AGEING

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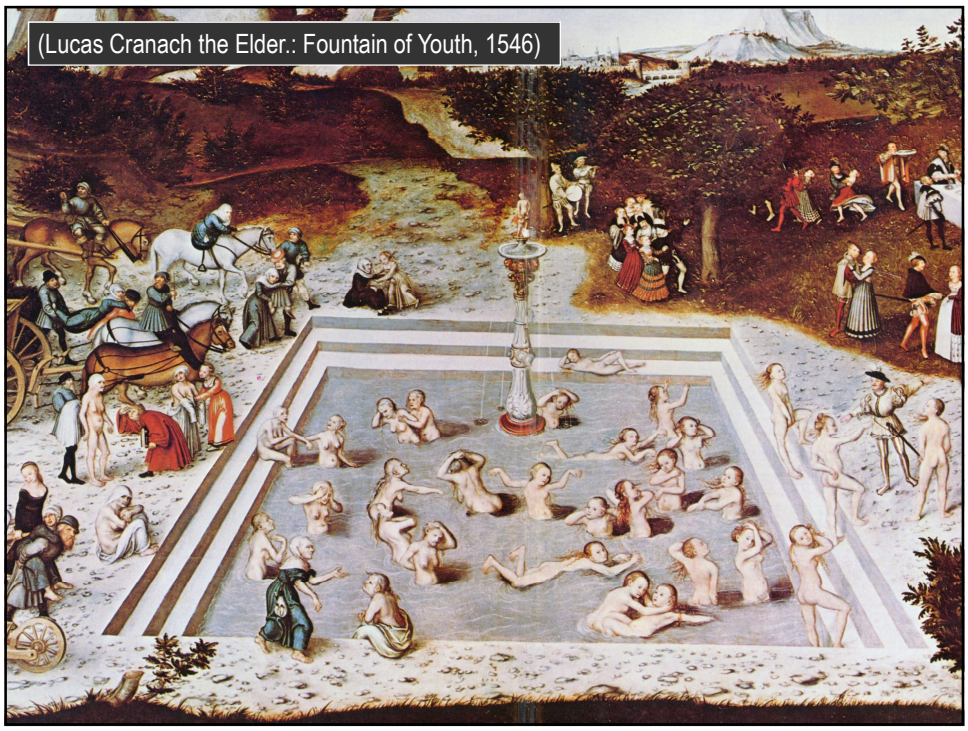
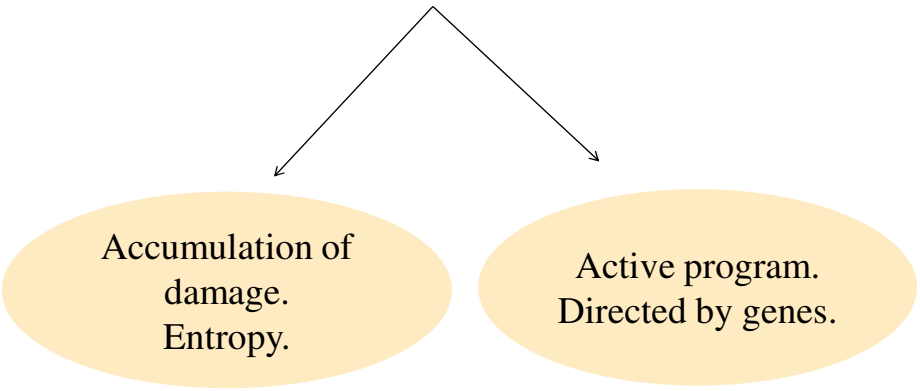


What is ageing

- Progressive loss of physiological capacity in the cells, increase in susceptibility to various diseases
- Not a disease as such
- Looks similar in various animals, but proceeds with variable speed … universal process
- Distinguish:
  - **Average life expectancy:**
    - 2000: men 71.7 years, women 78.4 years
    - 1920: men 47 years, women 49.6 years
  - **Maximum lifespan:**
    - For humans 115-120 years, does not change

To explain ageing, at least 28 theories formulated



What is ageing

- No evidence for program obtained so far.
- Stochastic process, unlike embryogenesis not directly programmed by genome
- On molecular level:

  inability to keep fidelity of biomolecules indefinitely

  „systemic molecular disorder“ (Hayflick)
- Consequence of accumulation of errors and organismal responses to them

Genes related to longevity

- Stress resistance (anti-heat shock, antioxidant defence…)
- Energy metabolism (insulin signaling, caloric intake, mitochondrial function…)
- Mutation prevention and repair
- Hormonal homeostasis
- Control of cell proliferation

Sources of errors/damage to biomolecules

- Errors in copying/expressing genomic and epigenomic information
- Biological side-reactions … spontaneous, permanent, non-enzymatic, exergonic processes
  - Oxidations
  - Glycations

Ionising radiation:

Hydroxyl radical originates from ionisation of water:

$H_2O + h\nu \rightarrow H^{\cdot} + OH^{\cdot}$

Reactive oxygen species in the body:

One-electron reduction of oxygen (mitochondria, NADPH oxidase) forms superoxide $O_2^{\cdot -}$

Dismutation of superoxide produces hydrogen peroxide:

$O_2^{\cdot -} + O_2^{\cdot -} + 2\,H^+ \rightarrow O_2 + H_2O_2$

Fenton reaction with Fe or Cu converts peroxide to hydroxyl radical:

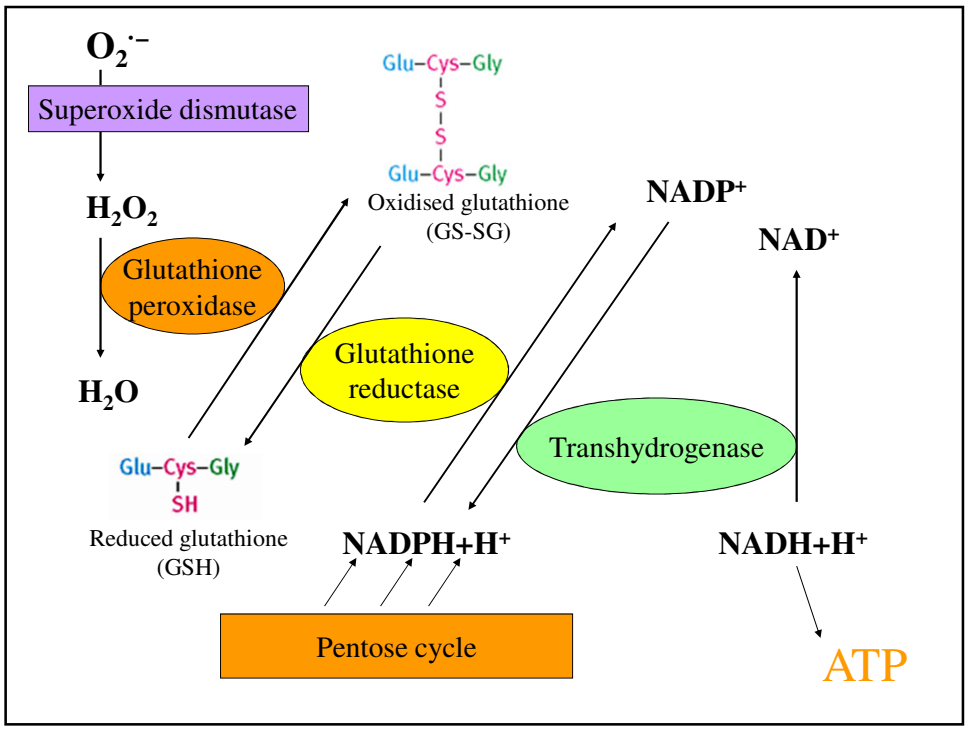
$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\cdot} + Fe^{3+}$

Oxidative damage to biomolecules

- **Lipids:** peroxidation of polyunsaturated fatty acids in membranes
- **Proteins:** oxidation of -SH, carbonylation of $-NH_2$, hydroxylation/nitrosylation of aromatic amino acids, cross-linking, degradation
- **Nucleic acids:** single/double strand breaks, hydroxylation of bases ... mutation, cancerogenesis…

Why is it difficult to completely prevent/repair damage by ROS?

- Chemistry too varied – some damage cannot be repaired
- Limited amount of energy is available for antioxidant defence and repair
- **ROS also essential in cell signaling and host defence!**



Antioxidant defence V

Stress response

Oxidation or nitrosylation of sensor -SH

↓

Transcription factors (NFκB, Nrf-2…):
activation, nuclear translocation

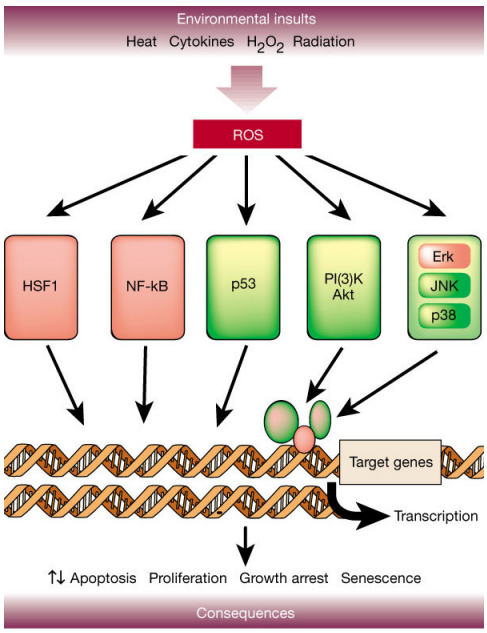
↓

Induction of gene expression:

- chaperones (heat shock proteins)
- antioxidant enzymes
- metallothionein
- hemoxygenase 1

…→ cell more resistant to further oxidative stress

…→ propagation of inflammation

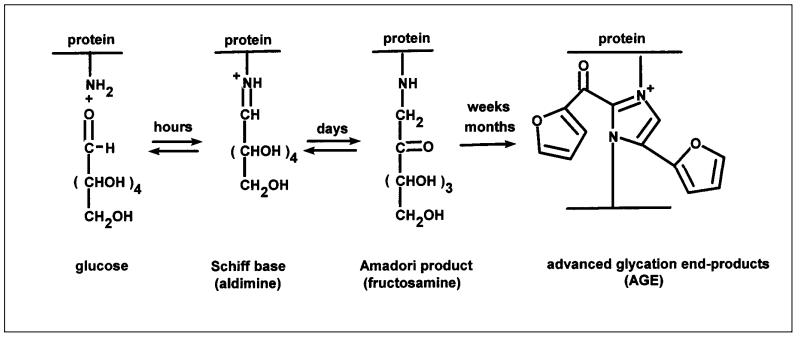


Adaptive homeostasis

- Endogenous ROS are critical mediators of cellular adaptation to various kinds of stress.
- Redox signaling: Oxidative stress activates protein kinases and transcription factors
- …Resistance to stressors, regulation of cell proliferation, apoptosis…

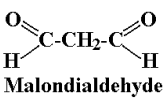
(T. Finkel & N.J. Holbrook, Nature 408 (2000), 239-247)

Protein Glycation/Carbonyl Stress

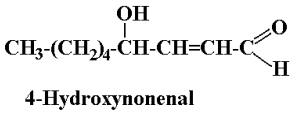


Maillard reaction. (According to Janebová et al., Remedia 1999).

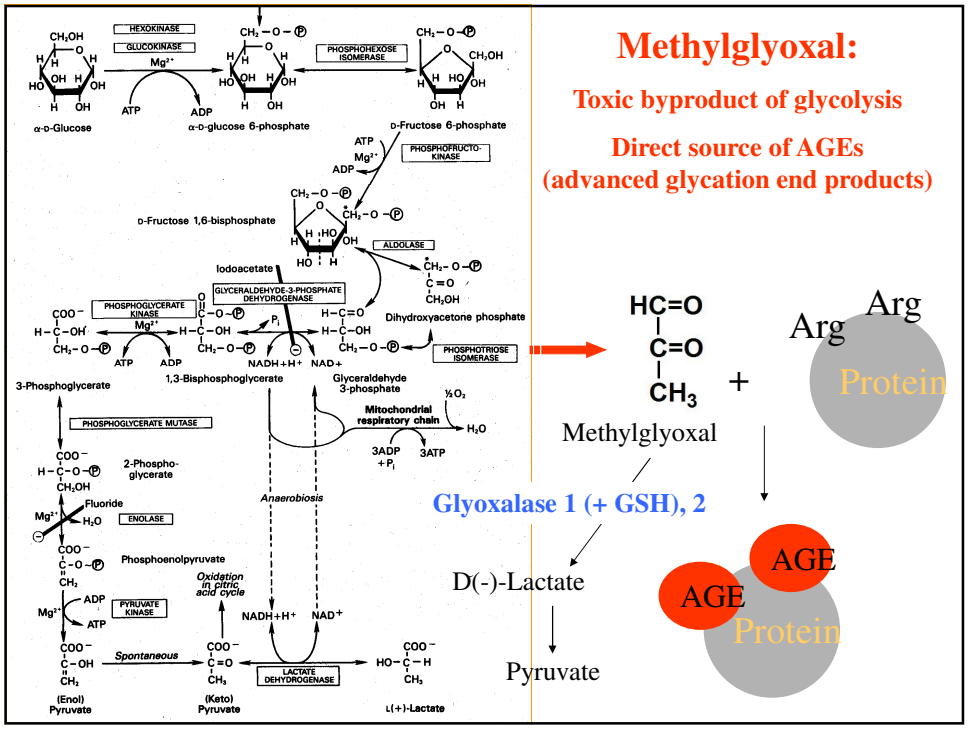
Reactive carbonyls (aldehydes) from lipid peroxidation:



Malondialdehyde



4-Hydroxynonenal



Effects of glycation (dicarbonyl stress):

- Cross-links of extracellular proteins → stiffness
- Protein modifications → proteasome ↓, misfolded proteins ↑, toxic aggregates
- Respiratory chain modification → ROS↑, ATP↓
- DNA modification → mutations, genomic instability
- RAGE (receptor for AGE) → inflammation

- BUT: methylgloxal also needed for induction of Nrf2 in stress response!

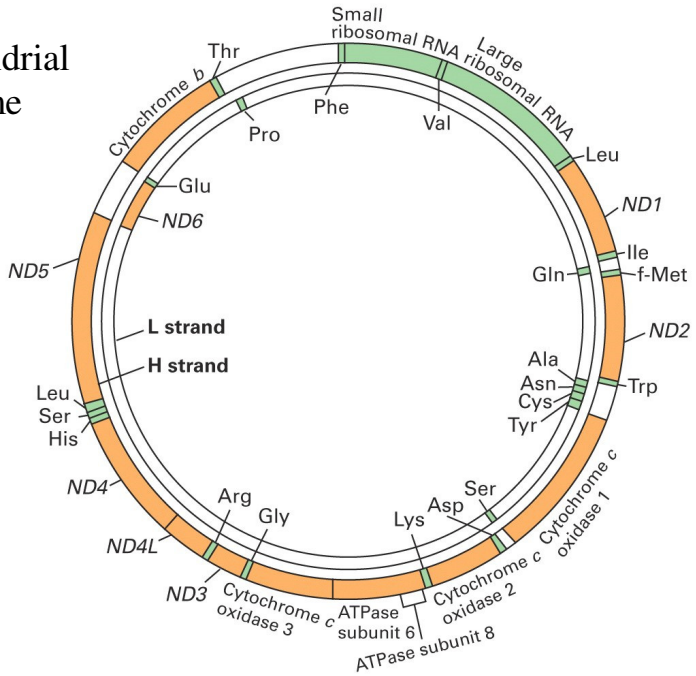
Ageing at cellular/tissue level

- Mitochondrial dysfunction
- Consequences of DNA damage
- Cellular senescence
- Alteration of epigenome
- Collapse of proteostasis
- Inflammation

Mitochondrial dysfunction

- Theoretical considerations:
  - Semiautonomous organelles with their own mtDNA
  - MtDNA:
    - Many copies per cells
    - Replication independent on cell cycle, no recombination
    - Somatic cell lacks mechanisms for selection of intact copies
  - Mitochondria produce ROS that can damage mtDNA
  - Life spans somehow related to metabolic rate

Mitochondrial genome



Mitochondrial theory of ageing: Vicious circle of oxidative damage

Production of oxygen radicals in mitochondria

↓

Accumulation of mtDNA mutations with age

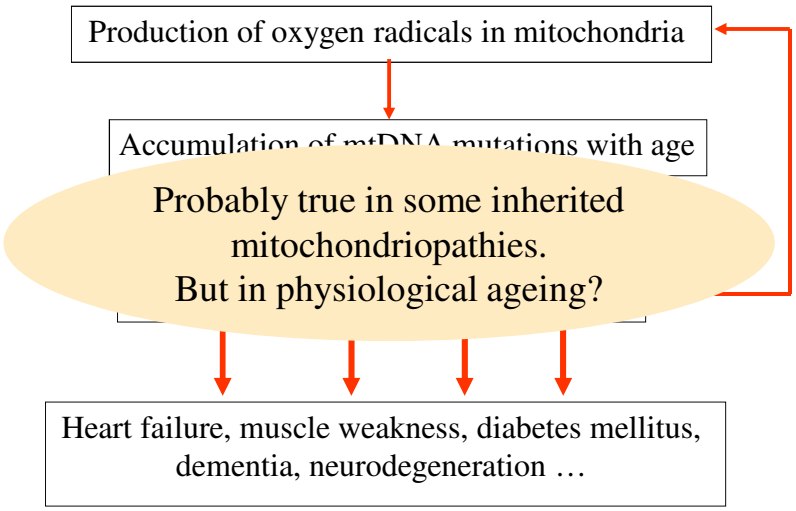
↓

Respiratory chain deficiency

↓

Heart failure, muscle weakness, diabetes mellitus, dementia, neurodegeneration …

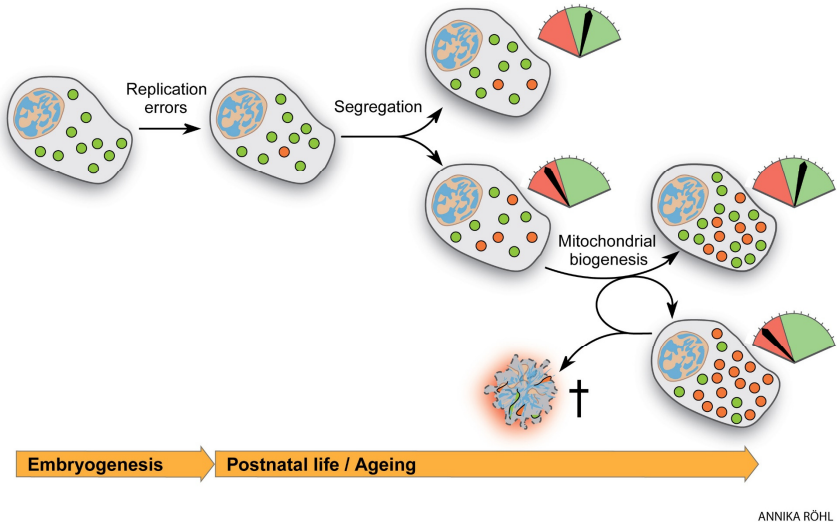
Mitochondrial theory of ageing: Vicious circle of oxidative damage



Mitochondrial dysfunction

- Mitochondrial number and function declines with age
- Point mutations and deletions in mtDNA do accumulate
- …but due to **replication errors** by mitochondrial DNA polymerase γ, rather than by ROS
- Mosaic OXPHOS deficiency develops

Clonal expansion of mutated mtDNA molecules leads to mitochondrial dysfunction in ageing



Cell Metabolism 2017 2557-71DOI: (10.1016/j.cmet.2016.09.017)

Most of DNA damage is endogenous

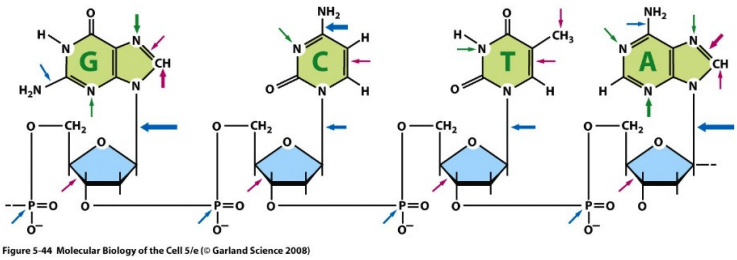


Figure 5-44 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- Depurination
- Spontaneous deamination of cytosine
- Oxidative modification of bases (G most sensitive)
- Reaction of bases with carbonyls (aldehydes from lipid peroxidation and glycation)
- Non-enzymatic alkylation (mostly methylation)
- Single- and double-strand breaks

How much damage?

- **Abasic sites (depurination, also by ROS!):**
  2,000-10,000 purines lost per cell and day
  Possibly steady state 50,000-200,000 lesions per liver cell
- **Hydrolytic deamination:**
  100-500 cytosines deaminated per cell and day,
  (for methyl-C more rapid and less effectively repaired)
- **Formation of 8OHdG:**
  Cumulative damage to DNA (8OHdG excreted into urine):
  … 140-200 G oxidized per cell and day
  Steady state 0.07 - 145.25 8OHdG per 10^6 nt
  … 168 - 348,000 8OHdG per cell (…cca 1:10^5 ?)

R. De Bont & N. van Larebeke: Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis (2004) 19, 169-185.
B. Halliwell & J.M.C. Gutteridge: Free Radicals in Biology and Medicine, 4th edition, Oxford University Press 2007

Double-strand DNA breaks

- Rare (10-50 per cell and day?)
- Dangerous: repair by NHEJ alters sequence
- If not repaired:
  → DNA Damage Response (DDR)

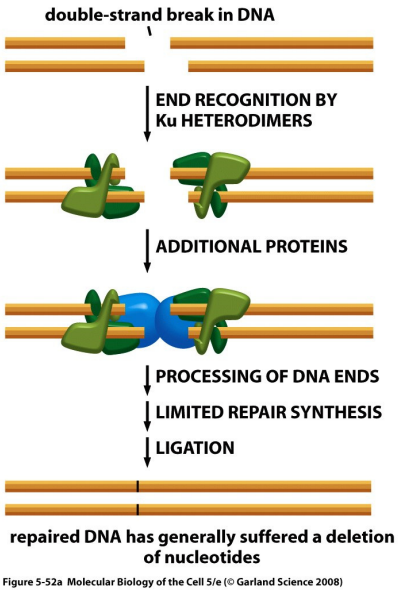


Figure 5-52a Molecular Biology of the Cell 5/e (© Garland Science 2008)

DNA Damage Response (DDR)

Persistent double-strand breaks

p53 protein

Halts cell cycle to provide time for repair

Apoptosis

DNA Damage Response (DDR)

Possible outcomes:

Cell survived, can divide again (mutated?)

….Cancer

Risk of cancer as function of age

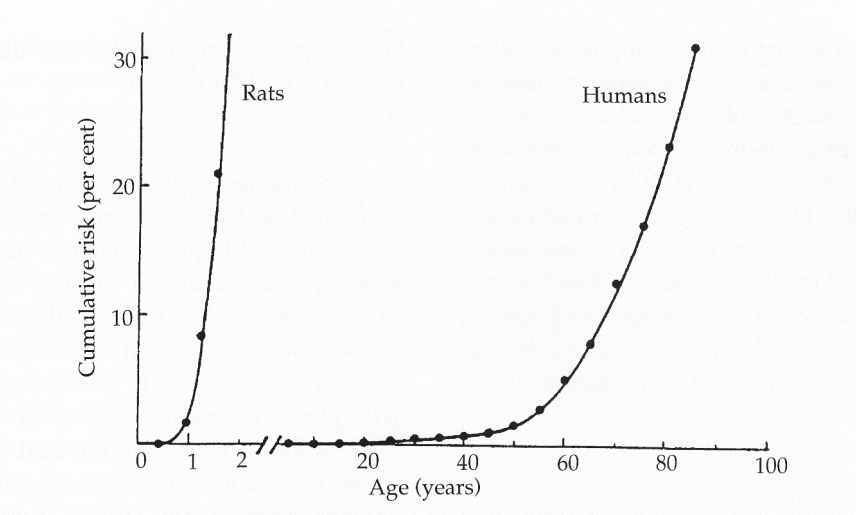


Figure from: B. Halliwell & J.M.C. Gutteridge: Free Radicals in Biology and Medicine, 4th edition, Oxford University Press 2007
(Original source: *Free Radic. Res. Commun.* (1989) 7, 122)

DNA Damage Response (DDR)

Possible outcomes:

Cell survived, can divide again (mutated?)

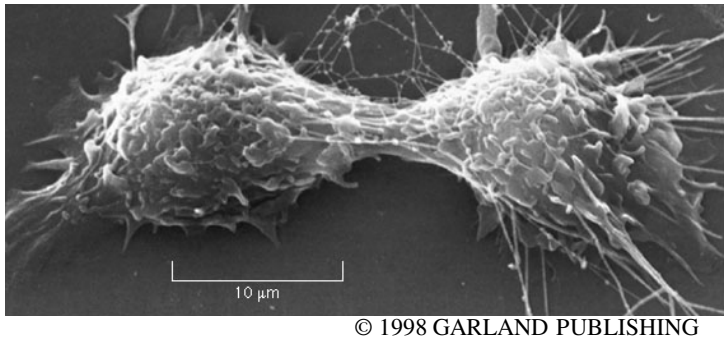
….Cancer

Cell eliminated by apoptosis

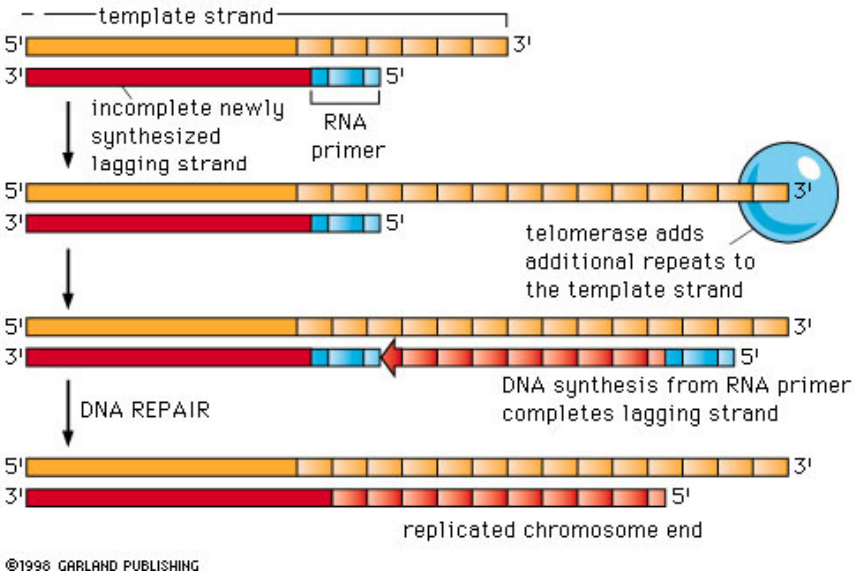
….(Stem) cell depletion, atrophy

Hayflick limit

- Human fibroblast in culture divide no more than 50-70 times, then enter **replicative senescence**
- Applies to all cultured somatic cells, but not to transformed cells or cancer cells
- Cells from an elderly donor divide fewer times



Telomerase



Telomerase is not the secret of eternal youth!

- Most cells of human body do not need telomerase (divide infrequently or not at all)
- Stem cells, germinal and activated immune cells possess telomerase
- Fibroblasts and epithelial cells do not regularly reach the Hayflick limit in vivo
- Murine somatic cells express telomerase, but still lifespan of mouse is much shorter than human
- K.O. of mouse telomerase gene: normal lifespan untill the 3rd generation, then accelerated ageing, occurence of human type cancer (carcinomas)

Real role of telomeres in ageing

- (TTAGGG)n…very sensitive to oxidation
- If telomere broken, shelterin inhibits DNA repair → DDR
- Telomeres are sensitive **detectors of oxidative stress**!

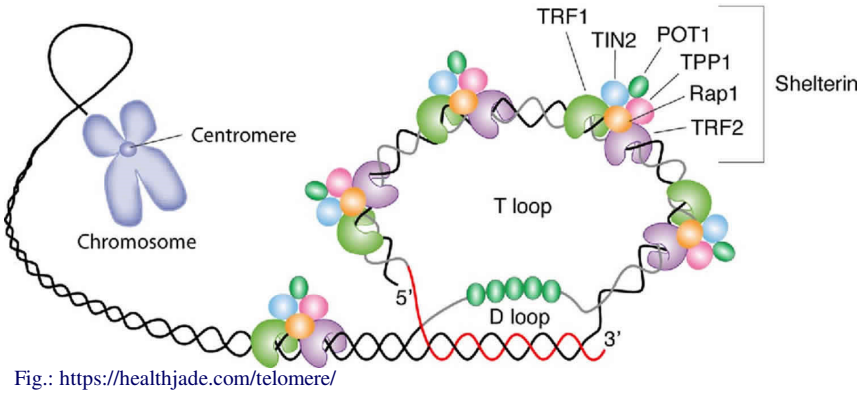
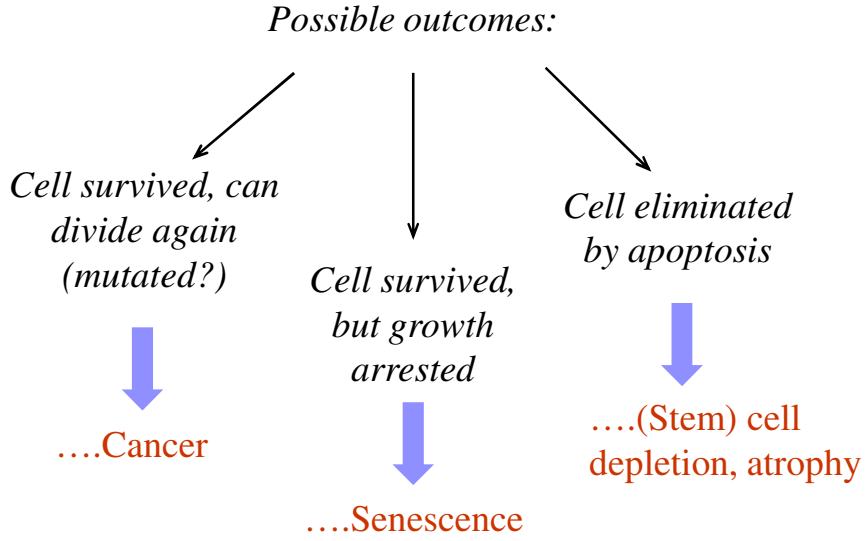


Fig.: https://healthjade.com/telomere/

DNA Damage Response (DDR)



Cellular senescence

- Stable growth arrest, but cells survive and change
- SASP (Senescence-associated secretory phenotype): proinflammatory factors
- Role in development and wound healing
- Accumulation in aged tissues (1-5%) detrimental
  - Organ dysfunction
  - Can support cancer growth
- Senolytics (drugs that kill senescent cells)?

Ageing epigenome

- Gene regulation: transcription factor binding, DNA methylation, histone marks, nucleosome positioning, non-coding RNA…
- Dysregulation with age, marks become lost or more random
- → Altered induction of genes
- → General loss of heterochromatin
  - Ectopic expression
  - Activation of transpozons

Collapse of proteostasis

- Ability to keep healthy proteome by synthesis, chaperoning, and removal
- **Theoretical considerations: Model of Kirkwood and Kovald (1995):**
  - Some ROS always escape mitochondria and damages other cellular structures
  - Prevention of ROS formation and repair systems are never 100 % effective
  - Slightly damaged mitochondria produce less energy than the cell would need
  - At some point lack of ATP causes sudden collapse of proteostasis in cytosol

Collapse of proteostasis

- Late event (last third of life) but dominant in ageing
- Oxidative protein damage ↑
- Synthesis of proteins ↓
- Degradation ↓
- Chaperones become more occupied with damaged proteins
- …Accumulation of misfolded proteins and toxic aggregates

Collapse of proteostasis:
Limits of autophagy

- Long-lived proteins, organelles removed by autophagy
- Digestion not complete…postmitotic cells accumulate ‚garbage'…lysosomal/mitochondrial isufficiency

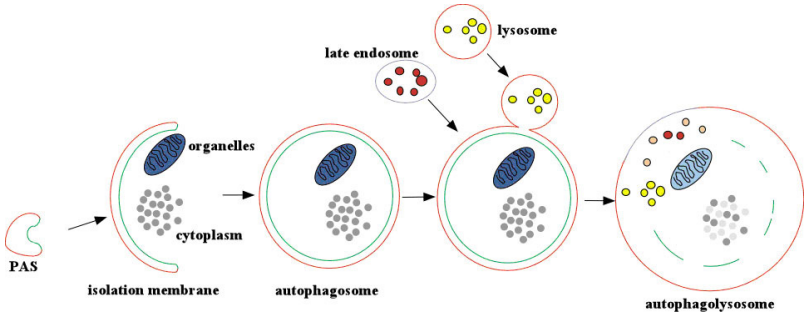
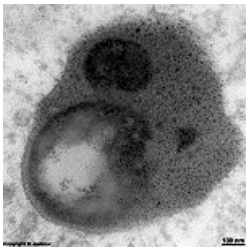


Fig.: http://cpmcnet.columbia.edu/dept/gsas/anatomy/Faculty/Kessin/autophagy.html

Ageing as a catabolic insufficiency

Incomplete digestion in lysosomes, release of Fe from mito, ROS, lipid peroxidation, cross-linking, aggregation and polymeration of oxidised proteins and lipids



↑ LIPOFUSCIN (in lysosomes)
In cytosol defective mito and indigestible protein aggregates

Loss of hydrolases delivered to lipofuscin-loaded lysosomes
Damaged and hypertrophic (giant) mito not degradable

+

Less ATP, more ROS, damaged mito & lysosomes can initiate apoptosis…

Fig.: http://www.uni-mainz.de/FB/Medizin/Anatomie/workshop/EM/EMtLyso.html

Inflammaging

- Increased baseline inflammation in ageing
- Reasons?
  - Secretions of senescent cells (SASP) and adipose tissue
  - ↑ mito ROS → stress response becomes permanent
  - Altered regulation of stress-related genes
  - AGE-RAGE interaction
  - …

Life renewal in nature

- Continuous cell division
- Genetic recombinance
- Selection against mutations

… Every organism dependent on postmitotic cells is mortal!

… Our highly specialised neuronal, skeletal and cardiac muscle cells are the most sensitive to ageing

… The ultimate limit of our lifespan

Is ageing inevitable in nature ?

Hydra (Cnidaria)

- Simple body plan, rapid and complete renewal from stem cells
- Nerve net, no recognizable brain or muscles
- Mostly asexual reproduction (budding)
- No signs of senescence or mortality in captivity

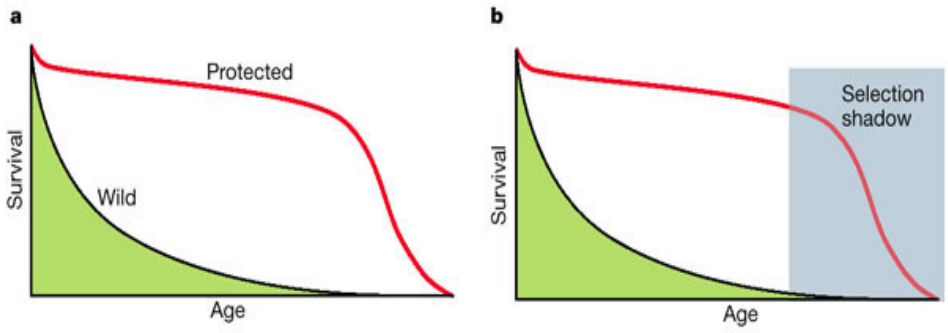
Even if achieved in humans by engineered stem cells, replacement of neuronal cells will change mind and wipe memories…

Evolution of ageing

Could ageing evolve as a programmable trait to promote evolutionary change by accelerating turnover of generations?
(August Weissman, 1891)

Ageing is rare in nature!

- Most wild animals never reach senescence
  ….No selection against mutations with negative effects late in the lifespan



(T.B.L.Kirkwood & S.N. Austad, Nature 408 (2000), 233-238)

Disposable soma theory
(throw-away body)

- **Bacteria:** do not age but for the price of high selection and mortality
- **Higher organisms:**
  - Specialisation

GAMETS: haploid (‚defect sieve'), huge redundance and high selection, but DNA passes to further generation.

SOMA: diploid, longer-lasting, but mortal structure, DNA not intended for further generation.

Disposable soma theory:
How long should the body be maintained?

- Evolution governed by success in reproduction !

- **Trade-off** between body maintenance and reproduction (metabolic energy limited!)
  - Extrinsic mortality high: better to invest in reproduction (r-strategy)
  - Extrinsic mortality low: better to invest in maintenance (K-strategy)

Catastrophic senescence: Pacific salmon

http://www.usgs.gov/features/lewisandclark/ChildrenWebSites.html

Animals living longer than predicted by ‚rate-of-living' theory : (relationship lifespan – oxygen consumption)

- Shell

- Wings

- Advanced brain

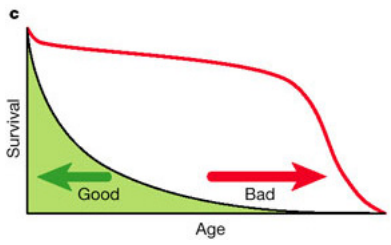
Fig.: http://www.discovergalapagos.com/tortoise.html
http://www.ctrl-c.liu.se/ftp/images/animals/misc/*.*
http://www.african-safari-pictures.com

Evidence for trade-off between longevity and fecundity

- Lifespan of Drosophila can double over ten generations of selective breeding:
  - Associated with later onset of reproduction, number of eggs remains the same
- Genealogical records of British aristocracy:
  - The longest-lived aristocrats tended to have had the greatest trouble with fertility

Theory of antagonistic pleiotropy

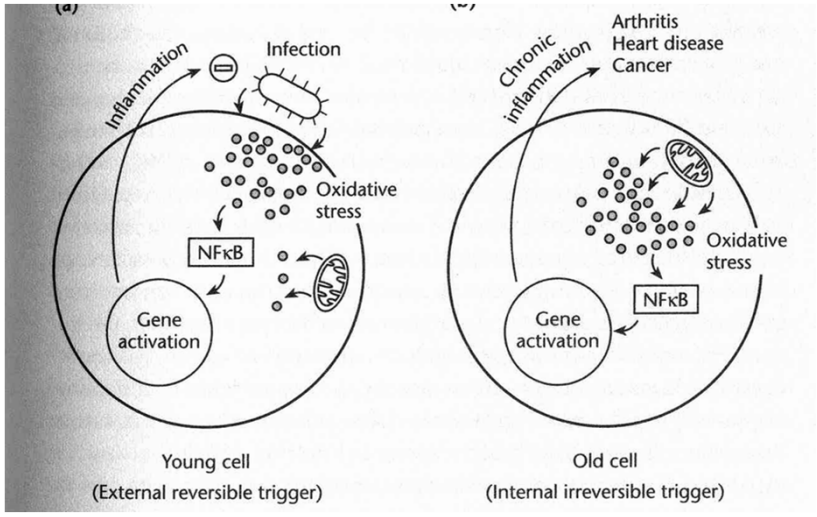
- Genes providing advantage for reproduction, but deleterious later in life



(T.B.L.Kirkwood & S.N. Austad, Nature 408 (2000), 233-238)

- Examples in humans:
  - Huntington disease
  - Hemochromatosis

Good defence against infection in youth
Chronic inflammation/oxidative stress later ?



Nick Lane: Oxygen. The Molecule that made the World. Oxford University Press 2002

Antioxidants as elixirs of youth ?

- Vitamin E (tocopherol)
- Vitamin C (ascorbate)
- β-carotene
- Selenium

Fig.: http://www.osel.cz

Antioxidant dietary supplements can even be harmful!

- Large meta-analysis of total mortality in 68 studies on administration of antioxidant supplements (232 606 participants, 385 publications):
  - β-carotene, vitamin A and vitamin E significantly **increase** mortality
  - Vitamin C and selenium have no effect

(Bjelakovic G et al., JAMA 2007; 297: 842-857)

Why the antioxidants do not help or even harm ???

- High doses are ineffective
- Suppress the beneficial oxidations
  - Inhibition of the stress response
  - Impair defence against infection, cancer, physiologic apoptosis?
- Have other effects in addition to antioxidant
  - tocopherols: anti-inflammatory
  - β-carotene: co-carcinogen (together with smoking or environmental toxins)

Caloric restriction extends lifespan

- Restricted amount of food with preserved quality
- Works also in higher animals with constant temperature (e.g. mice, rhesus monkeys)
  - Mouse lives for 28 months, but dietary restriction to 25% extends its lifespan to 47 months
- Really extends the maximum lifespan, decreases markers of oxidative stress, occurence of cancer, and slows down ageing

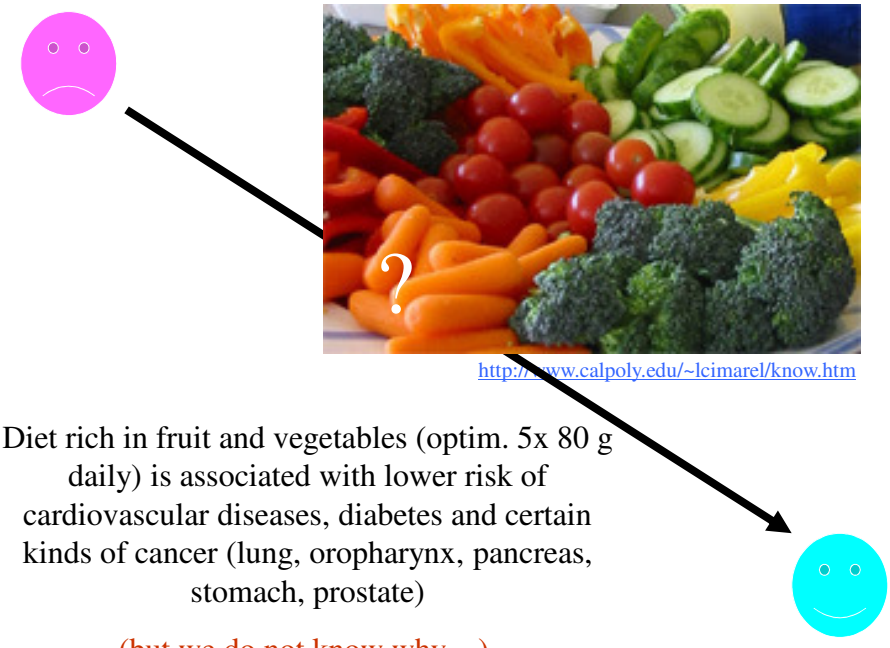
Caloric restriction extends lifespan

- Organism „waits out“and diverts metabolic energy from reproduction to maintenance functions.
- Mechanisms:
  - Some suppression of **IGF-I** and **insulin** signaling
  - Stimulation of autophagy
  - **Sirtuins** – enzymes deacetylating histones, p53 etc., inhibited by NADH
- CALERIE study in human volunteers:
  - Improvement in cardiometabolic health, lower markers of oxidative stress, no adverse effects on mood, sleep or sexual function

HORMESIS

- Mild stress (heat, cold, irradiation, ischemia, oxidants) enhances resistance to subsequent, more severe stress
  (…*what won't kill you, will make you strong*…)
- Mechanisms: adaptive homeostasis/stress response

- Example in humans: **physical activity**
  - ↑ ROS → stress response
  - ↓ ATP → stimulates biogenesis and renewal of muscle mitochondria
  - …



http://www.calpoly.edu/~lcimarel/know.htm

Diet rich in fruit and vegetables (optim. 5x 80 g daily) is associated with lower risk of cardiovascular diseases, diabetes and certain kinds of cancer (lung, oropharynx, pancreas, stomach, prostate)

(but we do not know why…)

Conclusion?

- Immortality and eternal youth are not at hand. Ageing appears to be inevitable consequence of intrinsic limits of body maintenance
- But the rate of ageing can be manipulated:
  - Balanced diet
  - Caloric restriction
  - Physical activity
- Future ?
  - Senolytics, stem cells, stimulators of autophagy, CR mimetics … ?

No one should get old
before first getting wise