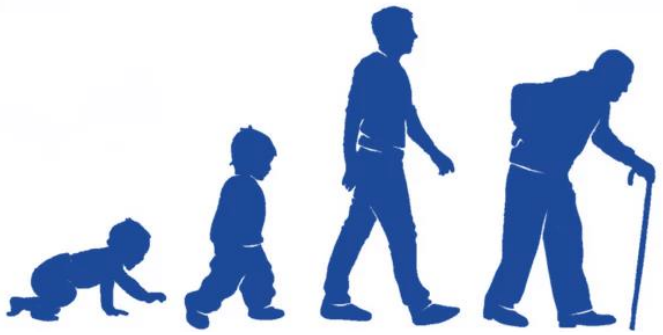
MUNI
MED

Pathophysiology of age-related processes,
aging, longevity, senescence, death

Petr Müller



What is ageing?

- Is ageing a disease?
- Which diasases are associated with ageing?

Mechanisms of ageing

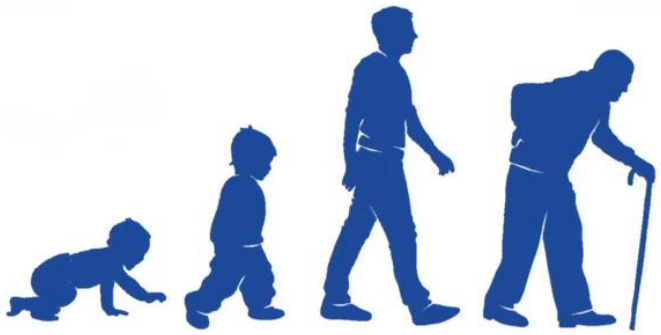
- Regulation of aging at different levels of the human body organization
- Ageing of DNA
  - Methylation
  - Telomeres
- Metabolism and ageing
- Cellular senescence
- Organ ageing

Evolutionary mechanisms of ageing

- Genetics of ageing

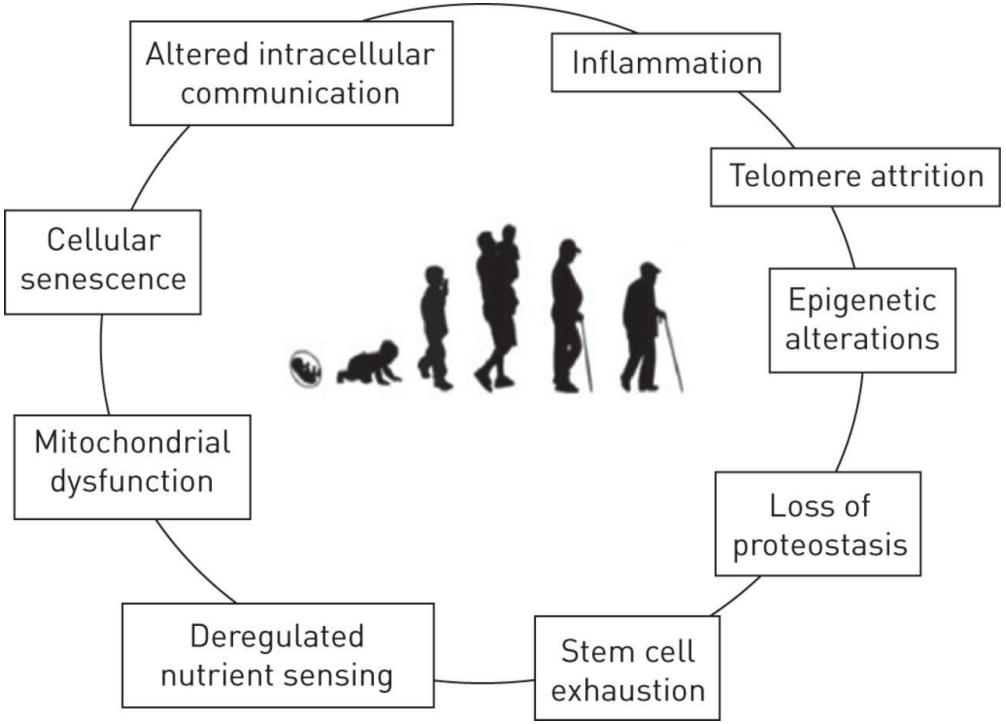
Can we treat/ slow down ageing

- Experiments on model organisms
- Implications for healthy ageing



Is ageing a disease ?

Aging is the sequential or progressive change in an organism that leads to an increased risk of debility, disease, and death.

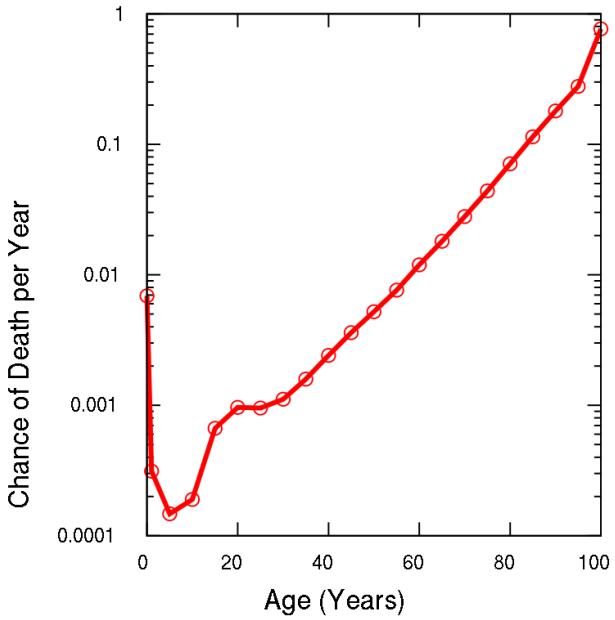


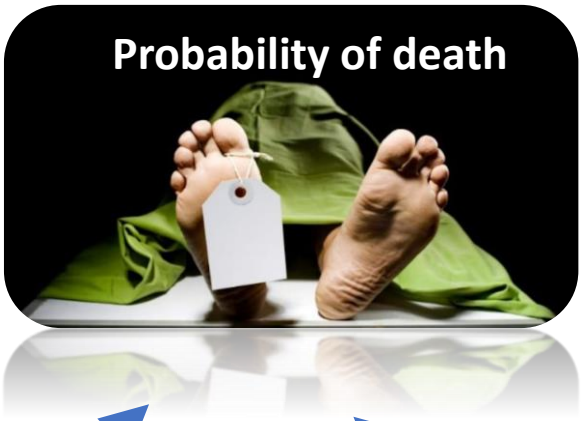
Programmed lifespan
Encoded in our genome

Ageing associated diseases

Gompertz–Makeham law of mortality

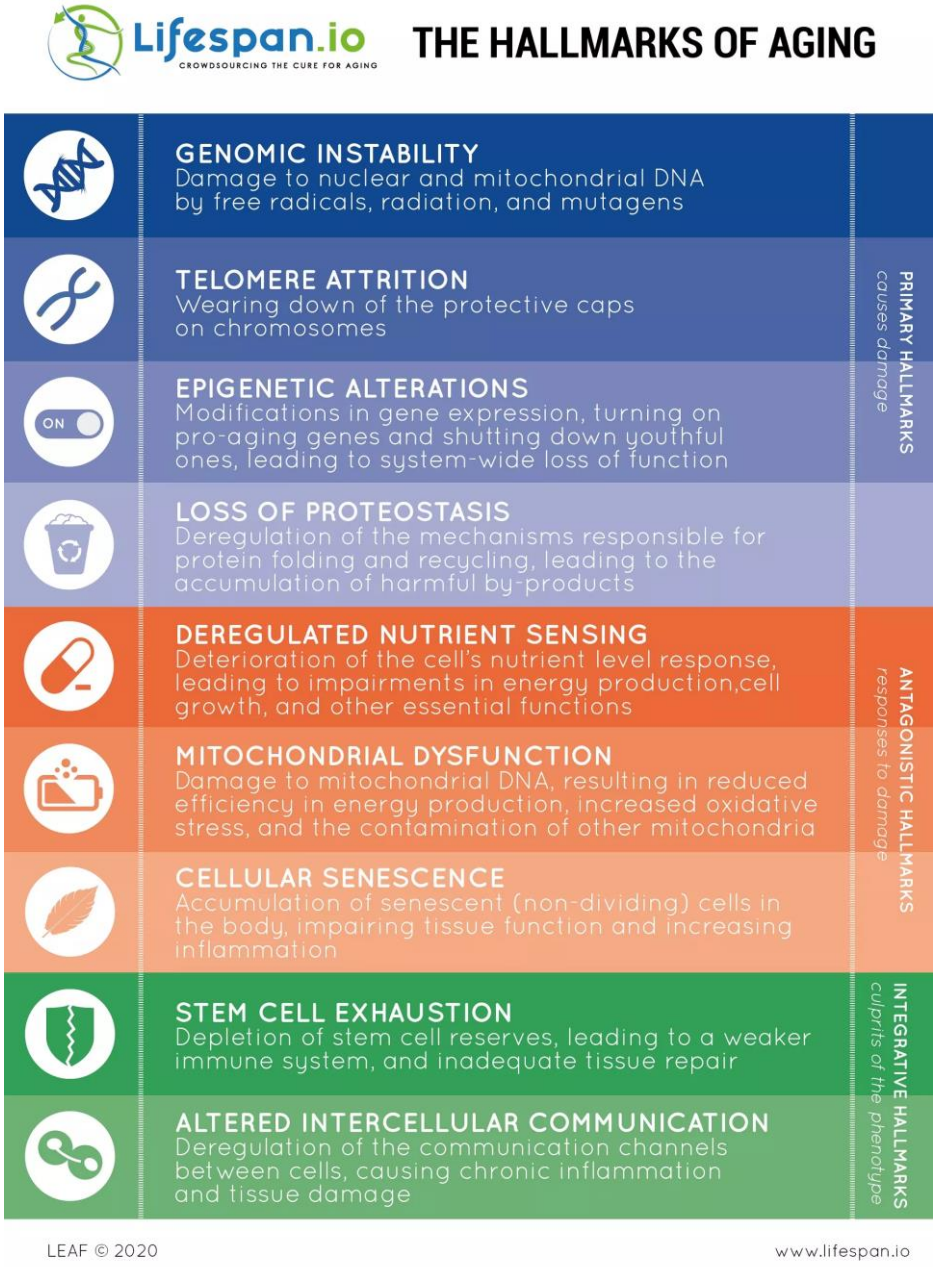
Estimated probability of a person dying at each age, for the U.S. in 2003. Mortality rates increase exponentially with age after age 30.





age-independent component

The Gompertz–Makeham law states that the human death rate is the sum of an **age-dependent component** (the Gompertz function, named after Benjamin Gompertz), which increases exponentially with age and an **age-independent component** (the Makeham term, named after William Makeham).



Cardiovascular system

- Hypertension
- Atherosclerosis
- Stroke, MI

CNS

- Dementia
- Neurodegenerative diseases

Musculoskeletal system

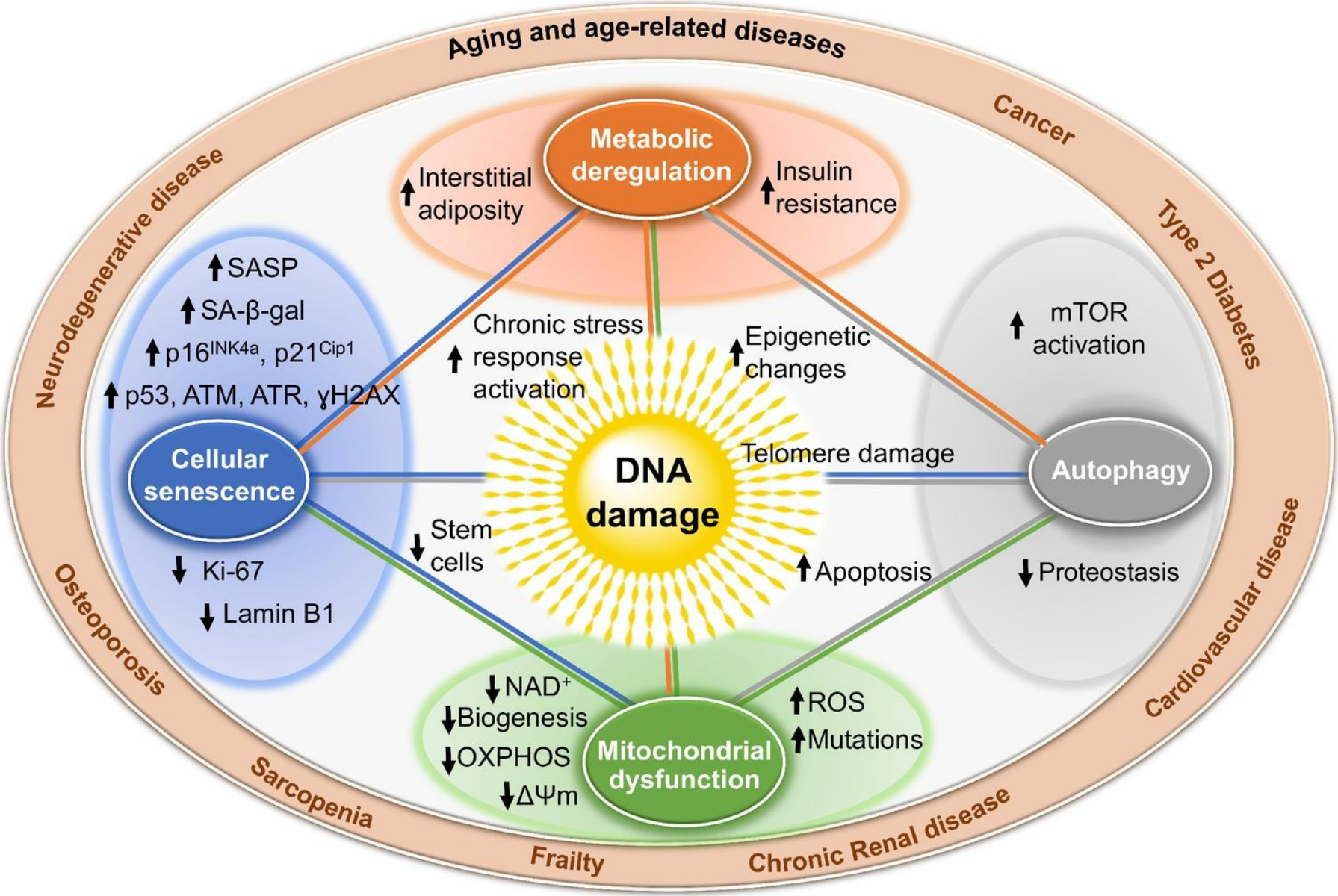
- Arthritis
- Muscle weakness

Cancer

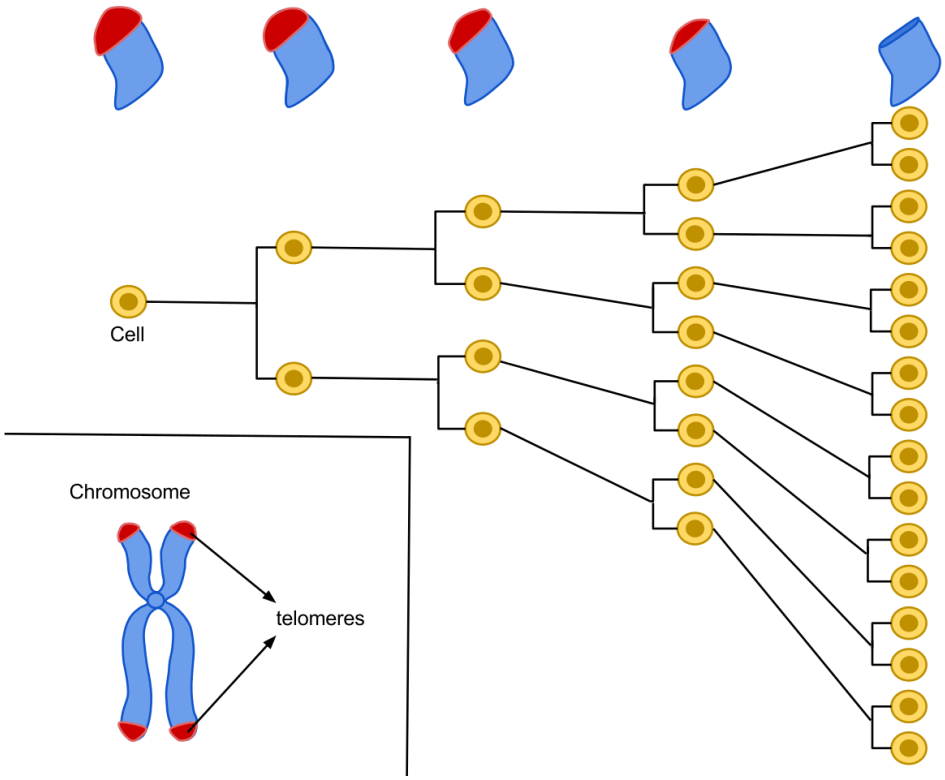
Metabolism

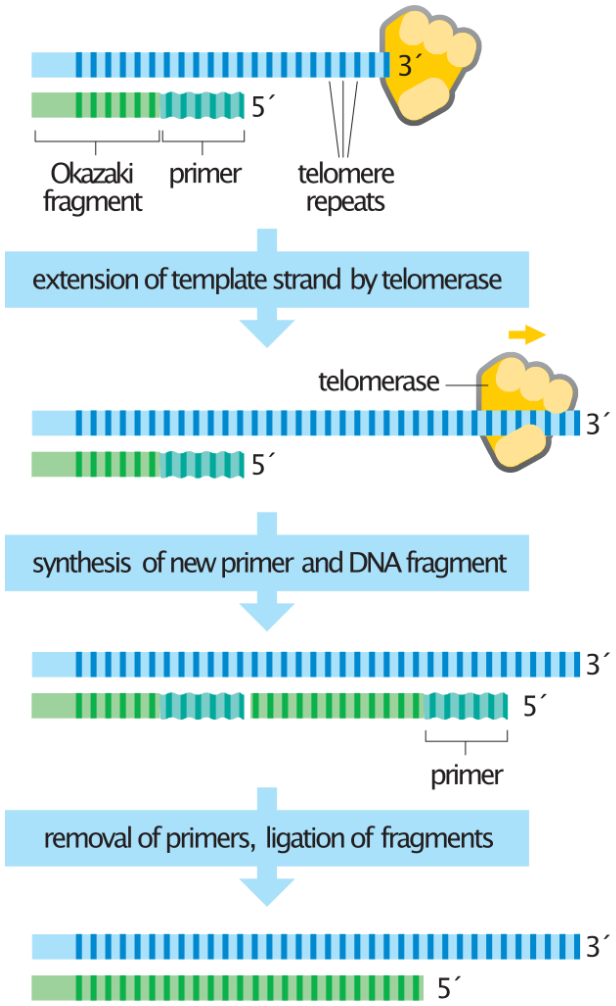
- Decreased basal metabolism
- Obesity
- Diabetes mellitus type 2

DNA damage theory of aging



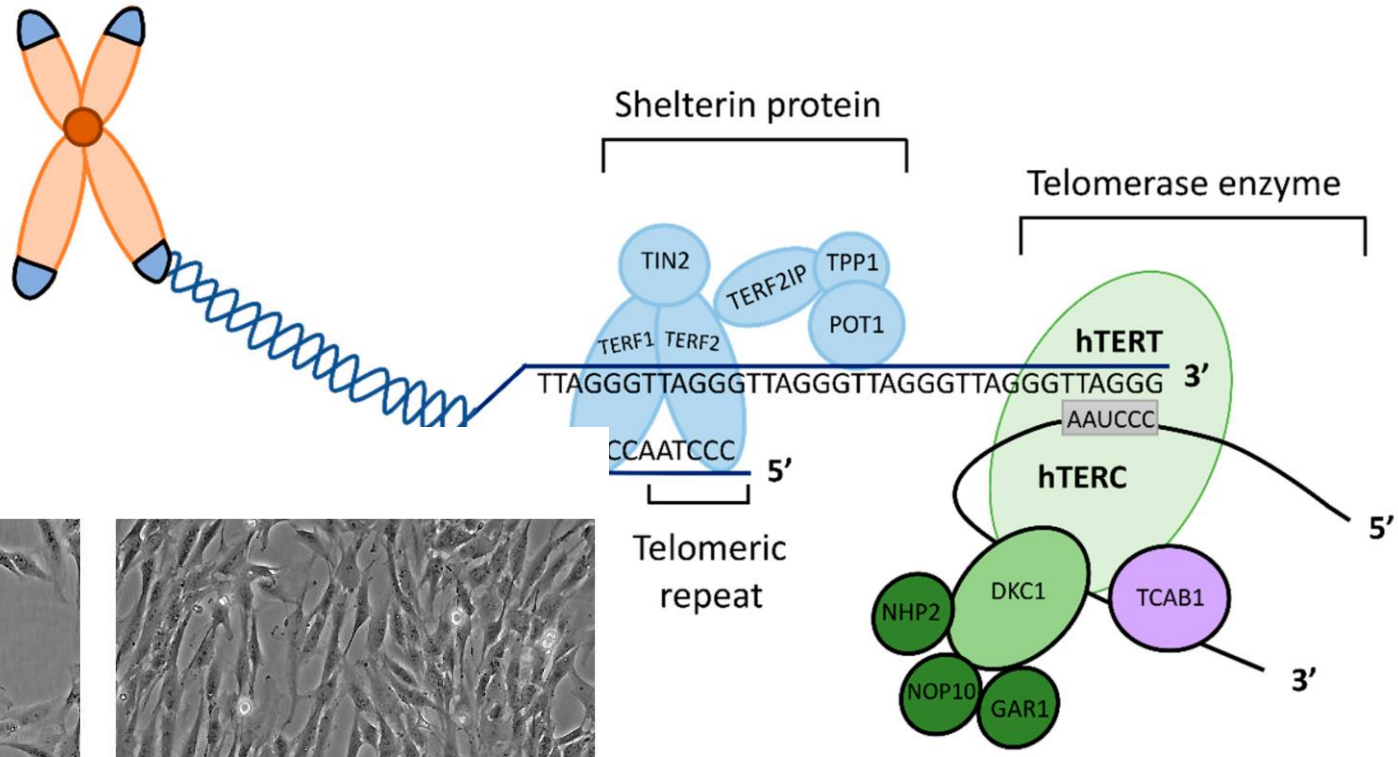
Telomere shortening and cellular senescence



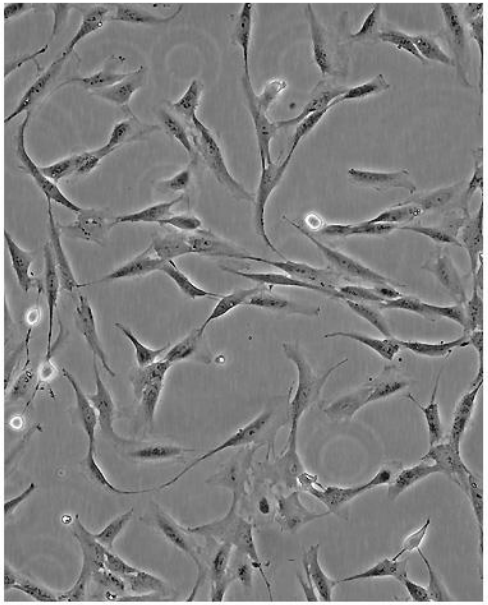


Hayflick limit he typical normal human fetal cell will divide between 50 and 70 times before experiencing senescence.

Telomerase hTERT and cell immortalization

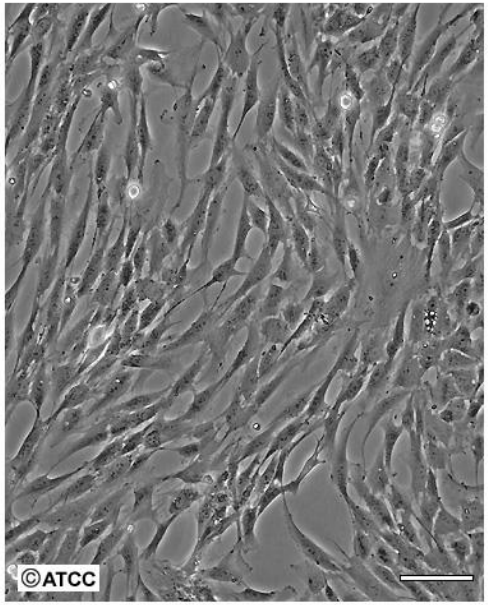


ATCC Number: **CRL-4000**
Designation: **hTERT RPE-1**



Low Density

Scale Bar = 100µm

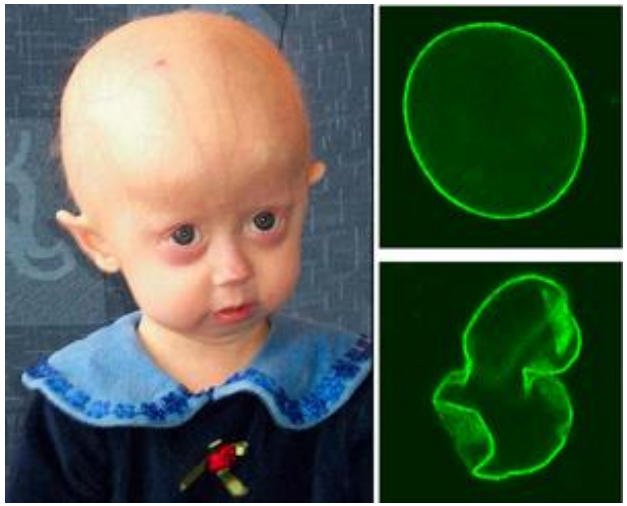


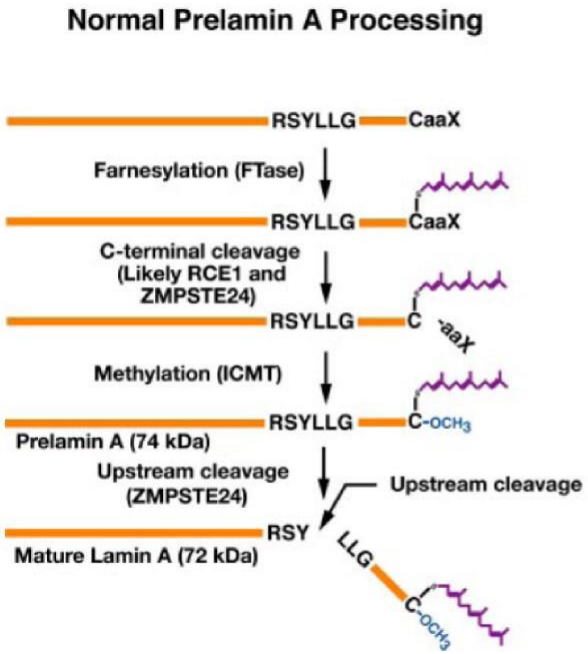
High Density

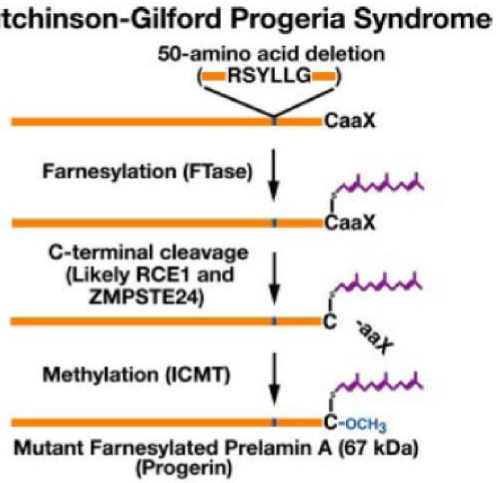
Scale Bar = 100µm

Progeria Hutchinson-Gilford syndrome

- Autosomal dominant disease
- Mutation in Lamin A
- Altered histone modifications a and chromatin structure
- Genomic instability



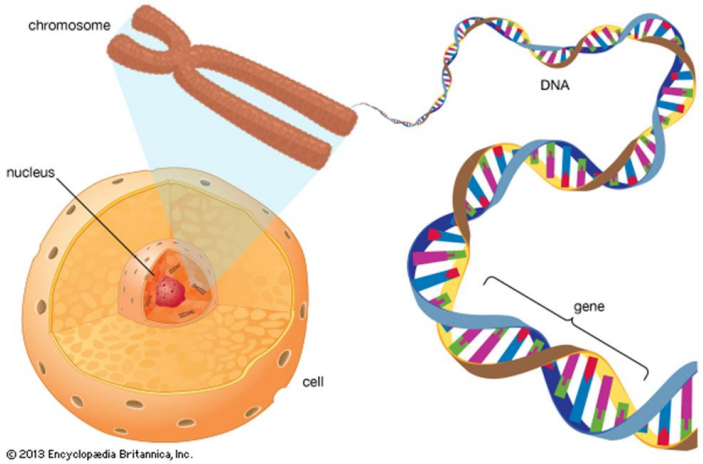




Other DNA damage related premature ageing:

- Werner syndrome
- Cockayne syndrome

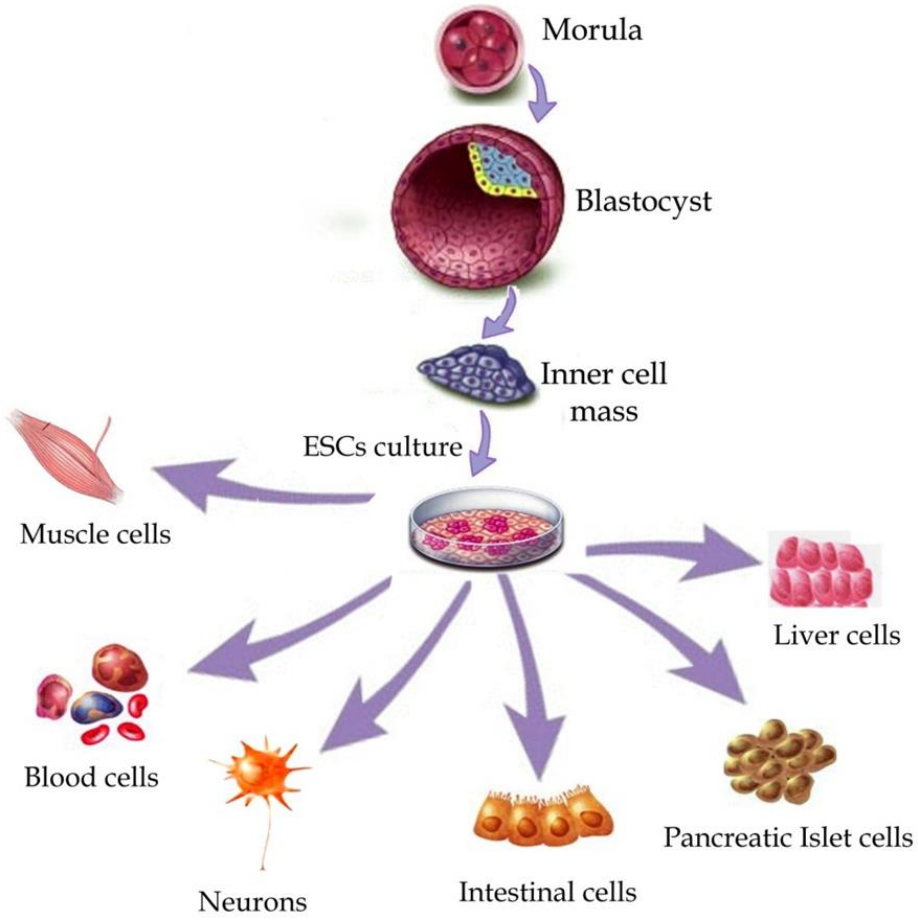
Ageing and epigenetics



All cells of the body retain complete genetic information that remains unchanged throughout life.

Differentiation

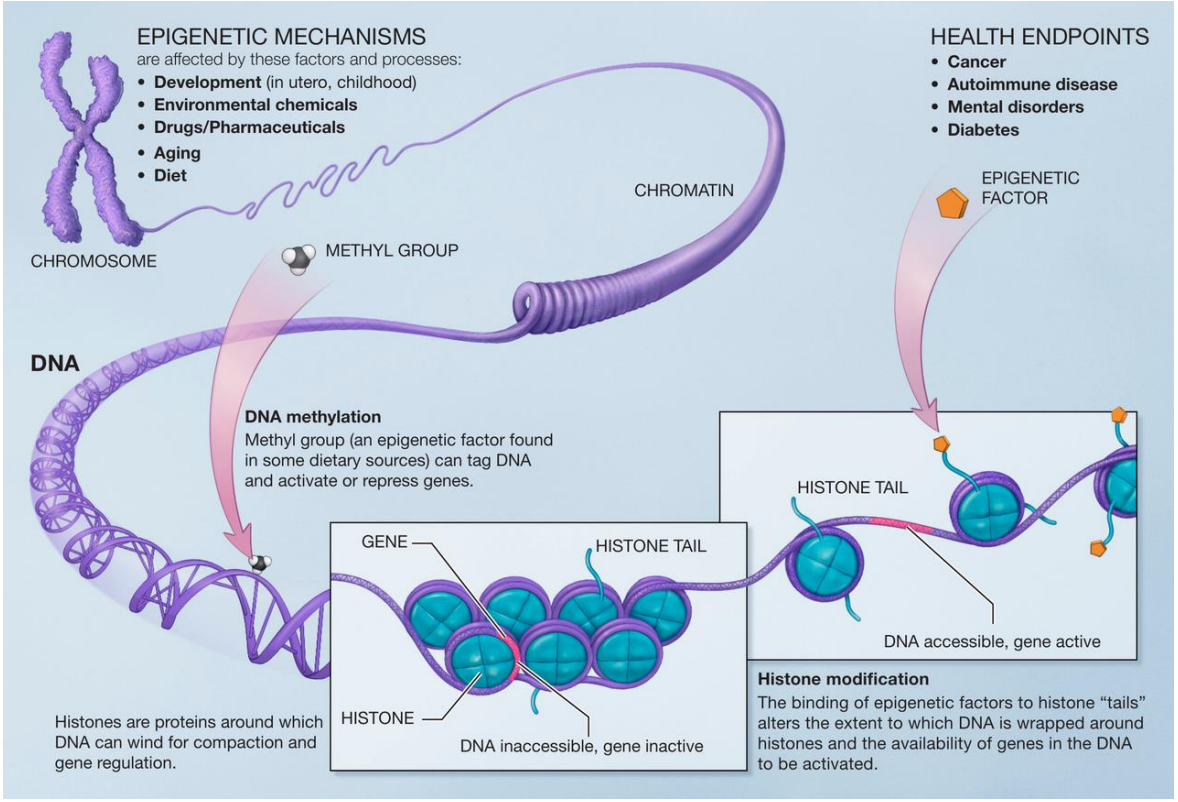
Human tissues are composed of differentiated cells
The daughter cells inherit the basic properties from parental cells



Epigenetics definitions and mechanisms

Epigenetics is the study of heritable phenotype changes that do not involve alterations in the DNA sequence.

Epigenetics most often involves changes that affect gene activity and expression, but the term can also be used to describe any heritable phenotypic change.

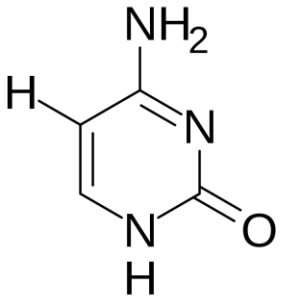


Mechanisms:

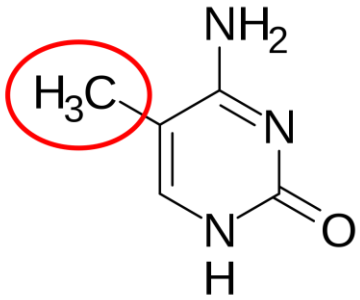
- Covalent modifications
- RNA transcripts
- MicroRNAs
- mRNA
- sRNAs
- Prions
- Structural inheritance
- Nucleosome positioning
- Histone variants
- Genomic architecture

DNA methylation

- process by which methyl groups are added to the DNA molecule.
- Methylation can change the activity of a DNA segment without changing the sequence



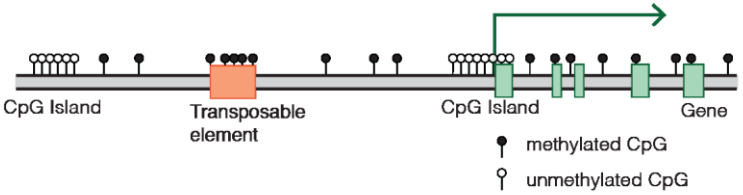
cytosine



methylated cytosine

In mammals however, DNA methylation is almost exclusively found in CpG dinucleotides, with the cytosines on both strands being usually methylated.

Typical mammalian DNA methylation landscape



CpG islands are usually defined as regions with:

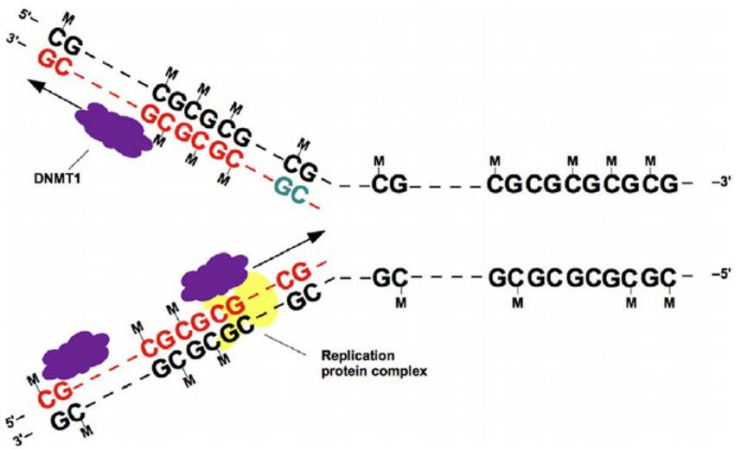
1) a length greater than 200bp,
2) a G+C content greater than 50%,
3) a ratio of observed to expected CpG greater than 0.6,

DNA methyltransferases (in mammals)

1. maintenance methylation (Maintenance methylation activity is necessary to preserve DNA methylation after every cellular DNA replication cycle).
2. *de novo* methylation

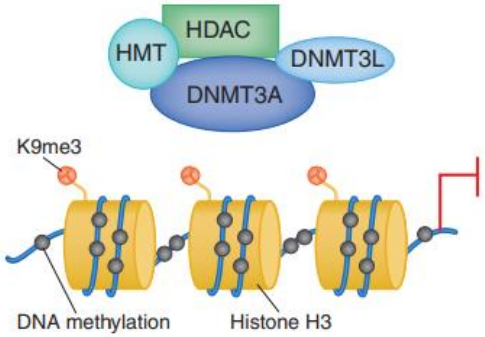
DNMT1

- maintanance



DNMT3a and DNMT3b

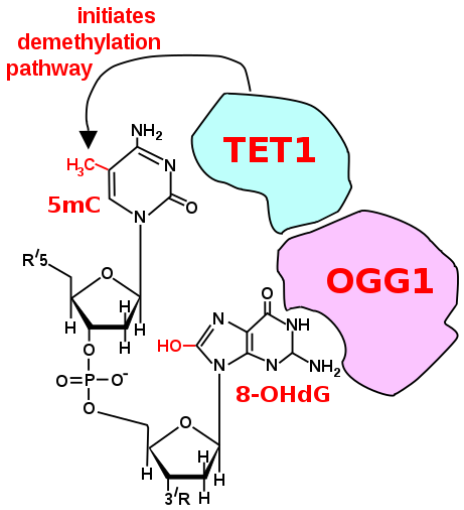
- the de novo methyltransferases that set up DNA methylation patterns



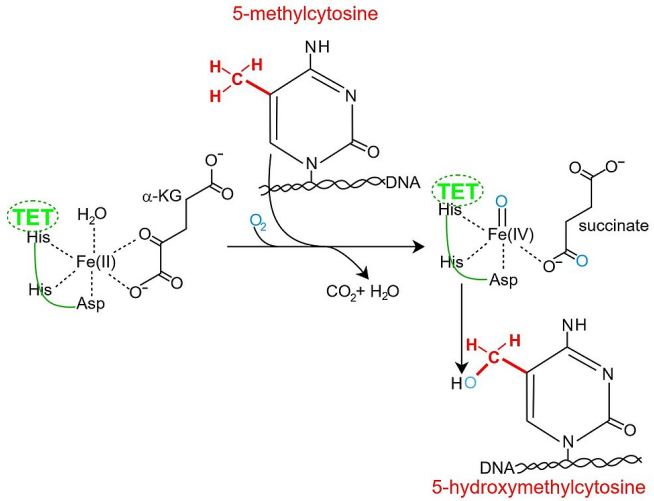
Model of DNMT3A activity. The DNMT3A protein complex is associated at promoters of silent genes in a complex with histone methyltransferase (HMT), histone deacetylase (HDAC) and DNA methyltransferase 3L (DNMT3L). These promoters are marked by DNA methylation, histone deacetylation and histone 3 lysine 9 methylation (K9me3).

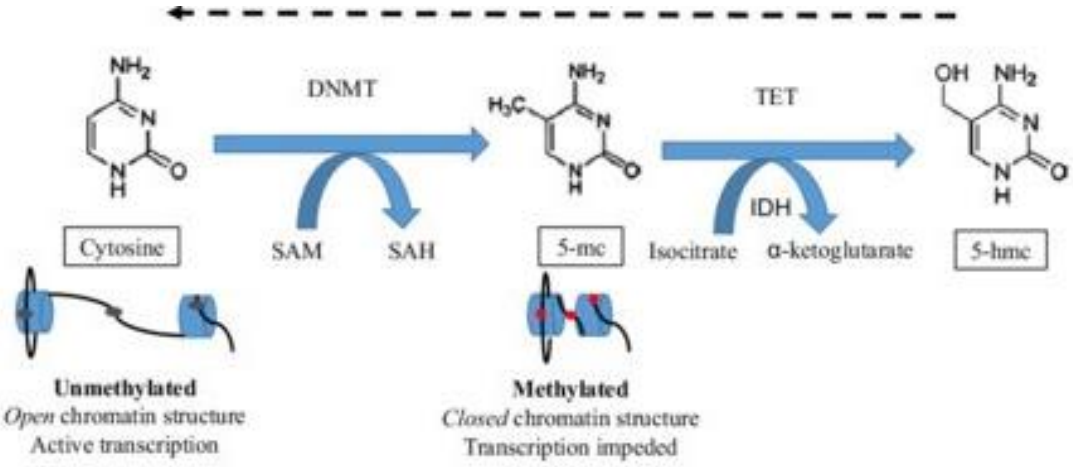
DNA demethylation

- TET enzymes are a family of ten-eleven translocation (TET) methylcytosine dioxygenases.
- They are instrumental in DNA demethylation.

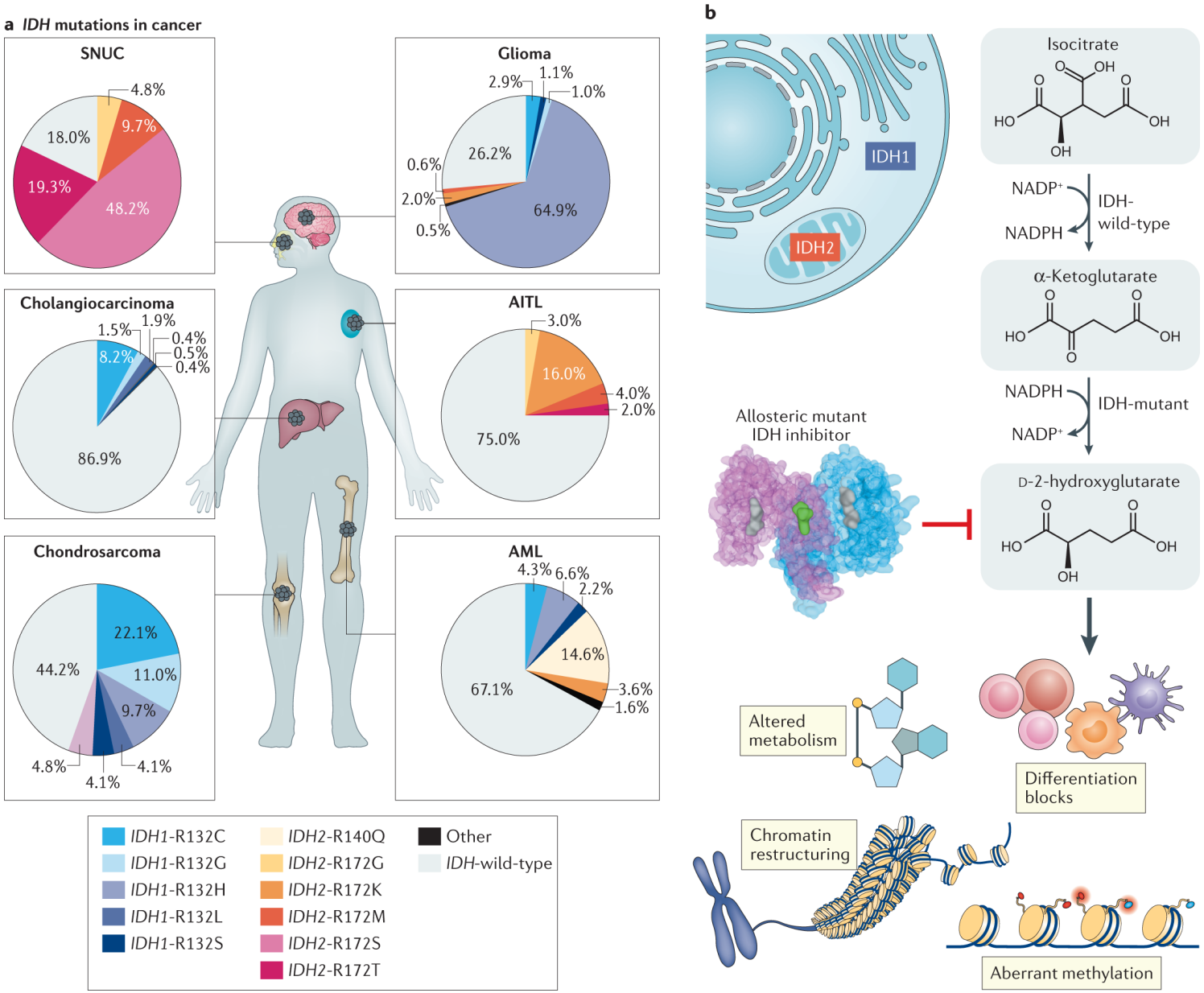


Oxoguanine glycosylase (OGG1) recruits TET enzyme





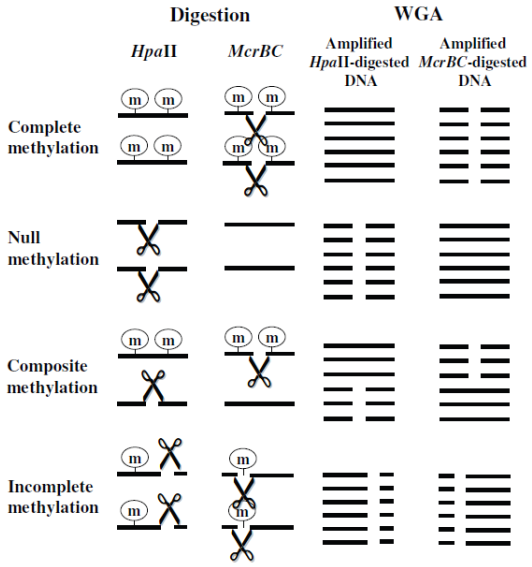
The implications of *IDH* mutations for cancer development and therapy



Nature Reviews Clinical Oncology **volume 18**, pages645–661 (2021)

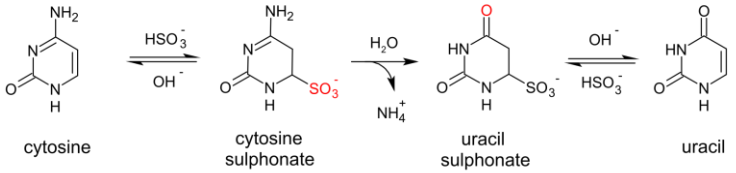
Detection of methylation

1) Using methylation sensitive restriction endonucleases

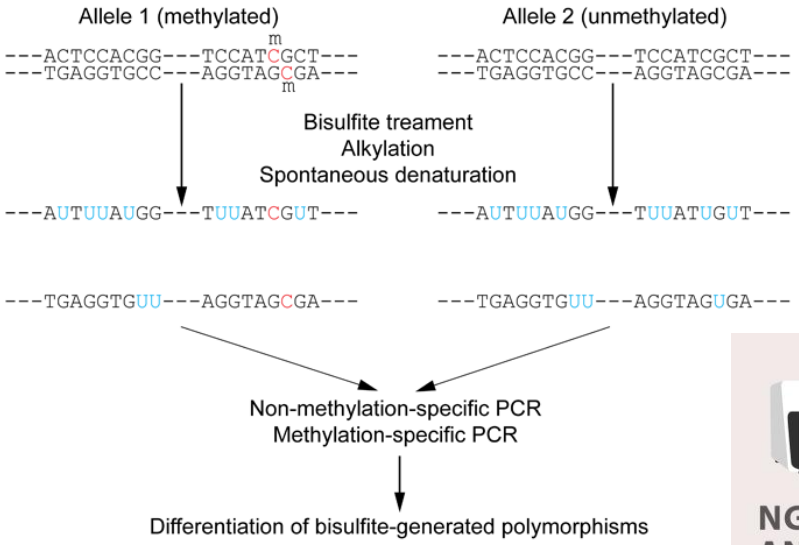


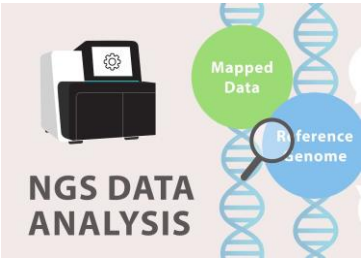
McrBC is an endonuclease which cleaves DNA containing methylcytosine* on one or both strands

2) Using bisulfite conversion

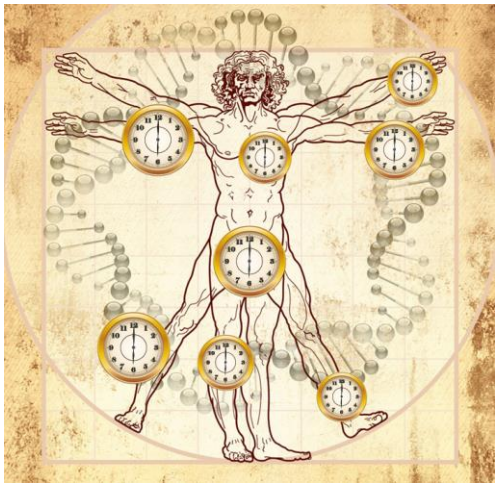


Outline of the chemical reaction that underlies the bisulfite-catalyzed conversion of cytosine to uracil.





Methylation and aging



Horvath's clock
Epigenetic clock

In humans and other mammals, DNA methylation levels can be used to accurately estimate the age of tissues and cell types, forming an accurate epigenetic clock

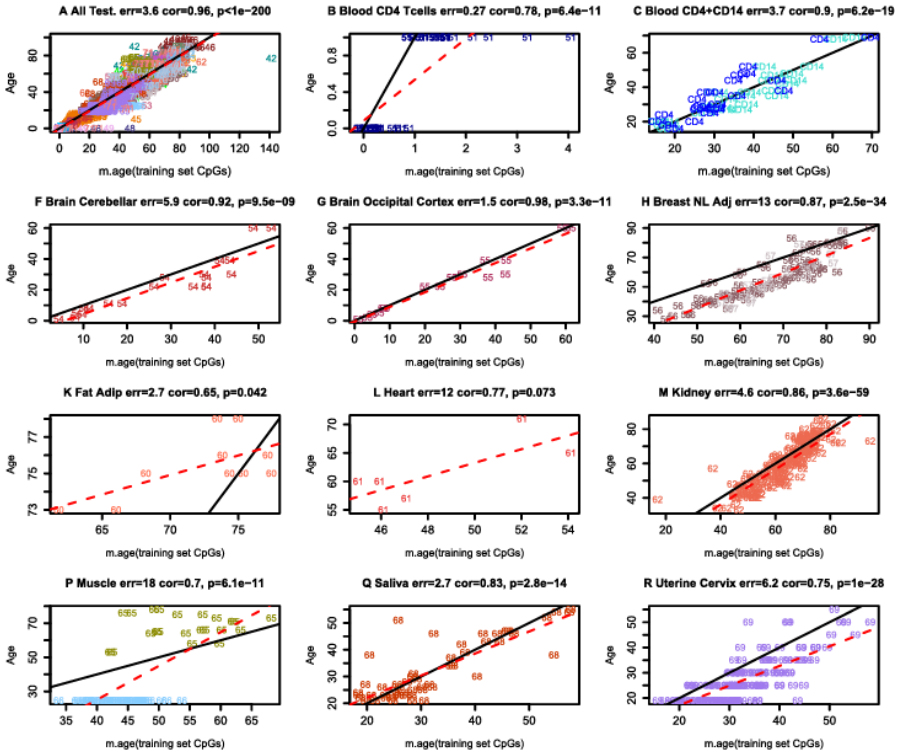
Horvath *Genome Biology* , **14**:R115
http://genomebiology.com//14/10/R115

Genome Biology

RESEARCH Open Access

DNA methylation age of human tissues and cell types

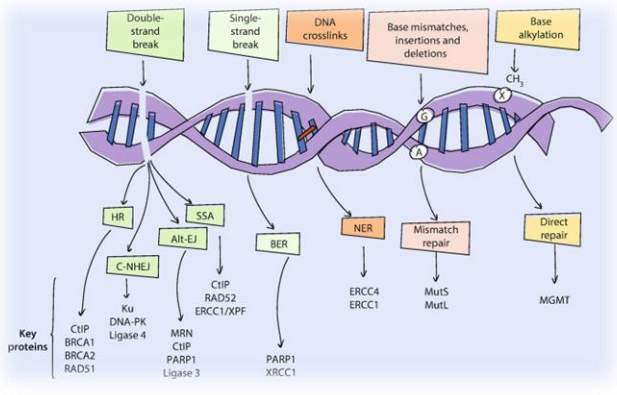
Steve Horvath[1,2,3]



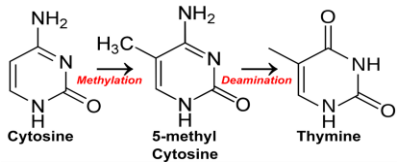
Chronological age (y-axis) versus DNAm age (x-axis) across different cells and tissues

Ageing methylation and cancer

Genomic instability

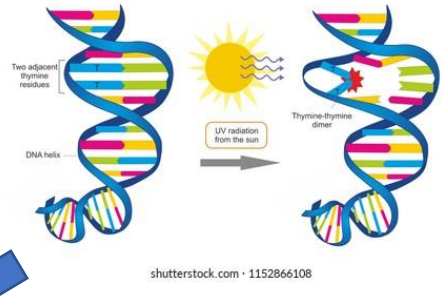


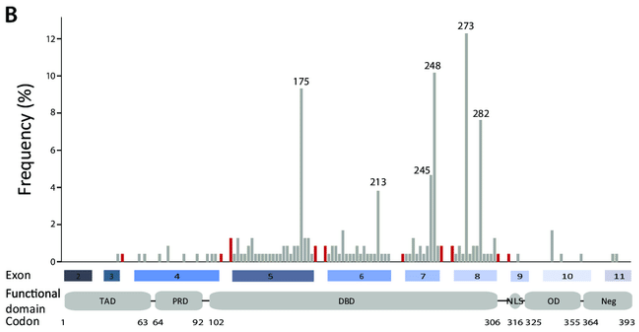
„Spontaneous“ mutations (aging and inflamation)



Exogenous mutagens

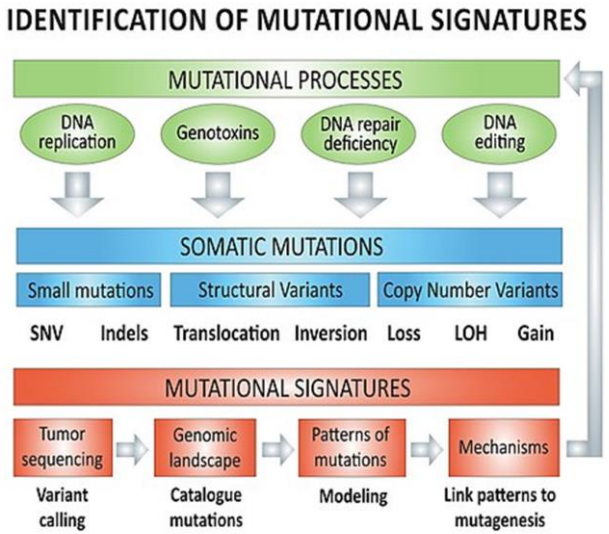
- Smoking
- UV light
- Alkylating agents
- aflatoxin

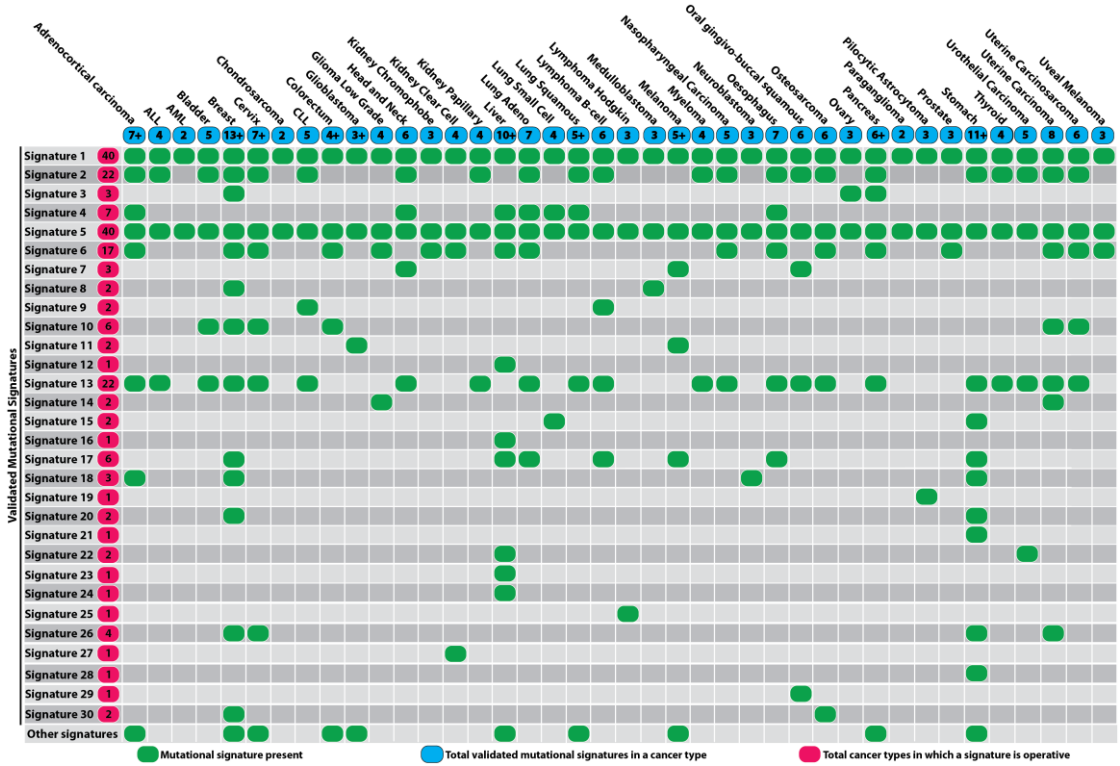


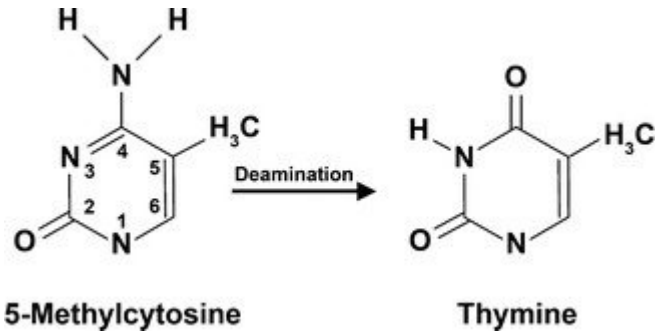


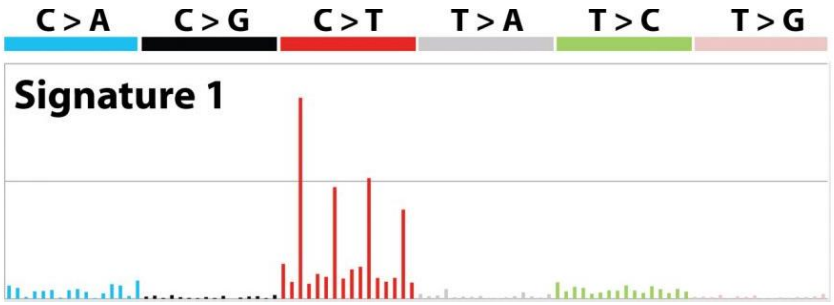
Mutation pattern
Mutational signatures

Mutational signature associated with ageing







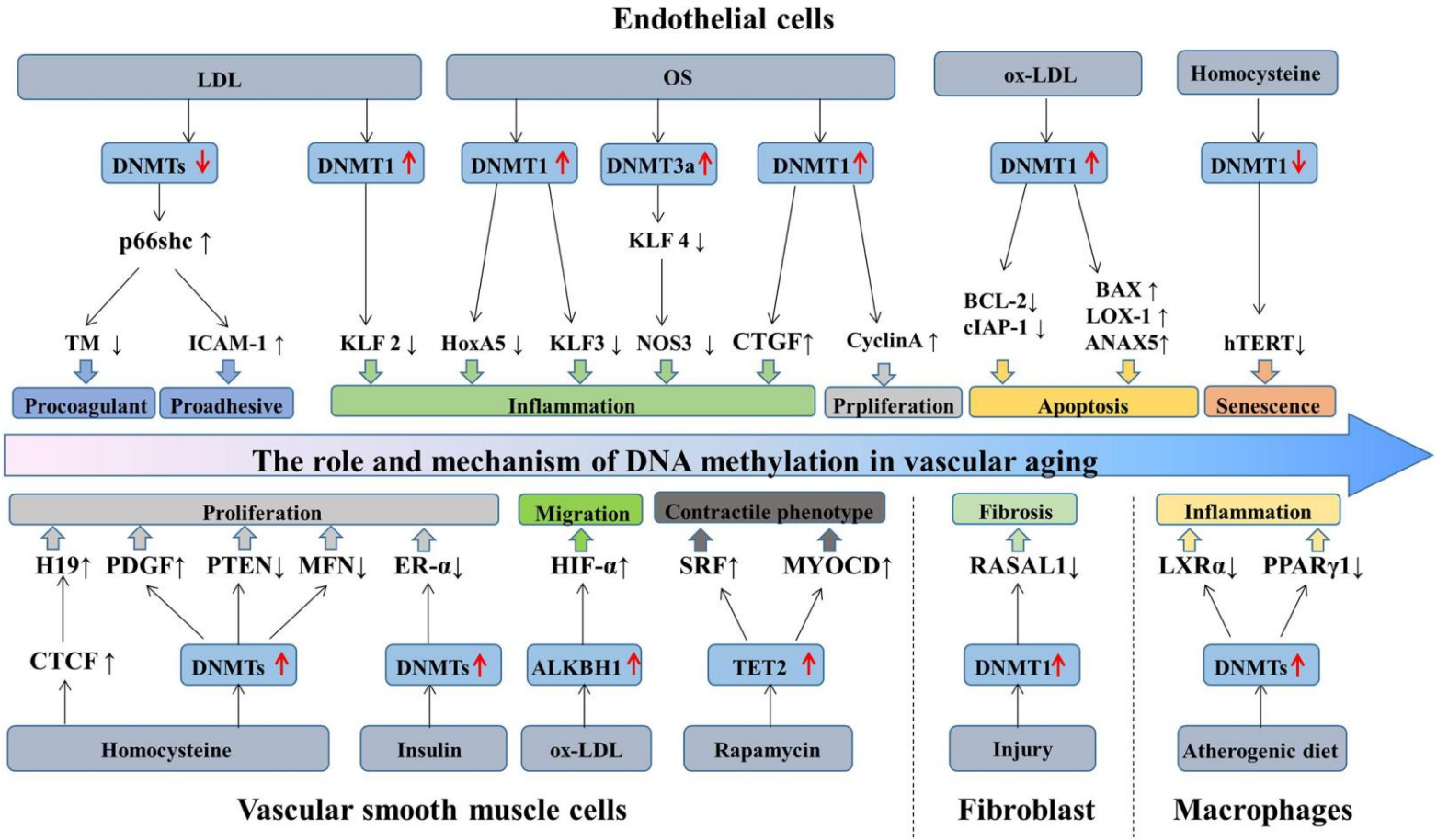


frontiers in Cell and Developmental Biology

REVIEW
published: 28 June 2021
doi: 10.3389/fcell.2021.699374

Roles and Mechanisms of DNA Methylation in Vascular Aging and Related Diseases

*Hui Xu[1,2], Shuang Li[1,2] and You-Shuo Liu[1,2]**



Article

Nature | Vol 588 | 3 December 2020

Reprogramming to recover youthful epigenetic information and restore vision

https://doi.org/10.1038/s41586-020-2975-4

Received: 31 July 2019

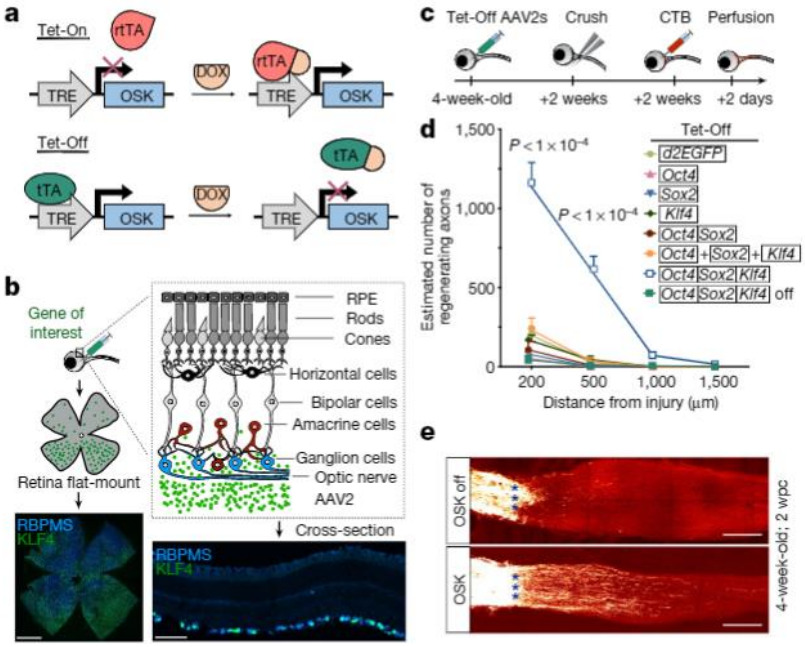
Accepted: 22 October 2020

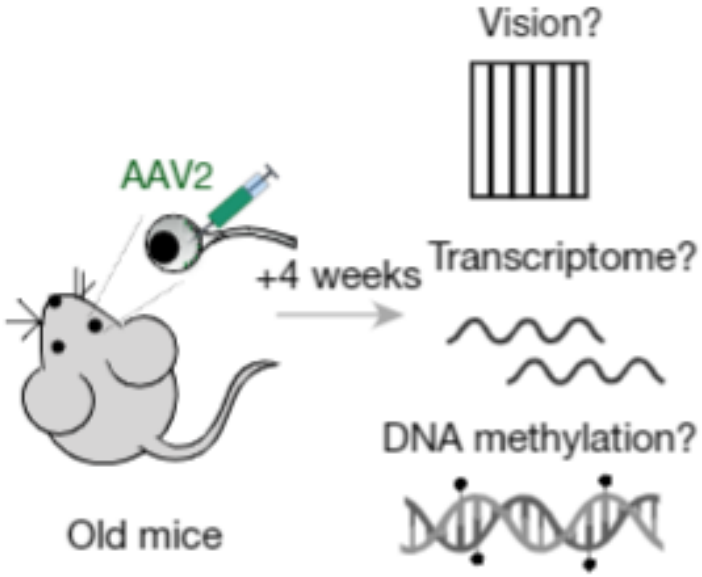
Published online: 2 December 2020

Check for updates

Yuancheng Lu[1], Benedikt Brommer[2,3,11], Xiao Tian[1,11], Anitha Krishnan[3,4,11], Margarita Meer[5,6,11], Chen Wang[2,3], Daniel L. Vera[1], Qiurui Zeng[1], Doudou Yu[1], Michael S. Bonkowski[1], Jae-Hyun Yang[1], Songlin Zhou[2,3], Emma M. Hoffmann[3,4], Margarete M. Karg[3,4], Michael B. Schultz[1], Alice E. Kane[1], Noah Davidsohn[7], Ekaterina Korobkina[3,4], Karolina Chwalek[1], Luis A. Rajman[1], George M. Church[7], Konrad Hochedlinger[8], Vadim N. Gladyshev[5], Steve Horvath[9], Morgan E. Levine[6], Meredith S. Gregory-Ksander[3,4,12], Bruce R. Ksander[3,4,12], Zhigang He[2,3,12] & David A. Sinclair[1,10,12]

Changes to DNA methylation patterns over time form the basis of ageing clocks, but whether older individuals retain the information needed to restore these patterns—and, if so, whether this could improve tissue function—is not known.





- Ectopic expression of Oct4 (also known as Pou5f1), Sox2 and Klf4 genes (OSK) in mouse retinal ganglion cells restores youthful DNA methylation patterns and transcriptomes, promotes axon regeneration after injury, and reverses vision loss in a mouse model of glaucoma and in aged mice.
- The beneficial effects of OSK-induced reprogramming in axon regeneration and vision require the DNA demethylases TET1 and TET2.

Chromatine remodelation to DNA methylation

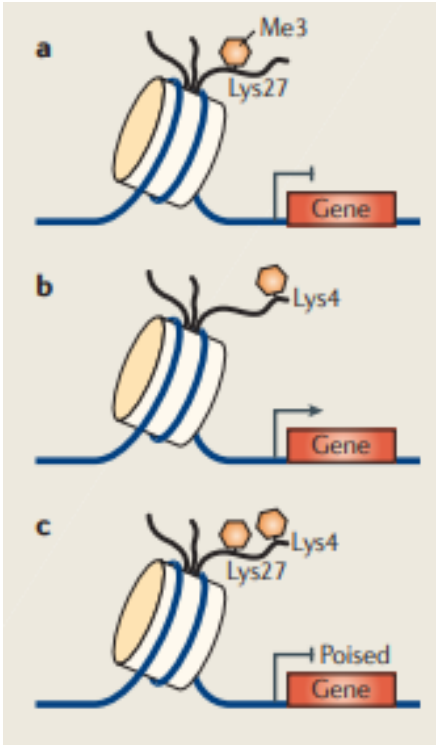
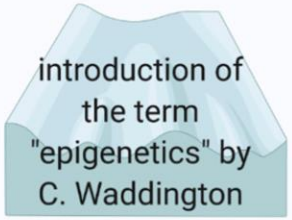


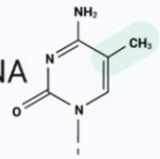
Table 1 | **Genes used to induce dedifferentiation, transdifferentiation or reprogramming**

Gene symbol*	Class	Role *in vivo*	Mouse knockout phenotype
Arf (*Cdkn2a*)	Protein kinase inhibitor	Negative regulator of proliferation	Increased tumorigenesis
Ascl1	Transcription factor	Neural lineage specification	Impaired development of various brain centres; neonatal lethality
Baf60c (*Smarcd3*)	Chromatin modulator	Neuron differentiation	Defective cardiogenesis and somitogenesis
Bcl11b	Transcription factor	Fetal thymocyte development and survival	Prenatal and perinatal lethality; haematopoietic defects
Brn2 (*Pou3f2*)	Transcription factor	Neuroectoderm specification	Perinatal lethality
Cebpa	Transcription factor	Broad target range	Neonatal lethality; multi-organ defects
Cebpb	Transcription factor	Immune and inflammatory response; brown fat specification	High neonatal hypoglycaemia and mortality
Fgf1	Growth factor	Angiogenic	Normal
Gata4	Transcription factor	Heart tube and foregut formation	Lethal; ventral defects
Klf4	Transcription factor	Differentiation of epithalial cells	Perinatal death owing to skin defects
Lin28	Transcription factor	Suppressor of microRNA biogenesis	Unknown
Mafa	Transcription factor	Activates insulin gene expression	Diabetes and pancreatic islet abnormalities
Mef2c	Transcription factor	Controls cardiac morphogenesis and myogenesis	Prenatal death and cardiovascular abnormalities
Myc	Transcription factor	Broad action on cell cycle and growth	Prenatal lethality and growth defects
Myt1l	Transcription factor	Pan-neural transcription factor with roles in neuronal differentiation	Unknown
Nanog	Transcription factor	Imposes pluripotency on embryonic stem cells and prevents their differentiation	Early embryonic death
Ngn3	Transcription factor	Neurogenesis and pancreatic endocrine cells specification	Deficiency of endocrine cells and insulin-producing cells; postnatal diabetes
p38 mapk (*Mapk14*)	Protein kinase	Inflammation and response to stress	Embryonic to perinatal lethal with multi-system defects
Pdx1	Transcription factor	Specifies early pancreatic epithelium	Postnatal lethality and abnormal pancreatic and liver development
Oct4	Transcription factor	Crucial for early embryogenesis and for embryonic stem cell pluripotency	Peri-implantation lethality; failure to develop the inner cell mass
Pu.1 (*Spi1*)	Transcription factor	Lymphoid-specific enhancer	Postnatal lethality and haematopoietic defects
Rb1	Transcription factor and chromatin modulator	Key regulator of entry into cell division	Prenatal lethality and neuronal and haematopoietic defects
Tbx5	Transcription factor	Mesoderm differentiation	Prenatal lethality and cardiovascular defects

Milestones in epigenetic aging research



1942 introduction of the term "epigenetics" by C. Waddington



1947 discovery of methylated cytosine in DNA

1948 caloric restriction extends life span of mice

1964 discovery of role of histone modifications for transcription

1993 single-gene mutation doubles life in C. elegans

2004 2006 unified theory of caloric restriction and longevity by D. Sinclair

2005 FDA approval of first epigenetic drug

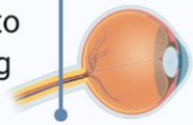
discovery of Yamanaka factors for iPSC generation

2013 DNA methylation clock by Horvath

2016 partial in vivo reprogramming to ameliorate aging

2019 single-cell multiomics of aging cells

2020 DNA methylation clock by deep learning



2021 partial reprogramming reverses vision loss in aged mice

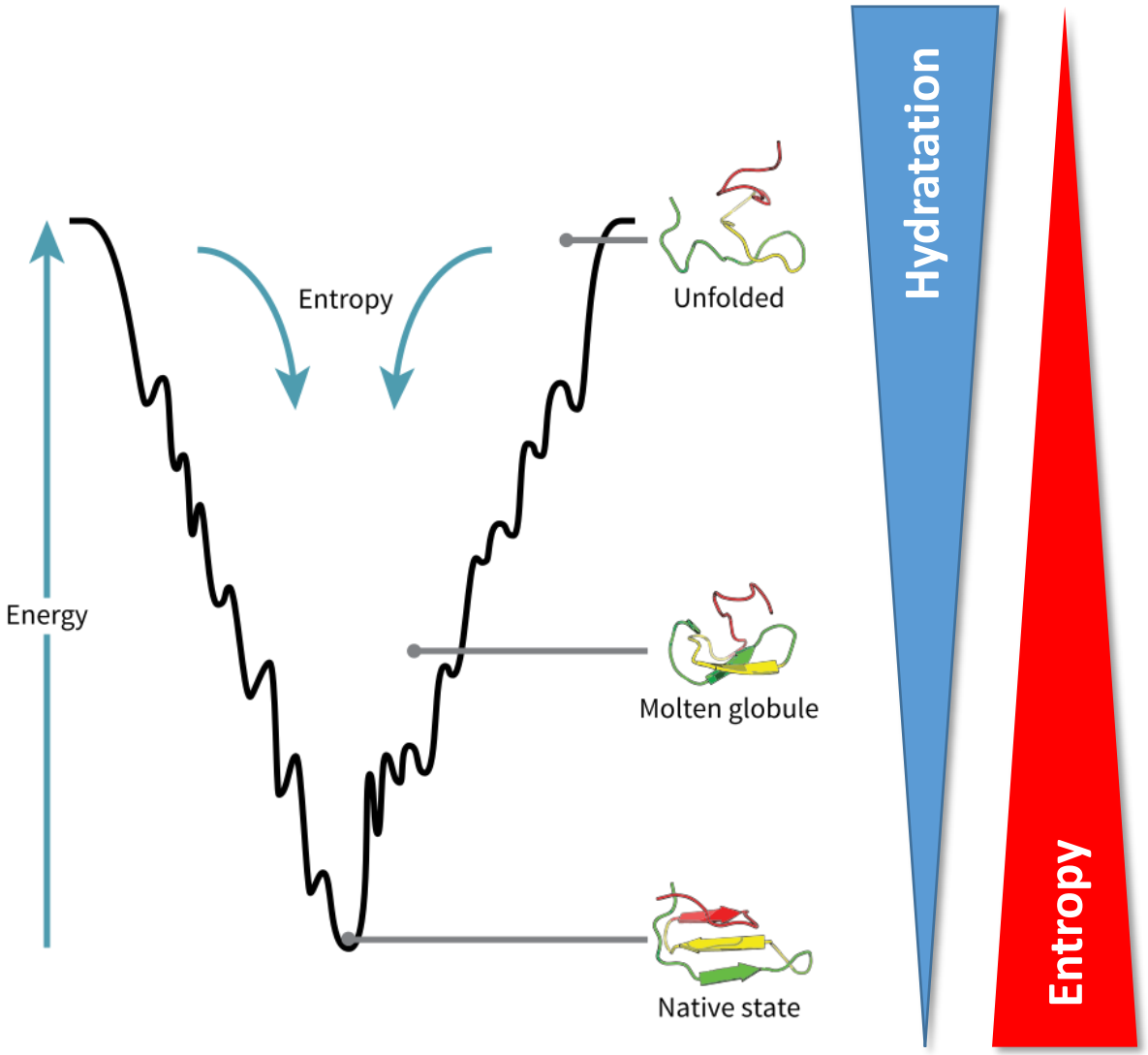
NATURE REVIEWS | GENETICS

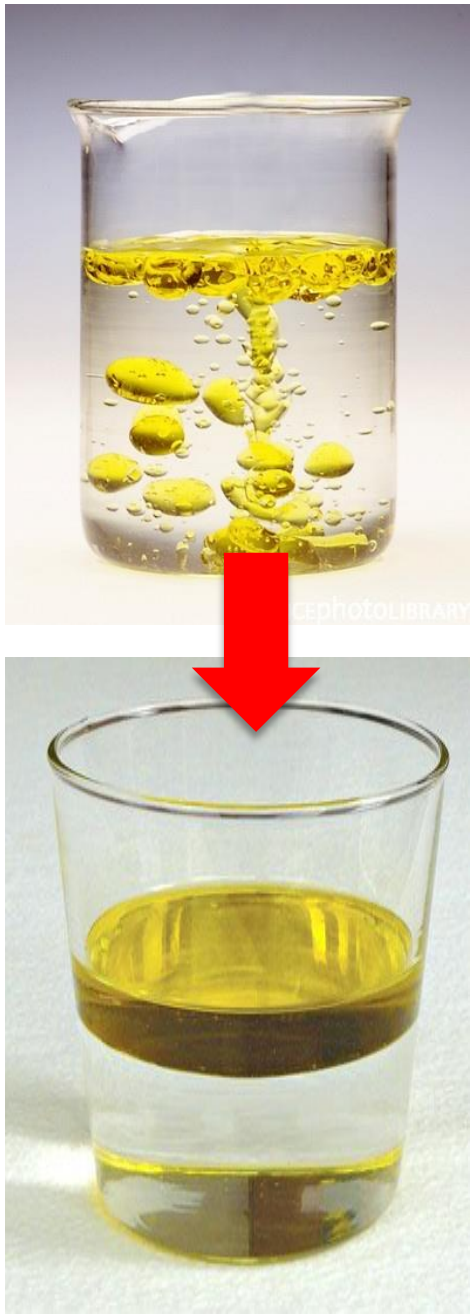
The genetics of human ageing

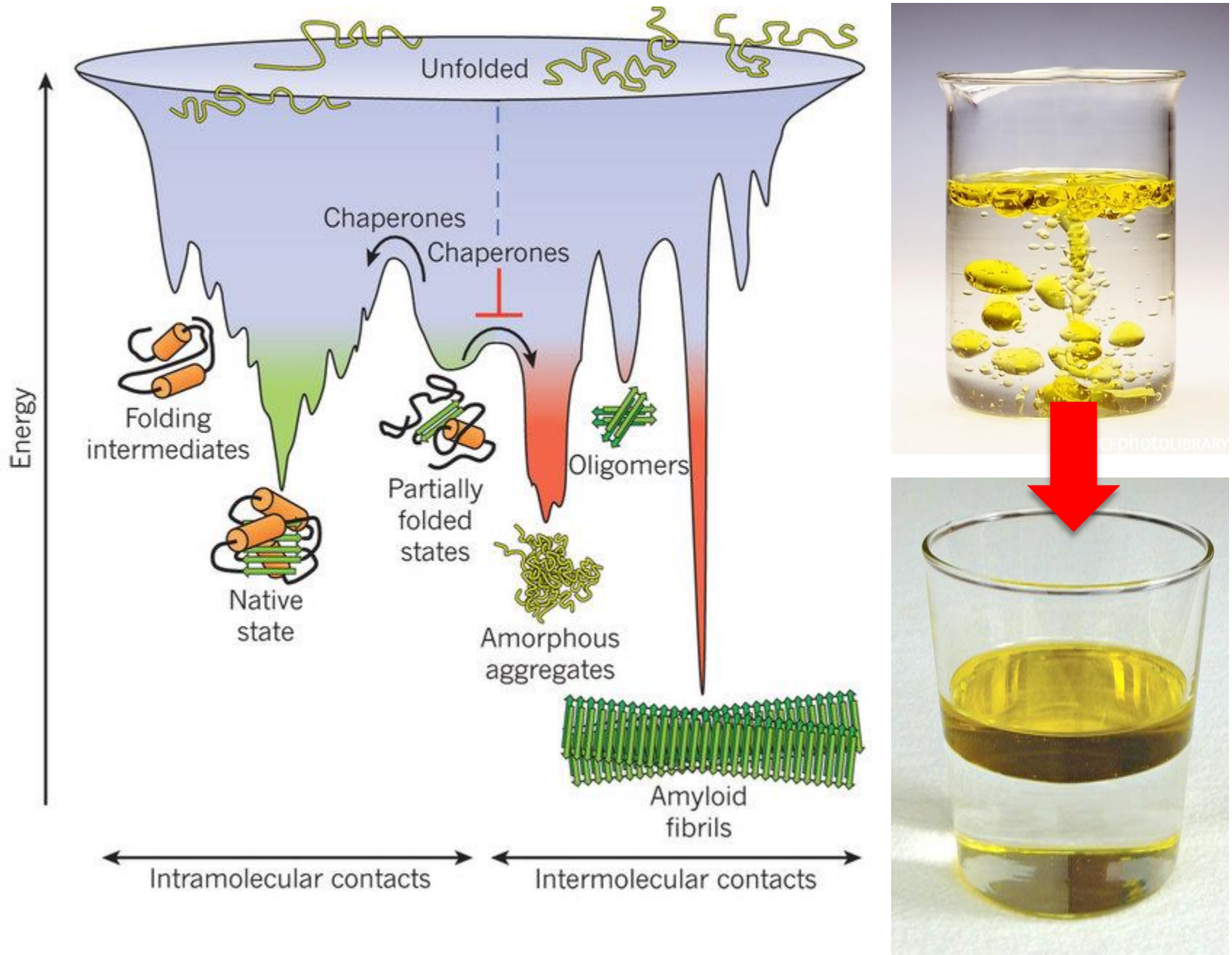
David Melzer[1,2], Luke C. Pilling[1,2] and Luigi Ferrucci[3]*

rsID (effect allele)	Effect[a]	Mapped genes	Gene name	Variant position	Associated disease
Loci significant in both[b] GWAS meta-analyses[31,32]					
rs429358 (T)	1.06	*APOE*	Apolipoprotein E	Missense	Cardiometabolic, dementia
rs10455872 (A)	0.76	*LPA*	Lipoprotein A	Intronic	Cardiometabolic
rs8042849 (T)[c]	0.44	*CHRNA3/5*	Cholinergic receptor nicotinic α3/5 subunit	Intronic	Smoking related
rs142158911 (A)	0.36	*LDLR*	Low-density lipoprotein receptor	Intergenic	Cardiometabolic
rs11065979 (C)[d]	0.28	*SH2B3, ATXN2*	SH2B adaptor protein 3, ataxin 2	Intergenic	Cardiometabolic, cancer, autoimmunity[e]
rs1556516 (G)	0.25	*CDKN2B-AS1*	CDKN2B antisense RNA 1	Intronic	Cardiometabolic, cancer[e]
Loci significant only in the UK Biobank and LifeGen cohorts[31]					
rs34967069 (T)	0.56	*HLA-DQA1*	Major histocompatibility complex, class II, DQ alpha 1	Intergenic	Autoimmune
rs1230666 (G)	0.32	*MAGI3*	Membrane associated guanylate kinase, WW and PDZ domain containing 3	Intronic	Autoimmune
rs12924886 (A)	0.28	*HP*	Haptoglobin	Intergenic	Cardiometabolic
rs1275922 (G)	0.26	*KCNK3*	Potassium two pore domain channel subfamily K member 3	Intronic	Cardiometabolic
rs6224 (G)[f]	0.25	*FURIN/FES*	Furin, paired basic amino acid cleaving enzyme	Intronic	Cardiometabolic
rs61348208 (T)	0.23	*HTT*	Huntingtin	Intronic	NR
Loci significant only in the UK Biobank and AncestryDNA cohorts[32]					
rs7844965 (G)[g]	0.25	*EPHX2*	Epoxide hydrolase 2	intronic	NR
rs4774495 (G)[g]	0.23	*SEMA6D*	Semaphorin 6D	intronic	NR
rs599839 (G)[g]	0.21	*CELSR2, PSRC1*	Cadherin EGF LAG seven-pass G-type receptor 2, proline and serine rich coiled-coil 1	intergenic	Cardiometabolic
rs3131621 (G)[g]	0.20	*MICA/B*	MHC class I polypeptide-related sequence A/B	intergenic	NR
rs15285 (G)[g]	0.18	*LPL*	Lipoprotein lipase	3′ UTR	Cardiometabolic
rs9872864 (G)[h]	0.14	*IP6K1*	Inositol hexakisphosphate kinase 1	intronic	NR

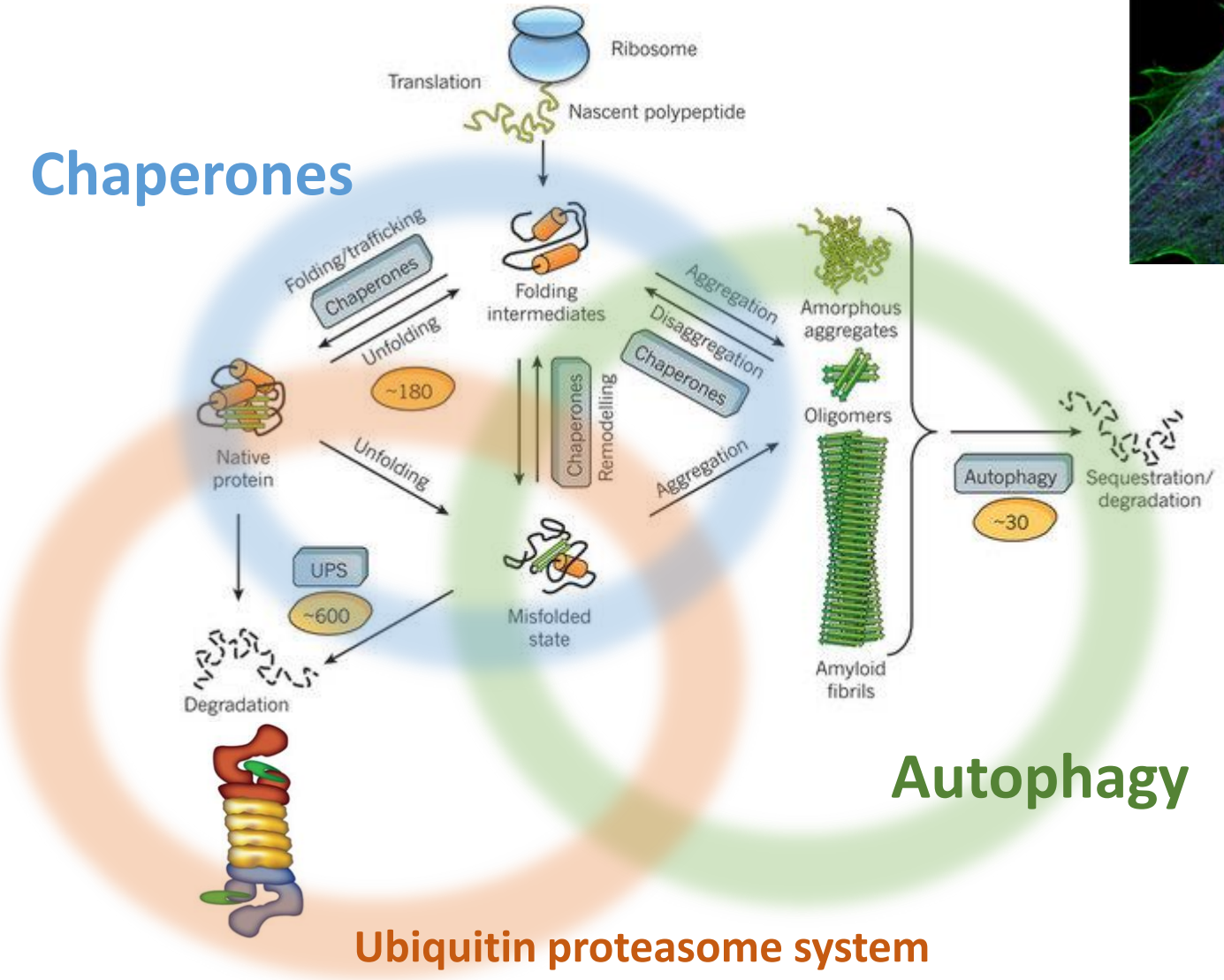
Folding is entropy driven process






Unfolded
Chaperones
Chaperones
Folding intermediates
Native state
Partially folded states
Oligomers
Amorphous aggregates
Amyloid fibrils
Energy
Intramolecular contacts
Intermolecular contacts

Protein homeostasis / proteostasis



Chaperones

Autophagy

Ubiquitin proteasome system

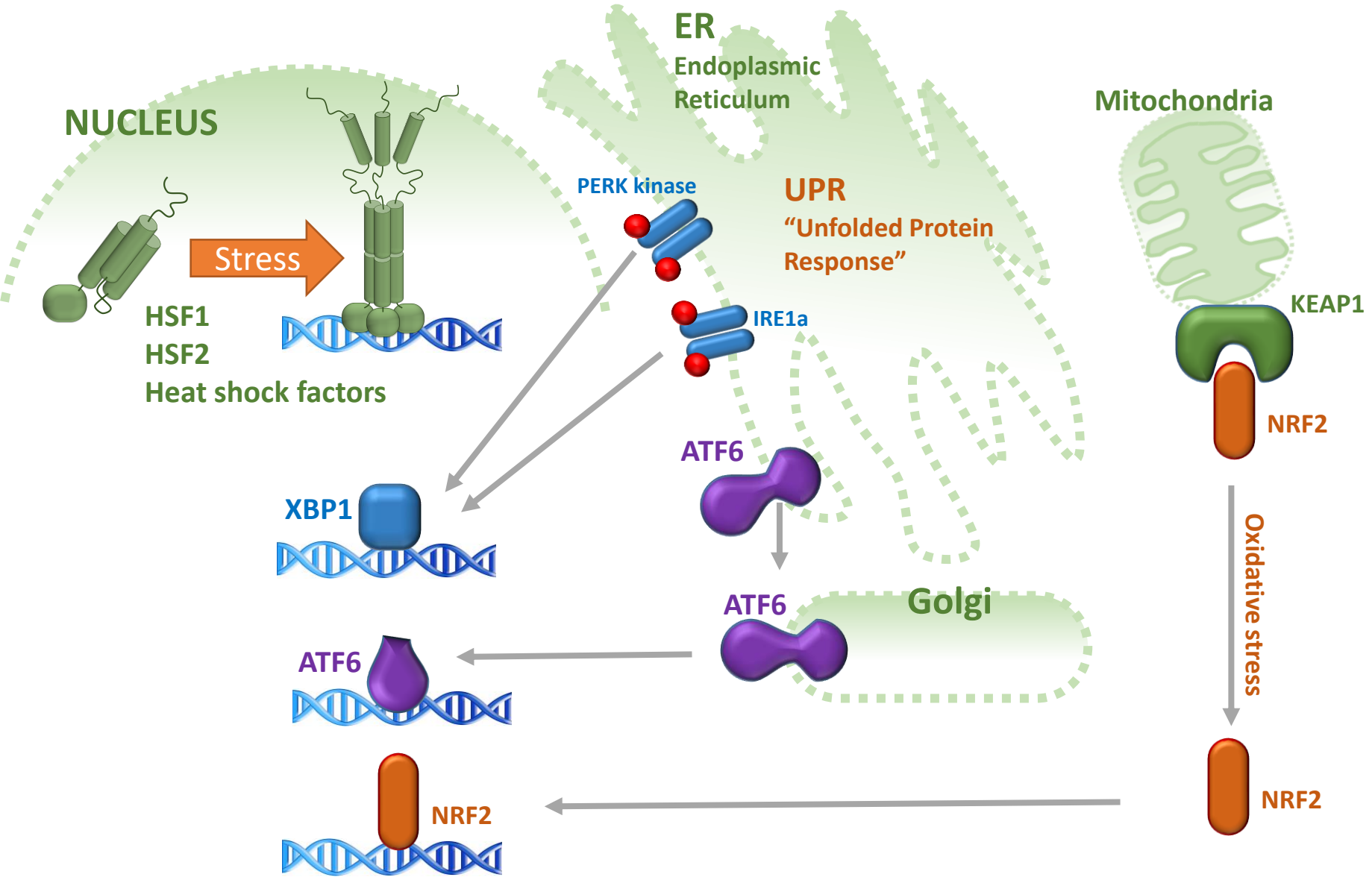
Sensors of proteotoxic stress

Metabolic stress

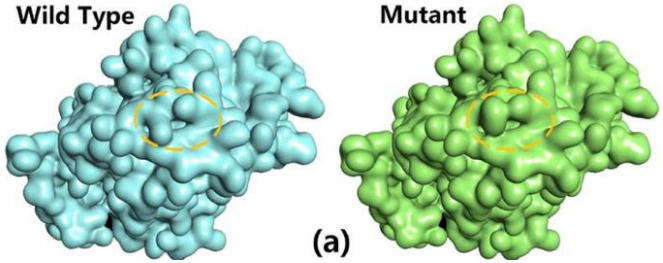
Increased temperature

Mutations and genomic instability

Oxidative stress

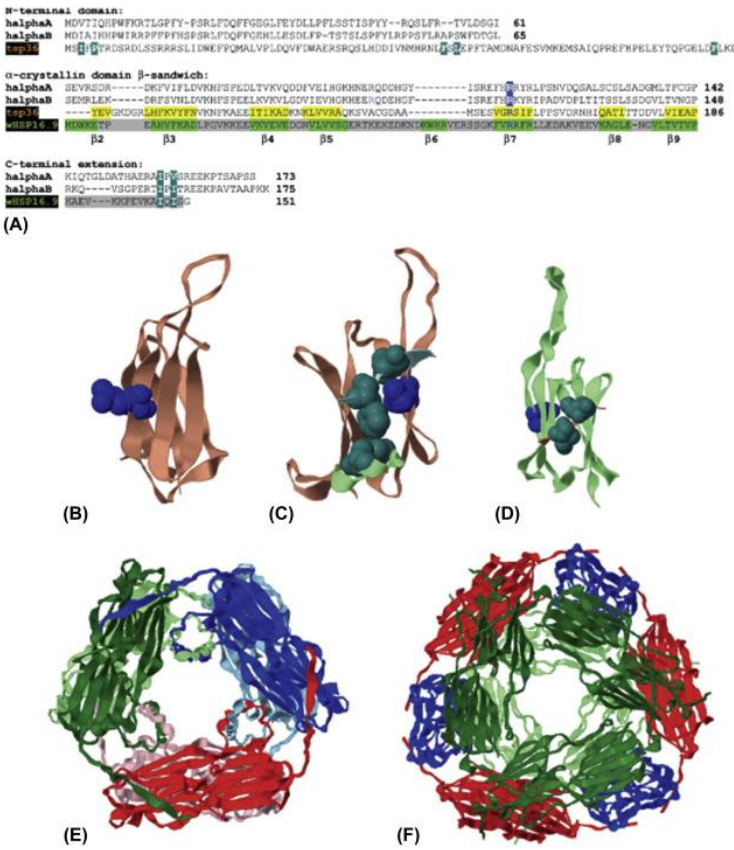


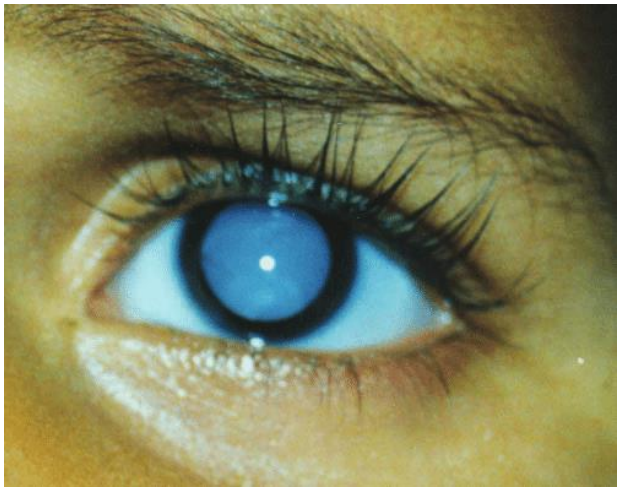
HSF4



Mutation in HSF4 leads to decreased expression of crystalline genes in the lens, resulting in congenital cataracts

Crystalline alpha/beta (CRYAB, CRYAA)

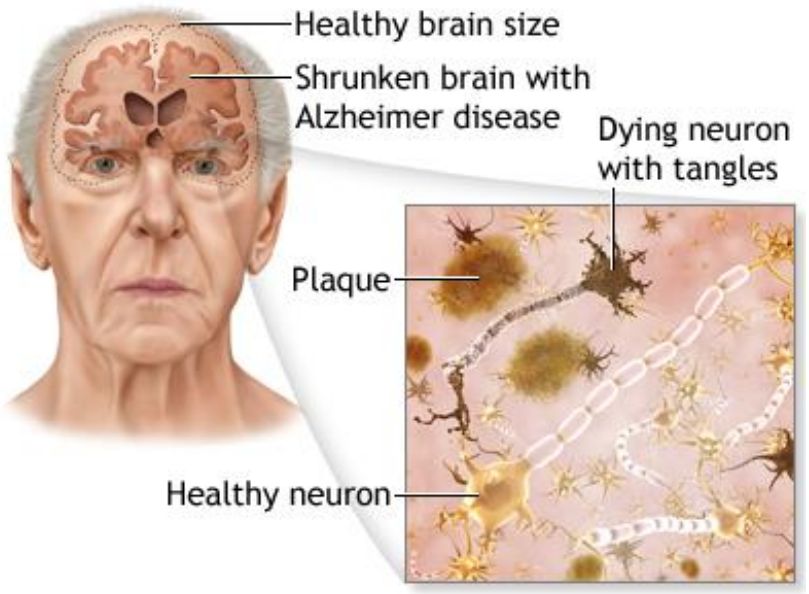


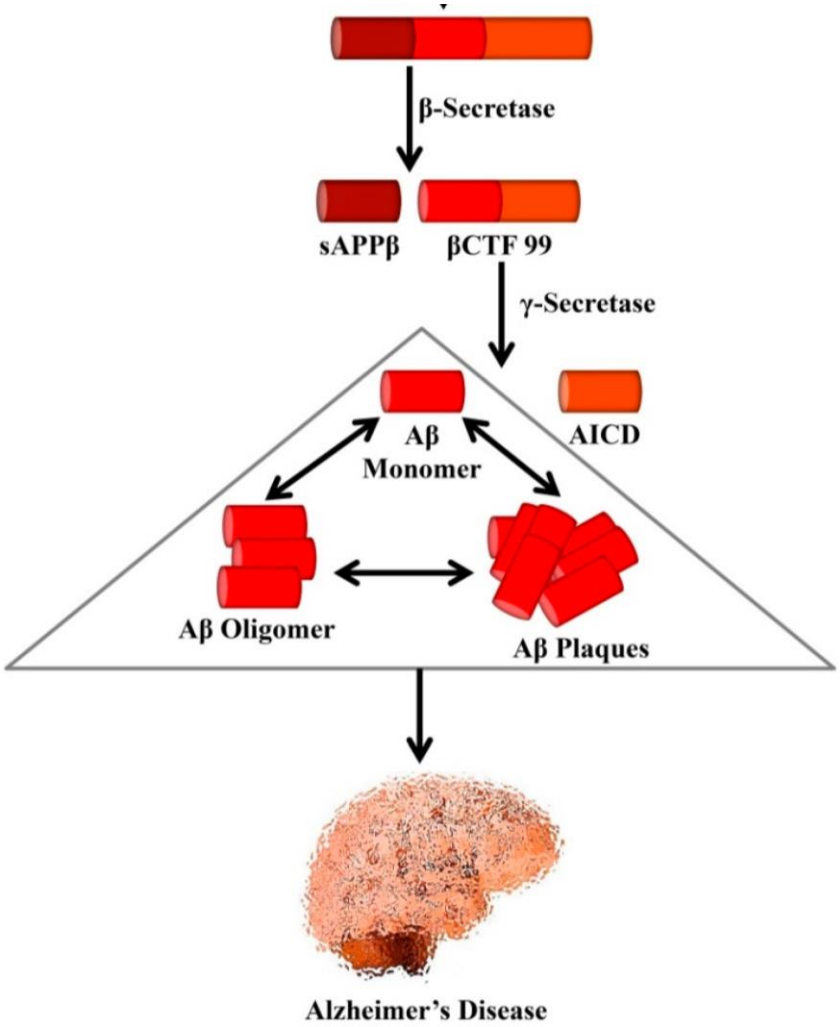


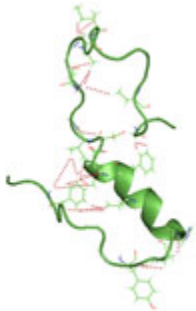
A Homozygous Splice Mutation in the HSF4 Gene Is Associated with an Autosomal Recessive Congenital Cataract

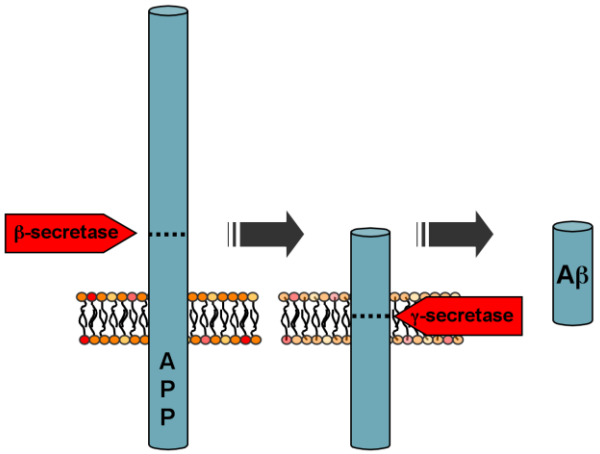
Congenital Cataract in Australian Shepard

Alzheimer's disease.

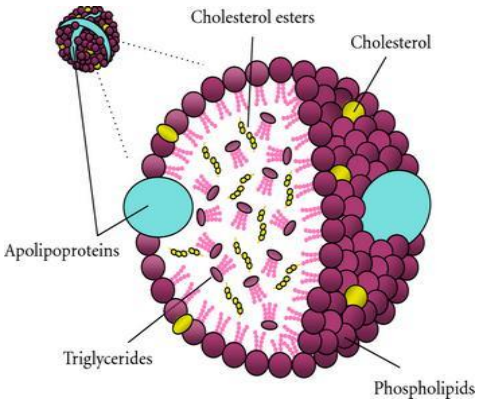


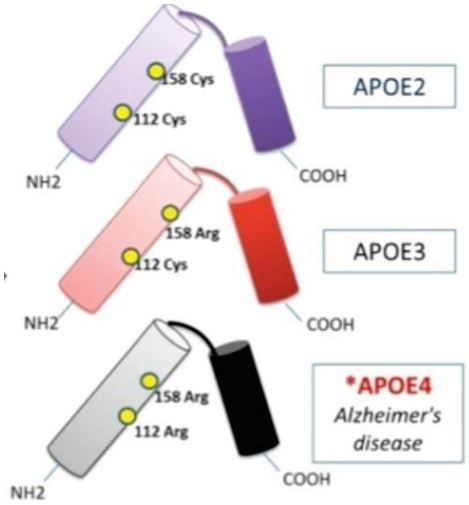


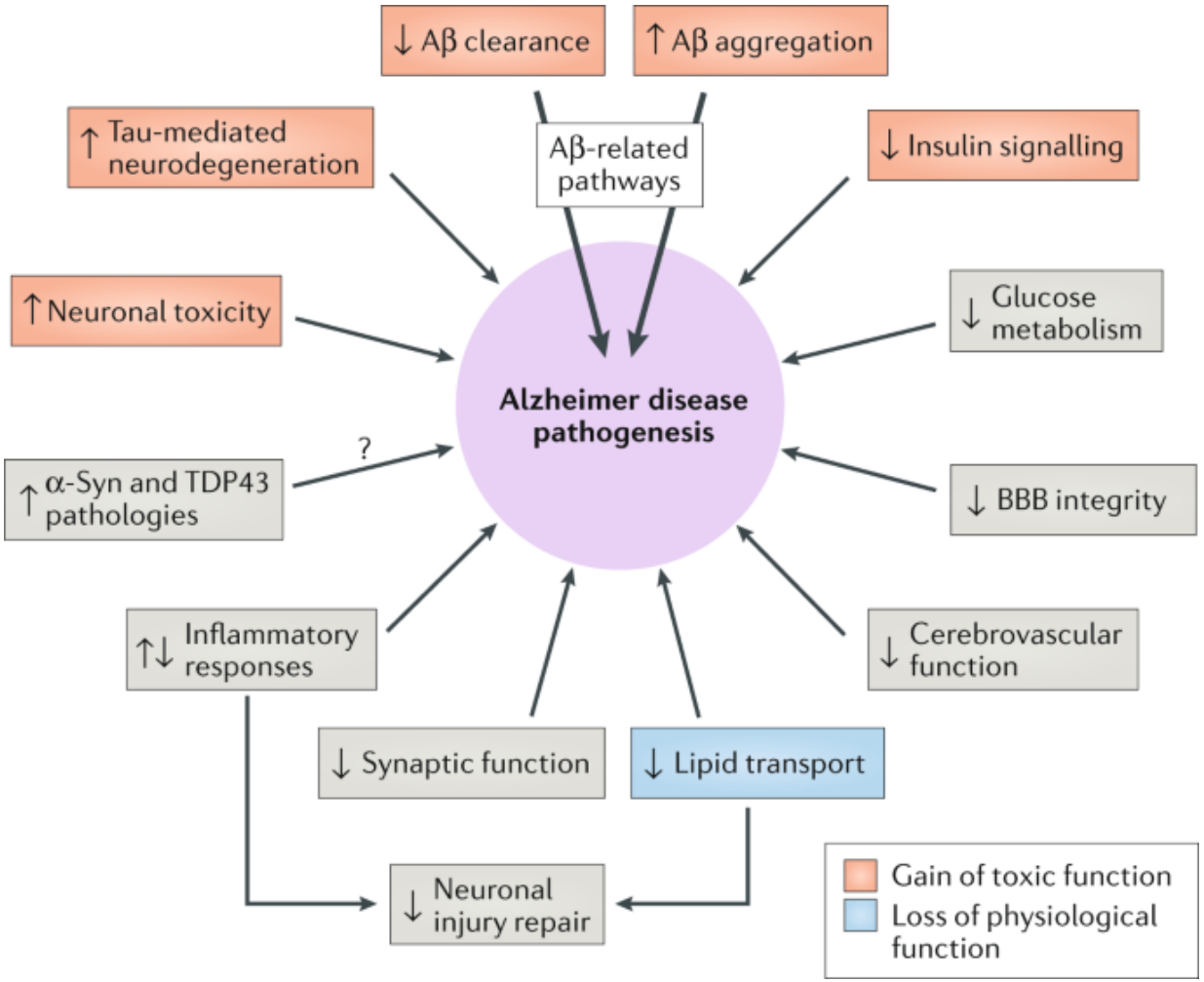




APOE4 is the strongest risk factor gene for Alzheimer's disease

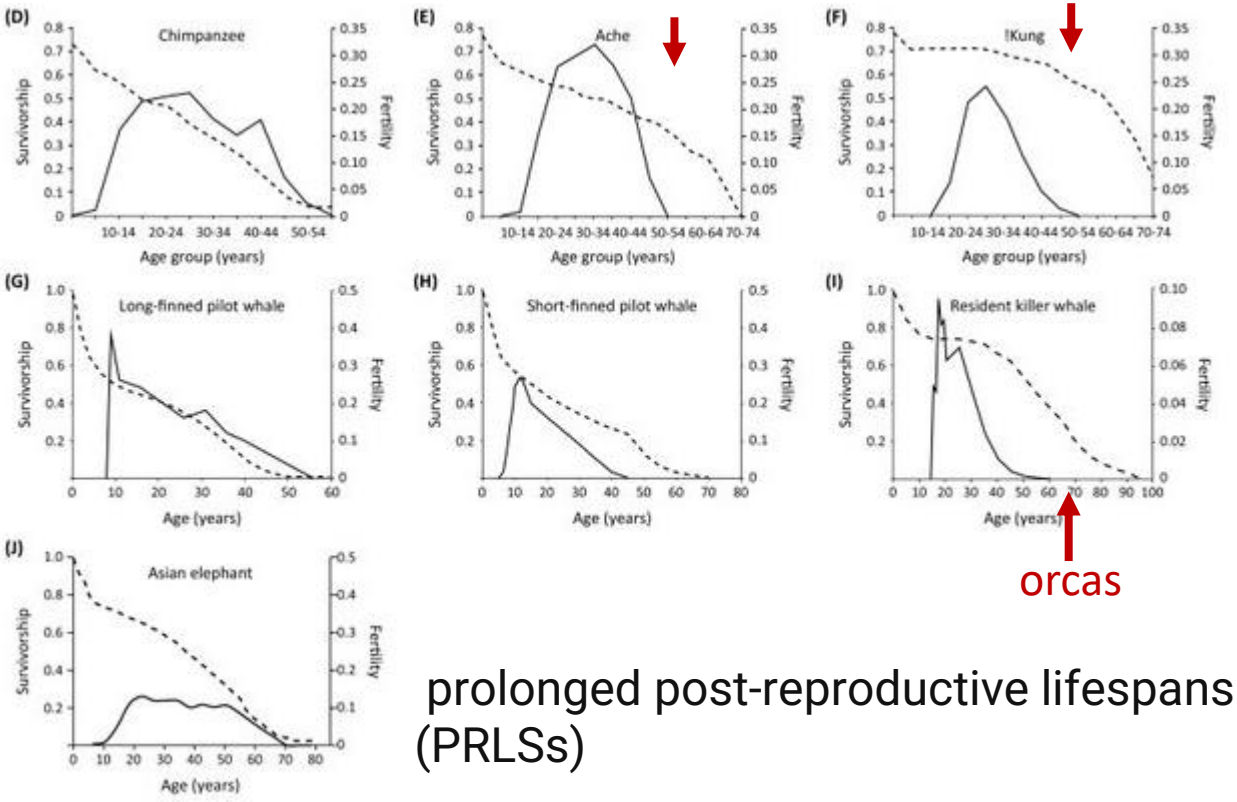






The evolution of prolonged life after reproduction

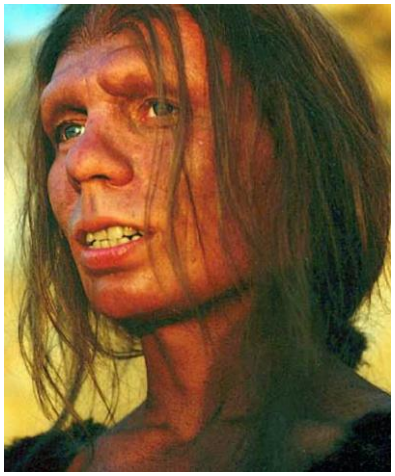
primitive indigenous people

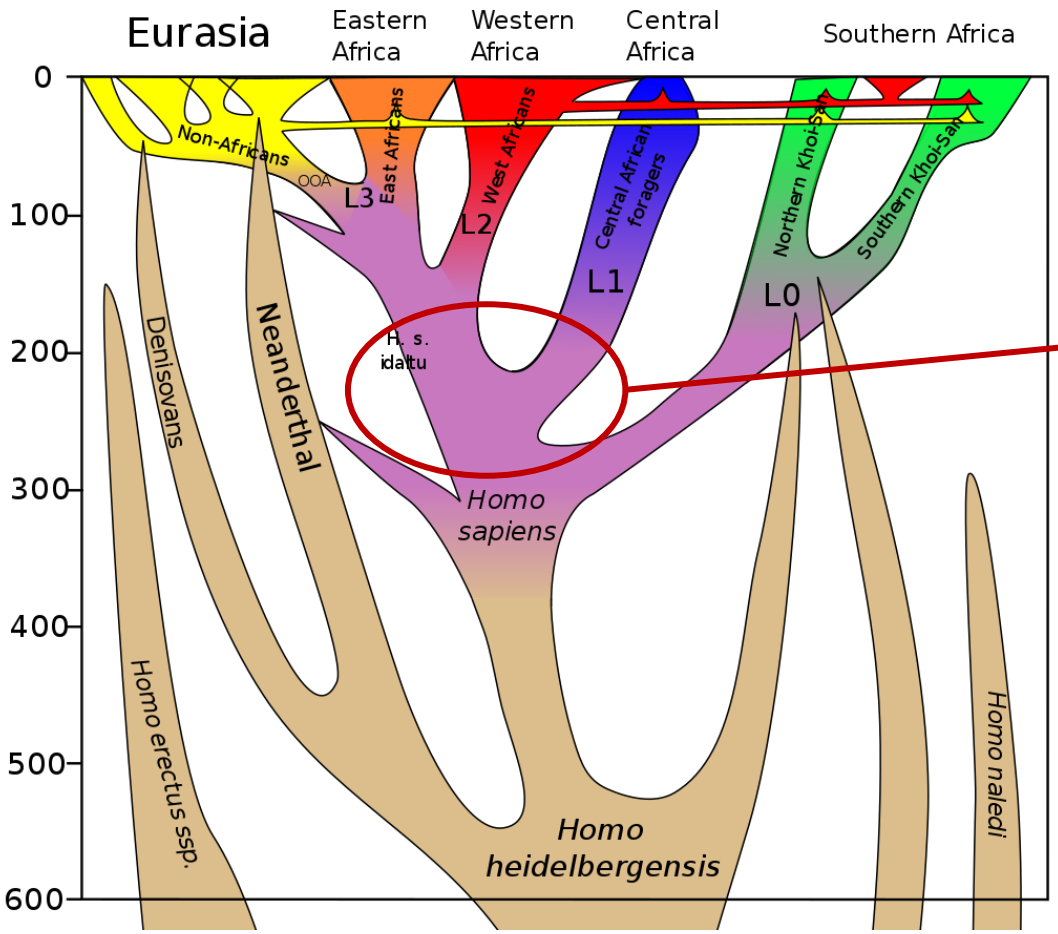


orcas

prolonged post-reproductive lifespans (PRLSs)

Cooperation and cultural evolution allowed the expansion of Homo sapiens species

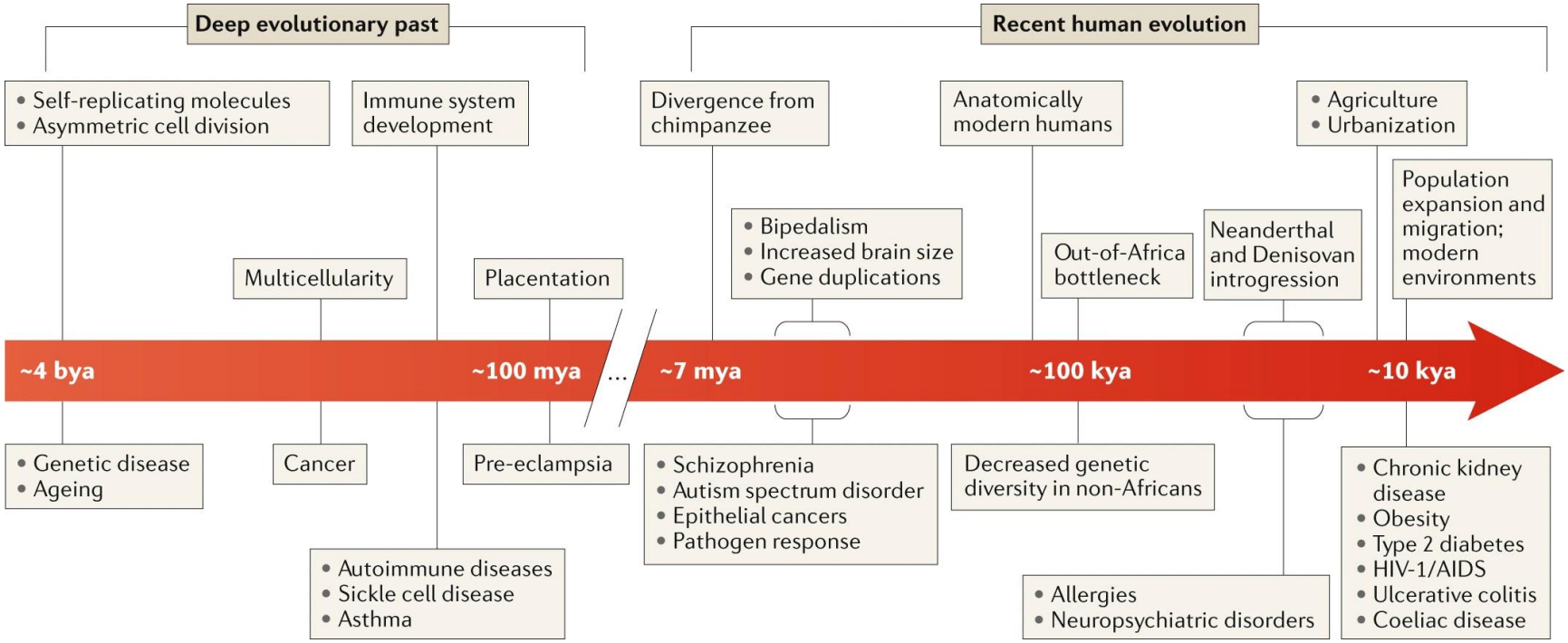




Higher genetic diversity
cohabitation of non-relatives
cooperation

A model of the phylogeny of *H. sapiens* over the last 600,000 years (vertical axis).

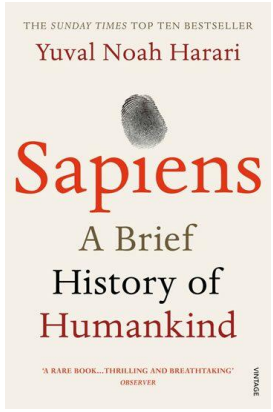
A timeline of evolutionary events →



A timeline patterns of human disease risk →

Cultural evolution

is the idea that human cultural change—that is, changes in socially transmitted beliefs, knowledge, customs, skills, attitudes, languages, and so on—can be described as a Darwinian evolutionary process



Slaves to wheat: How a grain domesticated us

Unlike animals, the survival of humans is currently much less determined by their genetic information.

Much more important to human evolutionary fitness has become information obtained non-genetically

Neolithic revolution, cooperation and cultural evolution

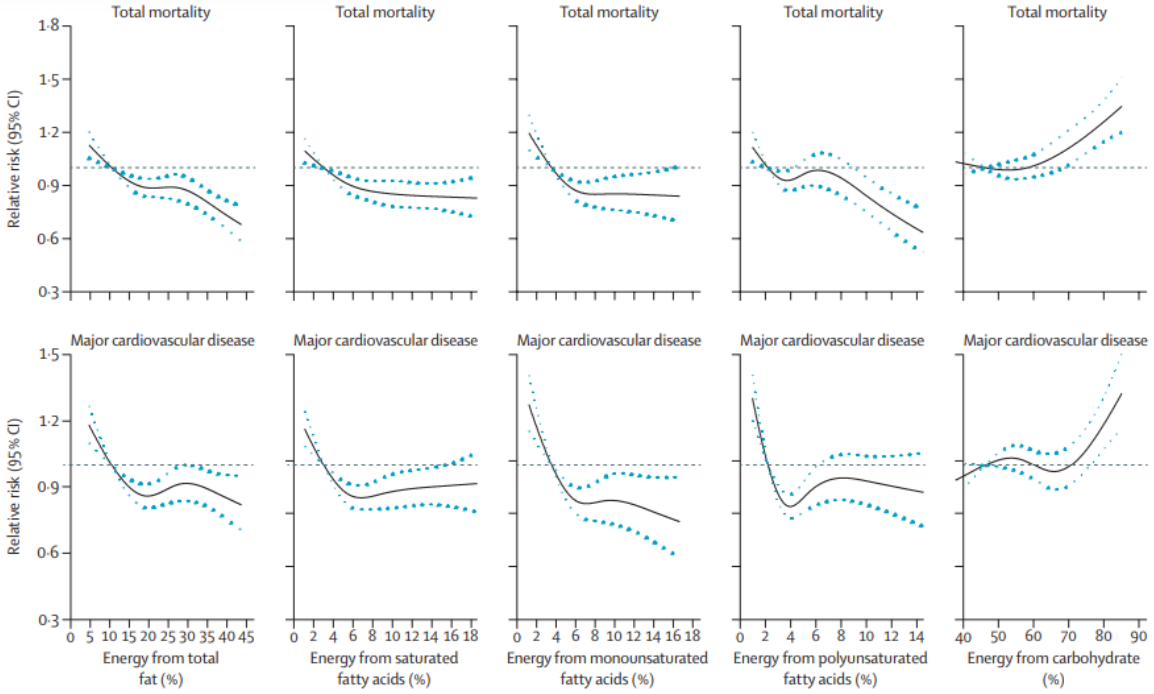
THE LANCET

Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis

Sara B Seidelmann, Brian Claggett, Susan Cheng, Mir Henglin, Amil Shah, Lyn M Steffen, Aaron R Folsom, Eric B Rimm, Walter C Willett, Scott D Solomon

Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study

Mahshid Dehghan, Andrew Mente, Xiaohe Zhang, Sumathi Swaminathan, Wei Li, Viswanathan Mohan, Romaina Iqbal, Rajesh Kumar,

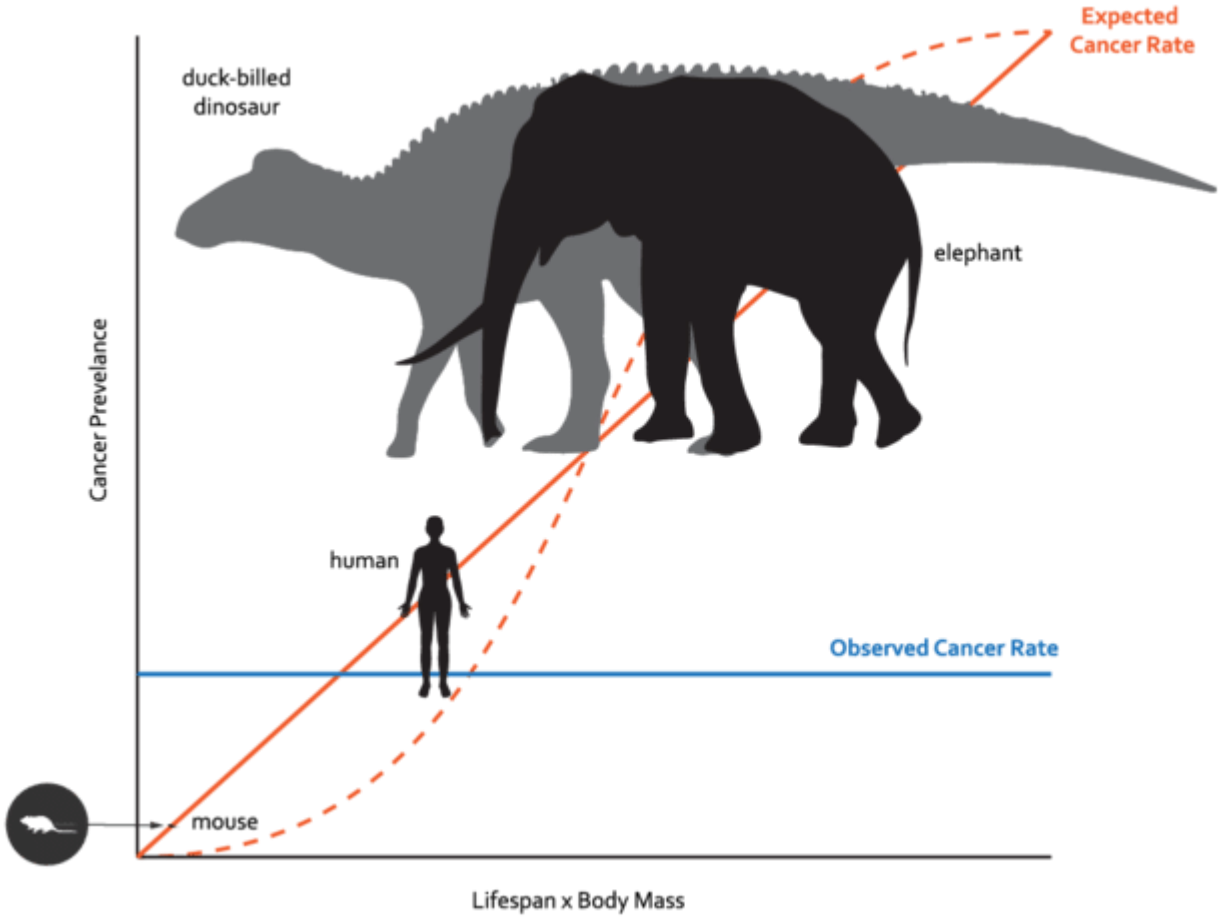


Mechanisms of evolutionary adaptations in different animal species
The traits related to common human diseases

- Cancer
- Ageing
- Pathogen/infection resistance

Cancer and Peto's paradox

- the incidence of cancer does not appear to correlate with the number of cells in an organism
- In order to build larger and longer-lived bodies, organisms required greater cancer suppression.



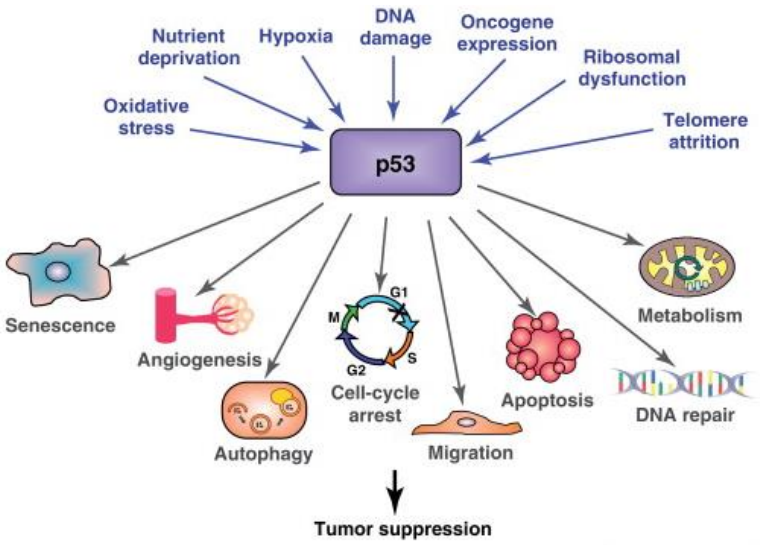
Evolutionary „trade off“:

Body size vs. risk of cancer

Gene Quantity in Cancer

HUMANS vs. ELEPHANTS

71 years	*average lifespan*	65 years
62 kg	*weight*	4800 kg
37.2 trillion	*number of cells*	3.72 quadrillion
11–25%	*cancer mortality*	4.81%
2	*copies of p53*	40



Mice altered to express "always-on" active TP53 exhibited increased tumor suppression ability, but also showed signs of premature aging. (TP53 cannot be the only explanation)

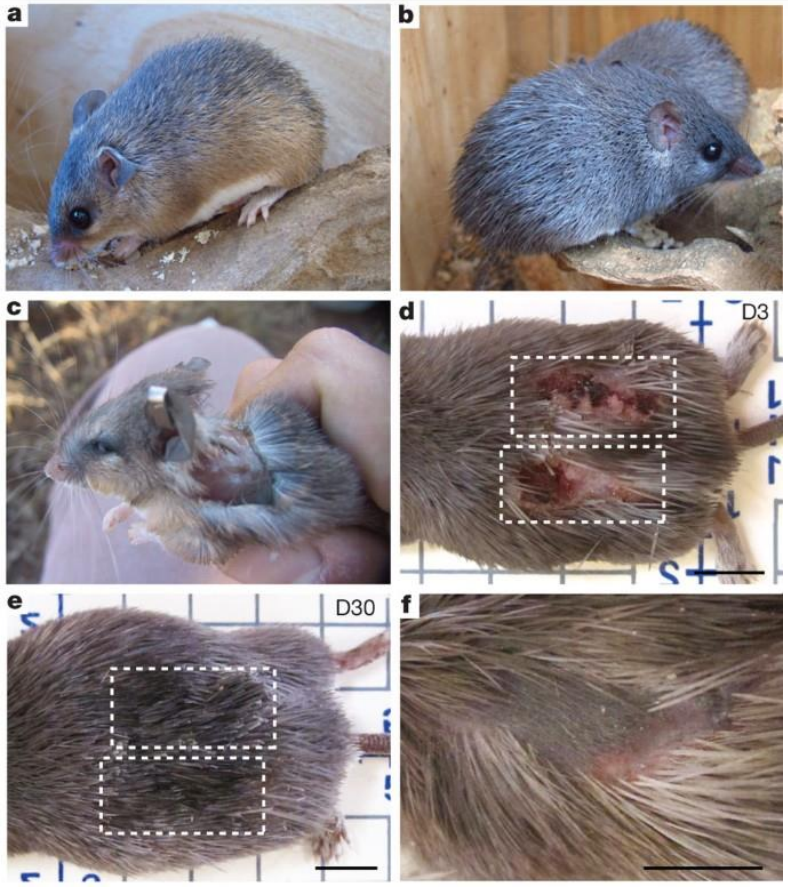
Available online at www.sciencedirect.com

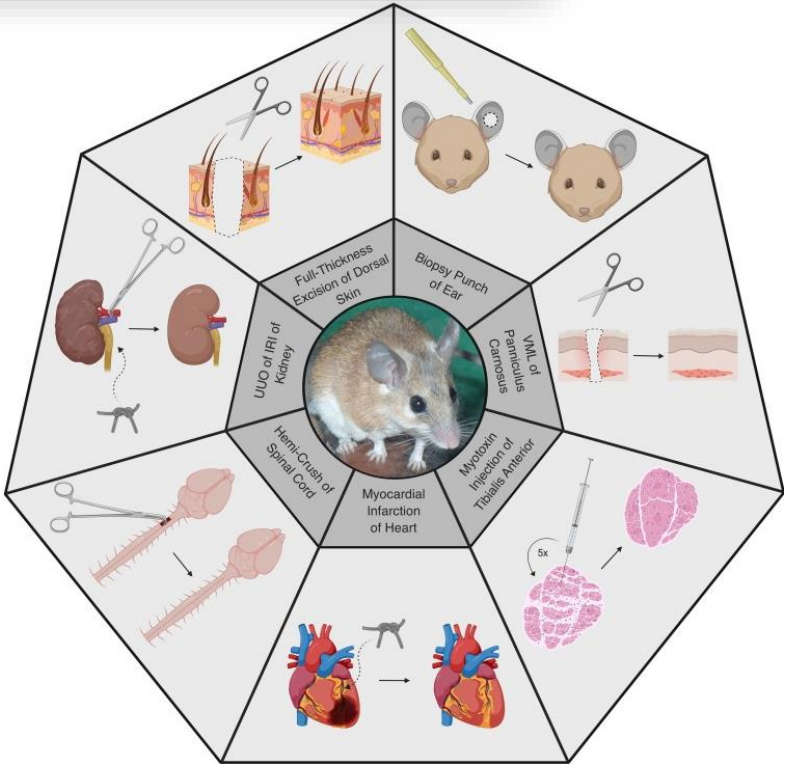
ScienceDirect

Current Opinion in Genetics & Development

Regeneration in the spiny mouse, *Acomys*, a new mammalian model

Aaron Gabriel W Sandoval and Malcolm Maden



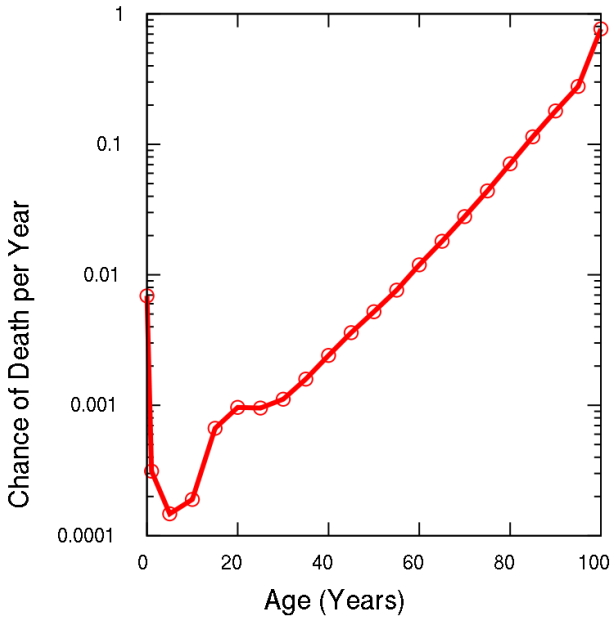


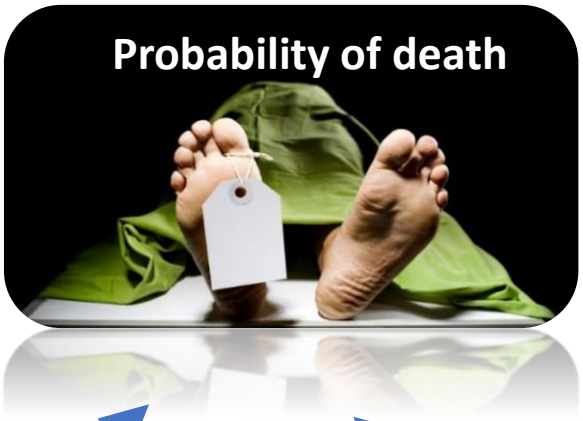
- Scar-less regeneration
- Role of macrophage M1<M2
- Prevent fibrosis

NATURE : 26 September 2012
Skin shedding and tissue regeneration in African spiny mice (Acomys)

Gompertz–Makeham law of mortality

Estimated probability of a person dying at each age, for the U.S. in 2003. Mortality rates increase exponentially with age after age 30.





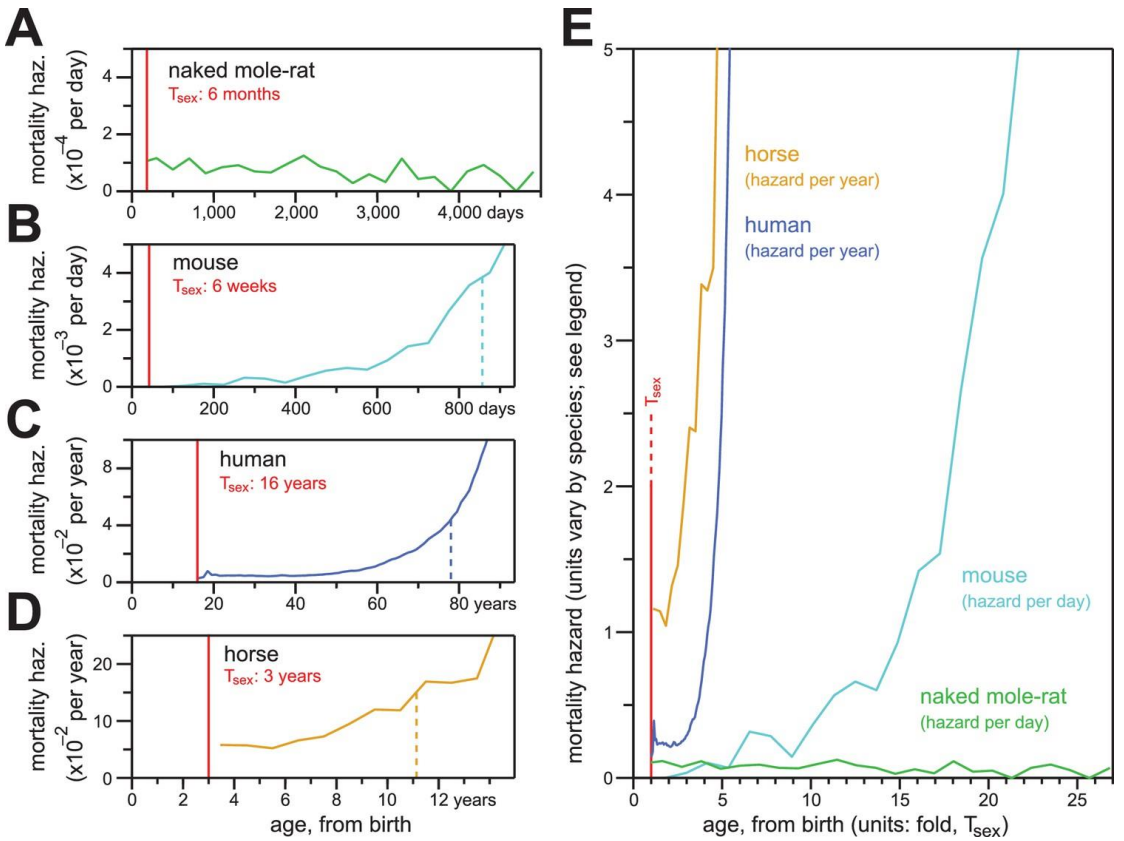
age-independent component

The Gompertz–Makeham law states that the human death rate is the sum of an **age-dependent component** (the Gompertz function, named after Benjamin Gompertz), which increases exponentially with age and an **age-independent component** (the Makeham term, named after William Makeham).

Naked mole rats defy the biological law of aging (Heterocephalus glaber)

- rarely get cancer
- resistant to some types of pain
- survive up to 18 minutes without oxygen.

In contrast to the mortality hazards of other mammals, which increased with chronological age, the mortality hazard of naked mole-rats remained constant.



Balance of protein production and its regulation

Injury

Infection

Interspecies and intraspecies competition

Lack of food

Growth factor

mTOR signalling

AMPK activation

Glucocorticoid signalling

Starvation Autophagy

Make more protein

Protein synthesis inhibition

Protein aggregation

Fitness

Adaptive immunity

Cancer

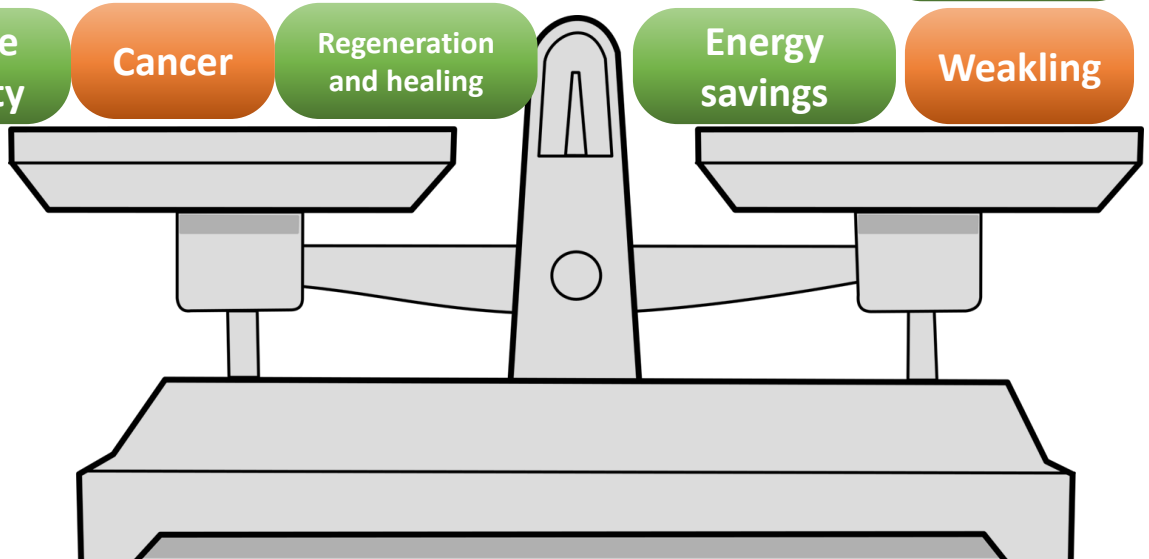
Regeneration and healing

Immunocompromised

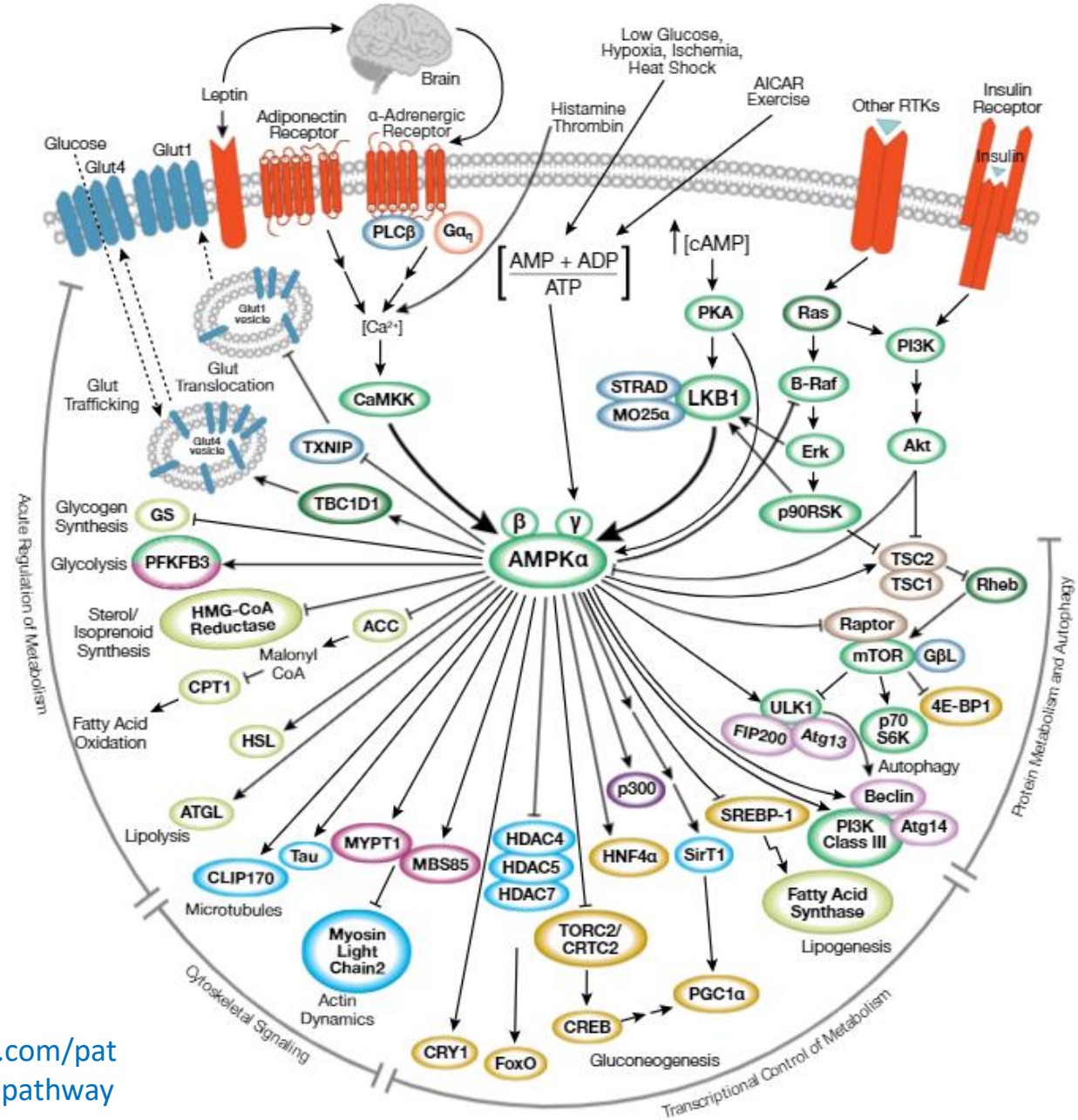
Longevity

Energy savings

Weakling



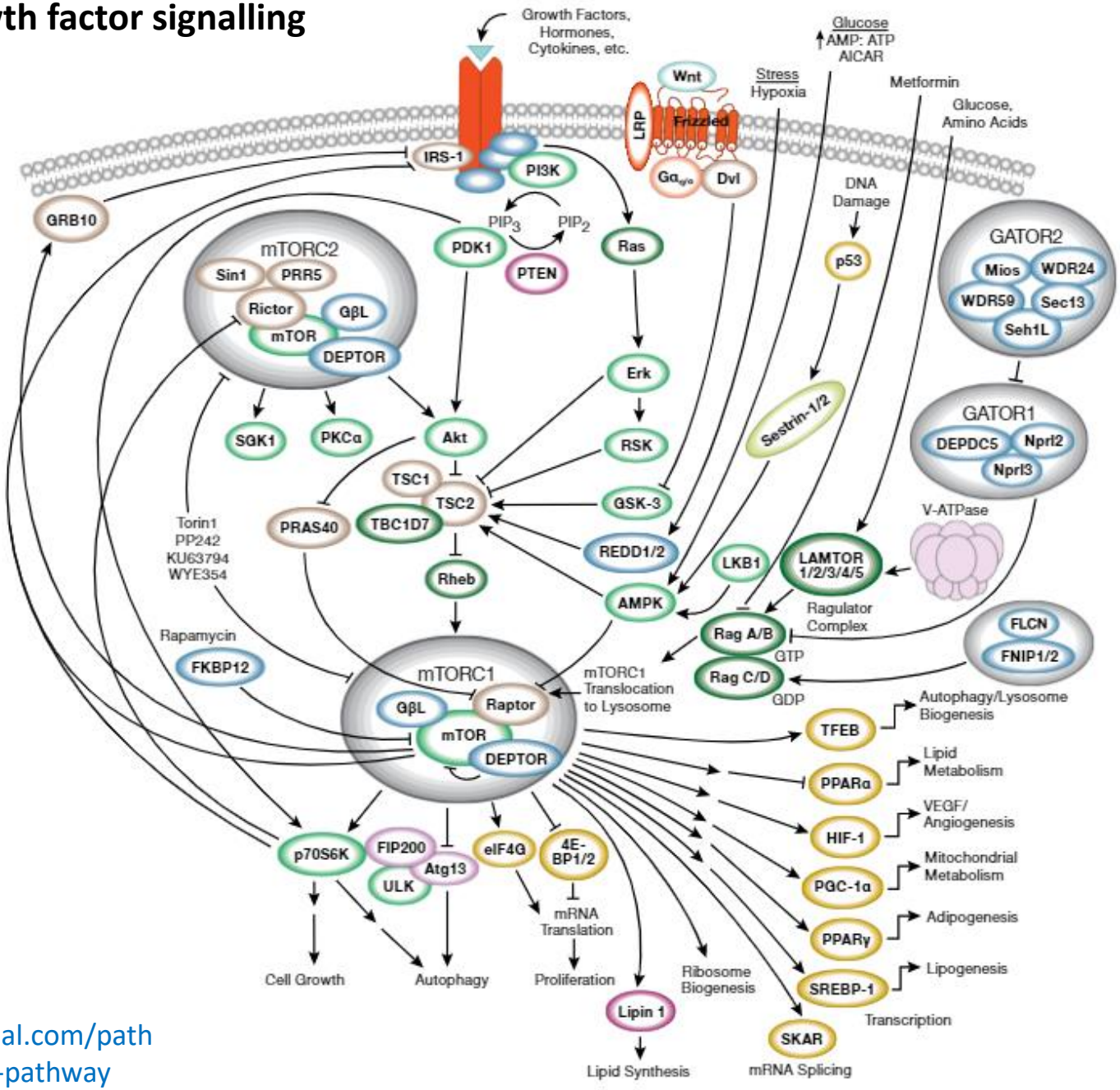
AMPK signalling



Autophagy

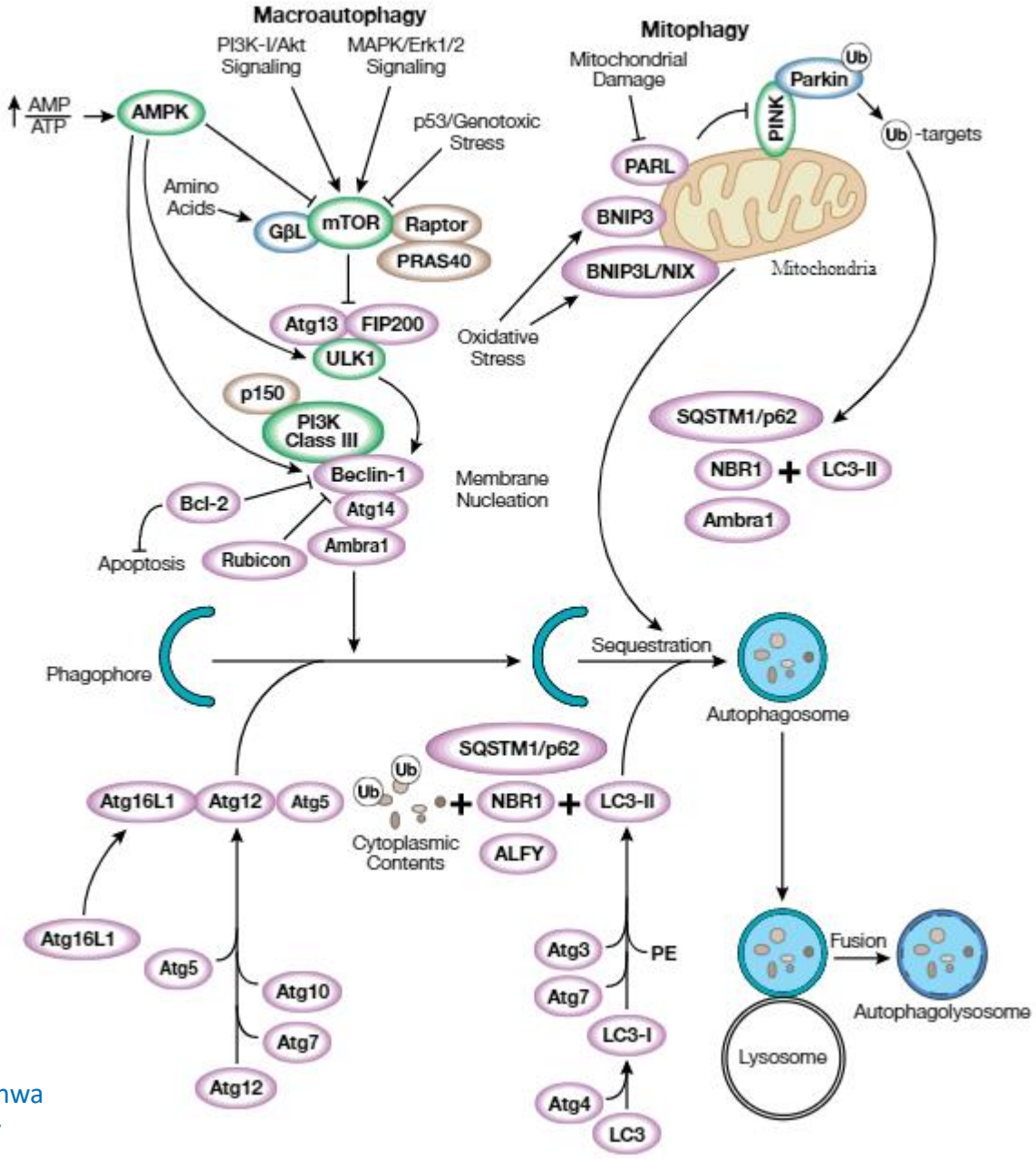
https://www.cellsignal.com/pathways/ampk-signaling-pathway

mTOR and growth factor signalling



https://www.cellsignal.com/pathways/mtor-signaling-pathway

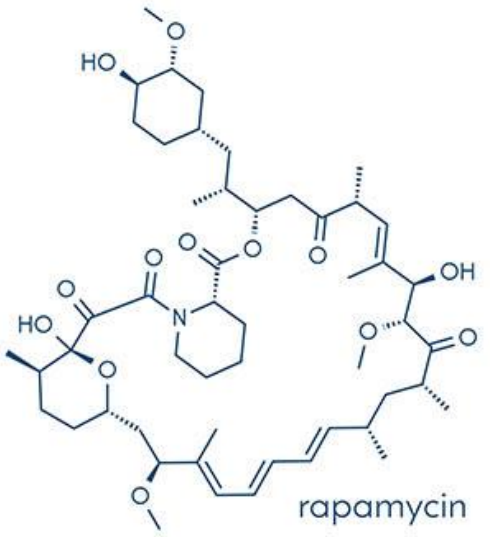
Autophagy

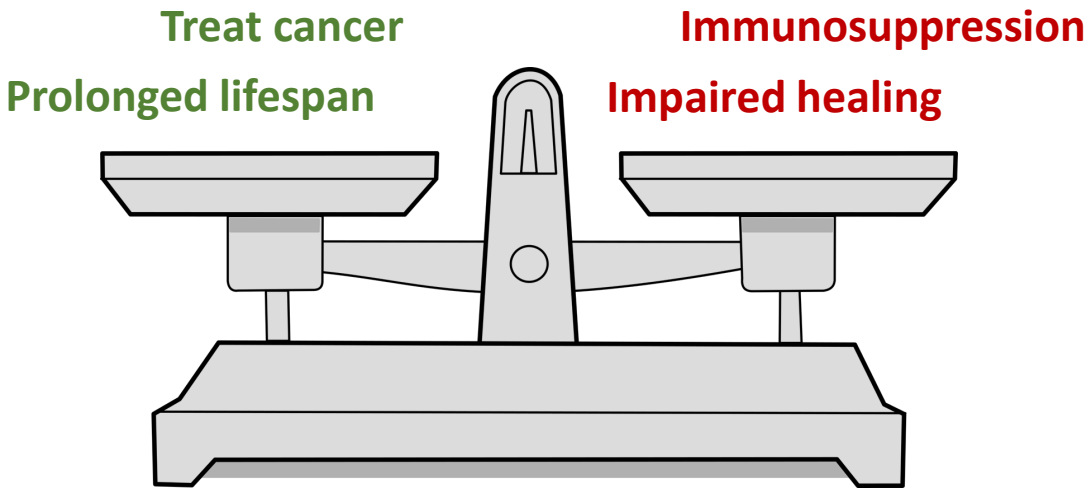


https://www.cellsignal.com/pathways/autophagy-signaling-pathway

How can we affect protein homeostasis ?

- Georges Nógrády
- The Ayerst Pharmaceuticals team was able to identify a new antifungal compound in the soil samples that was produced by the bacterium Streptomyces hygroscopicus
- **Identification of the mTOR Signaling Network**
- Rapamycin's eventual development into a clinical compound (Rapamune), used to prevent organ transplant rejection and treatment for some cancers



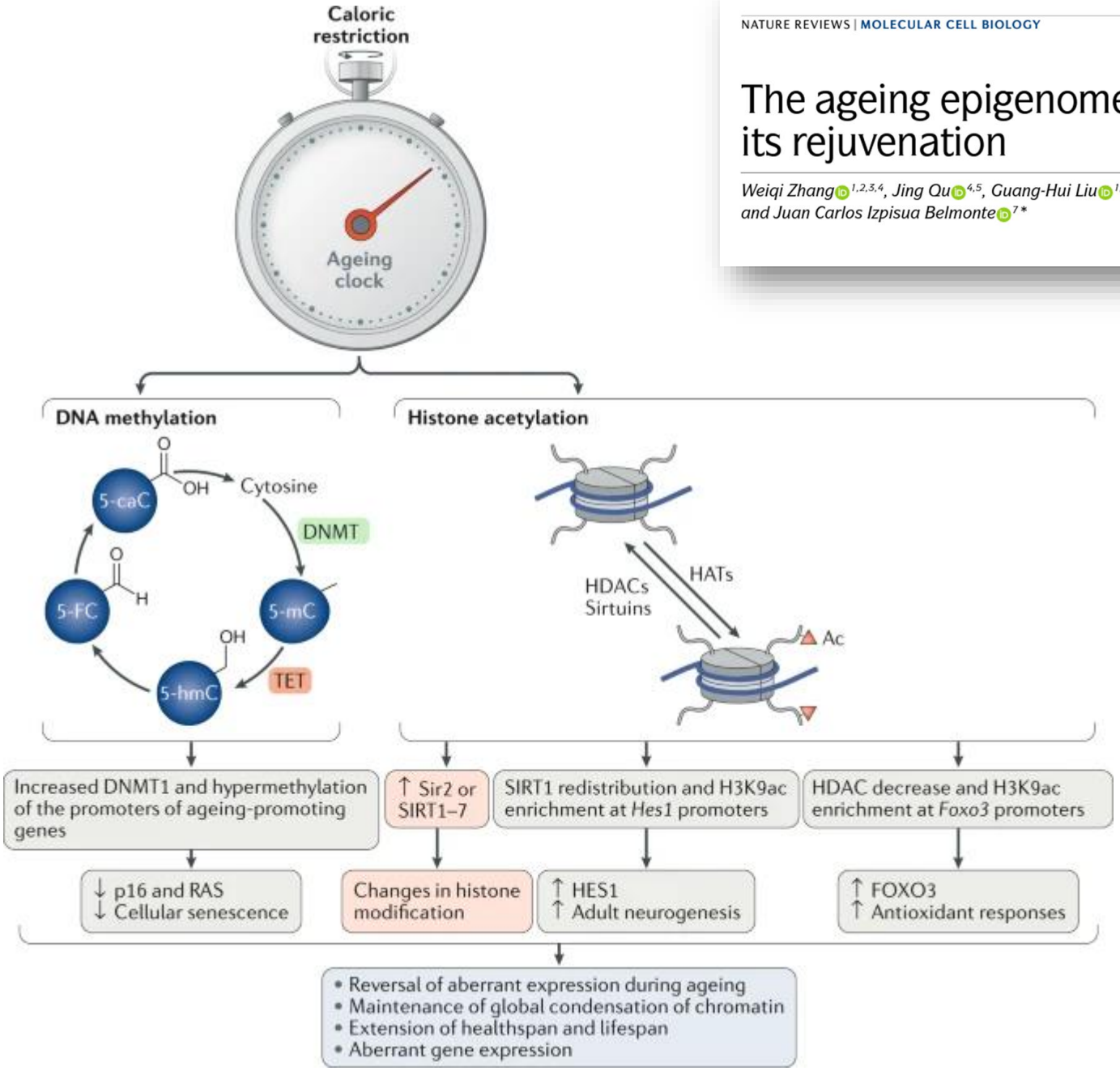


https://www.bio-rad-antibodies.com/blog/history-of-rapamycin.html

NATURE REVIEWS | MOLECULAR CELL BIOLOGY VOLUME 21 | MARCH 2020 | 137

The ageing epigenome and its rejuvenation

Weiqi Zhang[1,2,3,4], *Jing Qu*[4,5], *Guang-Hui Liu*[1,3,4,6]* *and Juan Carlos Izpisua Belmonte*[7]*



Contents lists available at ScienceDirect

Ageing Research Reviews

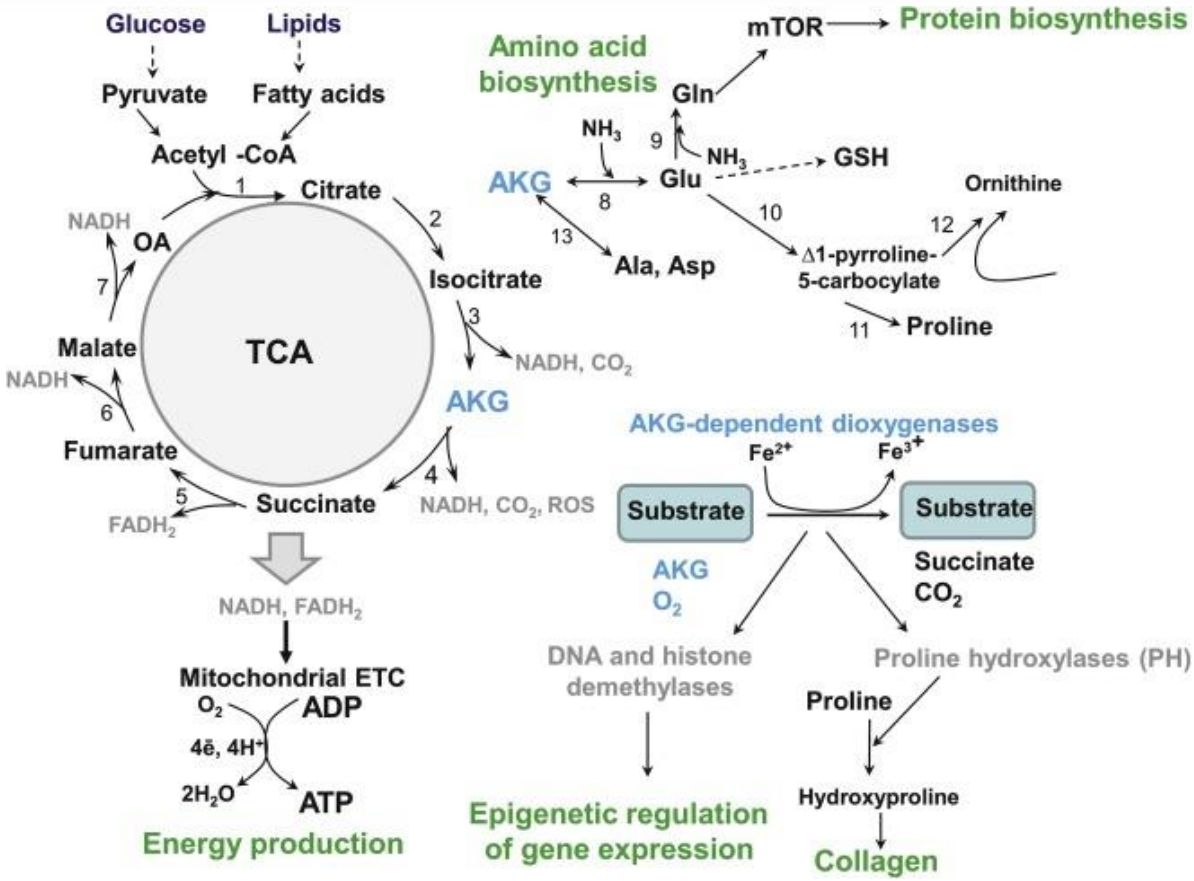
journal homepage: www.elsevier.com/locate/arr

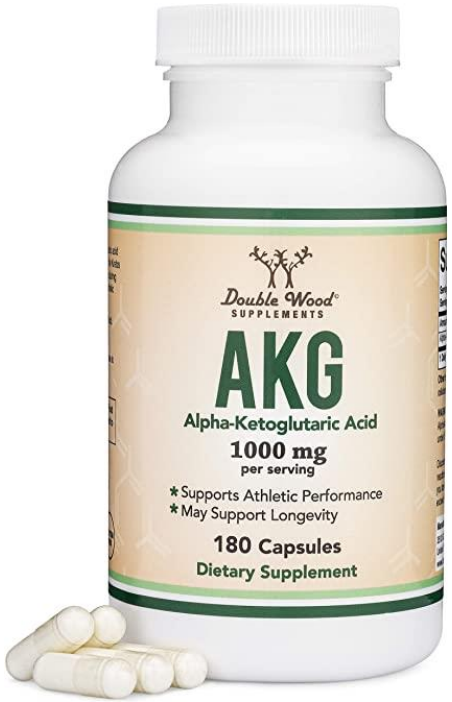
Review

Pleiotropic effects of alpha-ketoglutarate as a potential anti-ageing agent

Maria M. Bayliak [a,*], Volodymyr I. Lushchak [a,b,*]

[a] Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, 57 Shevchenko Str., Ivano-Frankivsk, 76018, Ukraine
[b] I. Horbachevsky Ternopil National Medical University, 46002, Ternopil, Ukraine





Contents lists available at ScienceDirect

Ageing Research Reviews

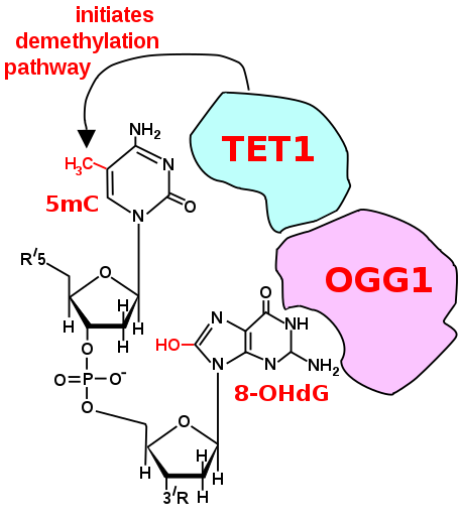
journal homepage: www.elsevier.com/locate/arr

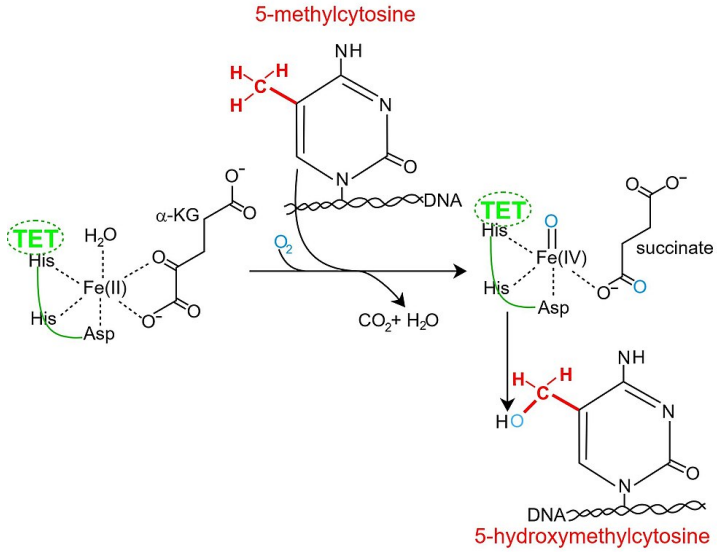
Review

Pleiotropic effects of alpha-ketoglutarate as a potential anti-ageing agent

Maria M. Bayliak [a,*], Volodymyr I. Lushchak [a,b,*]

[a] Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, 57 Shevchenko Str., Ivano-Frankivsk, 76018, Ukraine
[b] I. Horbachevsky Ternopil National Medical University, 46002, Ternopil, Ukraine





DNA demethylation

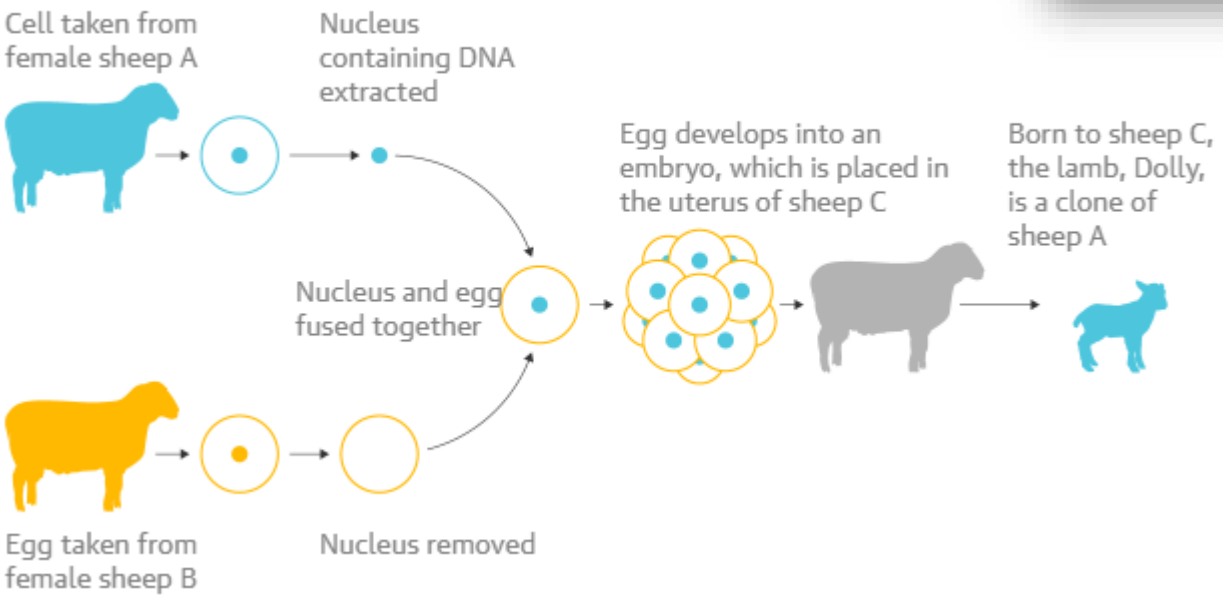
Resetting ageing clock by somatic cloning

somatic-cell nuclear transfer (SCNT) has no obvious detrimental long-term health effects in a cohort of 13 cloned sheep

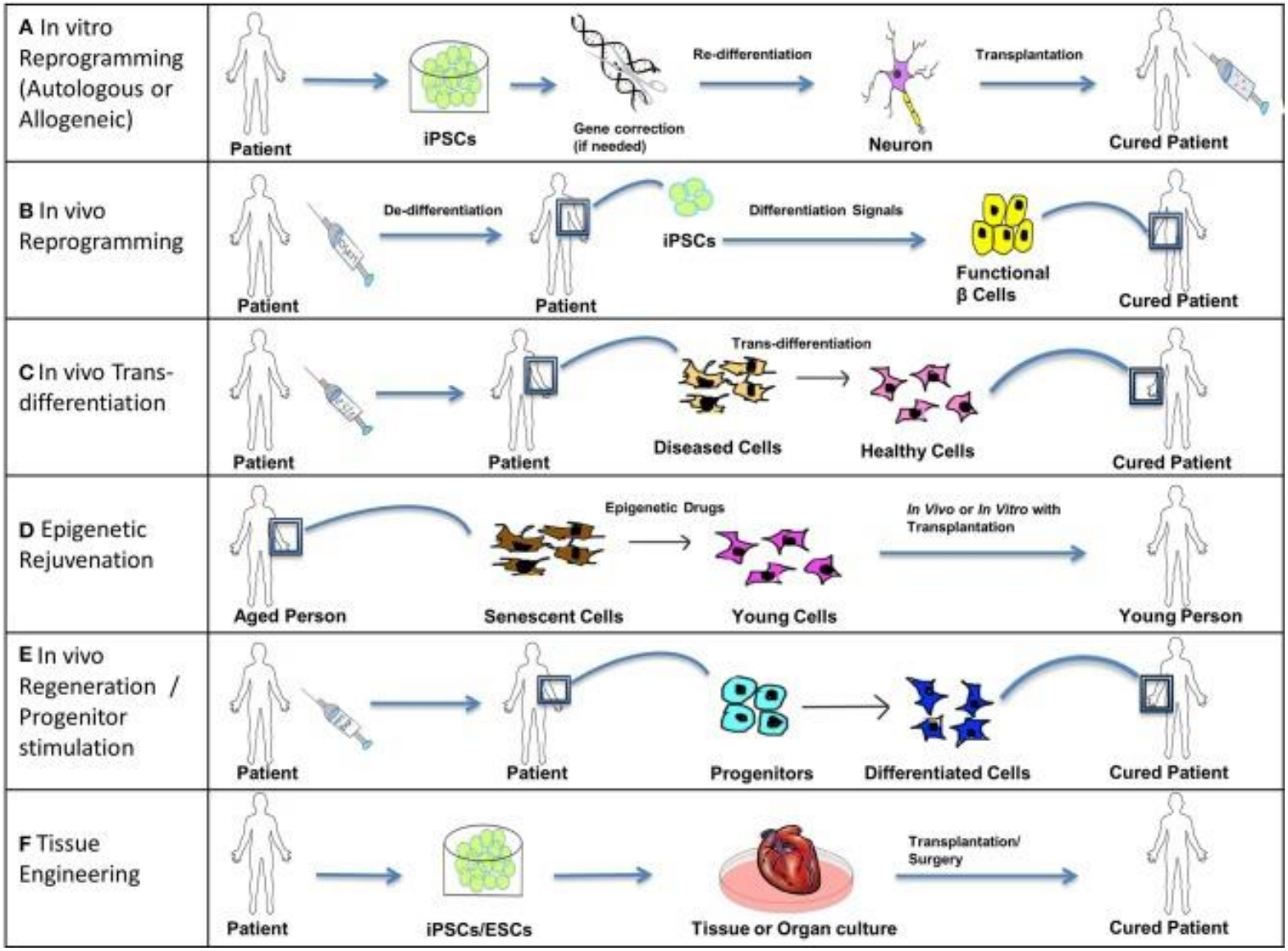
Dolly's clones ageing no differently to naturally-conceived sheep, study finds

Dolly the cloned sheep's early death left scientists wondering whether cloning causes premature ageing. Researchers now have their clearest answer yet

Debbie, Denise, Dianna and Daisy, who were born in July 2007 after being cloned from the same mammary gland cells used to make Dolly. Photograph: the University of Nottingham.

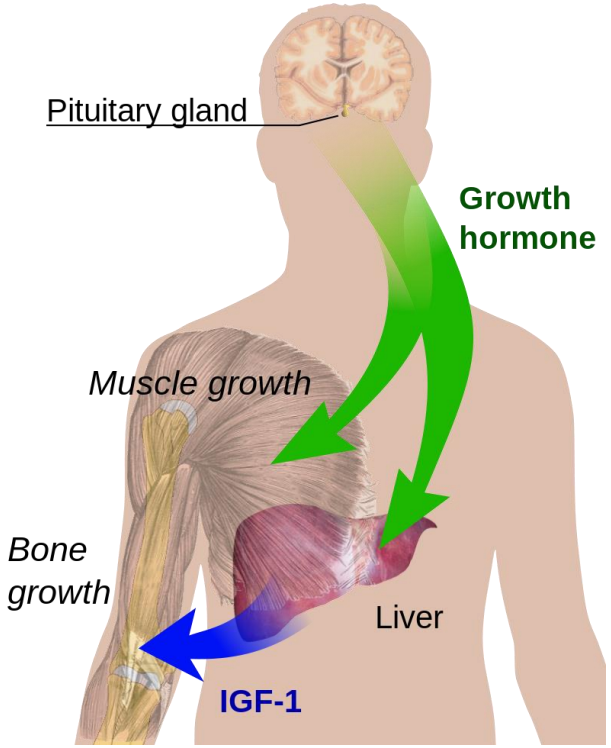


Epigenetic reprogramming and rejuvenation treatment



Growth hormone therapy

- **anabolic**
- **rejuvenation**



Received: 11 May 2019 | Revised: 16 July 2019 | Accepted: 4 August 2019
DOI: 10.1111/acel.13028

ORIGINAL ARTICLE

Aging Cell WILEY

Reversal of epigenetic aging and immunosenescent trends in humans

Gregory M. Fahy[1] | Robert T. Brooke[1] | James P. Watson[2] | Zinaida Good[3] | Shreyas S. Vasanawala[4] | Holden Maecker[5] | Michael D. Leipold[5] | David T. S. Lin[6] | Michael S. Kobor[6] | Steve Horvath[7]

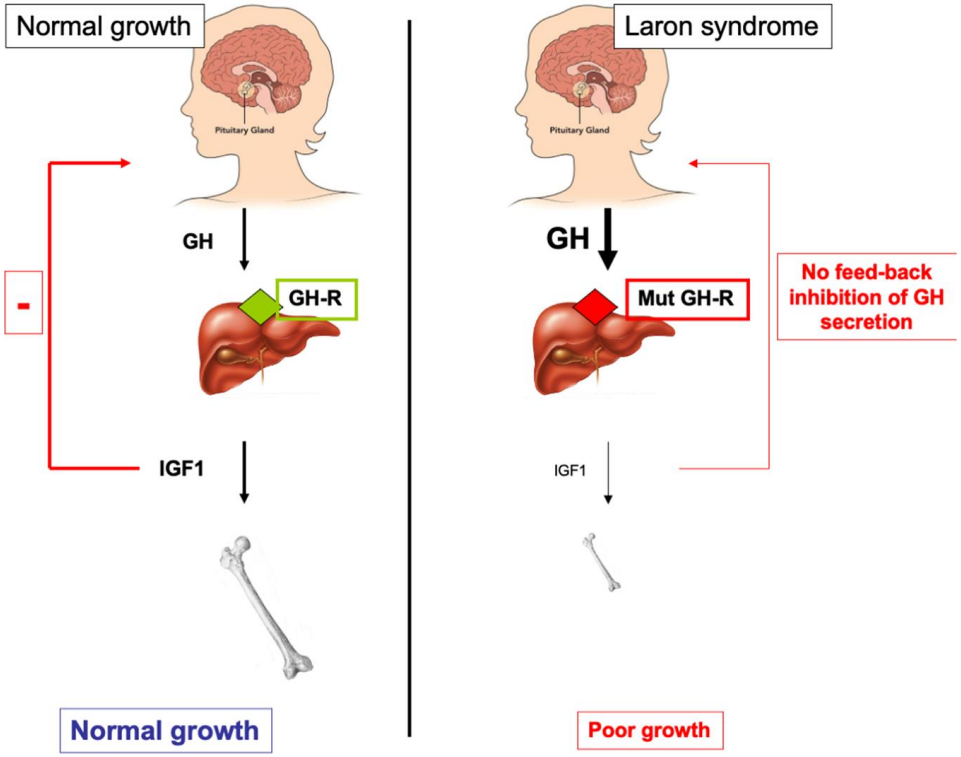
- Increases calcium retention, mineralization of bone
- Increases muscle mass
- Promotes lipolysis
- Increases protein synthesis
- Stimulates the growth of all internal organs
- Reduces liver uptake of glucose
- Promotes gluconeogenesis in the liver
- Contributes to the maintenance and function of pancreatic islets
- Stimulates the immune system
- Increases deiodination of T4 to T3

*It has been reported that **5% of male American high-school students used or have used hGH as an anabolic agent.***

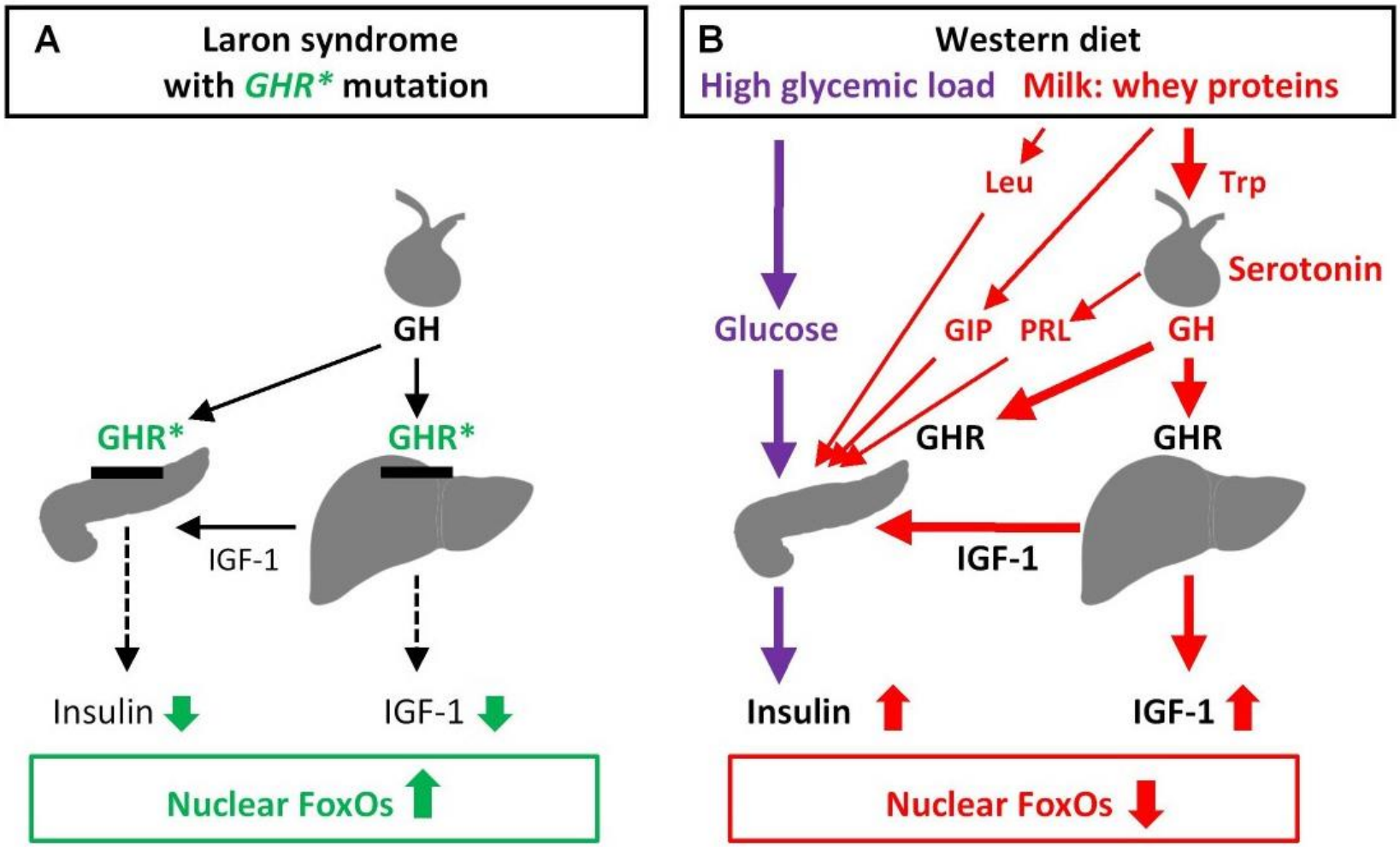
Laron syndrome

- growth hormone insensitivity
- growth hormone receptor deficiency (GHRD
- autosomal recessive disorder
- lack of insulin-like growth factor 1

Zvi Laron



Laron syndrome



Reduced linear growth, dwarfism
low oxidative stress, anti-aging signaling
low prevalence of acne, diabetes, cancer

Increased linear growth, tall people
high oxidative stress, pro-aging signaling
high prevalence of acne, diabetes, cancer