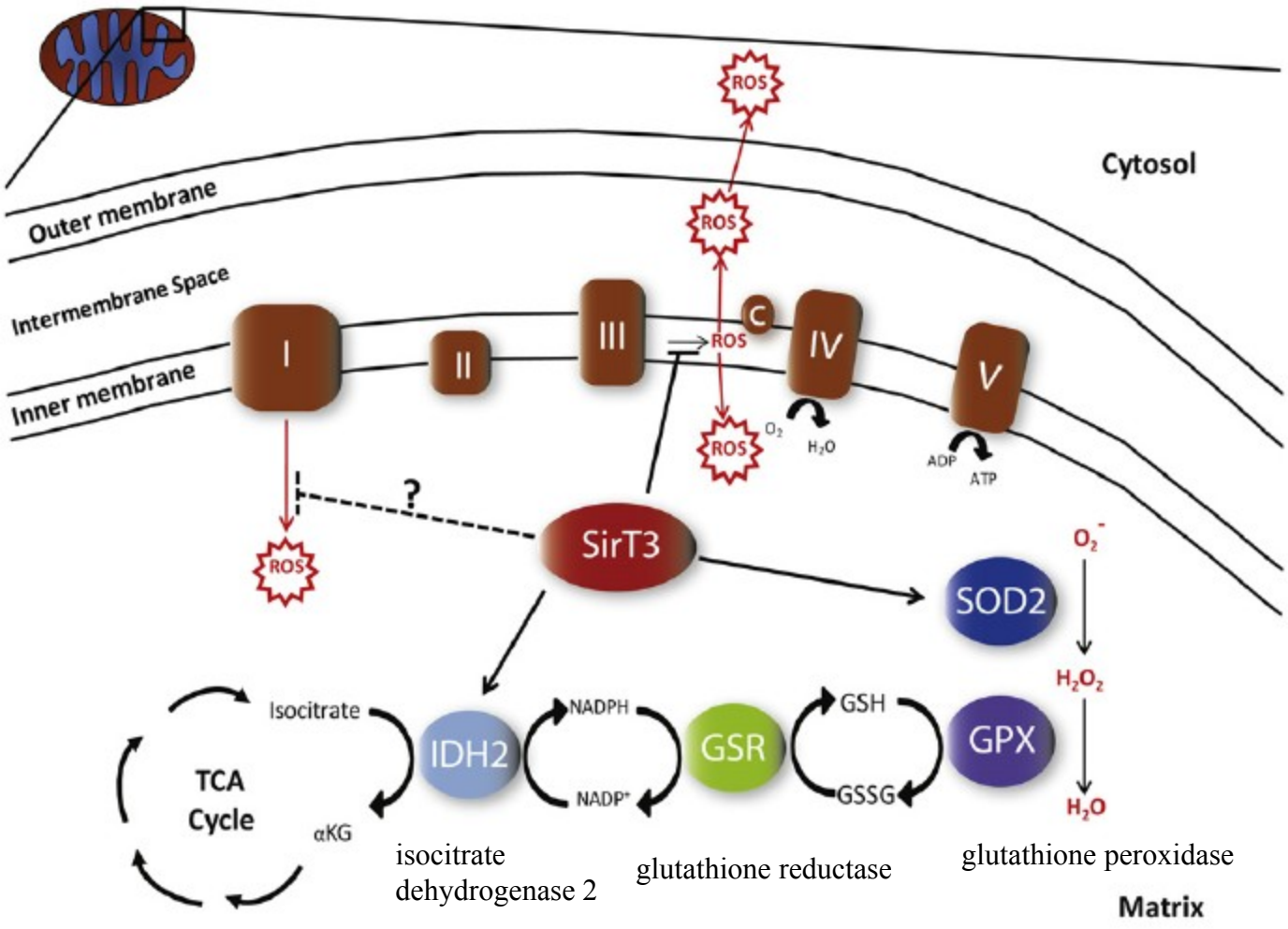
Table 1 | **SIRT1 mouse models and their effects on cancer and metabolism**

Mouse model	Cancer phenotypes	Metabolic phenotypes	Refs
Whole-body overexpression			
Sirt1-transgenic	Incidence of carcinomas and sarcomas reduced in ageing mice, as were metabolic syndrome-associated cancers. No effect seen on chemically induced fibrosarcomas or ageing-associated lymphomas	Mice were protected against developing glucose intolerance induced by ageing, diet-induced obesity and genetically induced obesity. They were also protected from fatty liver disease and inflammation in WAT and liver that is induced by a HFD	26,33, 34
Tissue-specific overexpression			
Sirt1 expressed in pancreatic β-cells	Not explored	Increased insulin secretion	31
Brain- and adipose tissue-specific expression of *Sirt1*	Not explored	Increased glucose tolerance and decreased body weight	32
Sirt1 expressed in the intestine of $Apc^{+/min}$ mice	Decreased intestinal polyp formation	Not explored	25
Whole-body deficiency			
Sirt1-null	No effect on $Apc^{+/min}$-induced intestinal polyps and no effect on chemically induced papillomas	Decreased insulin production, improved glucose tolerance and protection from fatty liver induced by LXR agonists	42,43, 62
$Sirt1^{+/-}$	Not explored	Increased fatty liver disease, body weight and liver inflammation induced by HFD	36
$Sirt1^{+/-}$;$Trp53^{+/-}$	Increased incidence of sarcomas and lymphomas associated with *Trp53* deficiency	Not explored	28
Tissue-specific deficiency			
Sirt1 liver-specific knockout	Not explored	Mice fed a HFD had increased fatty liver disease, inflammation and ER stress in liver, and increased body weight	40
Sirt1 liver-specific knockout	Not explored	Mice fed a HFD were protected from glucose intolerance and showed decreased liver and body weight	41
Sirt1 myeloid-specific knockout	Not explored	HFD induced increased glucose intolerance and inflammation in WAT and the liver	37
Sirt1 brain-specific knockout	Not explored	Increased glucose intolerance associated with ageing	38
Sirt1 POMC neuron-specific knockout	Not explored	Protection from HFD-induced obesity	39

ER, endoplasmic reticulum; HFD, high-fat diet; LXR, liver X receptor (also known as NR1H3); POMC, pro-opiomelanocortin; WAT, white adipose tissue.

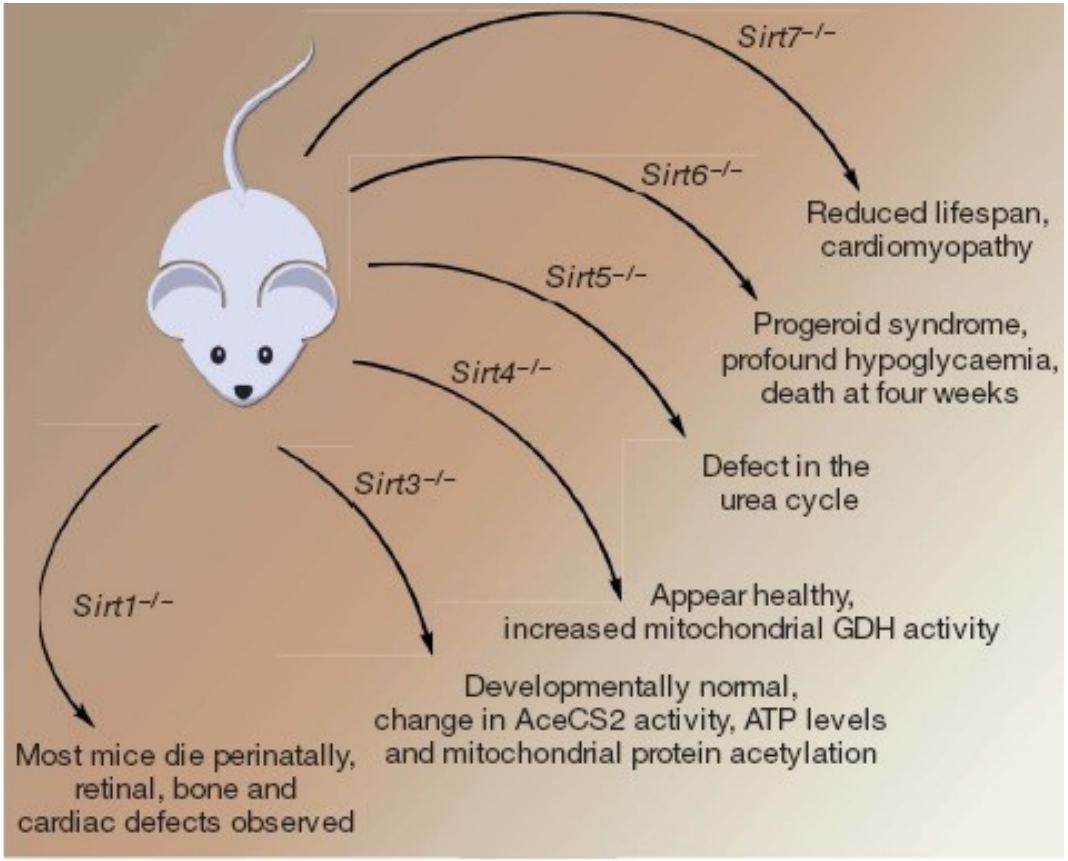
Herranz, D. and Serrano, M. 2010. SIRT1: recent lessons from mouse models. Nature Reviews 10, 819-823.

SirT3 protects against damage from mitochondrially derived ROS



Bell E.L. and Guarente, L. The SirT3 divining rod points to oxidative stress. 2011. Molecular Cell 42, 561-568.

SIRT targets in mammals

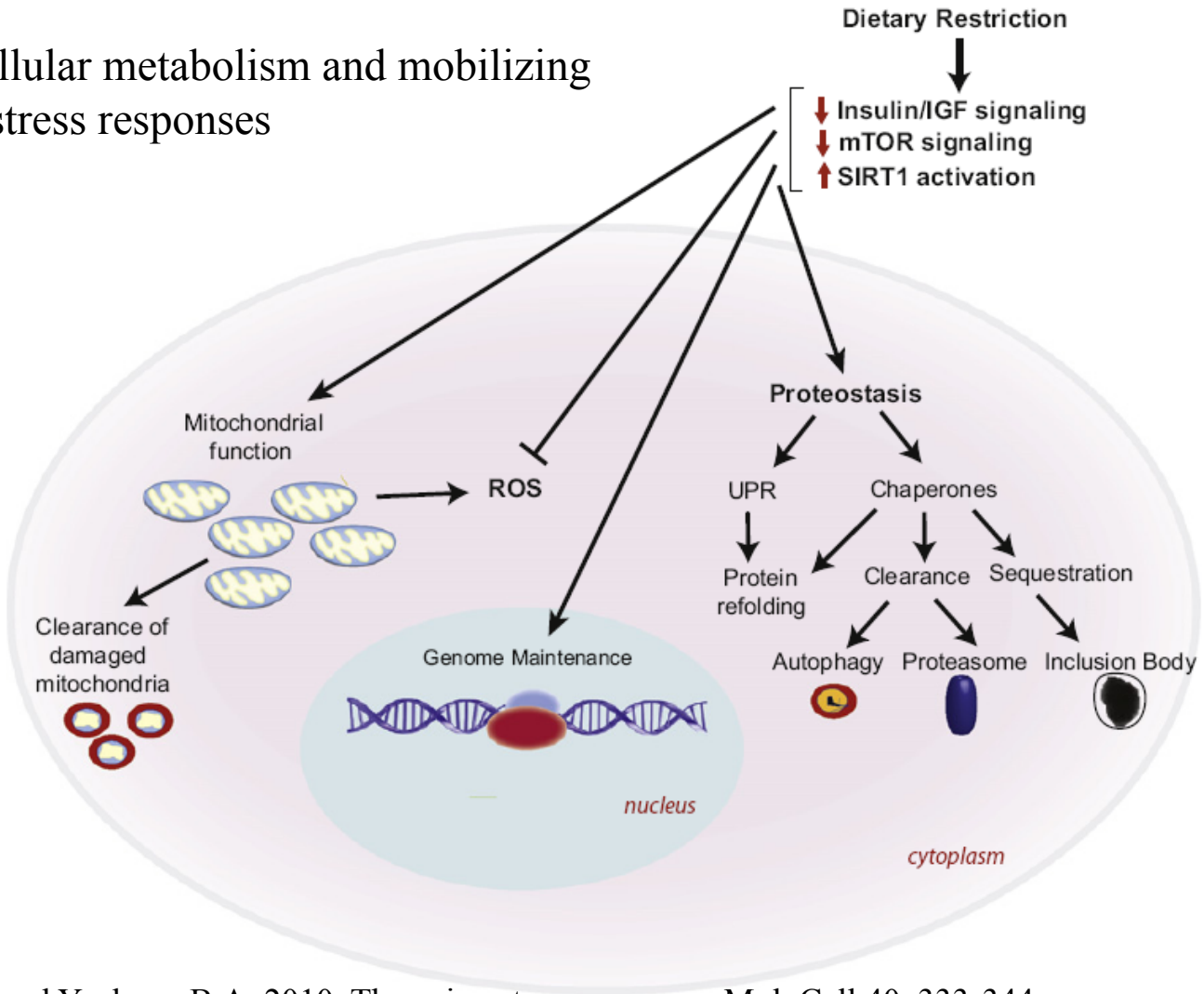


However, no definitive evidence that the SIR proteins play any direct role in mammalian lifespan regulation since neither pharmacological sirtuin activators nor overexpression of SIRT1 has been demonstrated to extend lifespan in mice; higher levels of overexpression or overexpression of several sirtuins?

Finkel, T. et al. 2009. Recent progress in the biology and physiology of sirtuins. Nature 460, 587-591.
Herranz, D. and Serrano, M. 2010. SIRT1: recent lessons from mouse models. Nature Reviews 10, 819-823.

Nutrional Regulation of Conserved Signaling and Stress Response Pathways

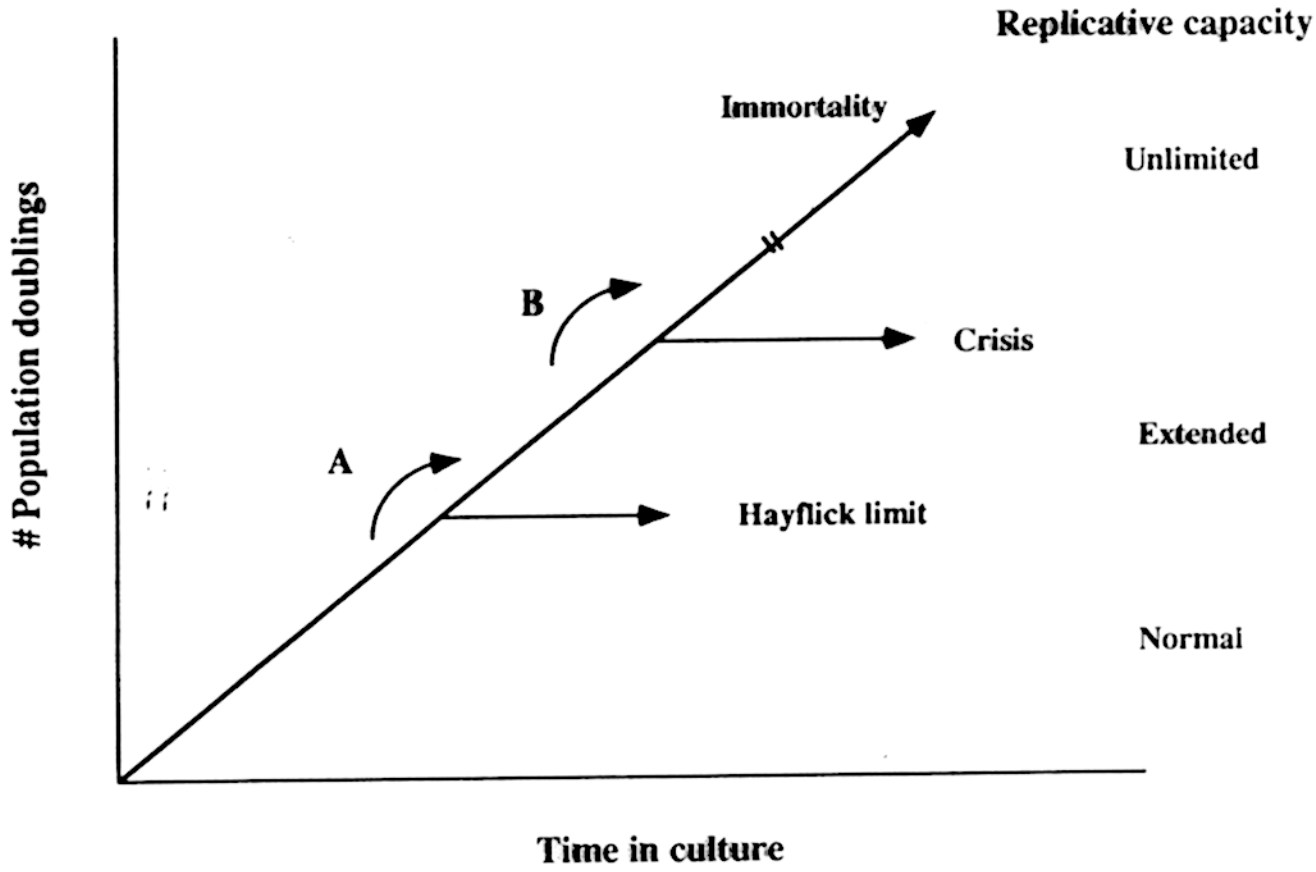
Altering cellular metabolism and mobilizing protective stress responses



Haigis, M.C. and Yankner, B.A. 2010. The aging stress response. Mol. Cell 40, 333-344

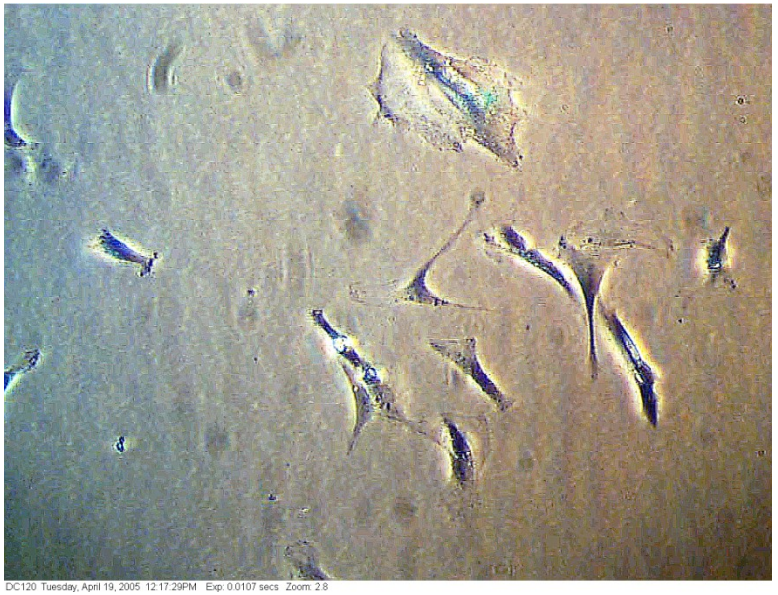
Replicative senescence and telomere theory of aging

Human primary cells such as fibroblasts divide a 'programmed' number of times before undergoing replicative senescence in culture. This limit on cell division is called the Hayflick limit.



Characteristics of senescent cells

Withdrawal from cell cycle, but not quiescent or terminally differentiated
Chromosomal instability
Morphological and biochemical changes (enlargement up to twofold relative to size of nonsenescent counterparts
Altered gene expression, increased p16INK4a
Metabolically viable
Senescence-associated β-galactosidase (some lysosome activities are elevated in senescent cells; lysosomal β-Gal may increase such that its activity is detectable at pH 6)
Senescence-associated heterochromatin (SAHF) which silence critical pro-proliferative genes

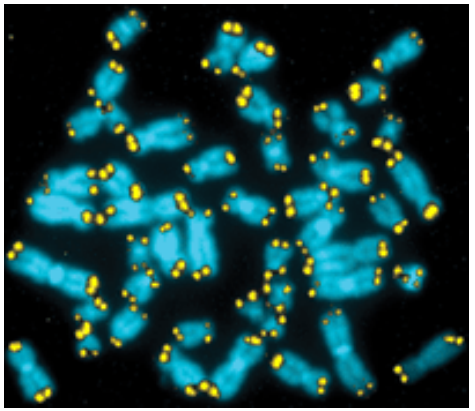


Human cell senescence *in vitro* and lifelong replication *in vivo*?

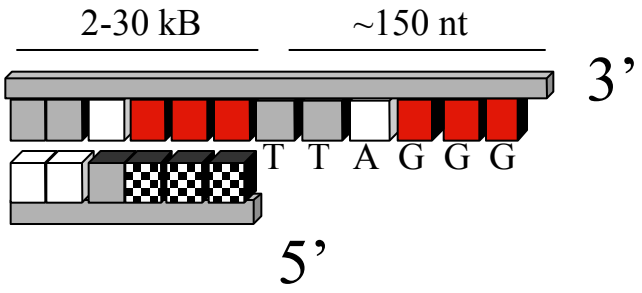
- **Cell from old donors divide fewer times in culture than cells from young donors**
- **Cells from different species have a Hayflick limit that correlates with species longevity**
- **Cell from patients with accelerated aging syndromes divide fewer times in culture than cells from age-matched controls**
- **Accumulation of senescent cells in older individuals**

Is there a genetic mitotic clock that counts the number of cell divisions and signals to exit the cell cycle?

- **Telomeres are ends of linear eukaryotic chromosomes**
- **Composed of many tandemly arranged copies of a short, G-rich DNA sequence (TTAGGG in humans)**
- **Contain a short ss G-rich 3'overhang that is important for telomere function**



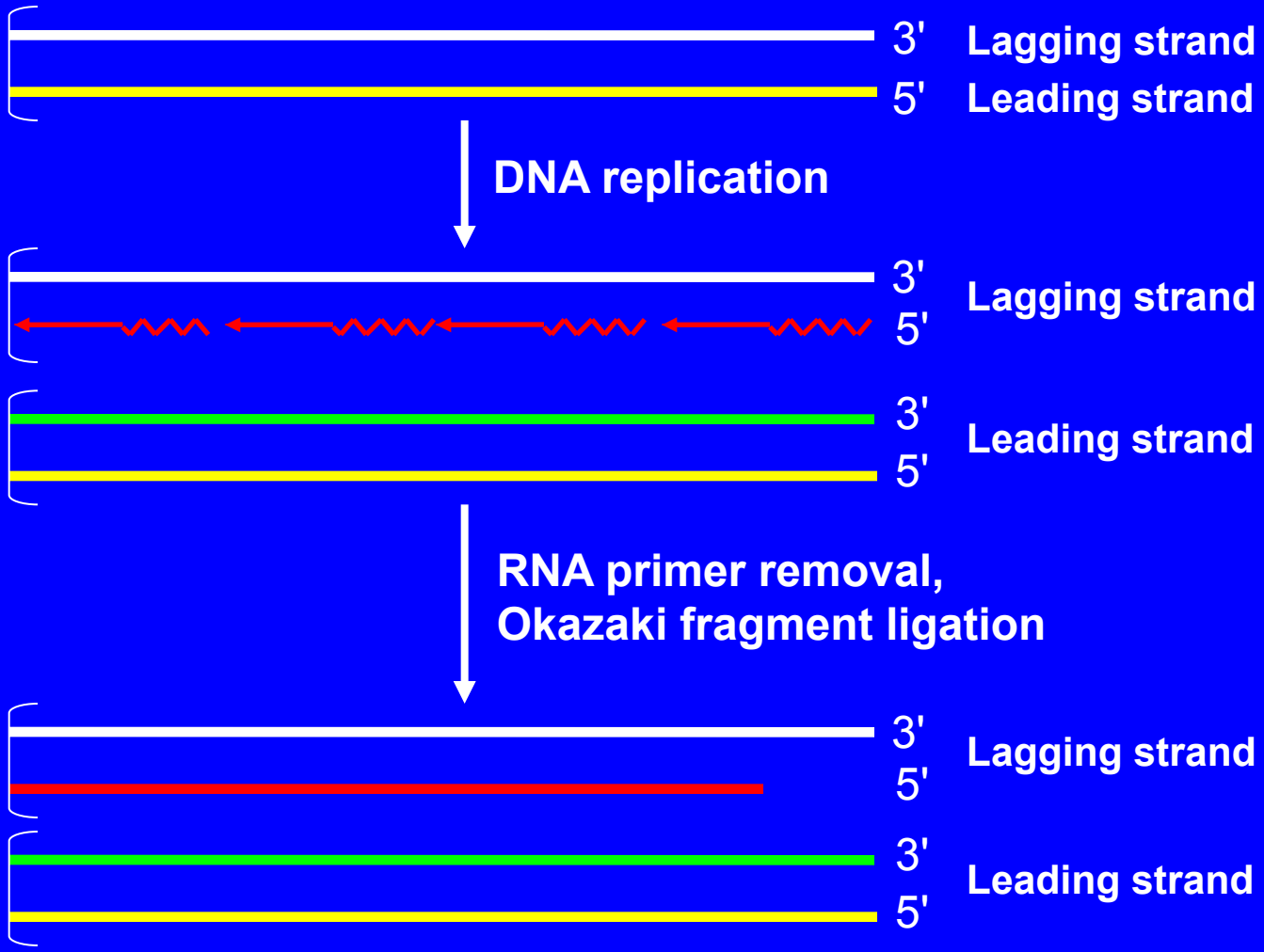
Ning et al., 2003



TELOMERIC SIMPLE SEQUENCE REPEATS

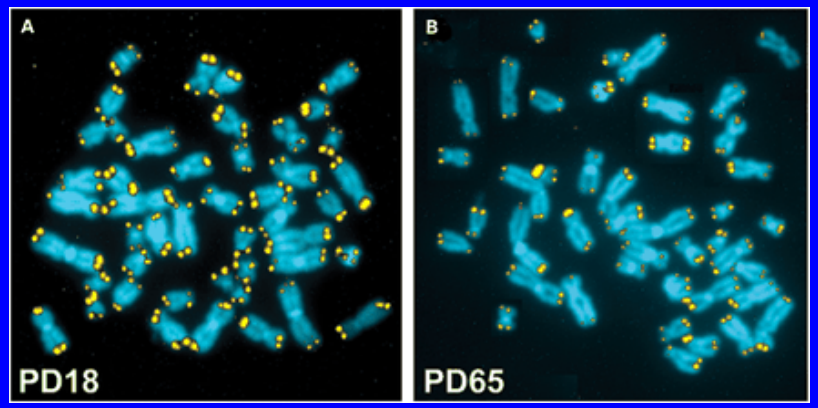
Organism	Repeat sequence
Tetrahymena	T_2G_4
Oxytricha	T_4G_4
Saccharomyces	$(TG)_{1\text{-}6}TG_{2\text{-}3}$
Kluyvermyces	$ACG_2AT_3GAT_2AG_2TATGTG_2TGT$
Arabidopsis	T_3AG_3
Homo sapiens	T_2AG_3

End-replication problem



The end replication problem causes telomere shortening

In the absence of a mechanism to counteract the end replication problem, telomeres shorten with each successive round of DNA replication.



Fluorescence in situ hybridization (FISH) with telomeric probe

Ning et al., 2003

Terminal restriction fragment (TRF) blot

Correlation between donor age and telomere length in human fibroblasts

TABLE 1 Effect of donor age on telomere length in human fibroblasts

Cell strain	Age *in vivo* years	Age *in vitro* MPD (MPD max)	Mean telomere length kb ± s.d. (*n*)
HSC172	Fetal	18–28 (88)	8.6 ± 0.5 (3)
A30S	0	33 (58)	7.3 (1)
A38	24	31–33 (68)	6.9 ± 0.3 (2)
A35	70	19 (41)	6.7 (1)
F001	71	21–29 (40)	6.5 ± 0.4 (5)
F002	91	18–20 (45)	6.2 ± 0.1 (3)

Mean telomere length (the length of the terminal restriction fragment) was determined as described in Fig. 2*a* for fibroblast cell strains at the earliest available mean population doubling (MPD) in separate experiments. Strains were derived from female fetal lung (HSC172), female newborn skin (A30S), male forearm skin (A38, A35) or female abdominal skin (F001, F002). MPD at time of assay and senescence (MPD max) are indicated. The correlation between increasing donor age and decreasing telomere length is statistically significant ($P < 0.05$).

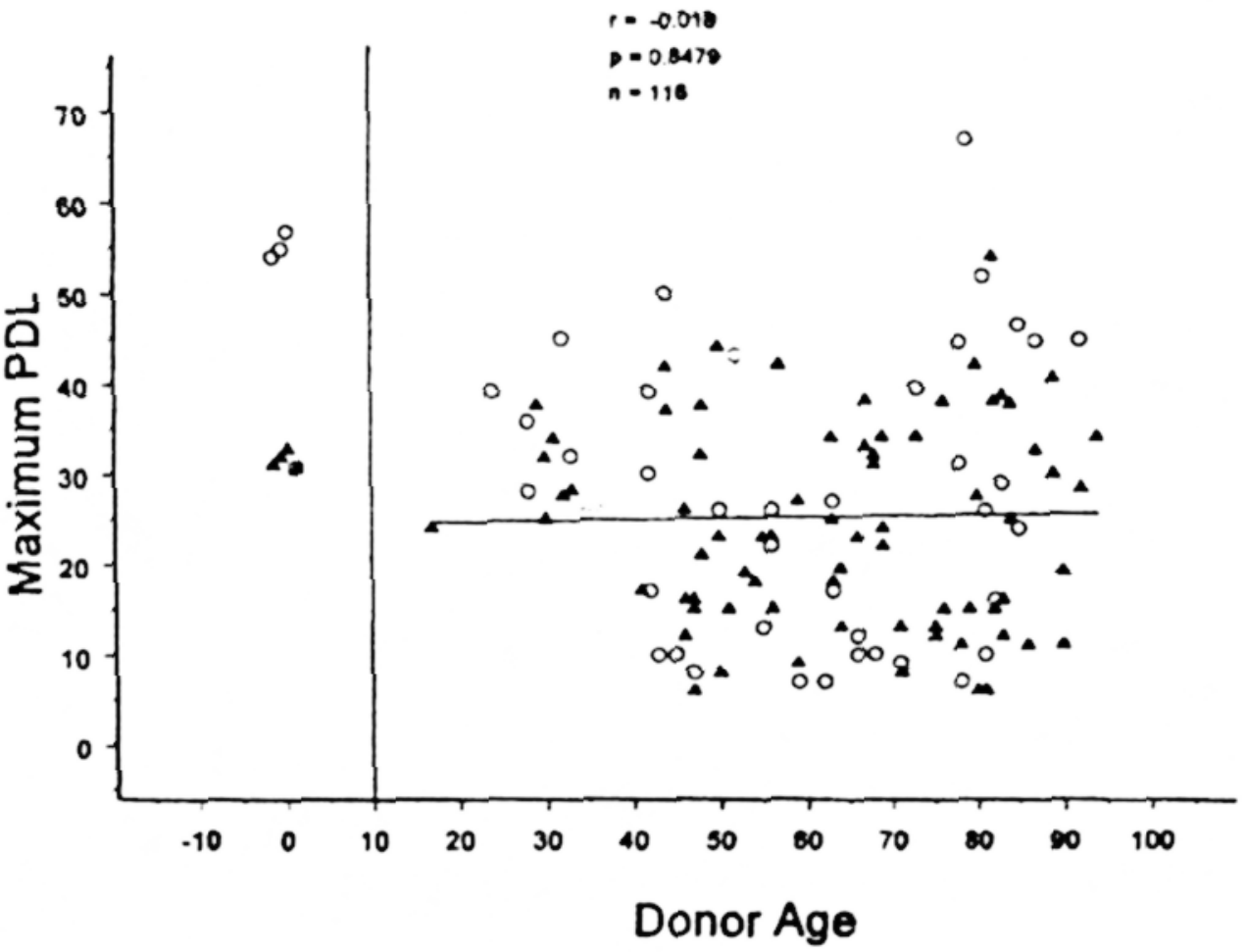
Harley, C.B. et al. 1990. Nature 345, 458-460.

Relation between replicative senescence of cells in culture and organismal lifespan?

- **There is a wide distribution of replicative potential of cells cultured from humans and animals**
- **The Hayflick limit applies only to the longest surviving clone**
- **Stem cells of intact renewing tissues undergo more divisions in a lifetime than the Hayflick limit in culture**
- **Morphological changes in vitro are not comparable to those in vivo**
- **Two clonal populations derived from a single mitosis may have different replicative potentials**
- **Correlation between donor age and replicative potential is difficult to reproduce**
- **Correlation between lifespan and Hayflick limit has several exceptions**

Rubin, H. 2002. The disparity between human cell senescence in vitro and lifelong replication in vivo. Nature Biotechnology 20, 675-681.

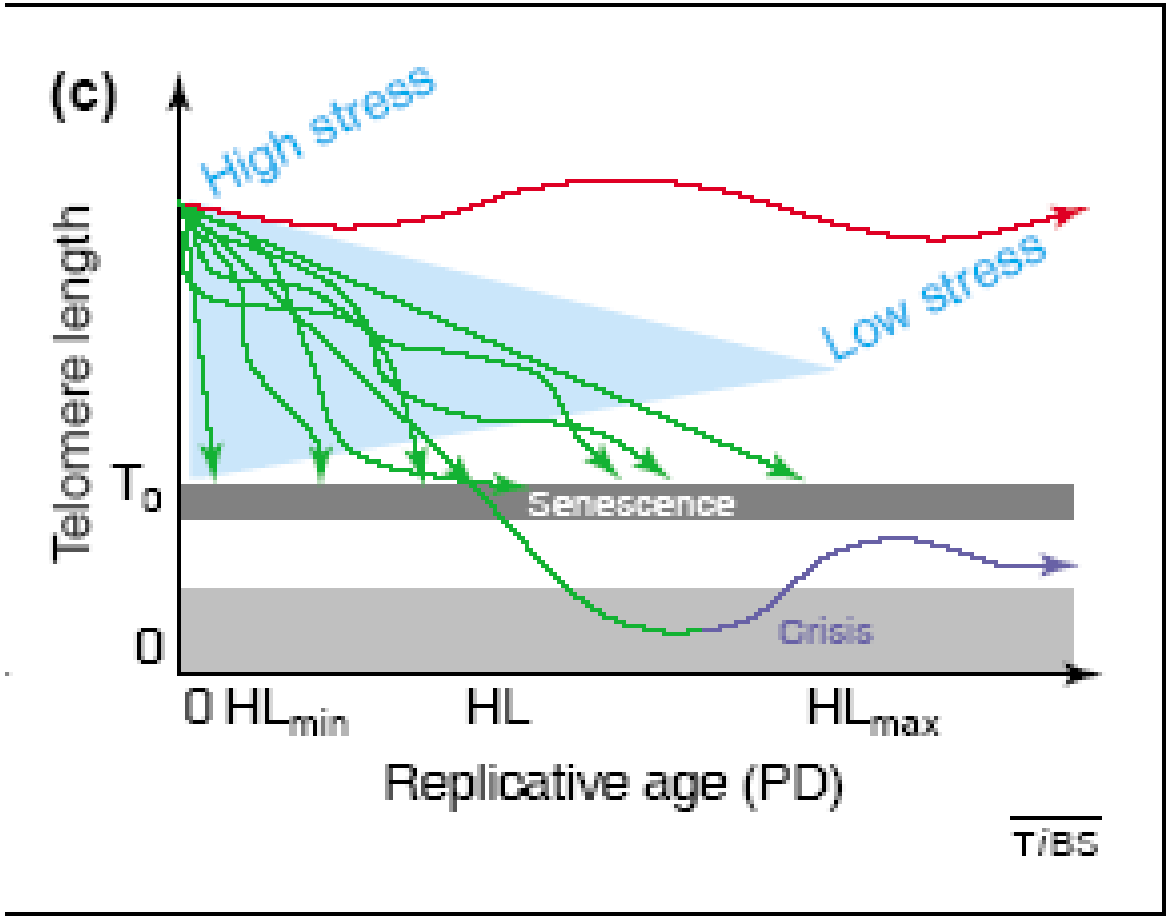
Relationship between in vitro proliferative capacity of postnatal skin fibroblast cell lines and donor age



Health status and biopsy conditions need to be considered

Cristofalo, V.J. et al. 1998. PNAS 95, 10614-10619.

Balance between oxidative stress and antioxidant defence modulates telomere length and replicative senescence



von Zglinicki, T. 2002. Oxidative stress shortens telomeres. Trends Biochem. Sci. 27, 339-344.

Aging, Rejunenation, and Epigenetic Reprogramming: Resetting the Aging Clock

Reprogramming during fertilization

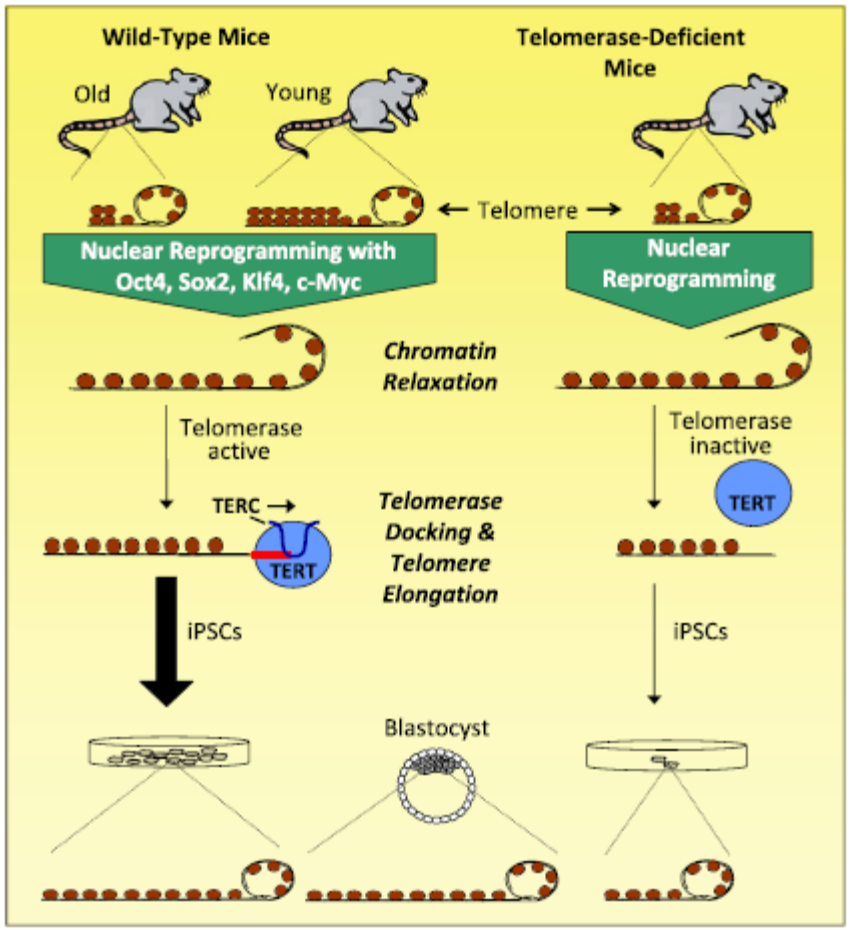
Somatic cell nuclear transfer exploits the reprogramming process during fertilization. Are there any age-related alterations of the transplanted nucleus?

Creation of induced pluripotent stem cells by the transcription factors Oct4, Sox2 and Klf4

Reprogramming is characterized by a reversal of the differentiation program and attainment of pluripotency, but not reversal of aging

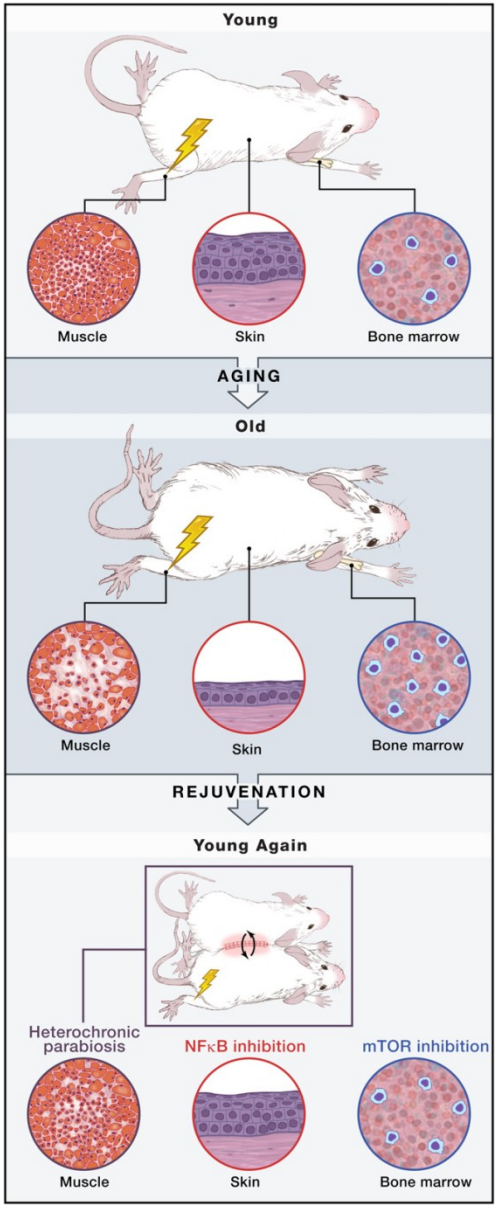
Rando, T.A. and Chang, H.Y. Aging, Rejuvenation, and Epigenetic reprogramming: resetting the aging clock. 2012. Cell 148, 46-57.

Induced pluripotent stem cells, epigenetic reprogramming and the role of telomeres



Davy and Allsopp, Cell Stem Cell, 4, 95-96, 2009

Is it possible to reset the aging clock without affecting the differentiation program?



- impaired regenerative responses in skeletal muscle
- thinning of the skin epithelium
- hypercellularity of the bone marrow.

Yes, environmentally, by heterochronic parabiosis (systemic circulations of two animals are joined together)

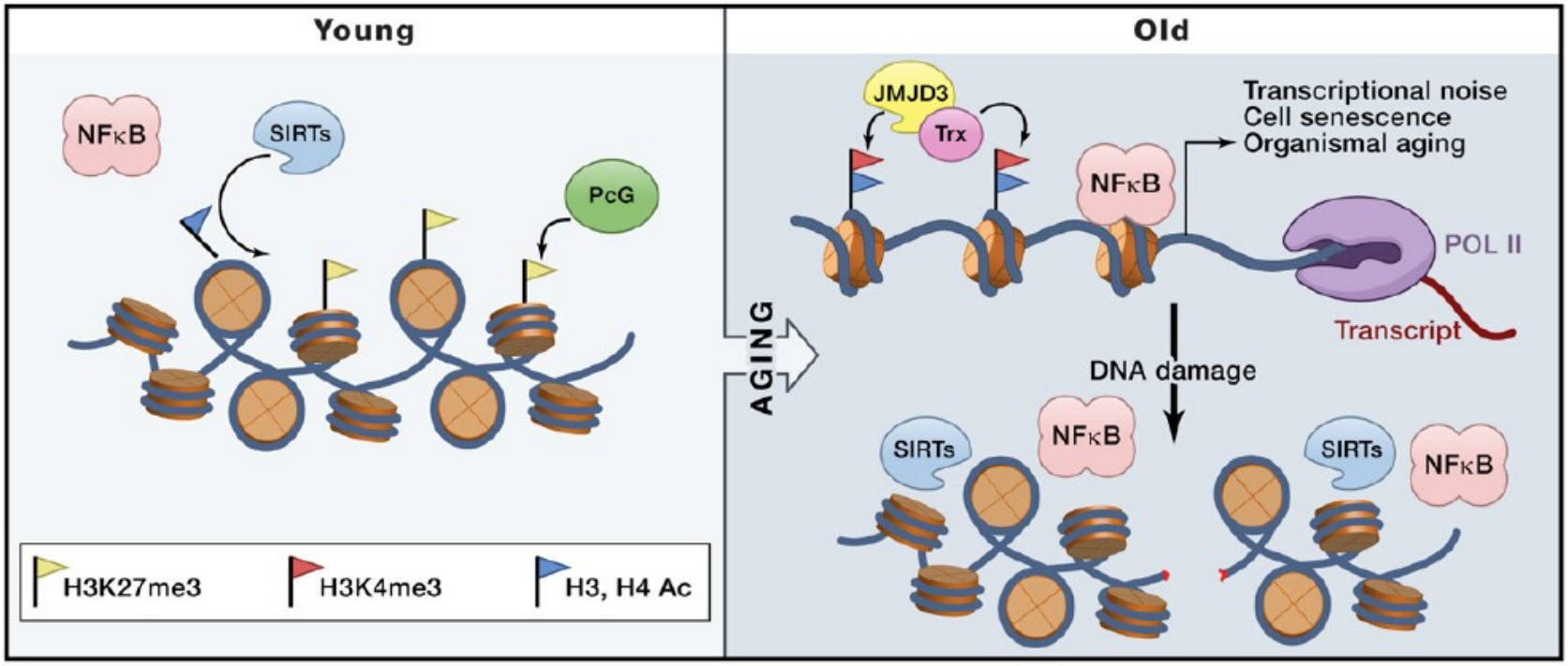
Genetically, by a conditional inhibition of NF-Kβ in the skin

Pharmacologically by administration of rapamycin, a mTOR inhibitor

Rando, T.A. and Chang, H.Y. Aging, Rejuvenation, and Epigenetic reprogramming: resetting the aging clock. 2012. Cell 148, 46-57.

Aging and Epigenetics

Is aging comparable to differentiation?
If aging is in part a manifestation of epigenetic changes, can young and old cells by characterized by specific epigenetic profiles?



PcG: Polycomb group proteins

Trithorax group proteins (Trx) and H3K27 demethylase JMJD3

Rando, T.A. and Chang, H.Y. Aging, Rejuvenation, and Epigenetic reprogramming: resetting the aging clock. 2012. Cell 148, 46-57.