Molecular basis of Aging

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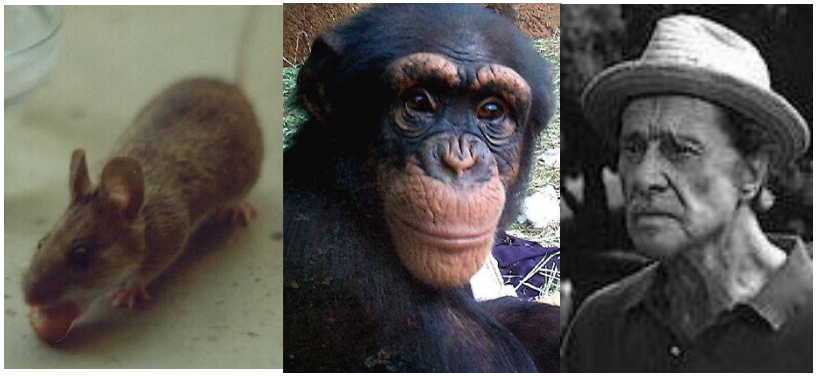
Aging: What is it?

- Aging, has been termed generally as a progressive decline in the ability of a physiological process after the reproductive phase of life.

Pinus longaeva **(Bristlecone pine) ~5000 years**

Maximum lifespans in mammals



3 years **59 years** **110 years**

Urticina felina **(Dahlia anemone) Non ageing**

Nihal Bitla, Face Of Progeria Campaign In India

Movie: Paa

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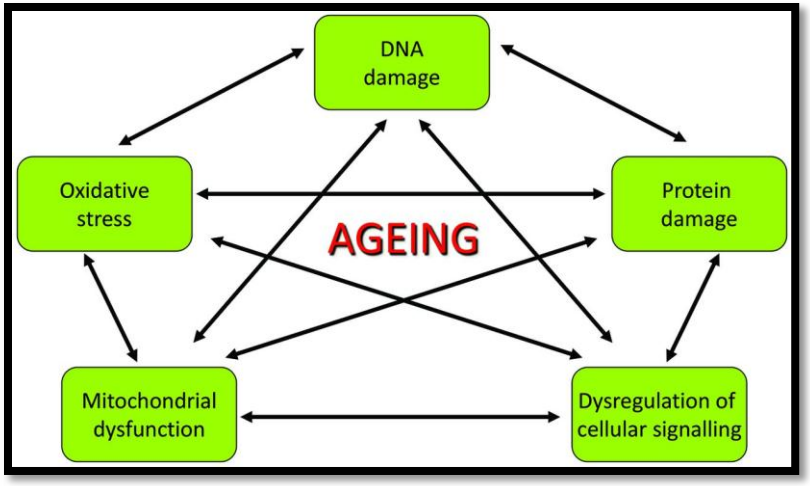
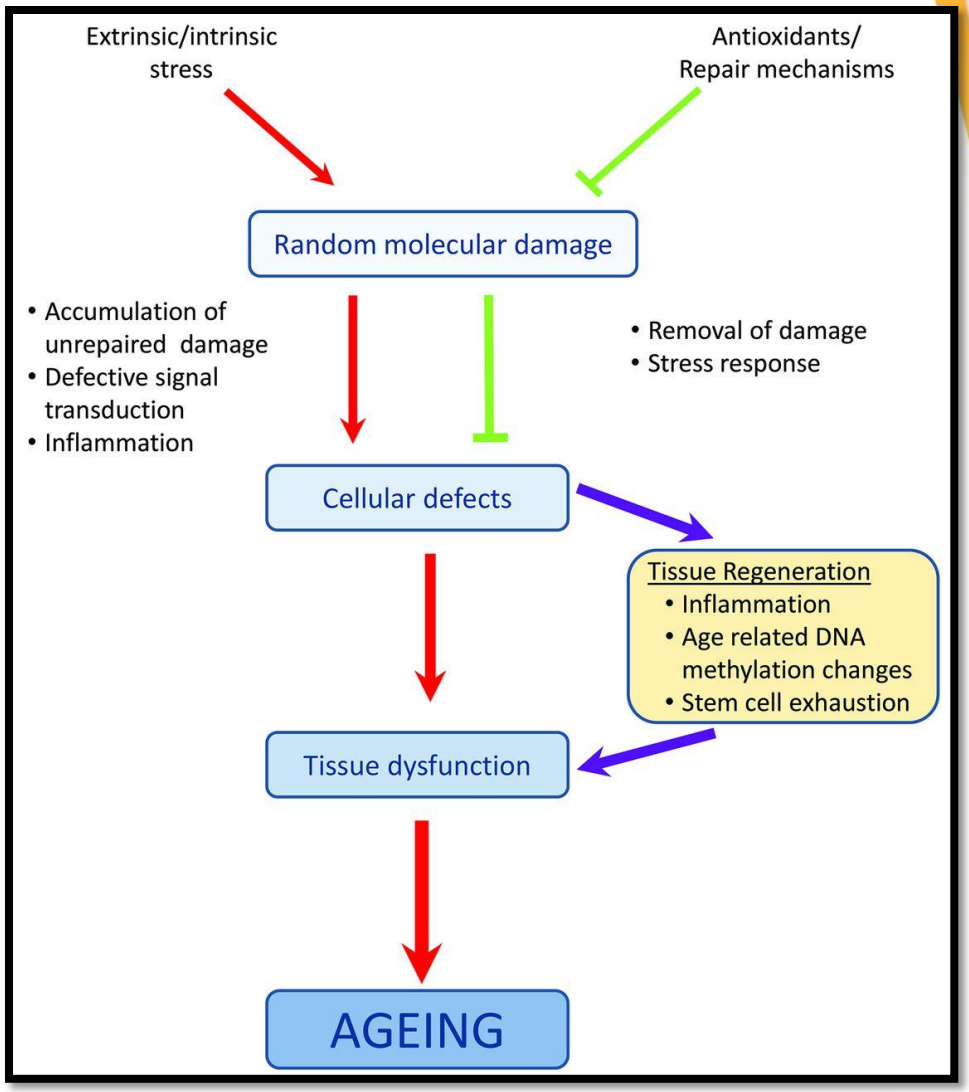
Process of Aging





Theories of Aging

- All aging begins with genetics
- Aging changes the biochemical and physiological processes in the body
- Cell and molecular biologists examine and propose theories to explain the aging process
  - What causes aging?
  - How can you influence aging …prolong life?

Theories of aging

- Genetic theories
  - Claim that aging is genetically determined
- Damage-accumulating theories
  - Claim that aging is a result of accumulating cellular damage to proteins, membranes…

A possible cause of cellular damage could be reactive oxygen species.

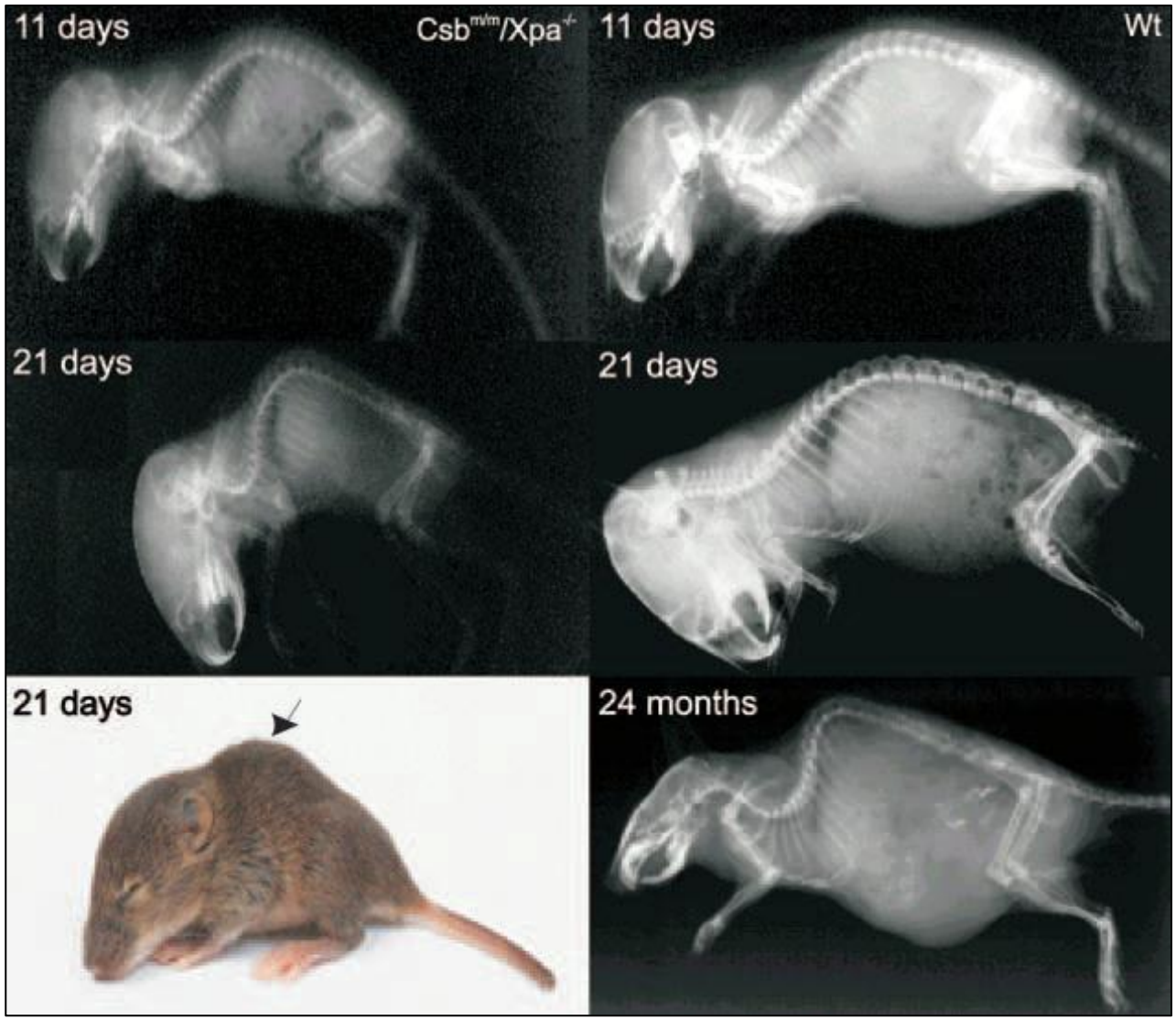
Genetic theories

- Genetic theories of aging propose that aging is a continuation of the process of development and differentiation, and is a sequence of events encoded into the genome.
- Some genes that alter or regulate life span (age-1, LAG1, RAS1 and RAS2) were identified in worm (C. elegans), fly (Drosophila) and fungi (yeast and Neurospora).
- Human disorders of premature aging (e.g., Werner's syndrome) were linked to specific genes encoding, for example, enzymes such as helicase.
- Aging was linked to the length of telomeres and to telomerase activity in aging cells.

Mutation accumulation theory

- Recurrent, deleterious, GERM LINE mutations occur
- Fewer bearers survive to express later-acting mutations
- The force of natural selection against them declines with age
- These mutations can therefore reach a higher frequency under mutation-selection balance

Deficiency of DNA repair enzymes



Damage-accumulating theories

- Damage-accumulating theories propose that aging is caused by progressive accumulation of cellular damage (different theories propose different damages, see table below).
- The accumulation of damaged molecules in cells is thought to result over time due to failure of repair and maintenance systems.

Theory	Summary
Free radical	Random deleterious effects of free radicals are produced during normal aerobic metabolism.
Immediate survival	Ageing occurs because nature selects for genes that have immediate survival value, but with long-term damaging consequences.
Cross-linkage Error catastrophe	Random cross-links of DNA and proteins disrupt function Cumulative random errors in protein synthesis.
Glycation	Formation of glycated proteins and other molecules leads to AGE formation and serious disruption of cell function
Longevity determinants	Ageing is caused by the products of cellular metabolism and the rate of ageing is governed by protection against these damaging products.
Membrane hypothesis	Membrane damage leads to decreased elimination of waste products, decreased protein synthesis and loss of water from the cytoplasm leading to decreased enzymic activities.
Entropy	Mechanism (e.g. caloric restriction) that reduce the rate of entropy production, liberating energy more slowly, delay molecular deterioration.

Somatic Mutation

- Genetic mutations occur and accumulate with age in the somatic cell causing the cell to:
  - Deteriorate
  - Malfunction
- Accumulation of mutations result in :
  - Damage to the DNA
    The theory states that aging is an imbalance between DNA's ability to repair itself and accumulating DNA damage.
  - When the damage exceeds the repair, the cell malfunctions and this can lead to senescence.

The Classical Genetic Approach

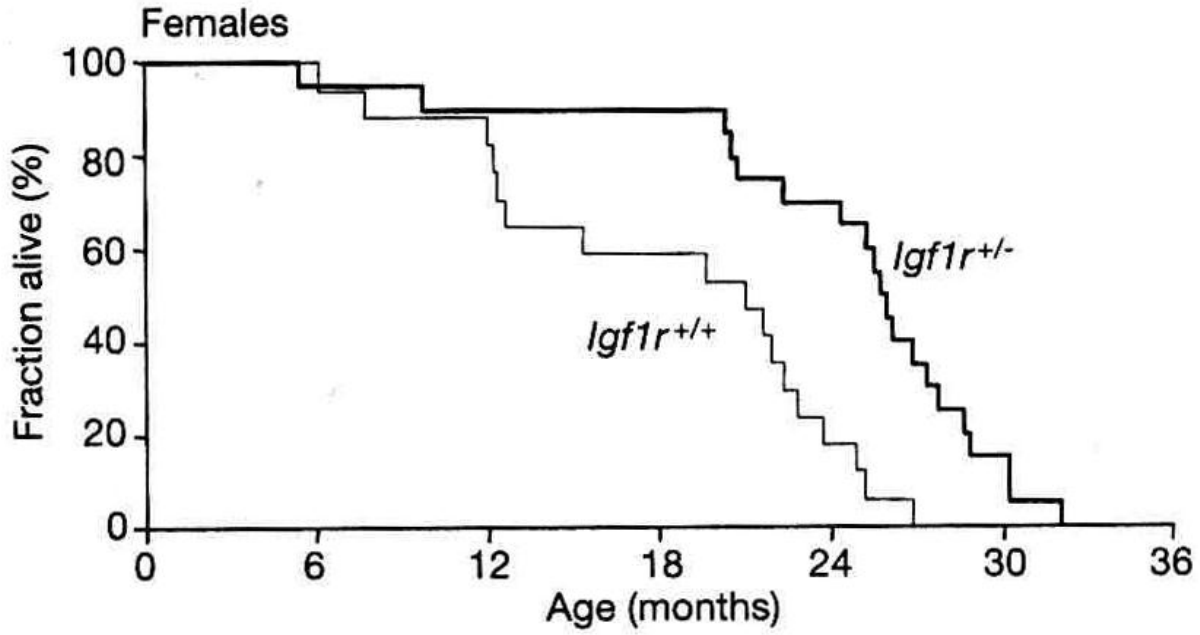
- Isolate mutants with altered rates of aging
  - Map, clone and sequence genes concerned
- Identify lifespan-determining proteins, biochemistry, etc
- Understand aging?

Physiological mechanism of Aging

- Insulin/IGF-1 signalling pathway genes are strongly implicated in aging effects - these genes regulate metabolism and stress responses, affect maintenance functions
- Dietary restriction after adulthood reduces effects of aging & leads to increased lifespan, in lab animals (yeast, worms, Daphnia, Drosophila, mice, primates).
- Molecular basis of this effect is rapidly being uncovered

IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice

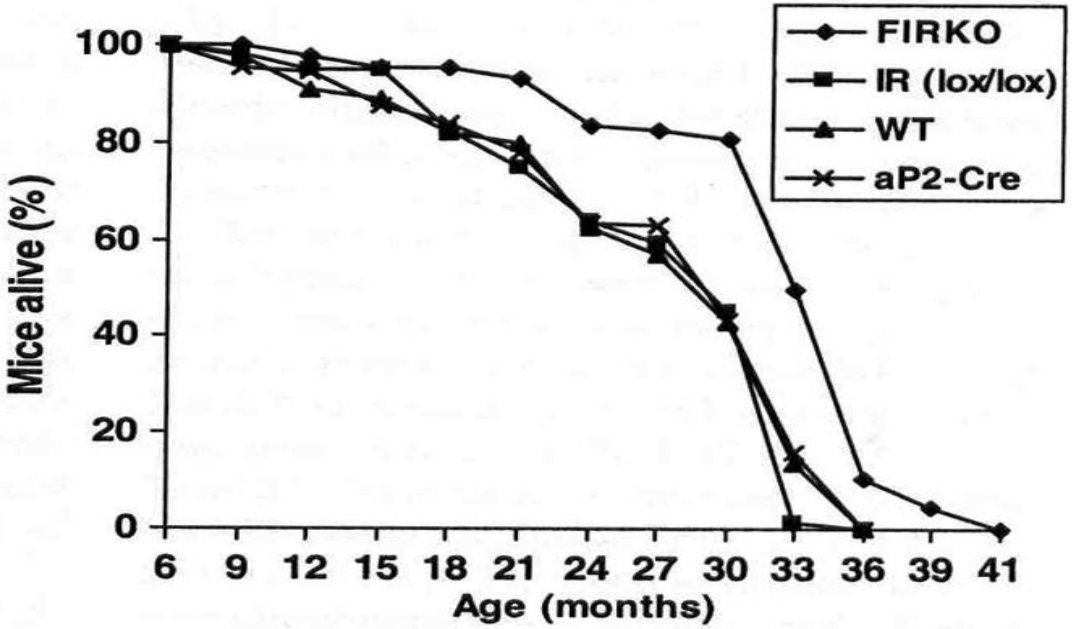
- Holzenberger (2002): Mice heterozygous for a deletion of the IGF-1 receptor gene
- Resistant to oxidative stress
- Increased mean lifespan (33% females, males not long lived)



Holzenberger *et al*. *Nature* 2002

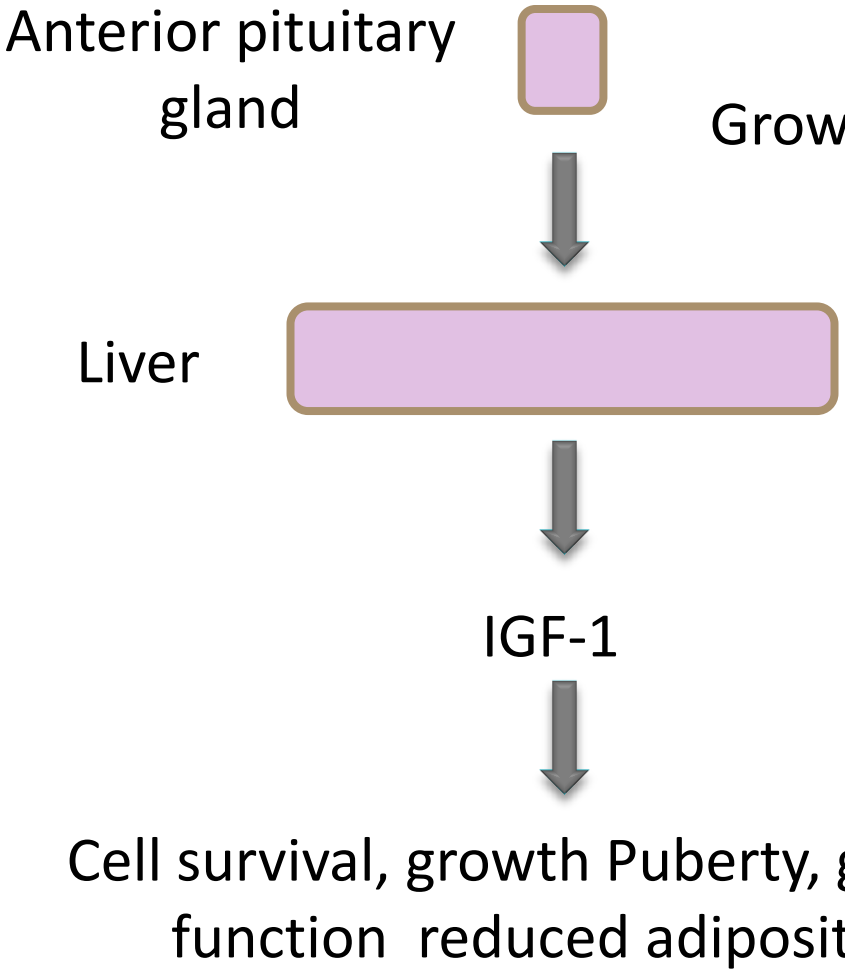
Extended longevity in mice lacking the insulin receptor in adipose tissue

- Ron Kahn (2003): fat-specific insulin receptor knockout (FIRKO) mouse
- Protected against age-related obesity
- 18% increase in mean lifespan in both sexes



Blüher et al. Science 2003

IGF-1, insulin-like growth factor 1



The somatotropic axis

Free Radical Theory

- During aging, damage produced by free radicals cause cells and organs to stop functioning.
- A free radical is a molecule with an unpaired, highly reactive electron. One type of very reactive free radical is the oxygen free radical, which may be produced during metabolism or as a result of environmental pollution.
  - Oxygen free radicals are formed in your cells, naturally, during the oxidation of food to water and carbon dioxide.

The free radical theory of aging

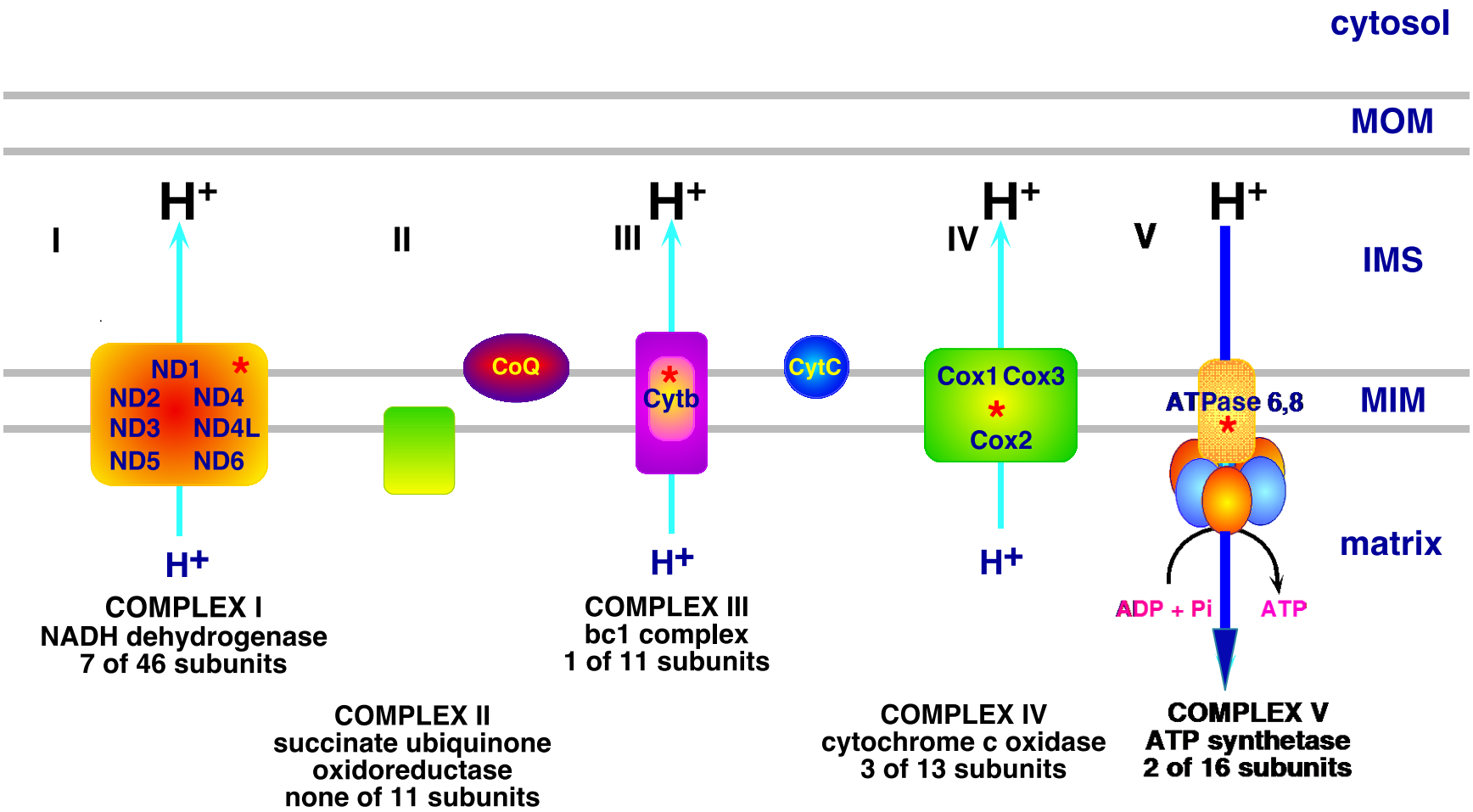
Denham Harman (1956)

"A free radical is any species capable of independent existence (hence the term 'free') that contains one or more unpaired electron"

$$O_2 + e^- \Rightarrow O_2^{\bullet -}$$

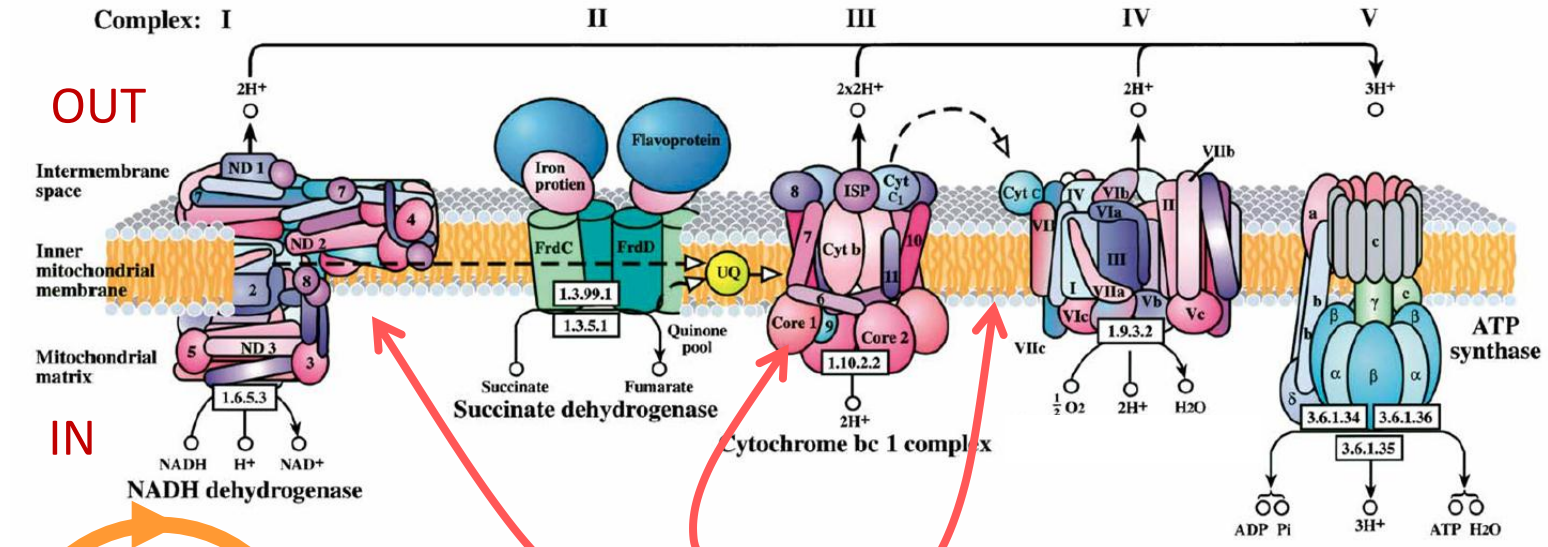
Superoxide

Mitochondrial genome encodes proteins of the oxidative phosphorylation pathway



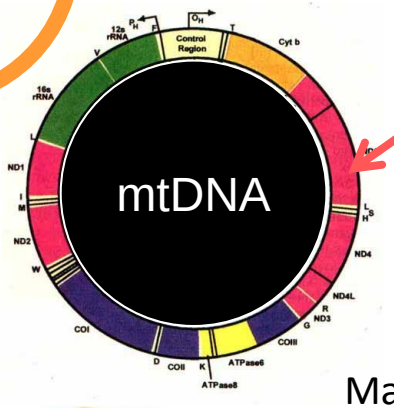
* subunits encoded by mtDNA

ROS can damage DNA, proteins and lipids



Krebs Cycle

·OH



hydroxyl radicals are highly reactive leading to damage of protein, lipids and DNA

Mandavilli et al, Mutation Research 509:127 (2002)

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Oxidative damage theory of aging

Radicals	Non-radicals
Superoxide, $O_2^{\cdot -}$	Hydrogen peroxide, H_2O_2
Hydroxyl, $OH^{\cdot}$	Hypochlorous acid, HOCl
Peroxyl, $RO_2^{\cdot}$	Ozone, O_3
Alkoxyl, $RO^{\cdot}$	Peroxynitrite, $ONOO^-$
Hydroperoxyl, $HO_2^{\cdot}$	

Reactive oxygen species (ROS)

Reactive nitrogen species (RNS)

Cellular Theories

The Hayflick Limit (1961)

Leonard Hayflick

Pre-1961: "All metazoan cells are potentially immortal. Ageing not cell autonomous"

Fibroblasts: connective tissue cells, e.g. from skin

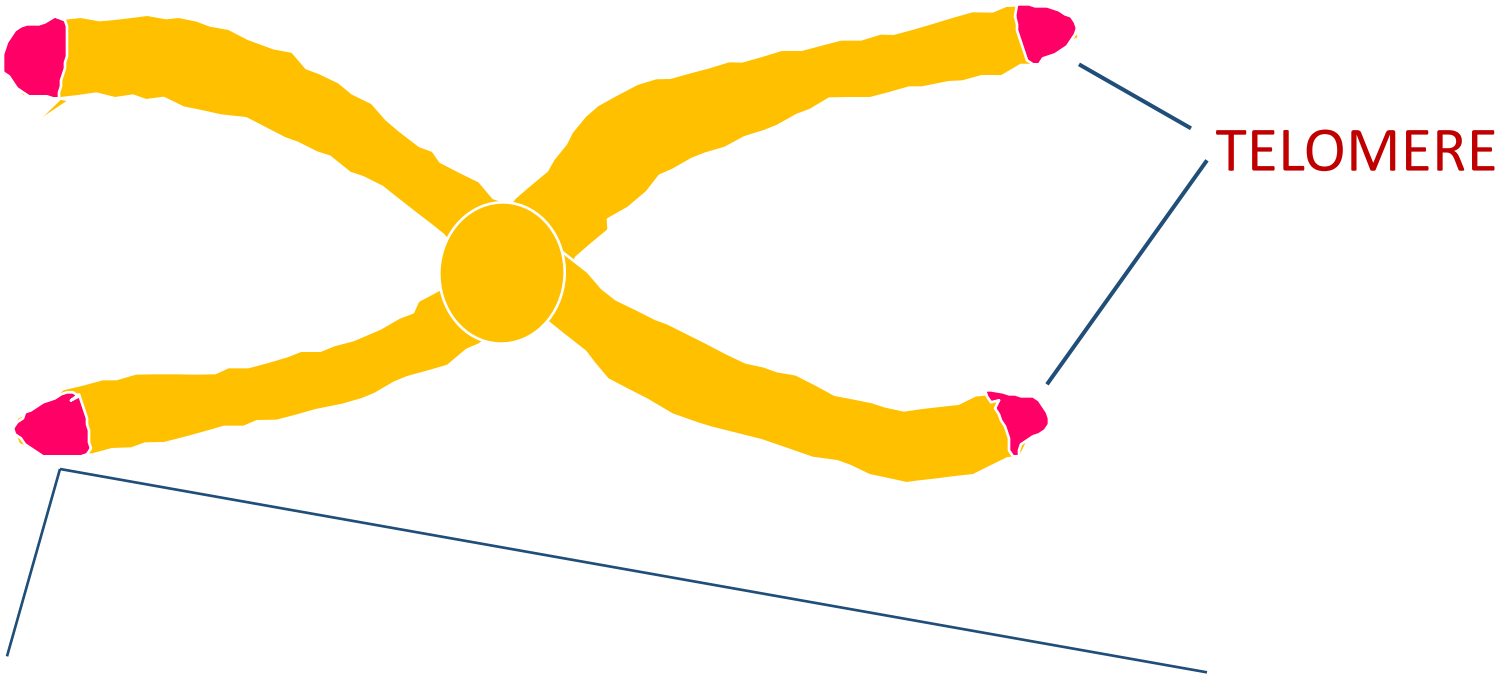
Hayflick and Moorhead (1961)

- Isolate cells from human tissue, place in culture vessel with nutrient medium
- Cells divide and form confluent layer on vessel surface
- Discard half the cells, allow remainder to grow to confluency = one passage
- Continue to passage the cells
- Cell replication slows and stops after 50 ± 10 passages: cells have reached the Hayflick limit and undergone replicative senescence

Telomeric Theory

- This is an extension of the “Hayflick Limit.”
- Telomeres are specialized DNA sequences at the end of chromosomes.
  - They shorten with each cell division.
  - When the telomeres become too short, the cell enters the senescence stage.
- In the normal process of DNA replication, the end of the chromosome is not copied exactly, which leaves an unreplicated gap.

Chromosome



TTAGGGTTAGGGTTAGGGTTAGGGTTAGGG$^{3'}$

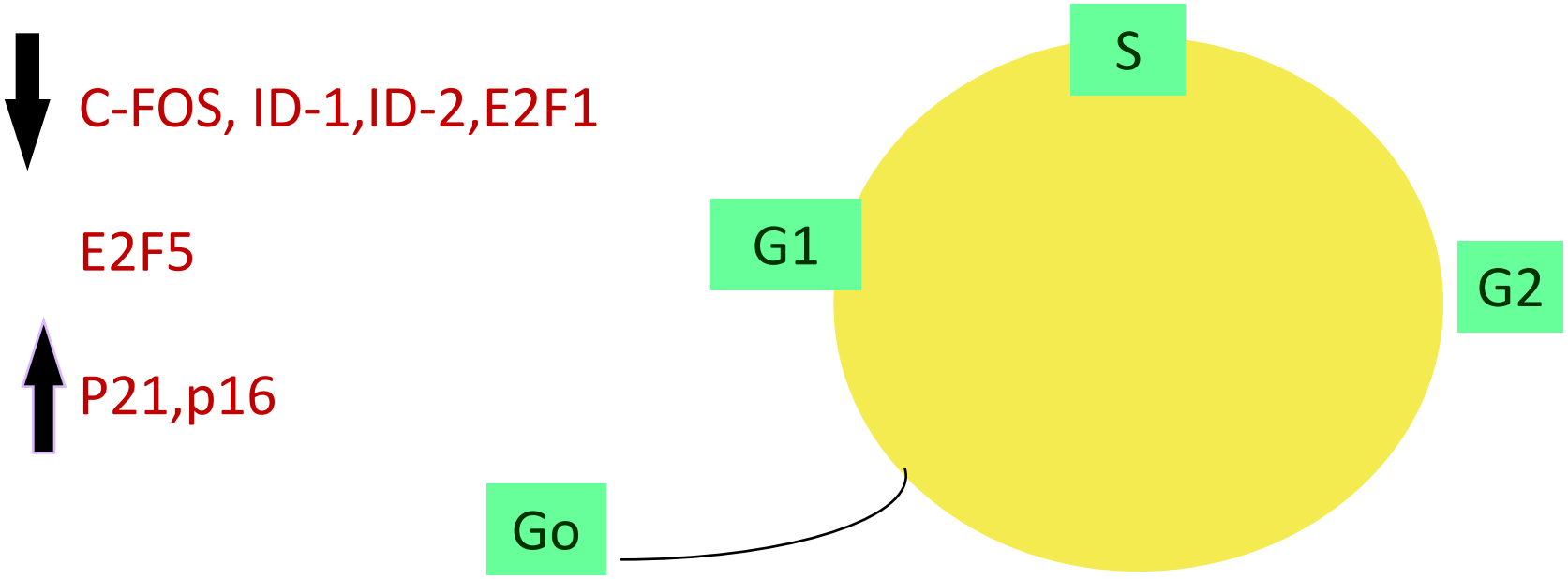
AATCCCAATCCC$^{5'}$

What are telomeres?

- Telomeres are…
  - Repetitive DNA sequences at the ends of all human chromosomes
  - They contain thousands of repeats of the six-nucleotide sequence, TTAGGG
  - In humans there are 46 chromosomes and thus 92 telomeres (one at each end)
  - Senescent cells have shorter telomeres
  - Length differs between species
  - In humans 8-14kb long
  - Telomere replication occurs late in the cell cycle

Replicative Senescence

- Telomeres shortens progressively with each cell division
- 100 base pair lost with each cell division
- Growth arrest



Telomere function…

- Telomeres are also thought to be the "clock" that regulates how many times an individual cell can divide.
- The enzyme, telomerase, fills the gap by attaching bases to the end of the chromosomes.
- As long as the cells have enough telomerase to do the job, they keep the telomeres long enough to prevent any important information from being lost as they go through each replication.
  - With time, telomerase levels decrease.
  - With decreasing telomerase levels, the telomeres become shorter and shorter.
- Once the telomere shrinks to a certain level, the cell can no longer divide. Its metabolism slows down, it ages, and dies

How Does Telomerase Work?

- Telomerase works by adding back telomeric DNA to the ends of chromosomes, thus compensating for the loss of telomeres that normally occurs as cells divide.
- Most normal cells do not have this enzyme and thus they lose telomeres with each division.

Telomeric Theory

Shortened telomeres are found in:

- Atherosclerosis
- Heart disease
- Hepatitis
- Cirrhosis

Telomeric Theory and Cancer

- 90% of cancer cells have been found to possess telomerase.
  - Telomerase prevents the telomere from shortening.
  - This allows the cancer cells to reproduce, resulting in tumor growth.
- Research areas
  - Measuring telomerase may help detect cancer.
  - Stopping telomerase may fight cancer by causing death of cancer cells.
  - Telomerase may be used to help with wound healing or the immune response.

Endocrine Theory

- Biological clocks act through hormones to control the pace of aging. Hormones effects growth, metabolism, temperature, inflammation and stress.
- Examples - Menopause
  - Decreased level of estrogen & progesterone
  - Hot flashes, insomnia

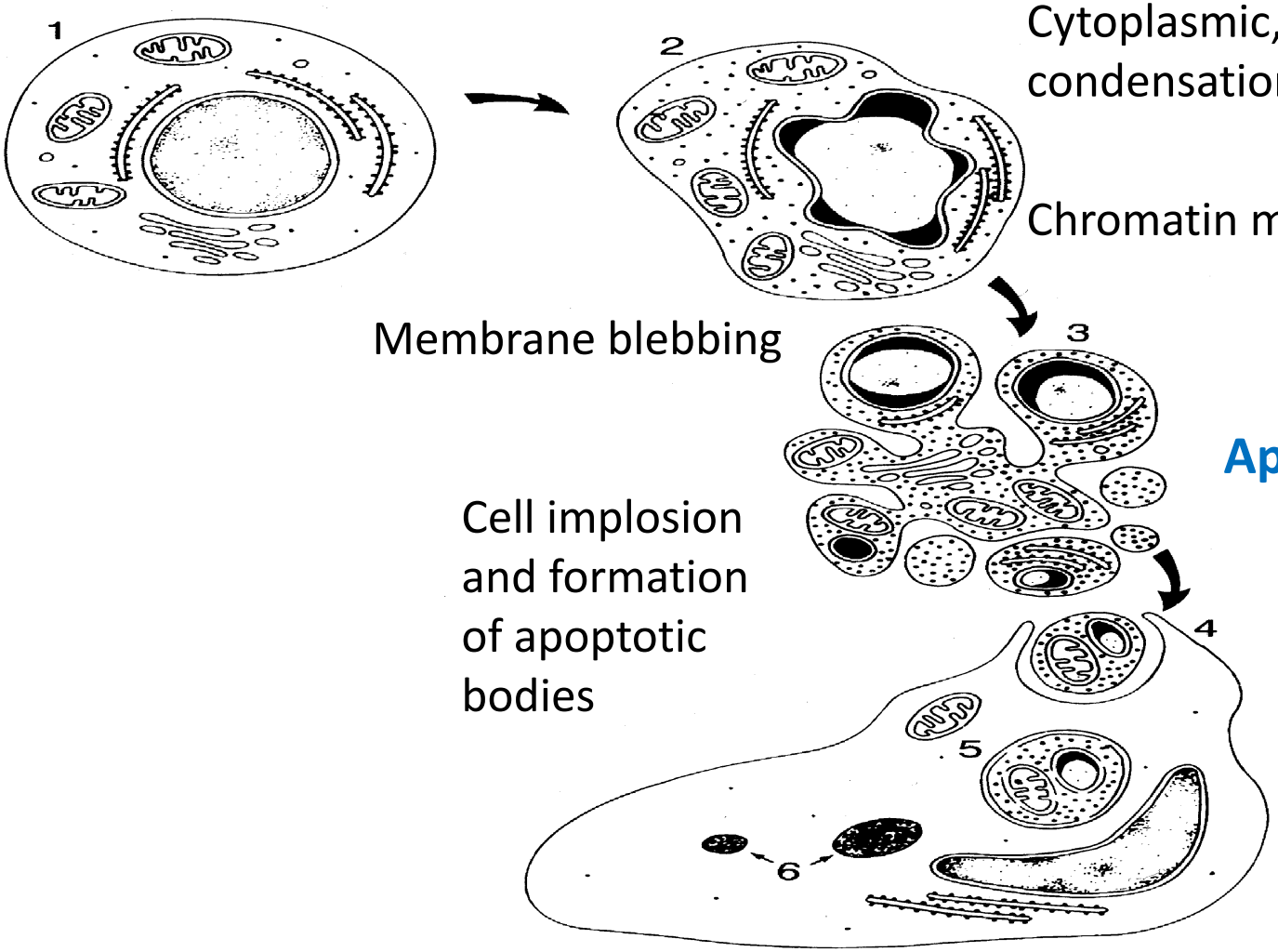
Immunologic Theory

- A programmed decline in the immune system leads to an increased vulnerability to disease, aging and death
- Example- Decreased T cells (helper cells) in adults
  - Increased diseases in older adults
  - Increased autoimmune diseases in adults

Cell Death

More than one way to die:
Necrosis and Apoptosis

More than one way to die: Necrosis and Apoptosis



Cytoplasmic, nuclear condensation

Chromatin margination

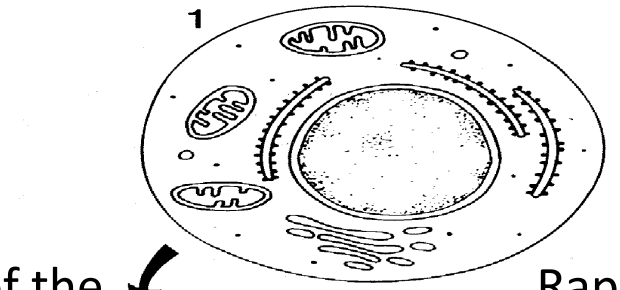
Membrane blebbing

Apoptosis

Cell implosion and formation of apoptotic bodies

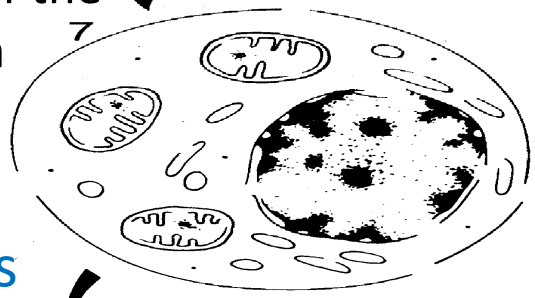
Recognition and engulfment of apoptotic bodies by phagocytic cells

More than one way to die: Necrosis and Apoptosis



Swelling of the cytoplasm

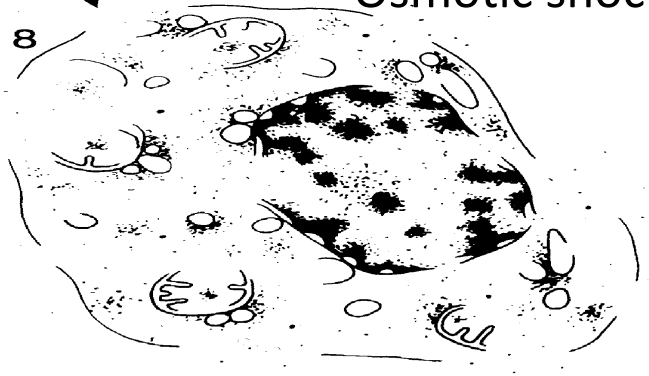
Rapid membrane permeablization



Swelling of the nucleus

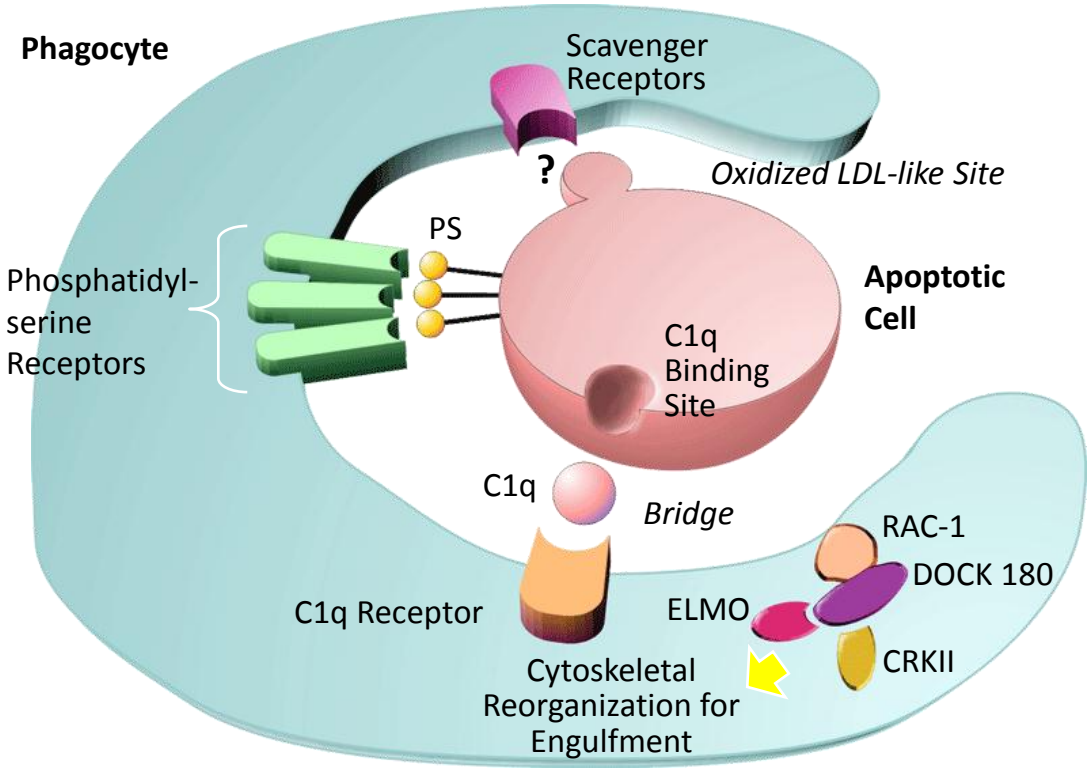
Necrosis

Osmotic shock



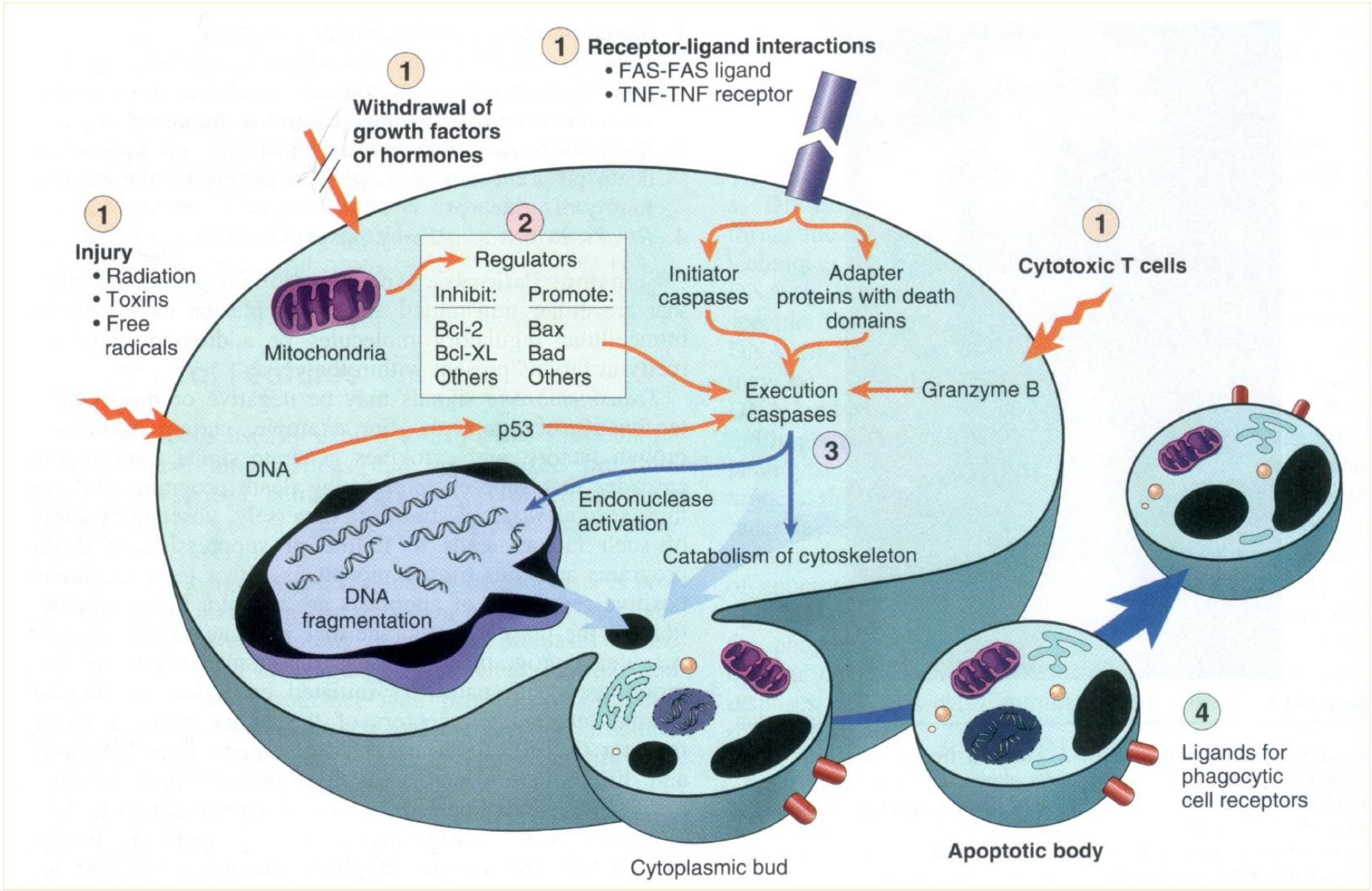
Release of intracellular content

Apoptosis and Phagocytosis



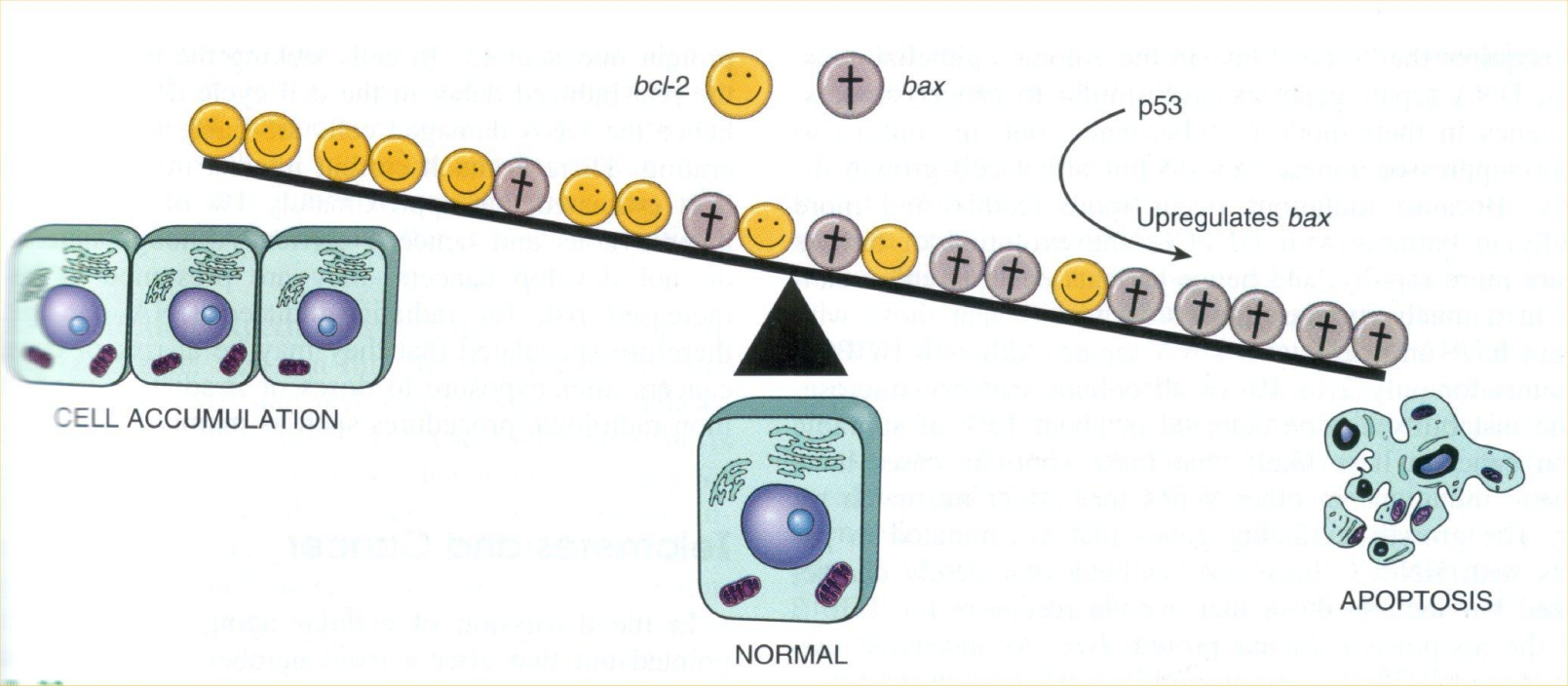
- Phagocytes recognize “eat-me” or cell corpse signals on the apoptotic cell surface. These signal the phagocyte to activate cellular engulfment machinery.
- Phosphatidylserine exposure on the target cell surface and the phosphatidylserine receptor on the phagocyte are essential for phagocytosis.
- Defining other receptors, bridge molecules, “eat-me” signals and signaling molecules involved in initiating the cytosolic changes needed for engulfment are very active areas of research. The articles listed below review current knowledge and are the sources for this diagram.

Savill, J. and Fadok, V. 2000. Nature. 407:784.
Canradt, B. 2002. Nature Cell Biol. 4:E139.

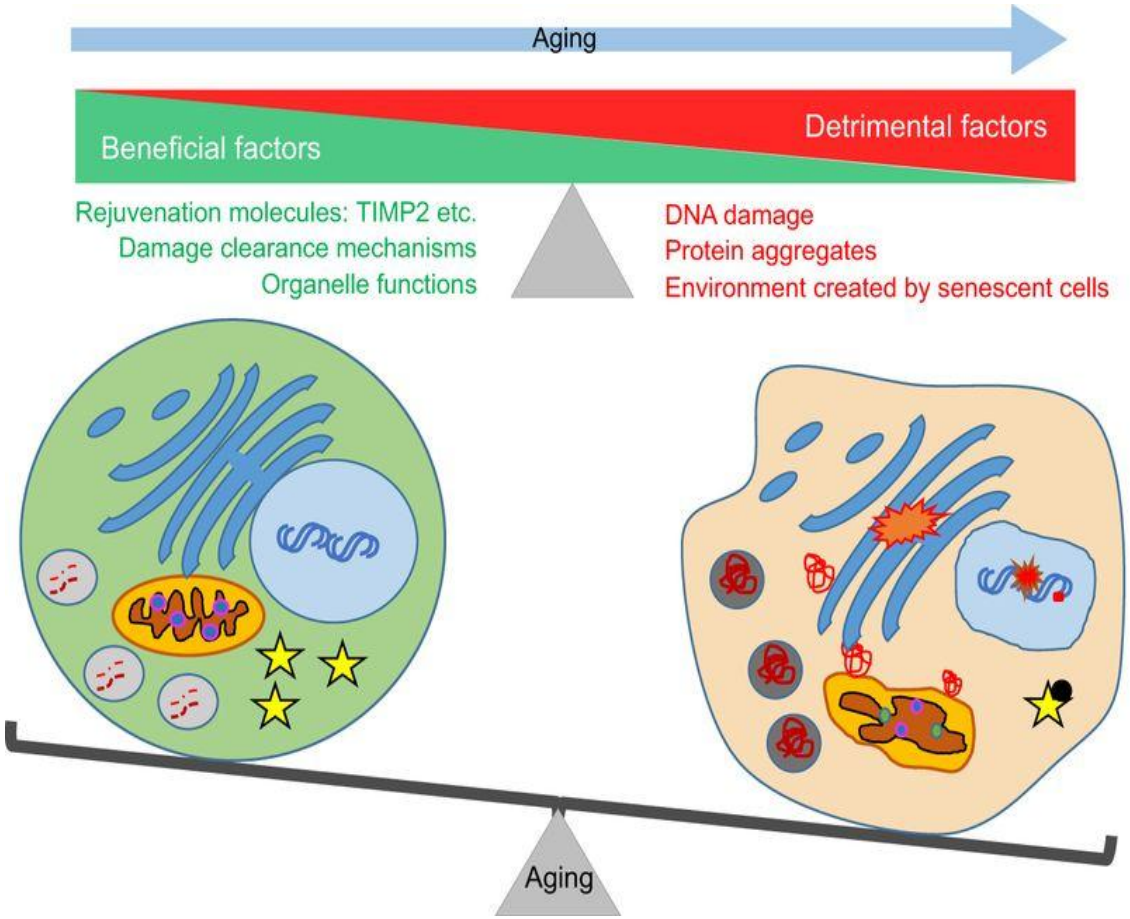

1
Receptor-ligand interactions
• FAS-FAS ligand
• TNF-TNF receptor
1
Withdrawal of growth factors or hormones
1
Injury
• Radiation
• Toxins
• Free radicals
2
Regulators
Inhibit: Promote:
Bcl-2 Bax
Bcl-XL Bad
Others Others
Mitochondria
Initiator caspases
Adapter proteins with death domains
1
Cytotoxic T cells
Execution caspases
Granzyme B
p53
DNA
3
Endonuclease activation
Catabolism of cytoskeleton
DNA fragmentation
Cytoplasmic bud
Apoptotic body
4
Ligands for phagocytic cell receptors

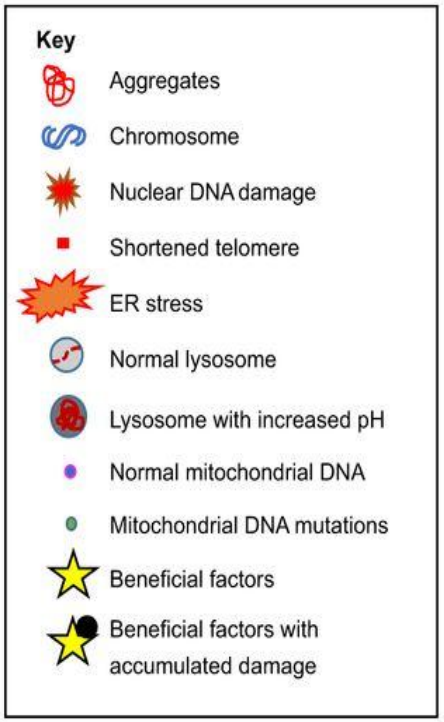
The Bcl-2 family of proteins

	Anti-apoptotic	Pro-apoptotic
Mammalian	Bcl-2 Bcl-X_L Bcl-W Mcl-1 A1	Bcl-X_s Bax Bad Bak
C. Elegans	Ced-9	
Viral	E1B-19K BHRF1	


bcl-2
bax
p53
Upregulates bax
CELL ACCUMULATION
NORMAL
APOPTOSIS

Beneficial & Detrimental factors of Aging





Ruan L, Zhang X, Li R. Recent insights into the cellular and molecular determinants of aging. J Cell Sci. 2018 Feb 2;131(3)

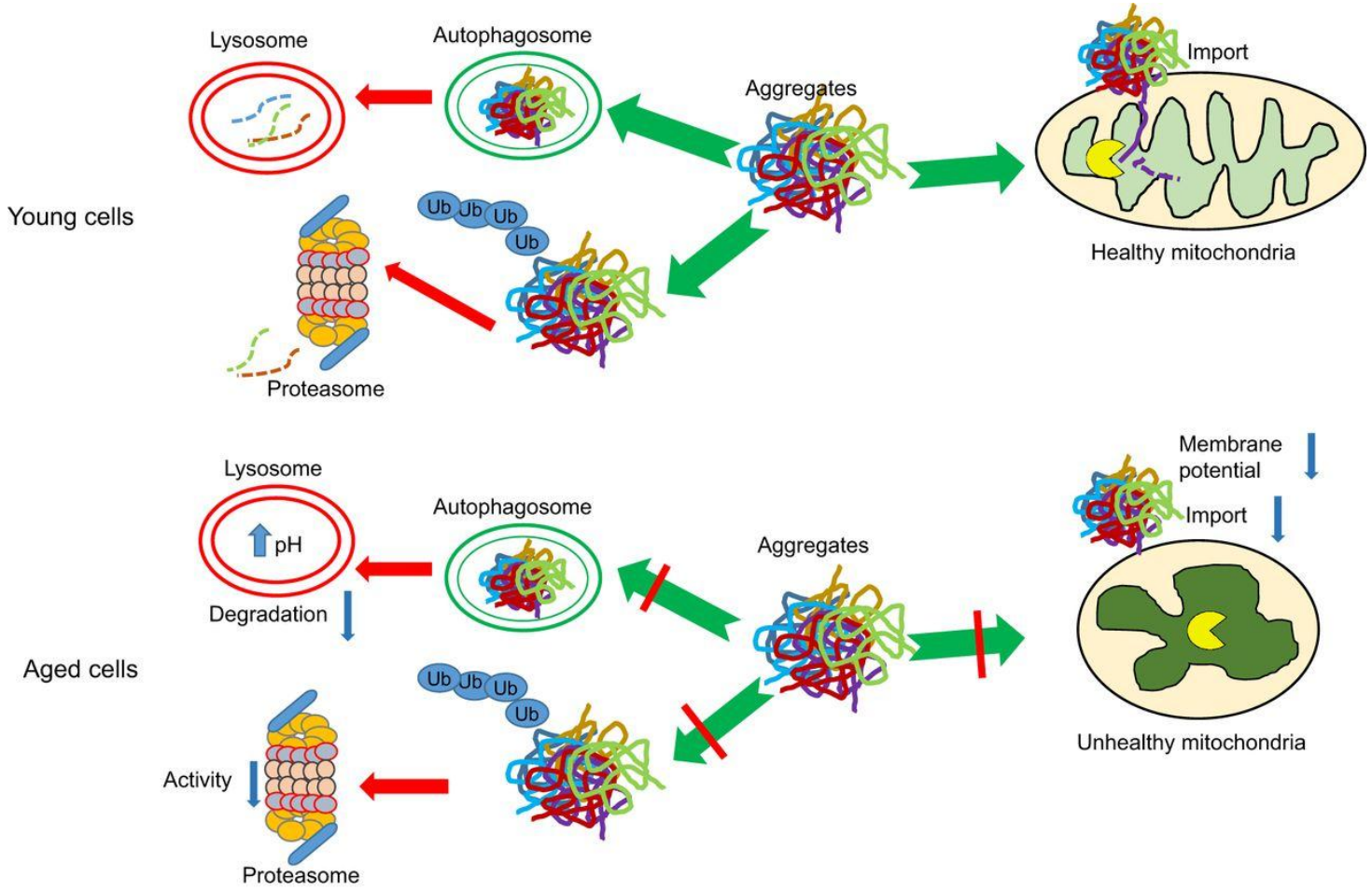
Overview of Beneficial factors

Beneficial molecules	Source	Effect	Reference
Blood from young mice	Heterochronic parabiosis of young and old mice	Reverse brain aging, improve learning and memory in aged mice	Villeda et al., 2014
TIMP2	Injection of human umbilical cord plasma to old mice	Rejuvenate the hippocampus and improve cognitive function in aged mice	Castellano et al., 2017
GnRH	Injection of GnRH to old mice	Promote adult neurogenesis at hypothalamus and hippocampus	Zhang et al., 2013
GDF11	Daily injection of GDF11 to old mic	Enhance neurogenesis	Katsimpardi et al., 2014
REST	Conditional knockout in the mouse brain	Repress genes that promote cell death and Alzheimer's disease pathology	Lu et al., 2014
SIRT3	Overexpression via lentiviral transduction in old mice cells	Reduce oxidative stress and rejuvenate aged HSCs	Brown et al., 2013
SIRT1	Brain specific overexpression in transgenic mice	Increase lifespan	Satoh et al., 2013
RanGAP	Overexpression of RanGAP in the eyes of a fly model that expresses C9orf72 repeats	Reverse age-dependent degeneration of fly eyes	Zhang et al., 2015
BubR1	Overexpression in transgenic mice	Maintain genomic integrity and extend lifespan	Baker et al., 2013
ABT263	Oral administration of ABT263 that targets senescent cells	Eliminate senescent cells in aged mice and rejuvenate the stem cells	Chang et al., 2016

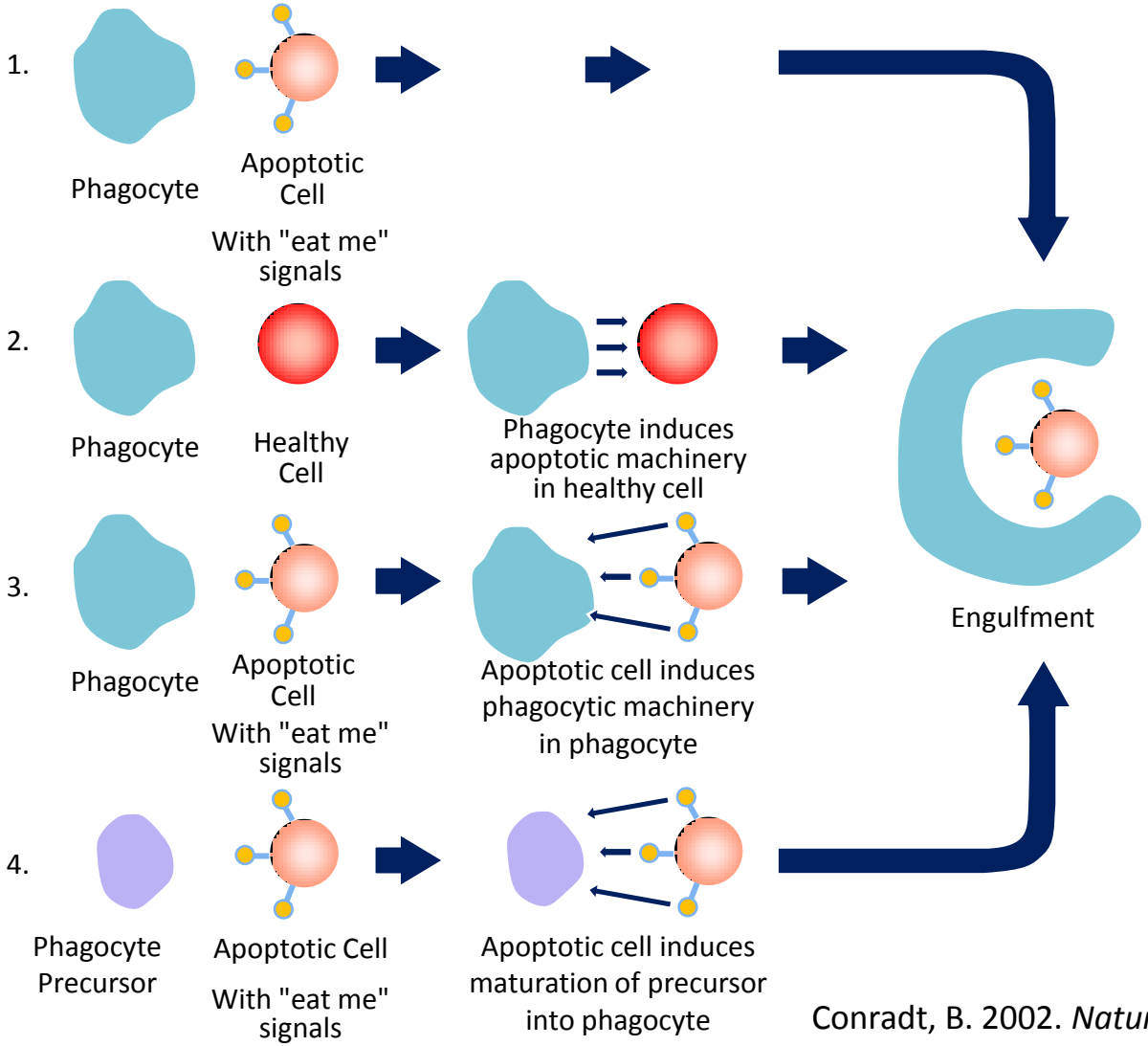
Conclusion

- Genetic basis of aging
- The oxidative damage theory seems to link all other aging theories.
- IGF-1 receptor regulates lifespan
- Telomerase theory
- The only experimentally proven treatment that can improve lifespan in mammals (and most likely humans) is restricted calorie intake

How protein aggregate are removed?

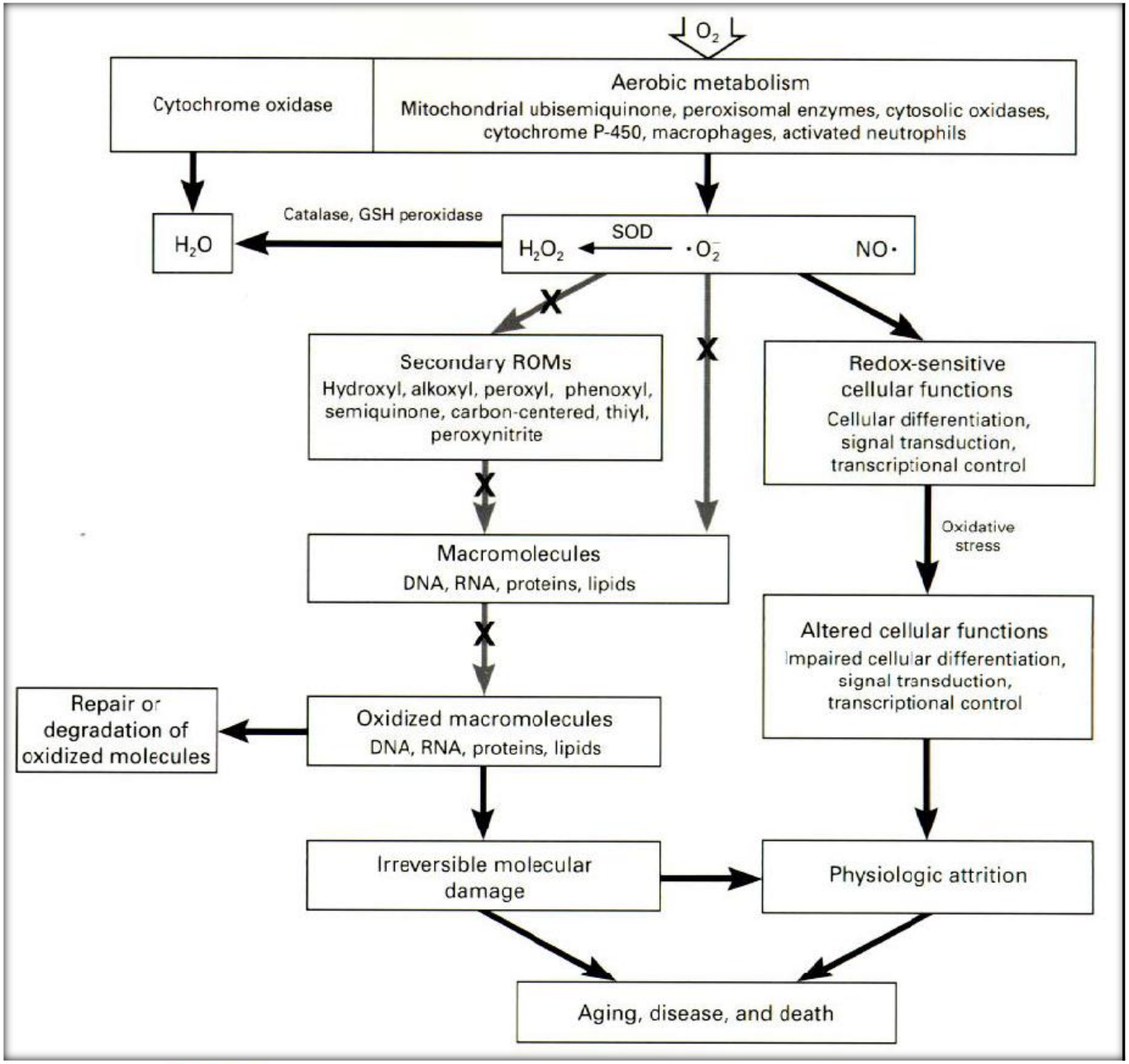


Apoptosis and Phagocytosis

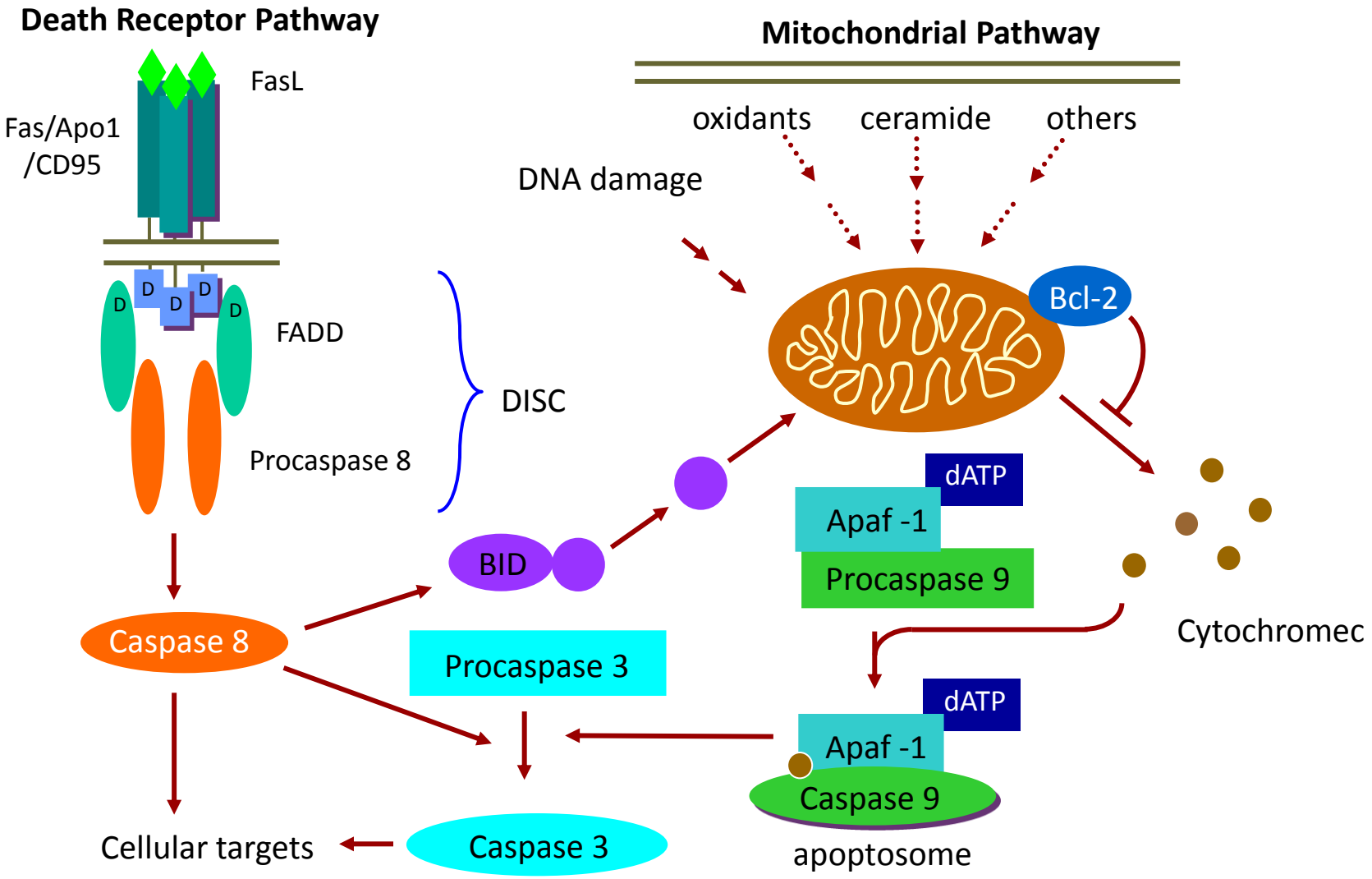


- The first pathway shows the engulfment of an apoptotic cell exposing “eat-me” signals.
- Data from mammalian systems and genetic studies from Caenorhabditis elegans have shown that phagocytes and target cells have several types of interactions.
- Conradt has proposed several models (2-4) to indicate the more complex phagocyte-target interactions.

Conradt, B. 2002. *Nature Cell Biol*. **4**:E139.
Greene, D.R. and Beere, H.M. 2001. *Nature*. **412**:133.


O_2
Cytochrome oxidase
Aerobic metabolism
Mitochondrial ubisemiquinone, peroxisomal enzymes, cytosolic oxidases, cytochrome P-450, macrophages, activated neutrophils
Catalase, GSH peroxidase
H_2O
SOD
H_2O_2
$\cdot O_2^-$
NO·
Secondary ROMs
Hydroxyl, alkoxyl, peroxyl, phenoxyl, semiquinone, carbon-centered, thiyl, peroxynitrite
Redox-sensitive cellular functions
Cellular differentiation, signal transduction, transcriptional control
Oxidative stress
Macromolecules
DNA, RNA, proteins, lipids
Altered cellular functions
Impaired cellular differentiation, signal transduction, transcriptional control
Repair or degradation of oxidized molecules
Oxidized macromolecules
DNA, RNA, proteins, lipids
Irreversible molecular damage
Physiologic attrition
Aging, disease, and death

Major Apoptotic Pathways in Mammalian Cells



Hengartner, M.O. 2000. *Nature*. **407**:770.

Green, D. and Kroemer, G. 1998. *Trends Cell Biol.* **8**:267.