Aging: The Biology of Senescence

Biological aging, termed **senescence** (process of ageing), is one of the most complex biological processes.

Aging can be defined as the time-related deterioration of the physiological functions necessary for survival and fertility.

Aging is defined as the progressive accumulation of damage over time, leading to disturbed function on the cellular, tissue and organ level and eventually to disease and death. It is a multifactorial process where genetic, endogenous and environmental factors play a role.

Aging has been associated with **a loss of complexity** in a wide range of physiological processes and anatomic structures.

Many evolutionary biologists would deny that aging is part of the genetic repertoire (prepared to perform) of an animal. Rather, they would consider aging to be the **default state** occurring after the animal has fulfilled the requirements of natural selection.

After its offspring are born and raised, the animal can die. Indeed, in many organisms, from moths to salmon, this is exactly what happens. As soon as the eggs are fertilized and laid, the adults die.

The **maximum life span** is a characteristic of the species. It is the maximum number of years a member of that species has been known to survive. The maximum human life span is estimated to be **121** years.

The life spans of **tortoises** and lake trout are both unknown, but are estimated to be more than **150** years.

The maximum life span of a domestic **dog is about 20** years, and that of a laboratory mouse is 4.5 years.

A ***Drosophila*** fruit fly survives to eclose (in the wild, over 90% die as larvae), it has a maximum life span of **3 months**.

Life expectancy is defined as the age at which half the population still survives.

It is the length of time an individual of a given species can expect to live, is not characteristic of species but of populations.

Life expectancy is the **key metric for assessing population health**.

In **1947**, when India became independent from British rule, life expectancy was around **32 years**. Improvements in public health and medical services have led to substantial control of specific infectious diseases which translated into significant decreases in mortality rates.

Life expectancy at birth rose steadily and by **1990** had reached 60 years (**60.51 for females and 60.31 for males**).

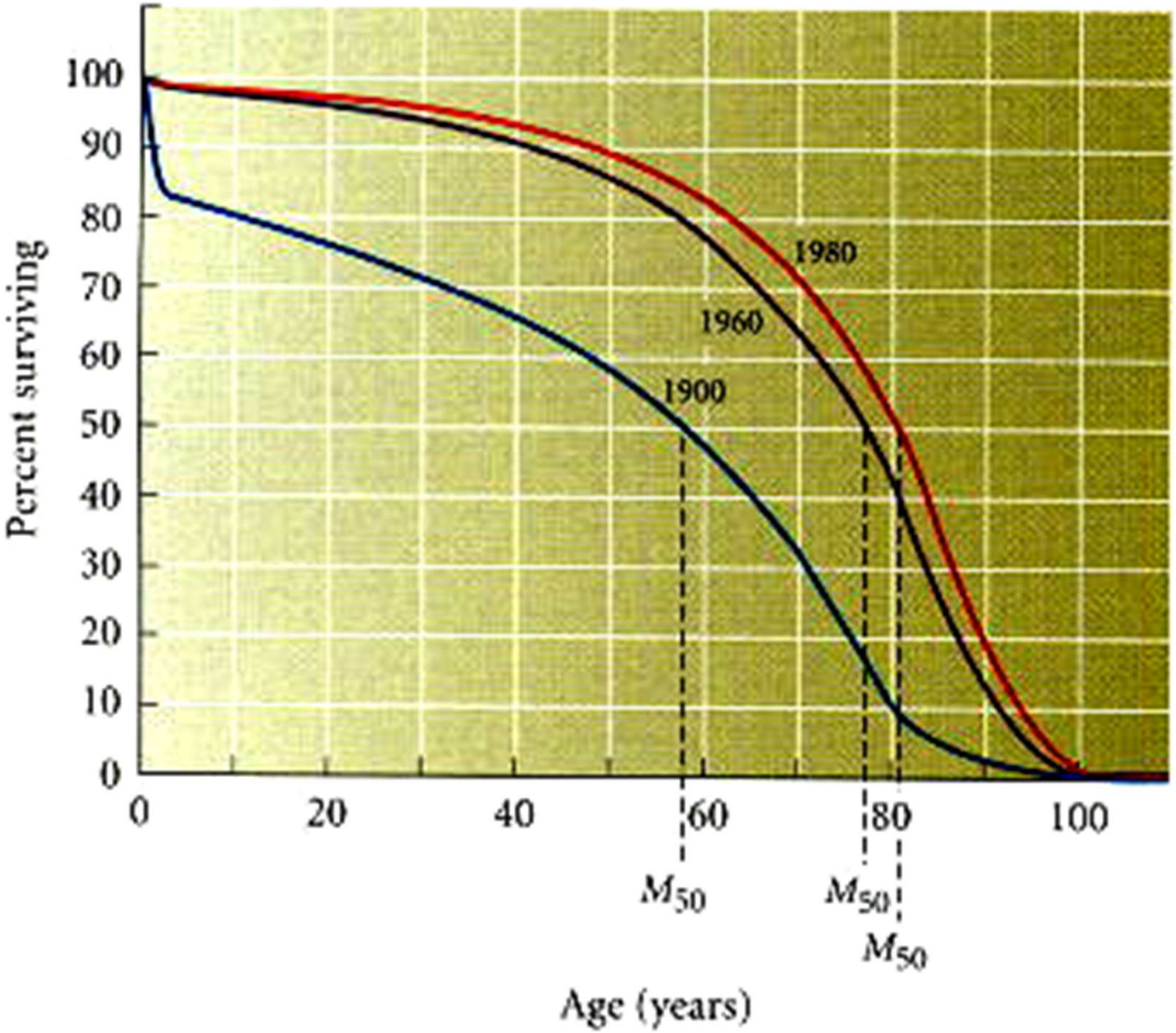
Population ageing is the most significant emerging demographic phenomenon in the world.

In 1950, the world population aged 60 years and above was 205 million (8.2 per cent of the population) which increased to 606 million (10 per cent of the population) in 2000.

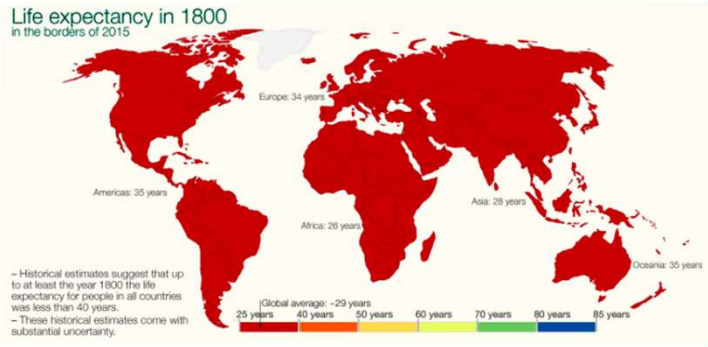
By 2050, the proportion of older persons 60 years and above is projected to rise to 21.1 per cent, which will be two billion in number. Asia has the largest number of world's elderly (53 per cent), followed by Europe (25 per cent).

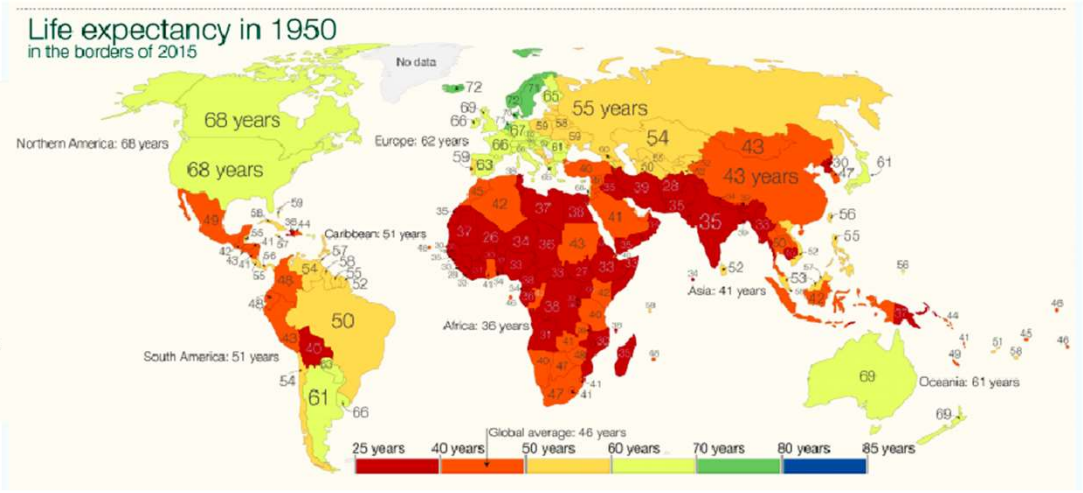
This pressure of increasing numbers of elderly will intensify.

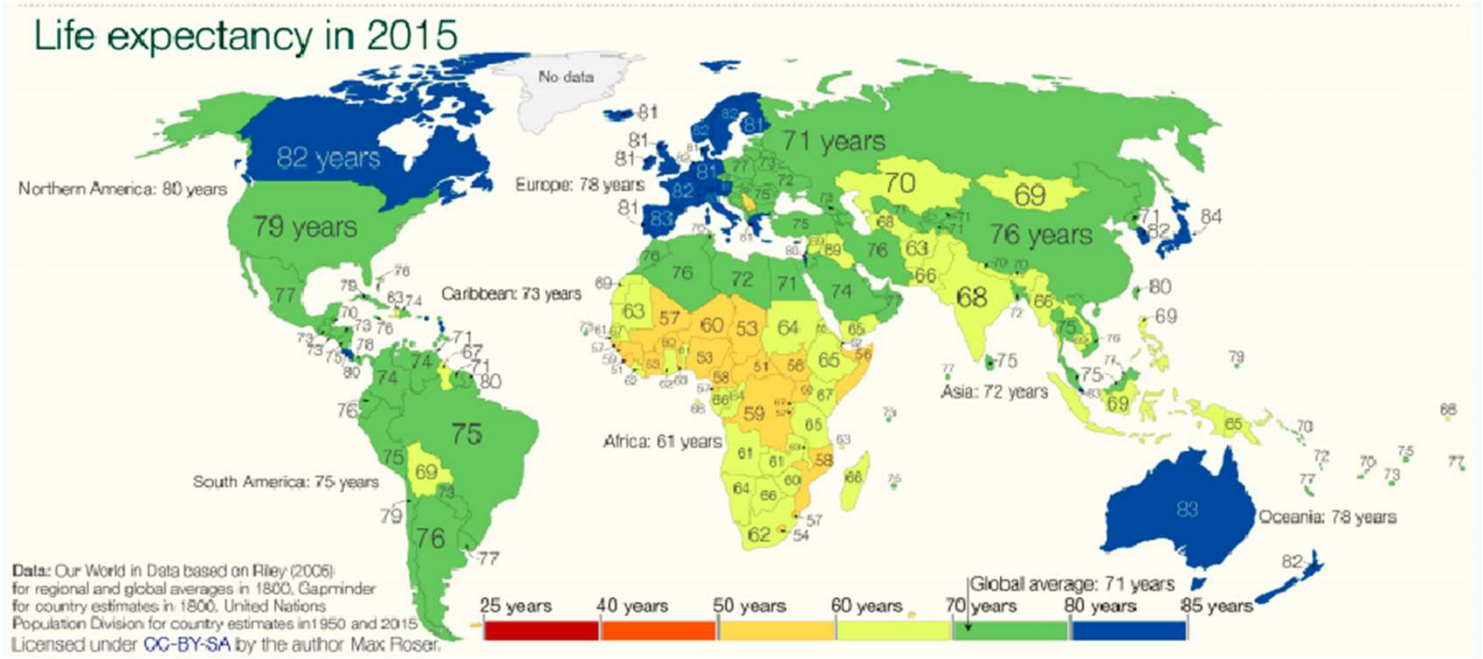
In 2050, 82 per cent of the world's elderly will be in developing regions of Asia, Africa, Latin America and the Caribbean while only 16 per cent of them will reside in the developed regions of Europe and North America.

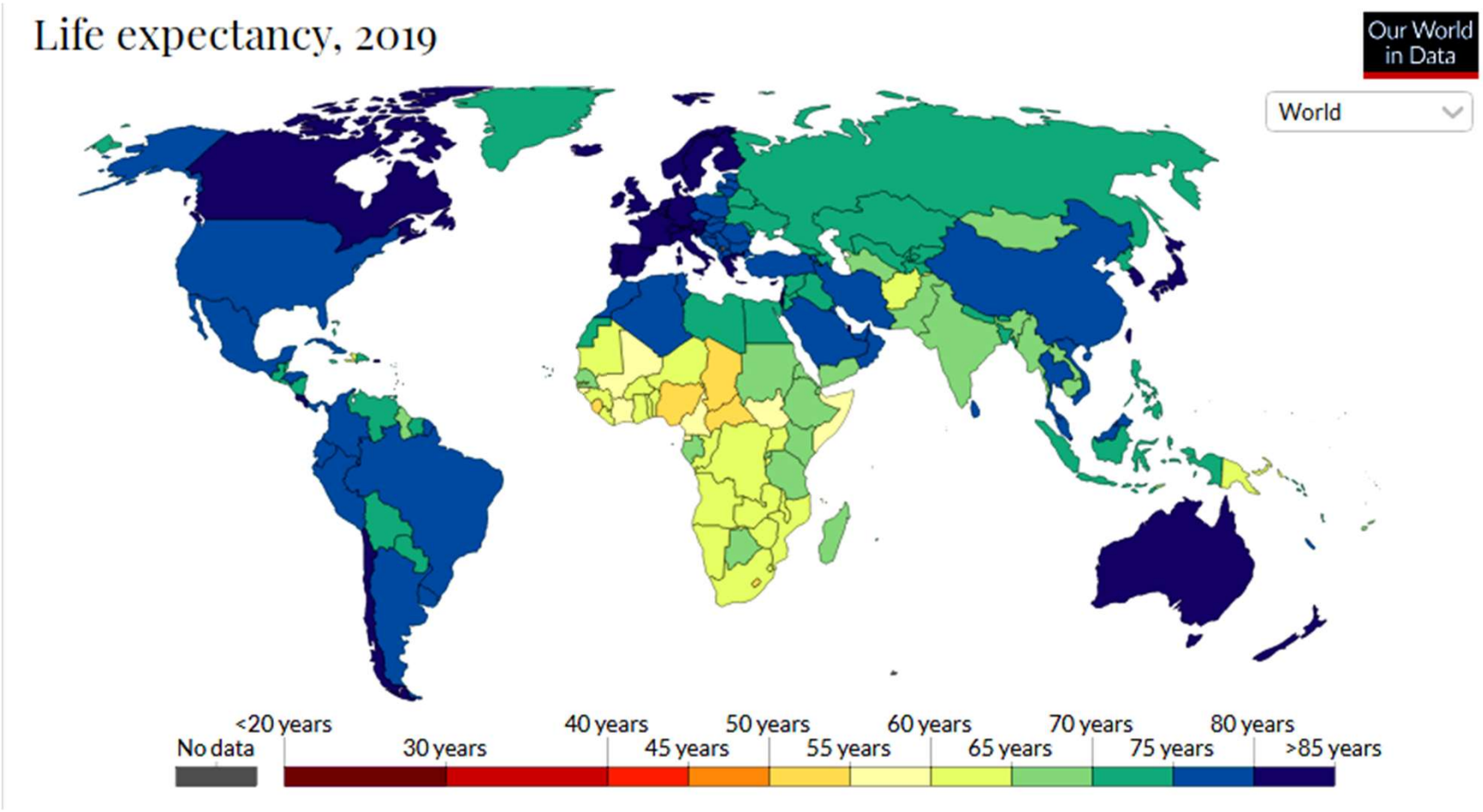


Survival curves for U.S. females in 1900, 1960, and 1980. M_{50} represents the age at which 50% of the individuals of each population survived.


Life expectancy in 1800
in the borders of 2015
Europe: 34 years
Americas: 35 years
Asia: 28 years
Africa: 26 years
Oceania: 35 years
– Historical estimates suggest that up to at least the year 1800 the life expectancy for people in all countries was less than 40 years.
– These historical estimates come with substantial uncertainty.
Global average: ~29 years
25 years
40 years
50 years
60 years
70 years
80 years
85 years


Life expectancy in 1950
in the borders of 2015
No data
Northern America: 68 years
Europe: 62 years
Caribbean: 51 years
South America: 51 years
Africa: 36 years
Asia: 41 years
Oceania: 61 years
Global average: 46 years
25 years
40 years
50 years
60 years
70 years
80 years
85 years


Life expectancy in 2015
No data
Northern America: 80 years
Europe: 78 years
Caribbean: 73 years
South America: 75 years
Africa: 61 years
Asia: 72 years
Oceania: 78 years
Global average: 71 years
25 years
40 years
50 years
60 years
70 years
80 years
85 years
Data: Our World in Data based on Riley (2006) for regional and global averages in 1800, Gapminder for country estimates in 1800, United Nations Population Division for country estimates in1950 and 2015
Licensed under CC-BY-SA by the author Max Roser.



Source: UN Population Division (2019)

Period life expectancy at birth, the average number of years a newborn would live if the pattern of mortality in the given year were to stay the same throughout its life

India=69.7years **Global average= 72.6**

Some species have been identified that apparently **do not age** or have *negligible senescence. Older individuals do not appear to be weaker, less agile, less reproductive, more susceptible to disease, or otherwise less fit than younger animals.*

(Ages of some wild animals can be determined by annual marks in scales or bones similar to tree rings.)

Determining the maximum age a long-lived animal can achieve is generally not possible because the vast majority of deaths are caused by external causes and very old individuals are very rare. Some species with age of oldest recorded specimen:–

Rougheye Rockfish 205
– **Lake Sturgeon 152 Years**
– **Aldabra Tortise 152 Years**
– **Koi 226 Years**
– **Bowhead Whale 211 Years**

Non-aging species tend to defeat simple deterioration theories and suggest dramatically longer human life spans are possible.

Rougheye Rockfish 205

Lake Sturgeon 152 Years

Aldabra Tortise 152 Years

Koi fish 226 Years

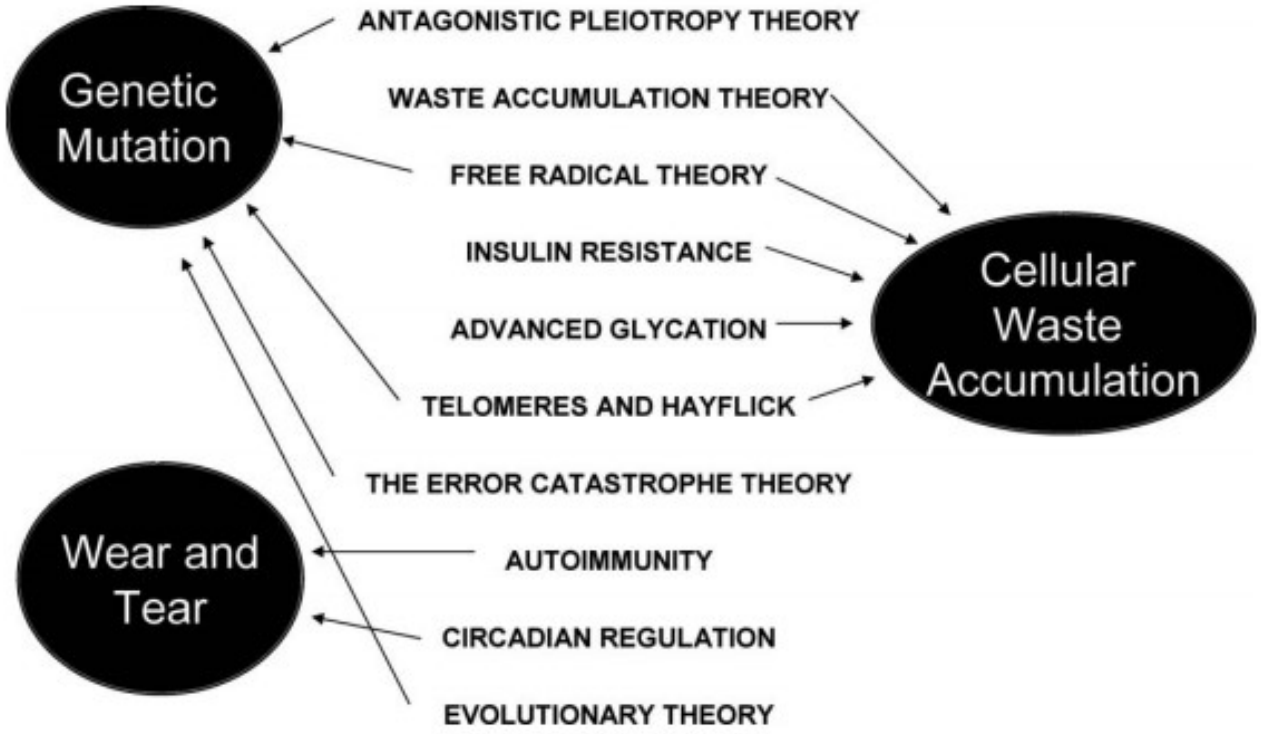
Bowhead Whale 211 Years

Theories of Ageing

Ageing is a multi-factorial process. Most hypotheses on the underlying mechanisms of the ageing process involve deterioration of the maintenance of homeostatic metabolic, inflammatory, and/or redox processes in cells and tissues.

There are more than **three hundred theories** of ageing. But , in general terms, all can be classified into three big groups: the **genetic mutation theories**, the **wear and tear theories**, and the **cellular waste accumulation theories.**

Some theories may be included in two groups, for instance the **free radical theory** of aging shares characteristics of the **genetic mutation**, as well as the cellular **waste accumulation** theories.



Classification and brief description of main theories of aging

Biological Level/Theory	Description
Evolutionary	
Mutation accumulation*	Mutations that affect health at older ages are not selected against.
Disposable soma*	Somatic cells are maintained only to ensure continued reproductive success; after reproduction, soma becomes disposable.
Antagonistic pleiotropy*	Genes beneficial at younger age become deleterious at older ages.
Molecular	
Gene regulation*	Aging is caused by changes in the expression of genes regulating both development and aging.
Codon restriction	Fidelity/accuracy of mRNA translation is impaired due to inability to decode codons in mRNA.
Error catastrophe	Decline in fidelity of gene expression with aging results in increased fraction of abnormal proteins.
Somatic mutation	Molecular damage accumulates, primarily to DNA/genetic material.
Dysdifferentiation	Gradual accumulation of random molecular damage impairs regulation of gene expression.
Cellular	
Cellular senescence-Telomere theory*	Phenotypes of aging are caused by an increase in frequency of senescent cells. Senescence may result from telomere loss (replicative senescence) or cell stress (cellular senescence).
Free radical*	Oxidative metabolism produces highly reactive free radicals that subsequently damage lipids, protein and DNA.
Wear-and-tear	Accumulation of normal injury.
Apoptosis	Programmed cell death from genetic events or genome crisis.
System	
Neuroendocrine*	Alterations in neuroendocrine control of homeostasis results in aging-related physiological changes.
Immunologic*	Decline of immune function with aging results in decreased incidence of infectious diseases but increased incidence of autoimmunity.
Rate-of-living	Assumes a fixed amount of metabolic potential for every living organism (live fast, die young).

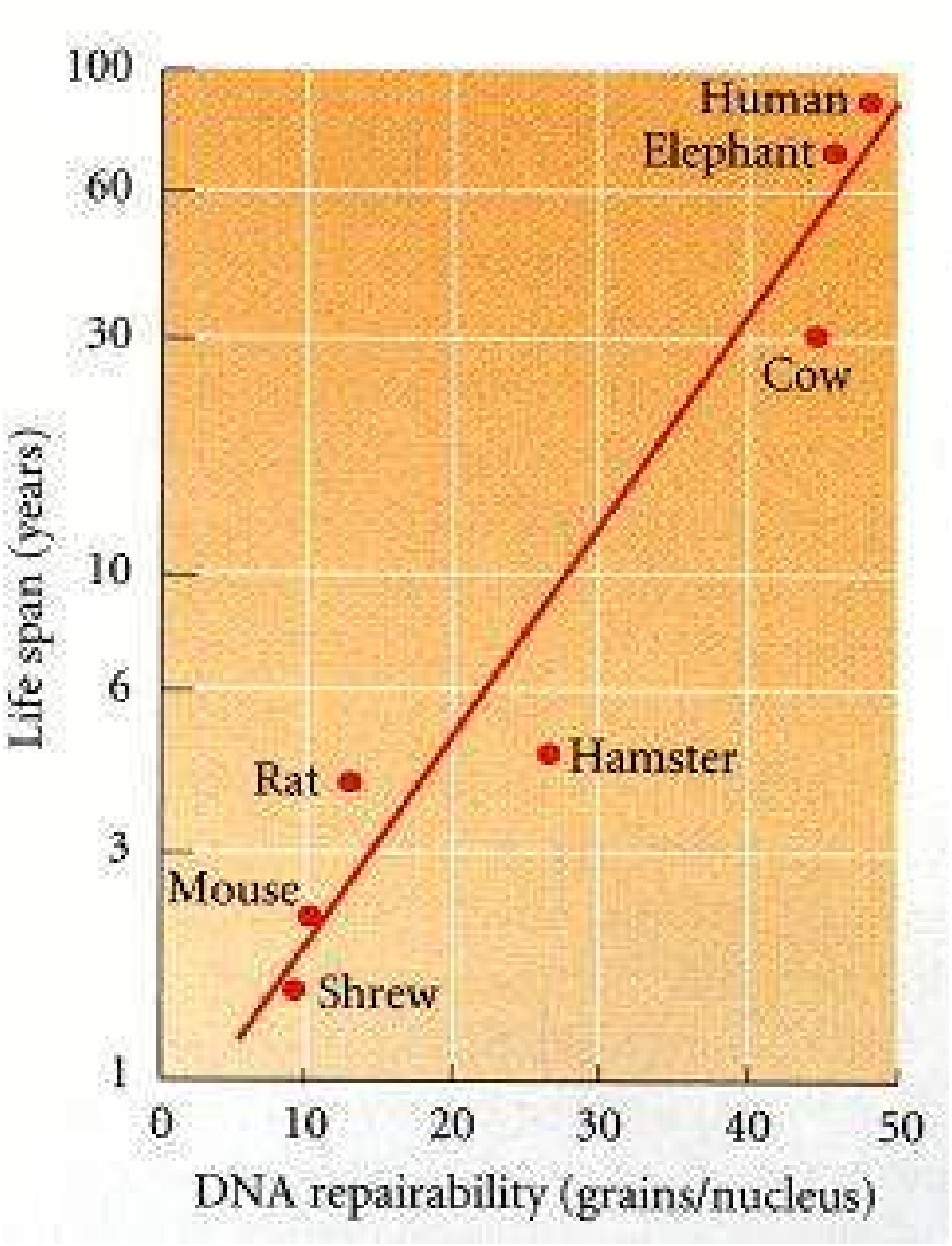
General wear-and-tear theory

Dr. August Weismann, a German biologist, first introduced this theory in 1882. He believed that the body and its cells were damaged by overuse and abuse. The organs and skin are worn down by toxins in the diet and in the environment. Wear and the tear are not confined to the organs; it also takes place at the cellular level.

As one gets older, small traumas to the body build up. Point mutations increase in number, and the efficiencies of the enzymes encoded by our genes decrease. Moreover, if a mutation occurred in a part of the protein synthetic apparatus, the cell would make a large percentage of faulty proteins.

If mutations arose in the DNA-synthesizing enzymes, the rate of mutations would be expected to increase markedly. Faulty DNA polymerases in senescent cells have been evident. Likewise, DNA repair may be important in preventing senescence, and species whose members' cells have more efficient DNA repair enzymes live longer .

Moreover, genetic defects in DNA repair enzymes can produce premature aging syndromes in humans.



Correlation between life span and the ability of fibroblasts from various mammalian species to repair DNA.

Capacity for repair is represented in autoradiography by the number of grains from radioactive thymidine per cell nucleus.

The Neuroendocrine Theory

This theory was described in the year 1954 by Dr. Vladimir Dilman, a Russian scientist. The neuroendocrine theory of aging states that "The effectiveness of the body's homeostatic adjustments declines with aging—leading to the failure of adaptive mechanisms, ageing and death."

This theory has also been referred to as the **aging clock theory**. Consistent with this theory, the **hypothalamic pituitary adrenal (HPA) axis,** the main regulatory system controlling homeostasis in humans, loses efficiency with aging. The HPA system works by the interplay of various hormonal signals that initiate reactions in target tissues coupled with a negative feedback mechanism (the produced substances inhibit their own production) to allow for fine control of body functions, such as blood pressure, fluid and electrolyte levels, and body temperature.

This theory proposes that **aging is due to changes in neural and endocrine functions that are crucial for**

1. Coordinating communication and responsiveness of all body systems with the external environment
2. Programming physiological responses to environmental stimuli; and
3. Maintaining an optimal functional state for reproduction and survival while responding to environmental demands.

Hormones are vital for repairing and regulating the bodily functions, and when aging causes a drop in the hormone production, it causes a decline in body's ability to repair and regulate itself as well.

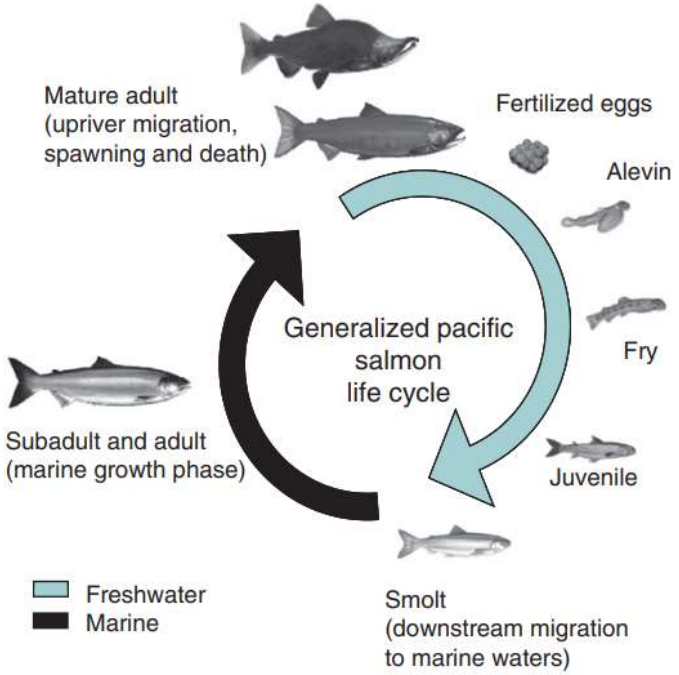
Thus, hormone replacement therapy, a frequent component of any anti-aging treatment, helps to reset the body's hormonal clock and so can reverse or delay the effects of aging and thus keeping young.

The Genetic Control Theory

This theory states that humans are born with a unique genetic code, a predetermined tendency to certain types of physical and mental functioning, and that the genetic inheritance has a great deal to say about how quickly one becomes aged and how long lives.

Each person has a biological clock. When that clock goes off, it signals the bodies first to age and then to die. However, the timing on this genetic clock is subject to enormous variation, depending on what happens with the growth and on how one actually lives (quality of life, feeding, sanitation and health care practices).

This theory should be restricted to cases where there is a specific control of the onset of ageing by an identifiable metabolic process and a functional role for senescence can be demonstrated for that species, e.g. **the rapid ageing and death of the Pacific salmon after spawning.**



Generalized life cycle for Pacific salmonids.

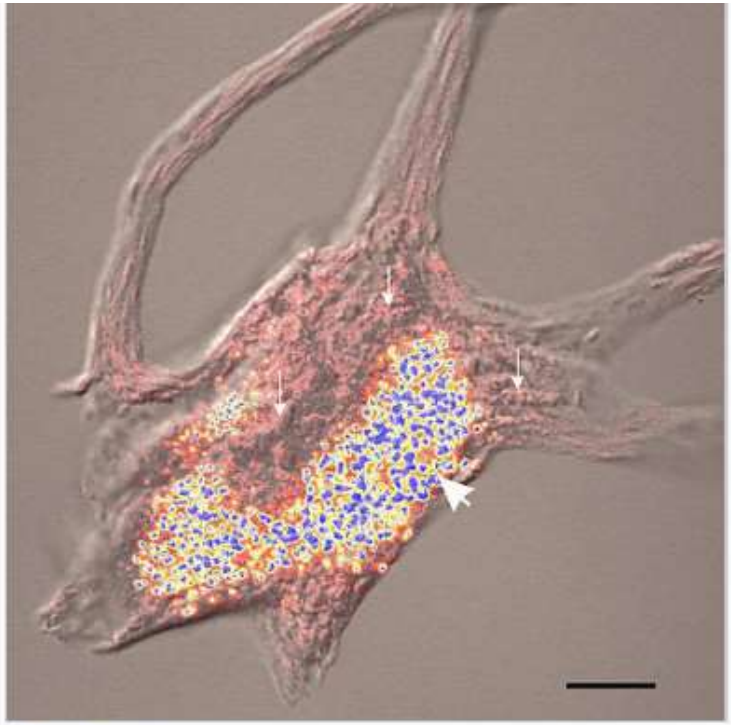
Waste (undegradable by-products of metabolism) Accumulation Theory of Ageing

In the course of life span the cells produce more waste than they can properly eliminate. This waste can include various toxins (free radicals, histones, aldehydes, lipofuscins) which when accumulated to a certain level, can interfere with normal cell function, ultimately killing the cell.

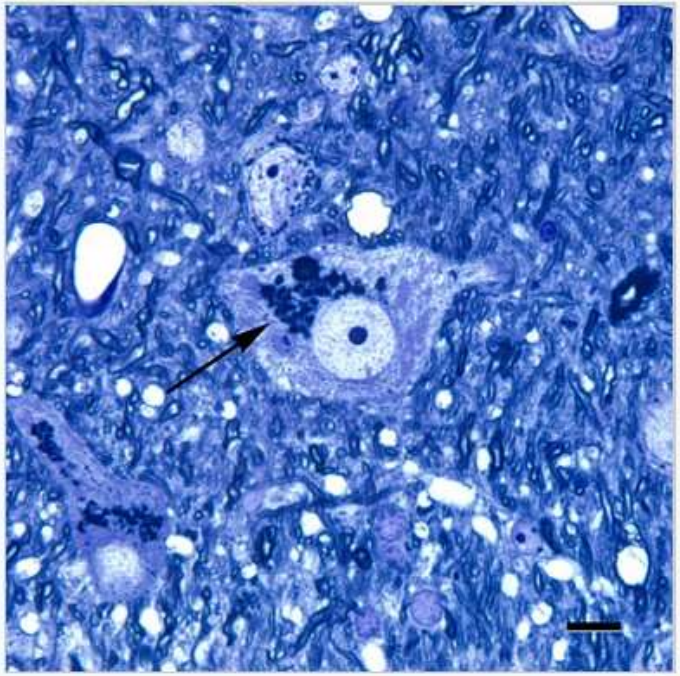
The only mechanism by which these agents are diluted in the cells **is cell division**. This only applies to replicative cells. The challenge for multicellular organisms such as the human organism is that many cell types lose replicative capacity or divide slowly, even though they remain active throughout the lifespan. These cells, including cardiomyocytes and brain neurons, accumulate metabolic waste that eventually affects normal cell functioning.

A common by-product of cellular metabolism seems to be lipofuscin (LF): Lpofuscin (age pigment) is a brown-yellow, electron-dense, autofluorescent material that accumulates progressively over time. LF is composed of highly oxidized cross-linked macromolecules (proteins, lipids, and sugars) with multiple metabolic origins. LF cannot be digested in the ubiquitin-proteasome system, because of highly oxidized proteins, polymeric and highly cross-linked nature. LF cannot be cleared by exocytosis, and accumulate within the lysosomes and cell cytoplasm of long-lived post-mitotic and senescent animal cells. LF inclusions from different animal and human tissues (myocardium, brain, liver, thyroid) are composed mainly of proteins and lipids, 30-70 and 20-50%, respectively. A small amount of carbohydrates (4.7%) and traces of metals are also found in LF granules.

The accumulation of non-degradable material can occur in the intra- and extracellular environments. Among the extracellular deposits found in humans, **cholesterol-containing plaques** and their oxidized derivatives in blood vessels, as well as protein polymers, such as **β-amyloid** in the central nervous system.



Confocal image of a spinal motor neuron showing stained lipofuscin granules in blue and yellow.

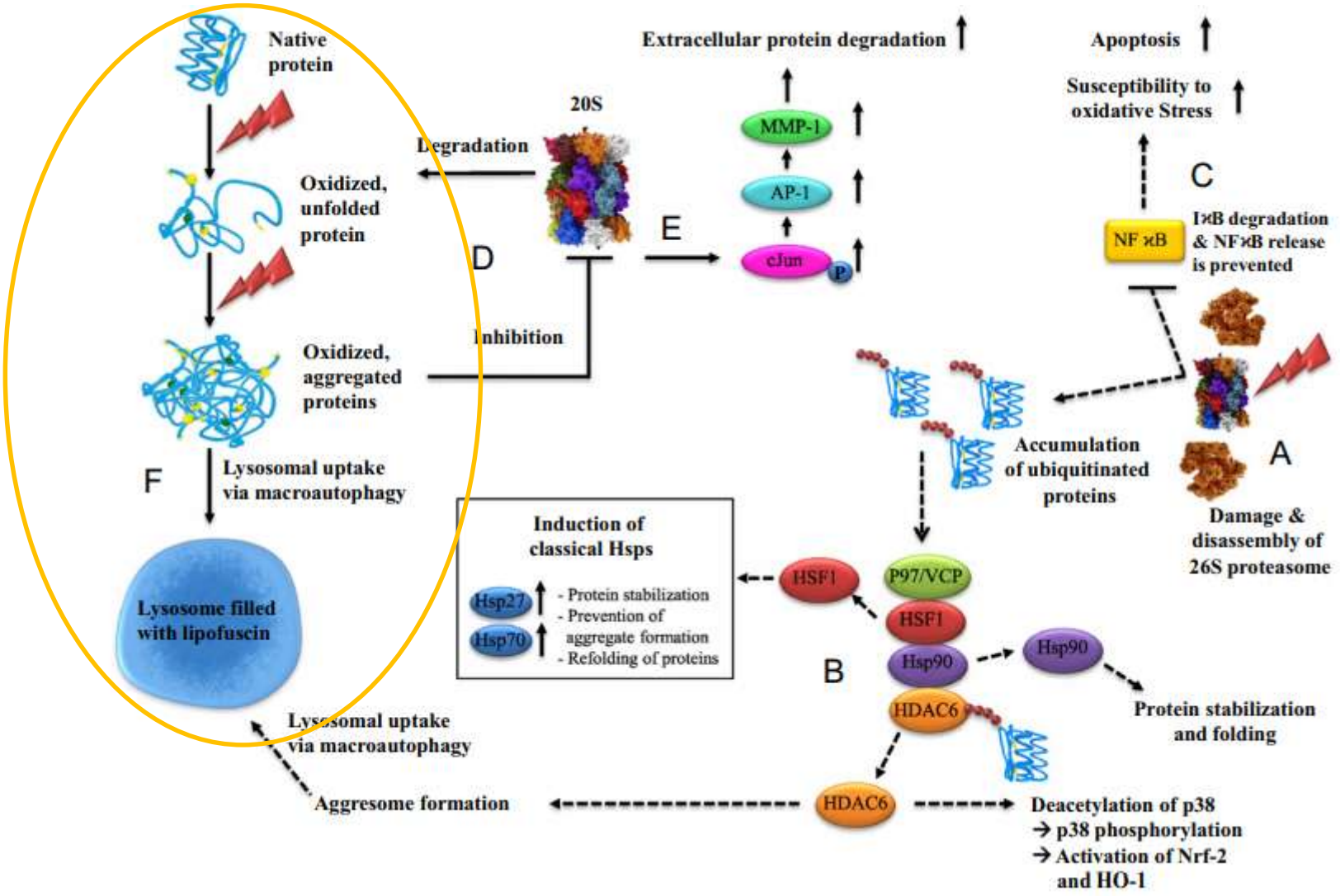


Micrograph showing a cluster of lipofuscin particles (arrow) in a nerve cell of the brain; toluidine blue stain; scale bar = 10 microns (0.01 millimeters)

In unstressed situations protein homeostasis is balanced by folding and stabilization of proteins by chaperones of the Hsp family and the controlled degradation of proteins by the proteasomal system. The proteasome exists in different forms, and its activity is modulated by multiple regulators. The 20S core proteasome contains the proteolytic activity and selectively degrades a multitude of oxidized proteins, as well as other substrates, in an ubiquitin- and ATP-independent manner. When the core 20S proteasome combines with two 19S regulators, the 26S proteasome is formed which selectively removes polyubiquitinated proteins.

Under stress conditions and, therefore, most notably during aging the balance between protein damage and clearance of damaged proteins is disturbed leading to a **malfunctioning of proteostasis** and an accumulating mass of **oxidized proteins**, aggregate and aggresome formation and finally to the accumulation of highly cross-linked materials such as **lipofuscin**, compromising cell viability.

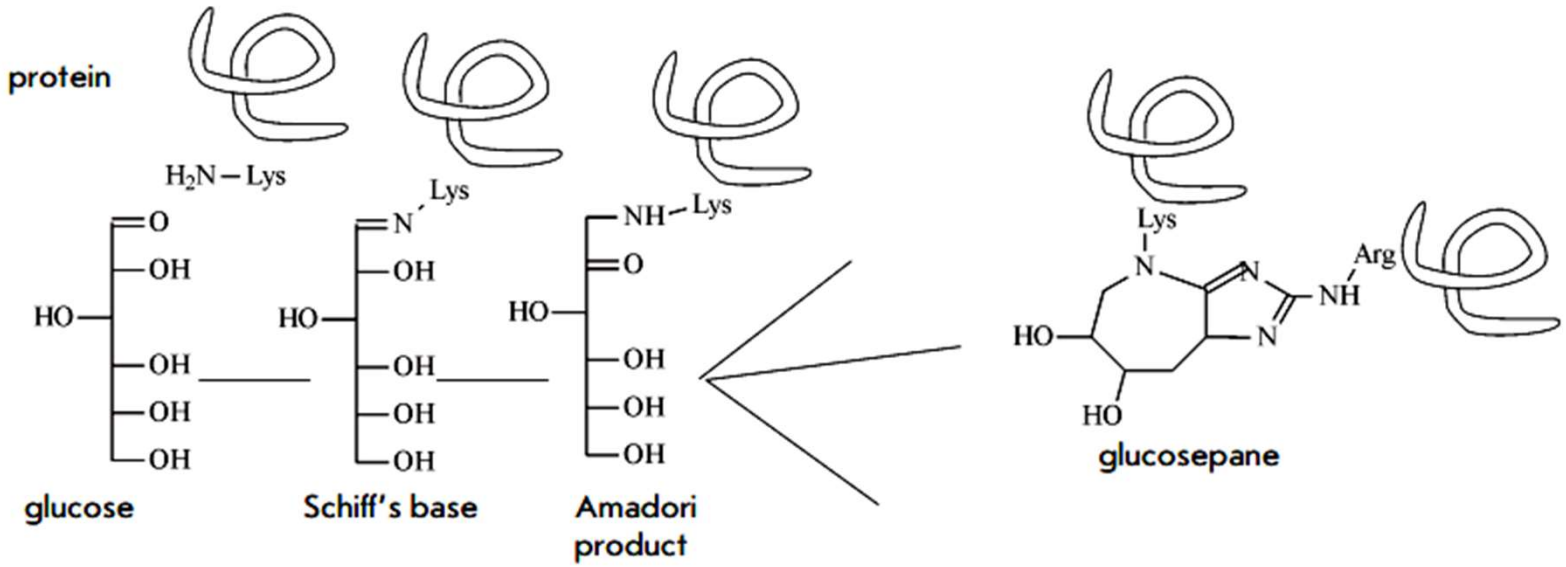
Accumulation of aggregates in postmitotic cells seems to be especially dramatic, since they are not able to dilute this material by cell division



Consequences of oxidative stress on proteins, the proteasomal system and transcription factors and formation of age pigment lipofuscin.

(F) When proteolytic capacity declines below a critical threshold of activity required to cope with oxidative stress, the final consequence is the accumulation of aggregated proteins which may, instead, cross-link with one another or form extensive hydrophobic bonds. This material might undergo further reactions and finally form the **age pigment lipofuscin** which has toxic properties and accumulates in the lysosomal system..

It is likely that a spontaneous modification of proteins also plays a role in the formation of metabolic debris.
Metabolic waste also includes, to a certain extent, spontaneously modified sugar-bound proteins mainly glucose molecules. Glycation involves interaction between the amino groups of lysine and the aldehyde groups of glucose via a Schiff base reaction. It is followed by rearrangement of the double C=N-bond, known as Amadori products, to yield a wide range of advanced glycation end-products such as **glucosepane**.
The main consequence of spontaneous glycation is impaired elasticity, which is essential to blood vessels. In addition, spontaneous glycation affects protein functioning. This process well describes the concept of accumulation of metabolic waste that promotes aging.



Spontaneous glycation of proteins

Errors and Repairs Theory

In 1963, Dr. Leslie Orgel, a British Chemist suggested that "an error in the machinery for making protein could be catastrophic." The production of proteins and the reproduction of DNA sometimes are not carried out with accuracy. The body's DNA is so vital that the natural repair processes kick in when an error is made. But the system is incapable of making perfect repairs on these molecules every time, and the accumulation of these flawed molecules can cause diseases and other age changes to occur.

Autoimmune Theory of ageing (immunosenescence)

This theory was proposed by an American scientist Dr. Roy Walford in the year 1969. Aging is associated with declines in adaptive and innate immunity established as immunosenescence. With age the system's ability to produce necessary antibodies that fight disease declines, as does its ability to distinguish between the antibodies and proteins. In a sense, the immune system becomes self-destructive and reacts against itself.

Thus elderly individuals usually present chronic low level inflammation, higher infection rates and chronic diseases.

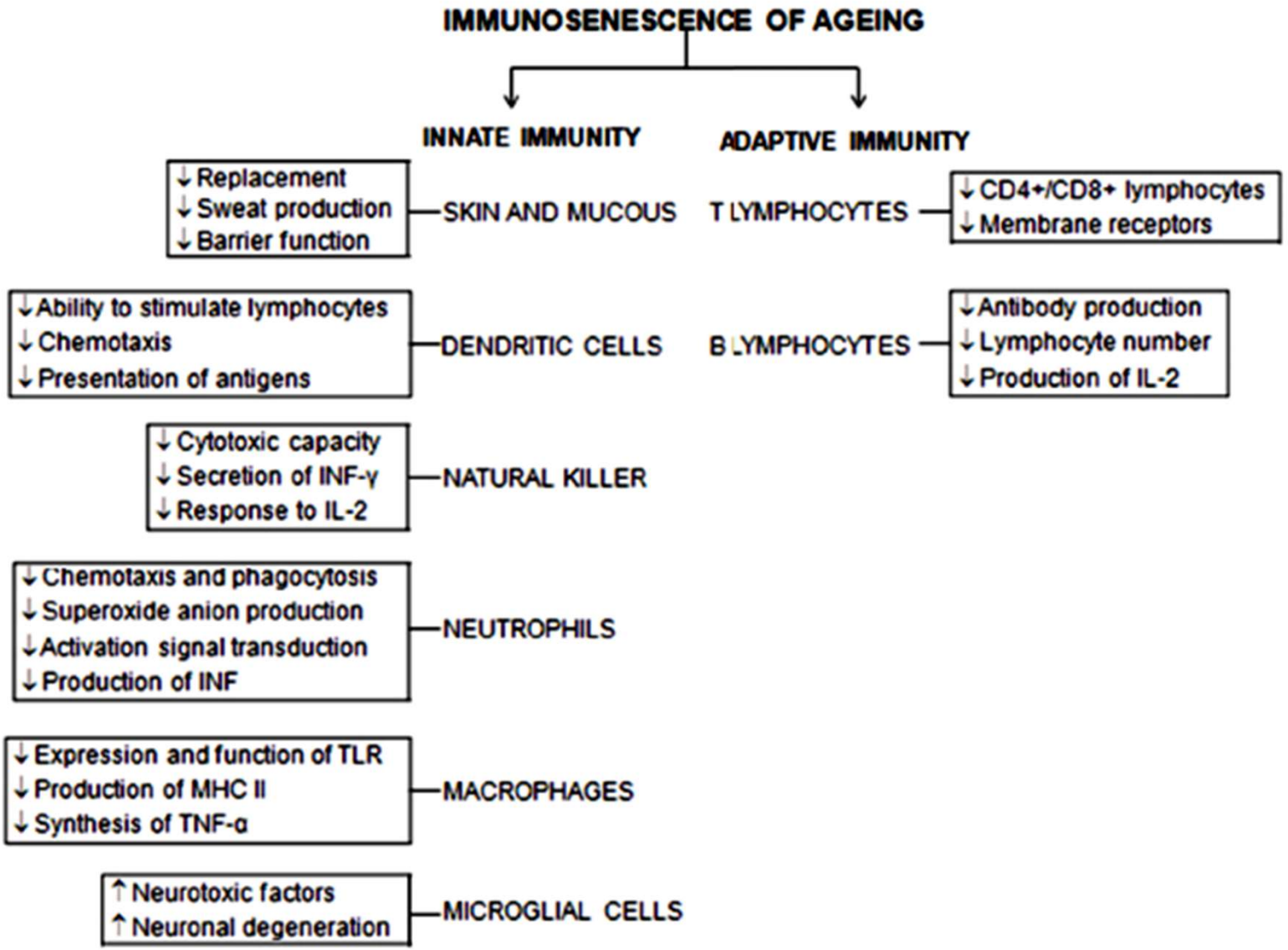
This theories suggest that with age comes decreased ability to recognize “self” from “nonself”.

Antibody structure becomes modified with ageing, causing them to acquire antigenic potential against normal body cells.

Cells hidden during embryonic development appear later in life.

Ab-Ag complexes from previous normal responses cause cumulative lesions characteristic of aging.

Medications may form complexes with body proteins that are identified as foreign.



Schematic representation of the main cells and their alterations involved in immunosenescence of ageing. INF: interferon, Il: interleukin, Tlr: toll like receptor, MHC: major histocompatibility complex, TNF: tumor necrosis factor.

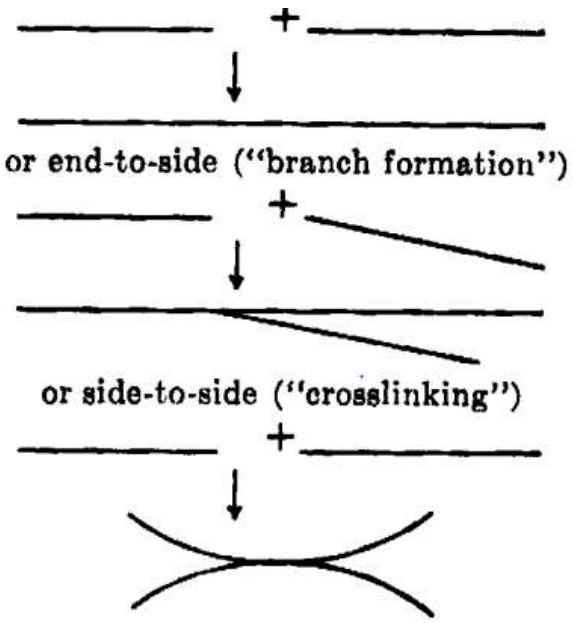
Cross-Linkage Theory of Ageing

Developmental aging and cross-linking were first proposed in 1942 by Johan Bjorksten, US Chemist. He applied this theory to aging diseases such as sclerosis, a declining immune system and the loss of elasticity in the skin.

The cross-linking hypothesis is based on the observation that with age, our proteins, DNA, and other structural molecules develop inappropriate attachments or cross-links to one another. These unnecessary links or bonds decrease the mobility or elasticity of proteins and other molecules.

Denatured proteins are irreversibly altered.

Denaturation is caused by cross-links between peptide strands within a protein or between proteins. With age comes new cross-links, leading to irreversible protein structural changes and altered protein functioning.



Different types of cross linking of molecules

It is thought that these cross-links begin to obstruct the passage of nutrients and waste between cells. Proteins that are damaged or are no longer needed are normally broken down by enzymes called proteases. However, the presence of cross-linkages inhibits the activity of proteases.

These damaged and unneeded proteins, therefore, stick around and can cause problems.

One of the main ways crosslinking occurs is through a process called **glycation**. Glucose molecules can stick to proteins, then transform into brownish molecules called **advanced glycation end products (AGEs)** When both of the sticky ends of AGEs adhere to neighboring proteins, they form permanent cross-links that disable the proteins' functions.

commonly affected body proteins

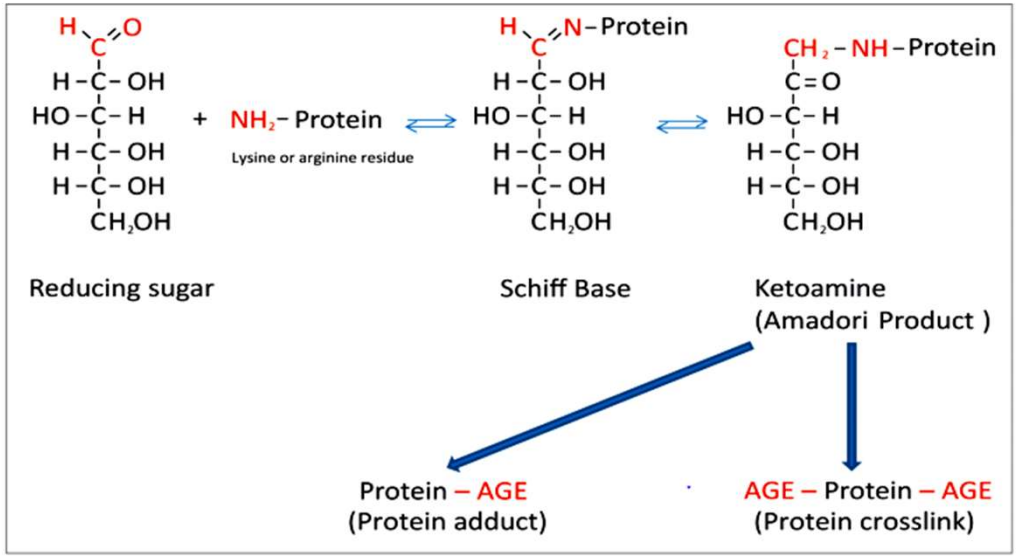
a. **Enzymes**
b. **Collagen (fibrosis)**
c. **Elastin**
d. **Ground substance**

More than 20 different AGEs have been identified in human blood and tissues and in foods.
AGEs can be divided into fluorescent and nonfluorescent AGEs.

The most important ones include carboxymethyl-lysine (CML), carboxyethyl-lysine (CEL), pyrraline (nonfluorescent AGEs),

pentosidine, and methylglyoxal-lysine dimer (MOLD) (fluorescent AGEs).

Glycation is the non-enzymatic reaction between reducing sugars, such as glucose, and proteins, lipids or nucleic acids. Glycation has to be distinguished from glycosylation, which is an enzymatic reaction. This was first described Maillard in 1912. Electrophilic carbonyl groups of glucose or other reactive sugars react with free amino groups of amino acids (especially of basic lysine or arginine residues), forming a non-stable Schiff base. Further rearrangement leads to formation of a more stable ketoamine (Amadori product). Schiff bases and Amadori products are reversible reaction products. However, they can react irreversibly with amino acid residues of peptides or proteins to form protein adducts or protein crosslinks. Alternatively, they can undergo further oxidation, dehydration, polymerization and oxidative breakdown reactions to give rise to numerous other AGEs. **AGEs are a very heterogeneous group of molecules.**



Schematic presentation of the Maillard reaction.
Reactive carbonyl groups of a reducing sugar react with neutrophilic free amino groups of proteins to form a **reversible Schiff base**. Through rearrangement a more stable **Amadori product** is formed. Dependent on the nature of these early glycation end products, protein adducts or protein crosslinks are formed.

The advanced glycation end products (AGEs) not only exert their deleterious actions due to their biological properties per se, but also through **their interaction with specific receptors.**

Receptor for AGEs (RAGE) is a multiligand member of the immunoglobulin superfamily of cell surface receptors.

The binding of ligands to RAGE stimulates various signaling pathways including the

mitogen-activated protein kinases (MAPKs)

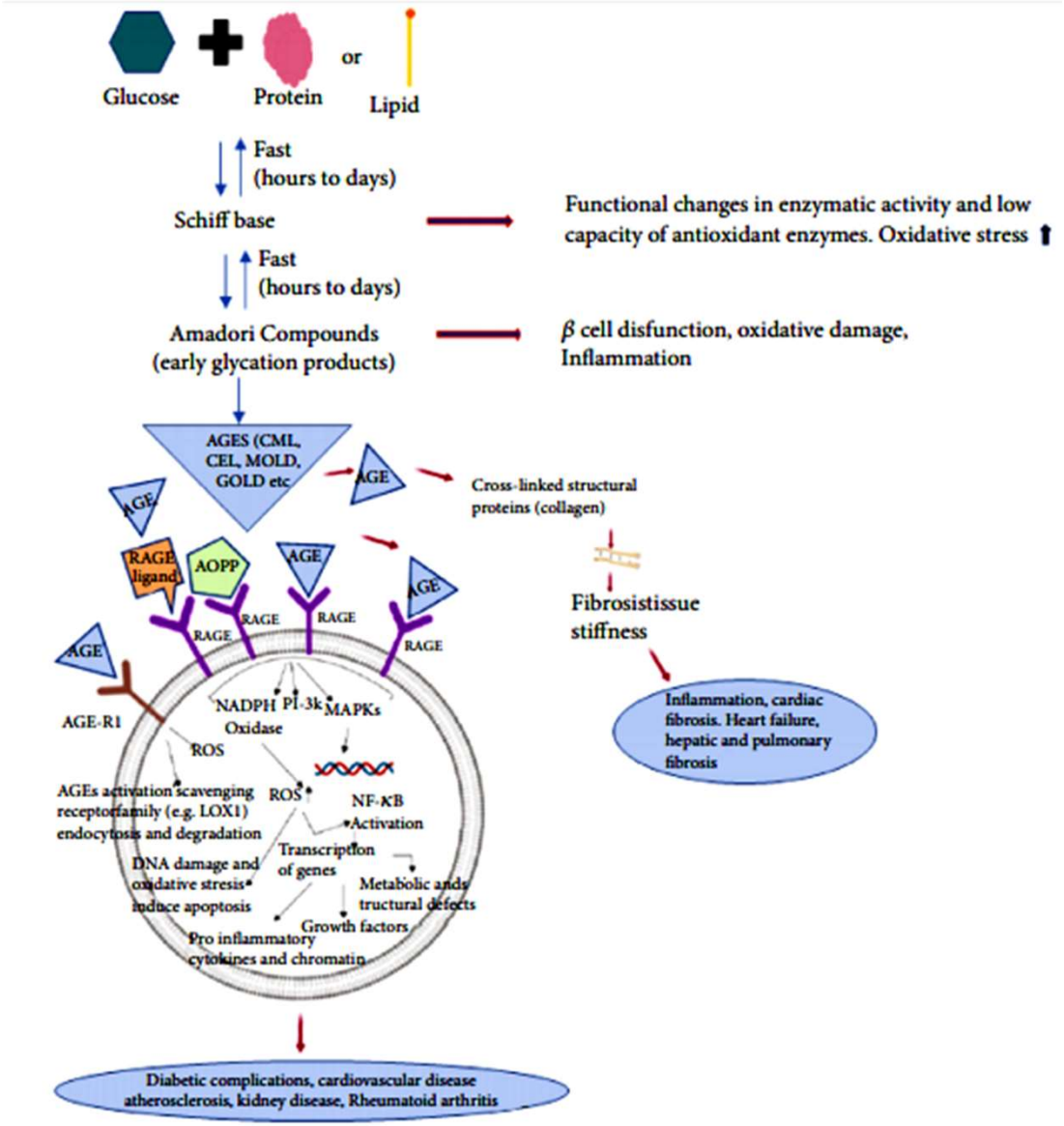
extracellular signal-regulated kinases (ERK) 1 and 2,

phosphatidyl-inositol 3 kinase, p21Ras,

stress-activated protein kinase/c-Jun-N-terminal kinase and

janus kinases

Stimulation of RAGE results in activation of the transcription factor nuclear factor kappa-B (NFκB) and subsequent transcription of many proinflammatory genes resulting formation of a vicious cycle of selfrenewing and perpetuating **proinflammatory signals.**



Biochemical formation of AGEs, their signaling, and molecular signal transduction that lead to pathological effects.

THE FREE RADICAL THEORY OR OXIDATIVE STRESS THEORY OF AGEING

The most popular theory of ageing is the free radical theory of aging. This theory was originally described by Denham Harman, the American scientist in the 1950s . It proposes that organisms age because they accumulate oxidative damage. This damage comes from reactive oxygen species (ROS), which are partially reduced metabolites of molecular oxygen generated as products of metabolic reactions or as by-products of various cellular processes, such as respiration. The damage can be attributed to an increased rate of free radical production in older organisms.

A free radical is any chemical species (atom, ion or molecule) that contains an unpaired or odd number of electrons and by far the most common source of free radicals in biological systems is oxygen. There are a number of ways in which free radicals can be formed, but their most abundant source are the mitochondria (which uses some 90% of the O_2 used by the human body) where oxygen is reduced in sequential steps to produce water.

This produces a number of short-lived intermediates including the formation of **superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^\bullet OH$).**

Both the superoxide and hydroxyl radicals have a free electron in their outer orbit and are highly reactive oxidants. Hydrogen peroxide is also toxic to cells and a cause of further free radical generation, particularly when reacting with reduced transition metals to form hydroxyl radicals.

In order to maintain proper cell signaling, number of radical scavenging enzymes maintain a threshold level of ROS inside the cell.

Low levels of ROS production are required to maintain physiological functions, including proliferation, host defense, signal transduction, and gene expression.

Antioxidant enzymes are proteins involved in the catalytic transformation of reactive oxygen species and their by-products into stable nontoxic molecules therefore representing the most important defense mechanism against oxidative stress induced cell damage.

Superoxide Dismutases Dismutation reactions are achieved by superoxide dismutases (SOD) (EC 1.15.1.1), and the catalytic reaction consists in the **transformation of the highly reactive superoxide ion into hydrogen peroxide,** with no unpaired electrons but still very reactive molecule.
Three different isoforms of this enzyme have been identified in human cells.
1. Copper Zinc Superoxide Dismutase
2. Manganese Superoxide Dismutase
3. Extracellular Superoxide Dismutase

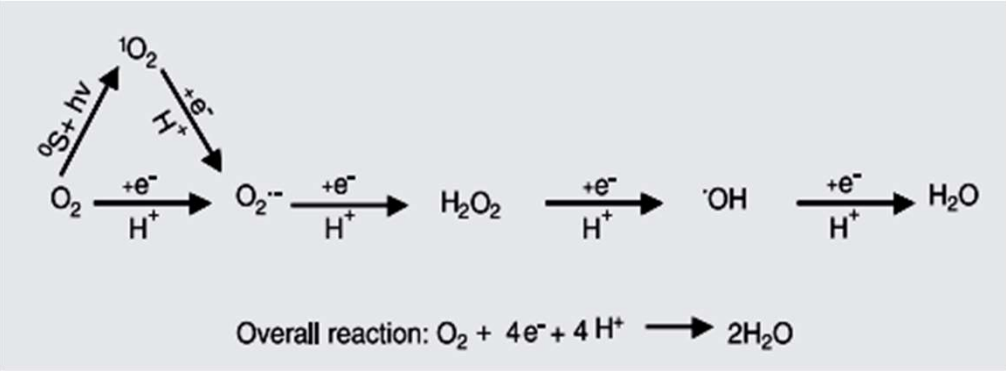
Catalase Catalase (EC 1.11.1.6) is a heme homotetrameric enzyme with a molecular weight of 240 kDa; its defense mechanisms consist in the decomposition of H_2O_2 to water and oxygen.

Glutathione Peroxidases (GPxs) (EC 1.11.1.19) are a superfamily of enzymes which catalyze the reduction of hydroperoxides (-ROOH) to alcoholic groups and water using reduced glutathione (GSH) as cosubstrate. In the reaction GSH is oxidized to GSSG.

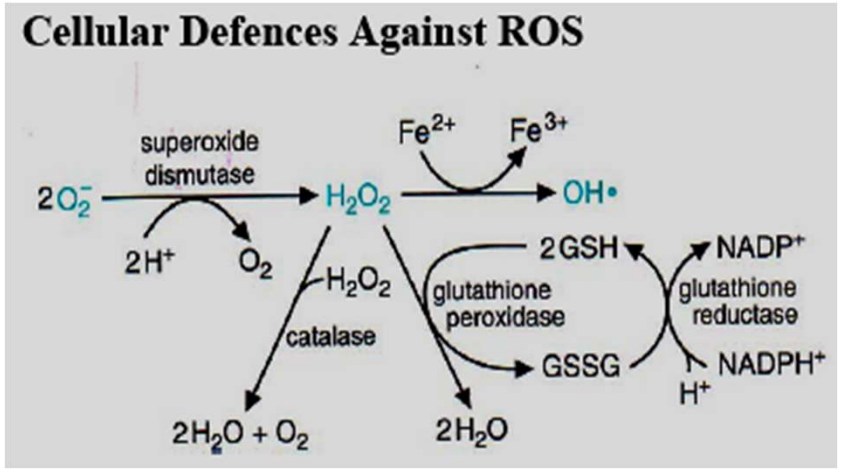
However, when the level of ROS exceeds this threshold, an increase in ROS production may lead to excessive signals to the cell, in addition to direct damage to key components in signaling pathways.

Thus, an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage is known as **Oxidative stress**.

The **continuous Reactive oxygen species** (ROS) generation by mitochondria during the whole life span, causes a **chronic oxidative stress**, associated with age, which plays a critical role in ageing.



Pathways in the univalent reduction of oxygen to water leading to generation of various intermediate reactive oxygen species (ROS).



SOD

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

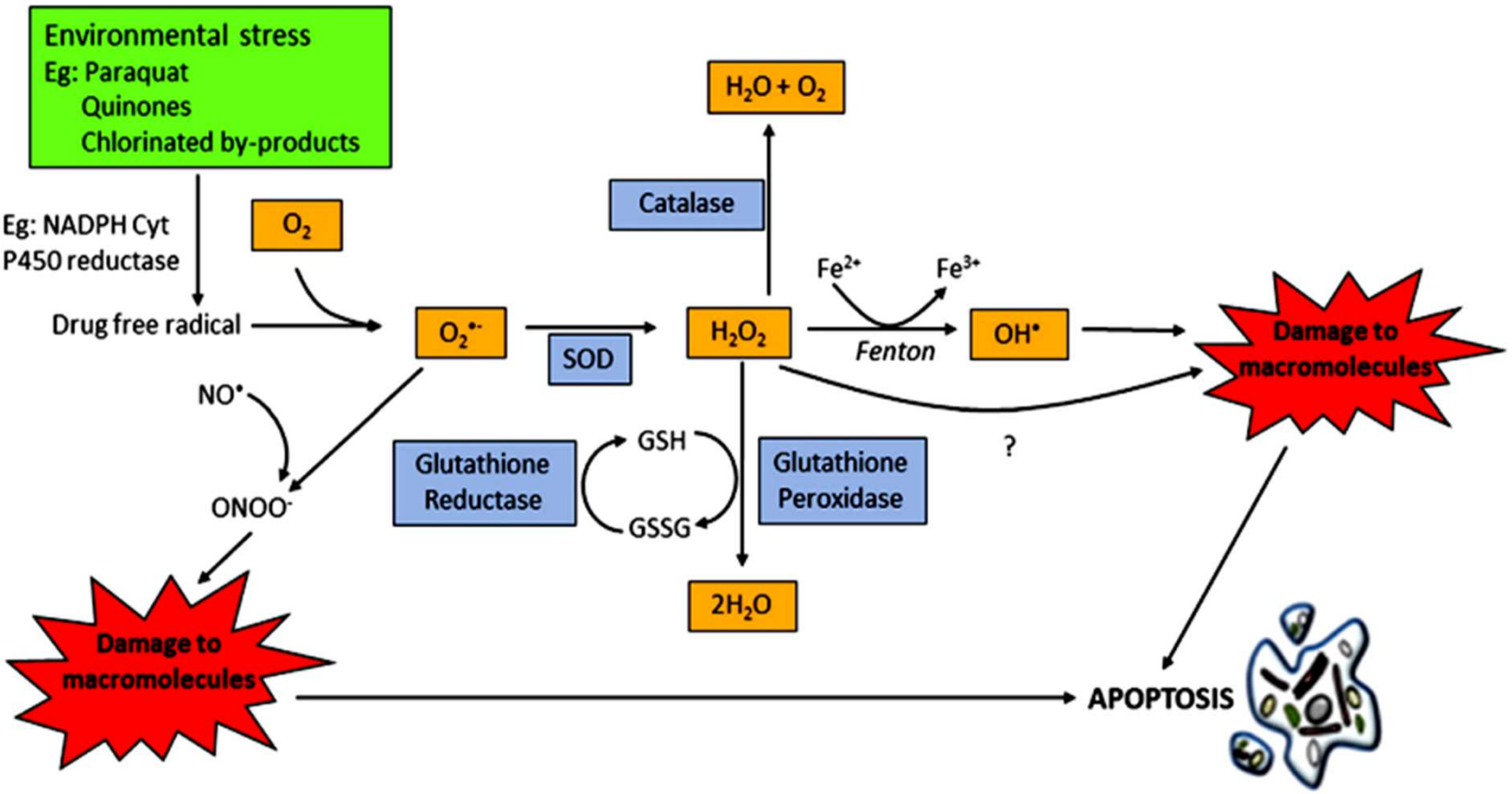
CAT

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

GPx

$$H_2O_2 + 2\ GSH \longrightarrow 2\ H_2O + GSSG$$

The antioxidant enzymes and the reactions they catalyze.
SOD: superoxide dismutase,
CAT: catalase,
GPx: glutathione peroxidase,
GSH : reduced glutathione (monomeric glutathione),
GSSG: oxidized glutathione (glutathione disulfide)


Environmental stress
Eg: Paraquat
Quinones
Chlorinated by-products
Eg: NADPH Cyt P450 reductase
Drug free radical
O_2
$O_2^{\bullet-}$
SOD
H_2O_2
$H_2O + O_2$
Catalase
Fe^{2+}
Fe^{3+}
Fenton
OH•
Damage to macromolecules
?
NO•
ONOO⁻
Glutathione Reductase
GSH
GSSG
Glutathione Peroxidase
$2H_2O$
Damage to macromolecules
APOPTOSIS

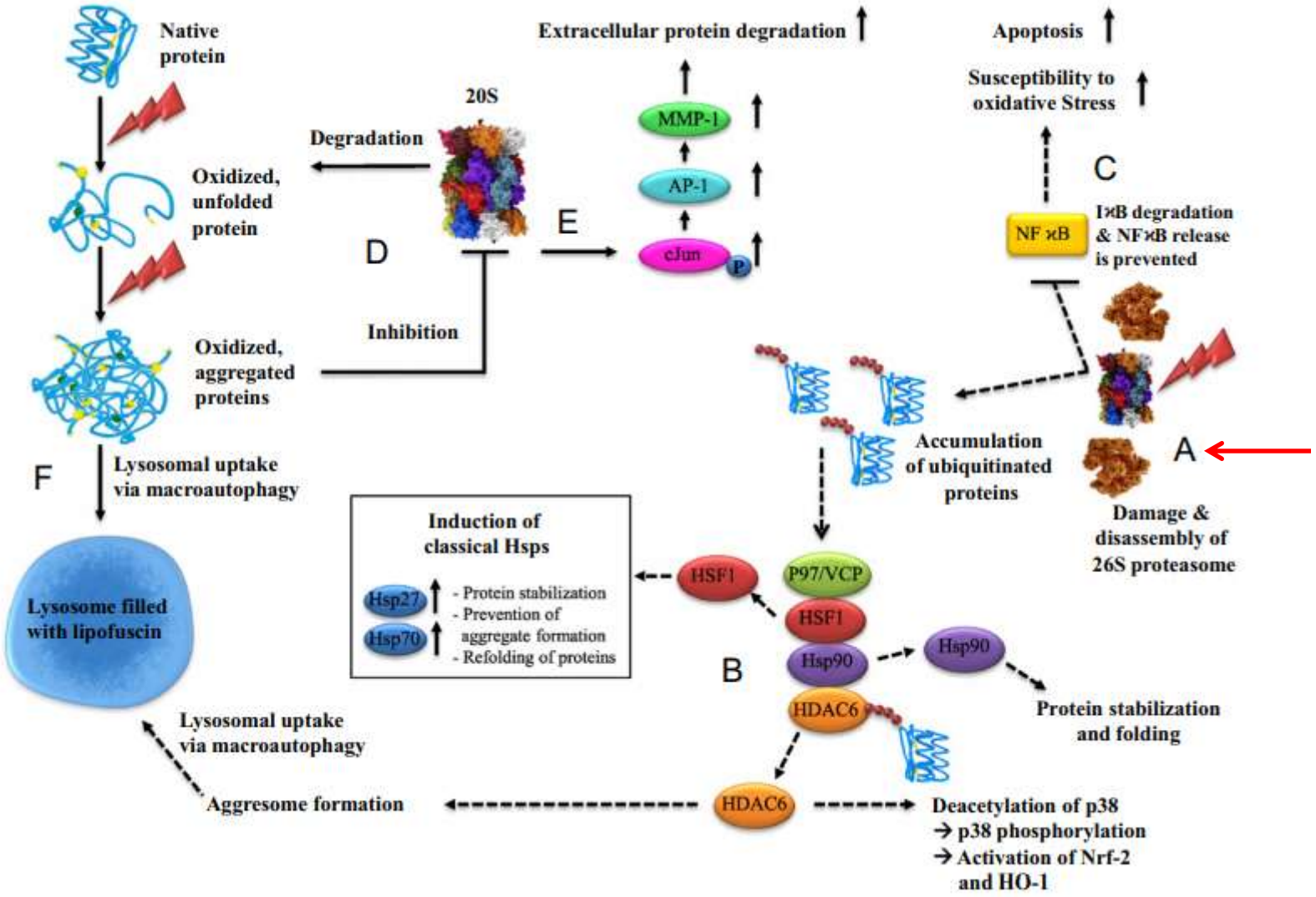
Oxidative damage by Reactive Oxygen Species (ROS)

ROS can oxidize and damage cell membranes, proteins, and nucleic acids.

Evidence for this theory includes the observation that *Drosophila* that overexpress enzymes that destroy ROS (catalase, and superoxide dismutase) live 30–40% longer than do controls.

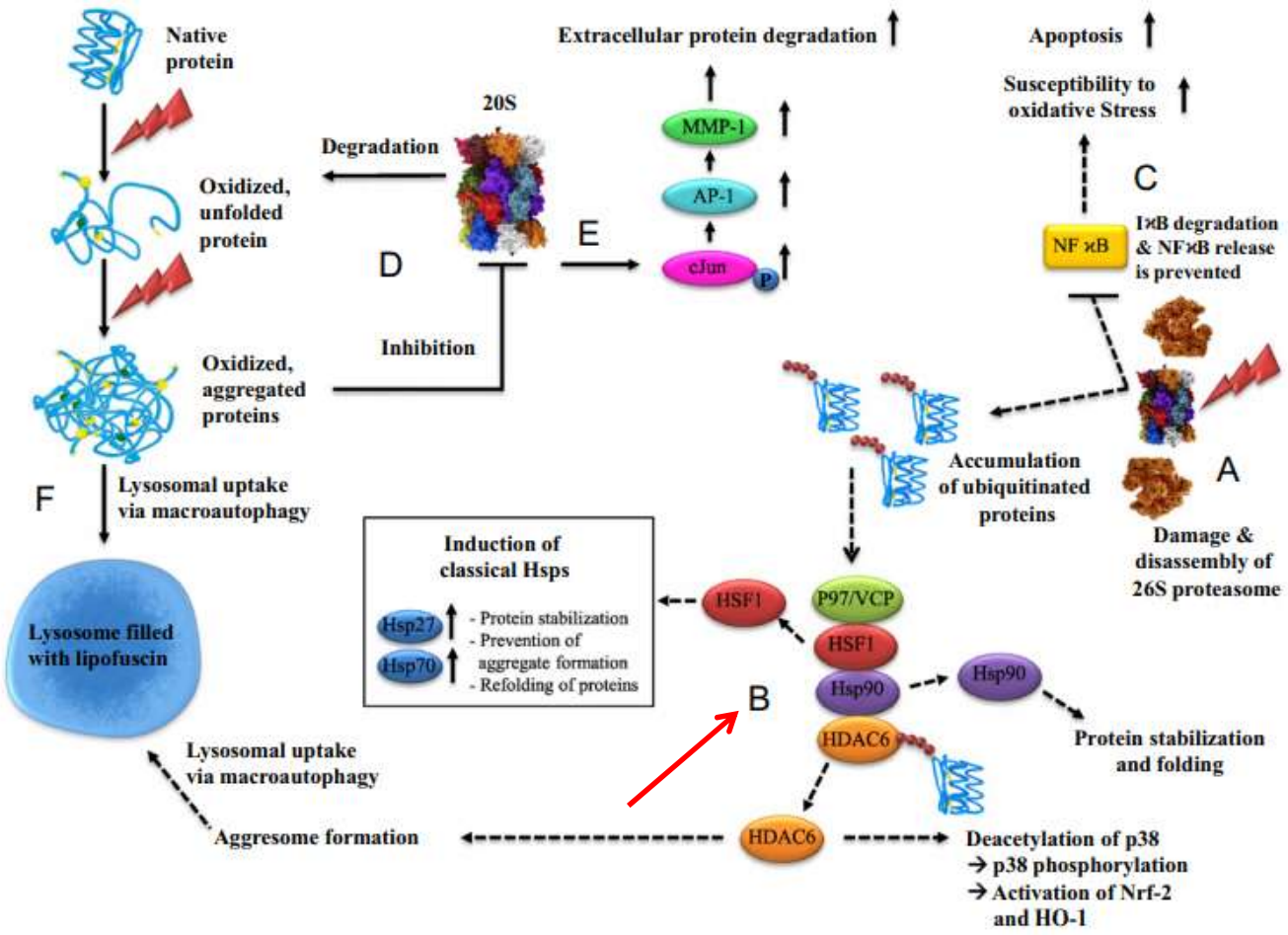
One of the most considered aspect of ROS-induced damage in DNA and aging is **DNA methylation** levels. These vary with age, and it is commonly considered that DNA hypomethylation is a typical aspect of the aging process.

ROS are active intermediates of DNA methylation, as well as of **histone modification**. These reactive oxygen species may play a role in epigenetic processes (physiological phenotypic variations caused by external or environmental factors that switch genes on/off) through reactions of **nucleophilic substitution** at the DNA level.

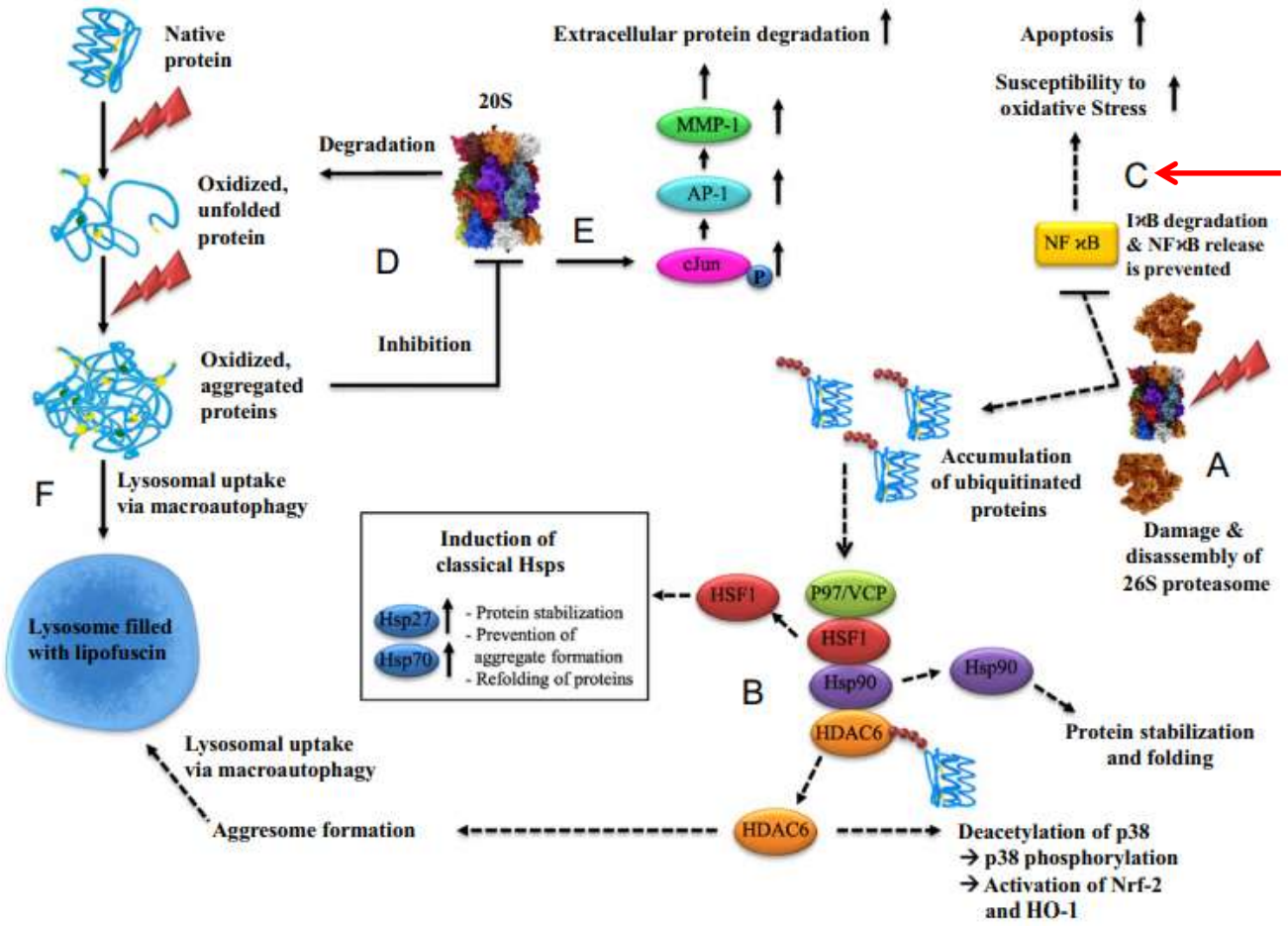


Consequences of oxidative stress on proteins, the proteasomal system and transcription factors

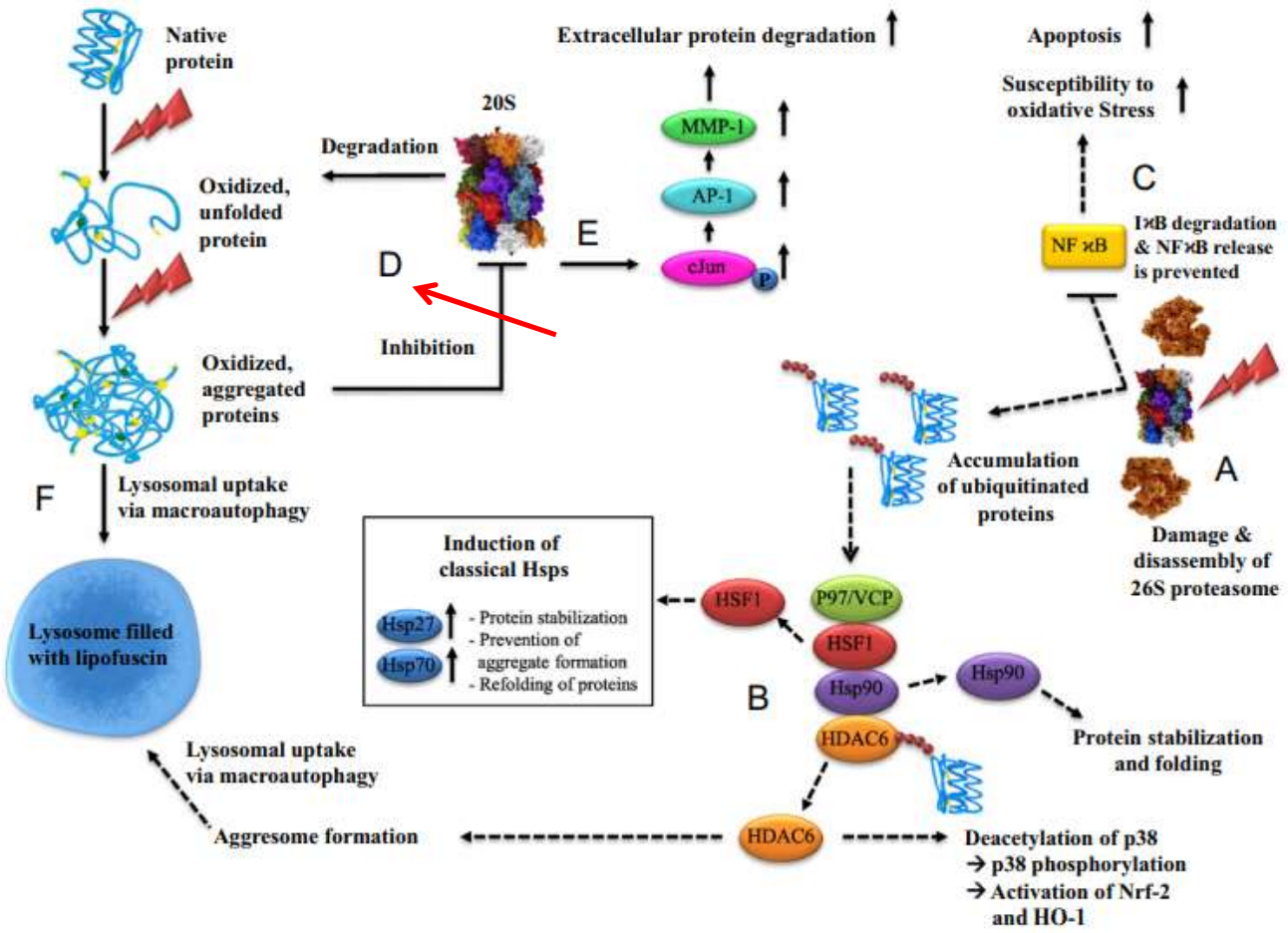
(A) The 26S proteasome is highly susceptible to inactivation during oxidative stress (figured as flash symbol) leading to an excessive accumulation of polyubiquitinated proteins.



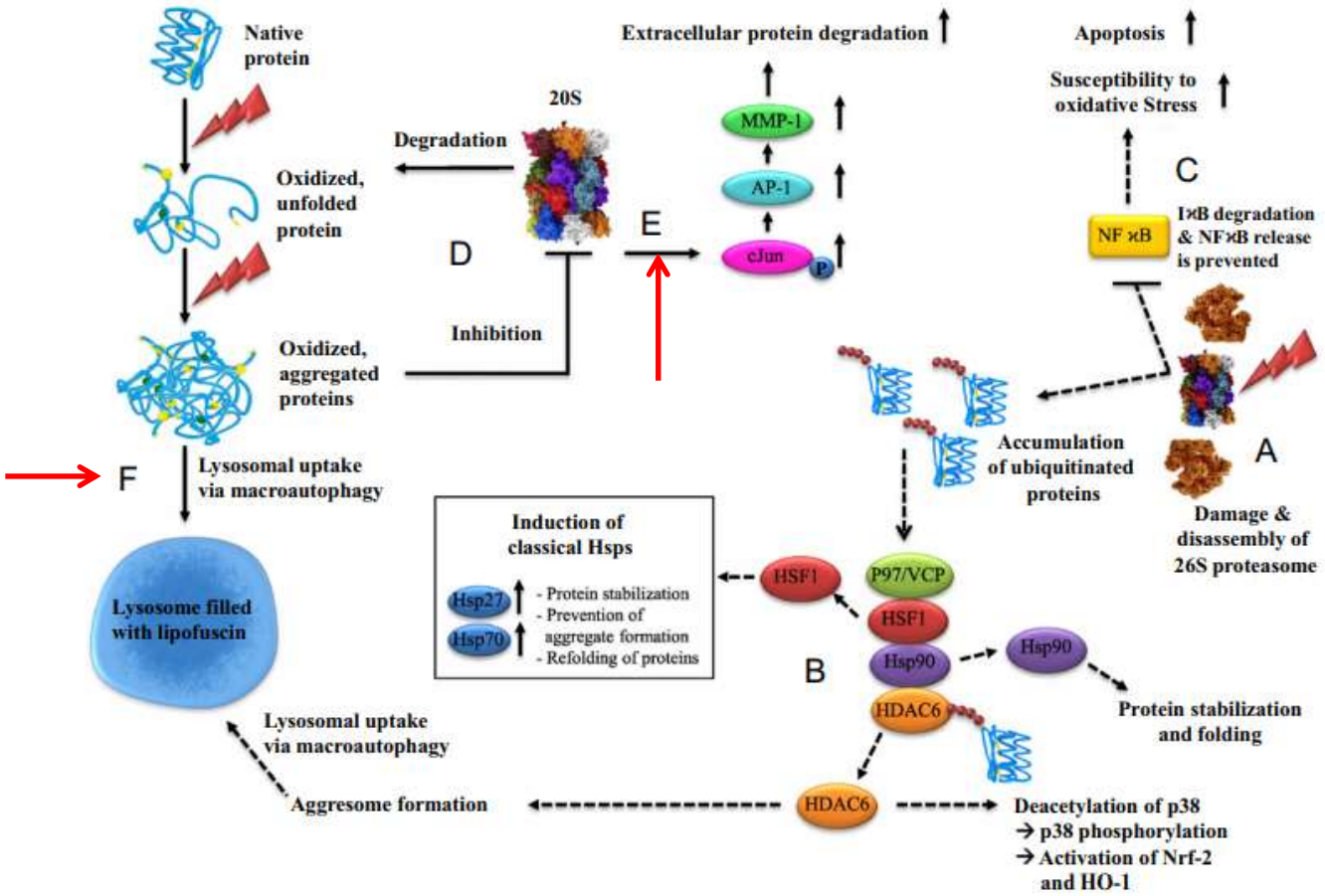
(B) Histone deacetylase 6 (HDAC6) has the ability to sense and bind ubiquitinated proteins via the BUZ (bound to ubiquitin zinc finger) domain which induces the dissociation of a complex formed out of Hsp90, HSF-1, p97/VCP and HDAC6. Following the liberation of HDAC6 and p97/VCP , the latter uses either its ATP dependent segregase activity to dissolve the bond between Hsp90 and HSF-1 directly or p97/VCP stimulates the Hsp90 ATPase activity, which results in the release of HSF-1 and the consequence up-regulation of several heat shock proteins, such as Hsp70 and Hsp 27 . Beyond up-regulation of classical Hsps upon proteasome inhibition, HDAC6 is also involved in an induction of HO-1 after proteasome inhibition . Initiation of this pathway is also the detection of ubiquitinated proteins and the release of HDAC6 and mediated by a p38/MAPK-dependent activation of Nrf-2, which is the most important transcriptional activator of HO-1 gene translation. Further HDAC6 has the ability to favor the accumulation of polyubiquitinated proteins in cellular aggresomes by interaction with ubiquitin and dynein motors. Aggresomes are inclusion bodies next to the nucleus at the proximity end of the microtubule organizing center, finally eliminated by an autophagy-mediated mechanism.



(C) Furthermore, a pivotal regulatory protein function is disrupted by 26S proteasomal inhibition, the transcription factor NFKB. This factor binds to multiple DNA sequences, initiating the transcription of gene products including various cytokines, angiogenesis factors, cell adhesion molecules, enzymes and antiapoptotic factors . NFKB is located in the cytoplasm in an inactive form, bound to an inhibitor molecule IKB. Stimulation of cells through a variety of mechanisms triggers a cascade of signaling events resulting in the degradation of IKB by the proteasome. This degradation releases active NFKB, which then translocates into the nucleus and binds to specific DNA sequences on its target genes . Proteasomal inhibition blocks NFKB activation and leads among others to increased susceptibility to oxidative stress and apoptosis.

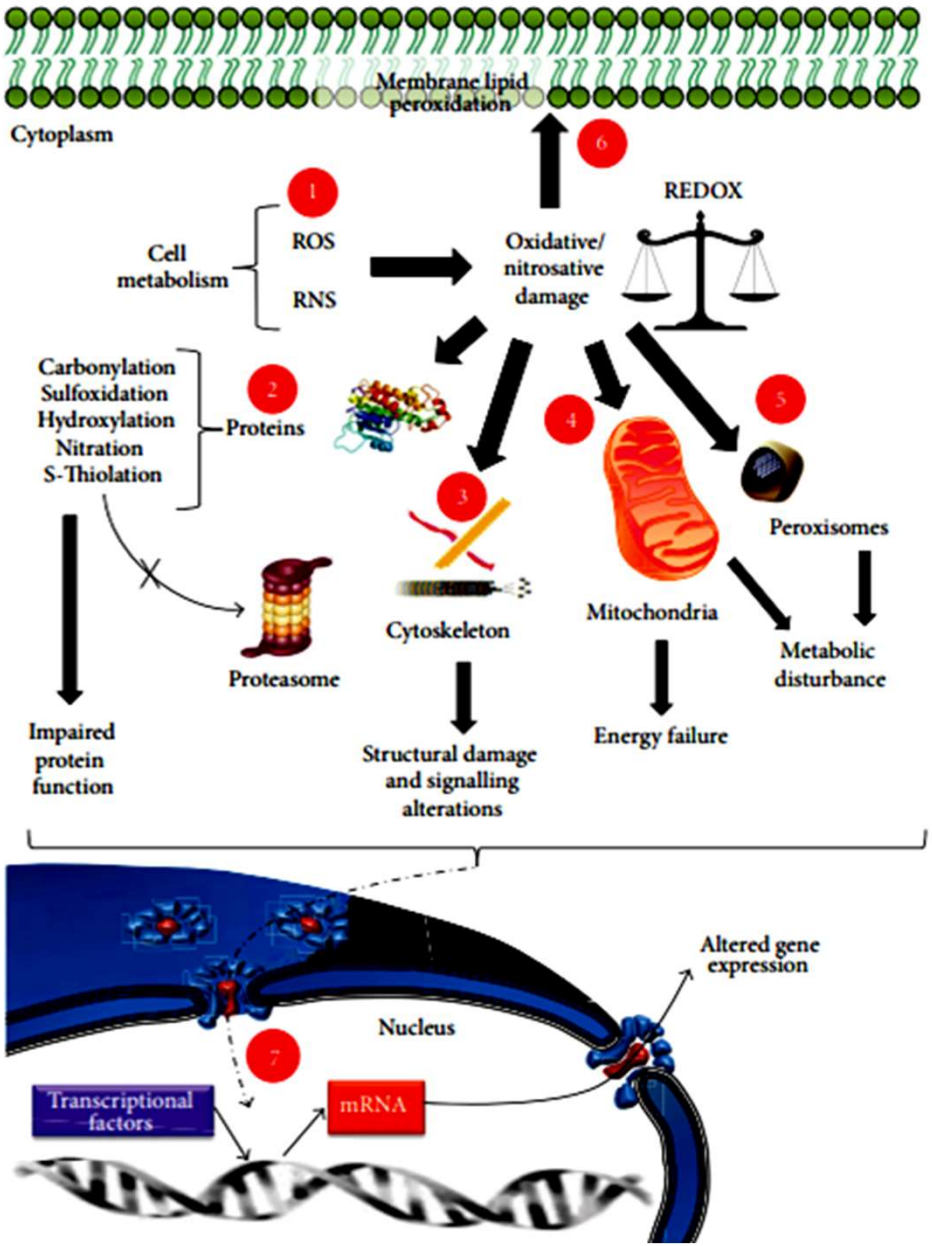


(D) The activity of the 20S proteasome, which is mostly responsible for the degradation of oxidized proteins and less susceptible to direct oxidative stress, can be nevertheless diminished by aggregated oxidized proteins. These protein aggregates are formed under stress conditions as complexes of unfolded proteins which do not normally interact with each other. It might require several steps depending on the nature of the initial conditions, leading to unfolding and aggregate formation. Due to the complex process of intermolecular interactions, such as during physiological aging, the process of aggregation is slow. The aggregate is independent from the original structure of the protein and introduces a new toxic element into cellular metabolism, partly by inhibiting 20S proteasome.



(E) Another side effect of proteasome inhibition is leading to a higher amount of phosphorylated c-Jun leading to an activation of AP-1, known to control the expression of MMP-1 and numerous other genes. Subsequently increased extracellular protein degradation can be assumed, **as observed in skin aging.**

(F) When proteolytic capacity declines below a critical threshold of activity required to cope with oxidative stress, the final consequence is the accumulation of aggregated proteins which may, instead, cross-link with one another or form extensive hydrophobic bonds. This material might undergo further reactions and finally form the **age pigment lipofuscin** which has toxic properties and accumulates in the lysosomal system..



Cellular dysfunctions in aging or in age-related diseases by oxidative stress imbalance.

(1) Cell metabolism generates reactive oxygen species (ROS) and reactive nitrogen species (RNS), which in turn causes oxidative/nitrosative damage.

(2) Proteins are the most affected macromolecules by oxidative stress, undergoing several modifications that avoid their being correctly degraded and recycled by the proteasome, thus generating impaired protein function.

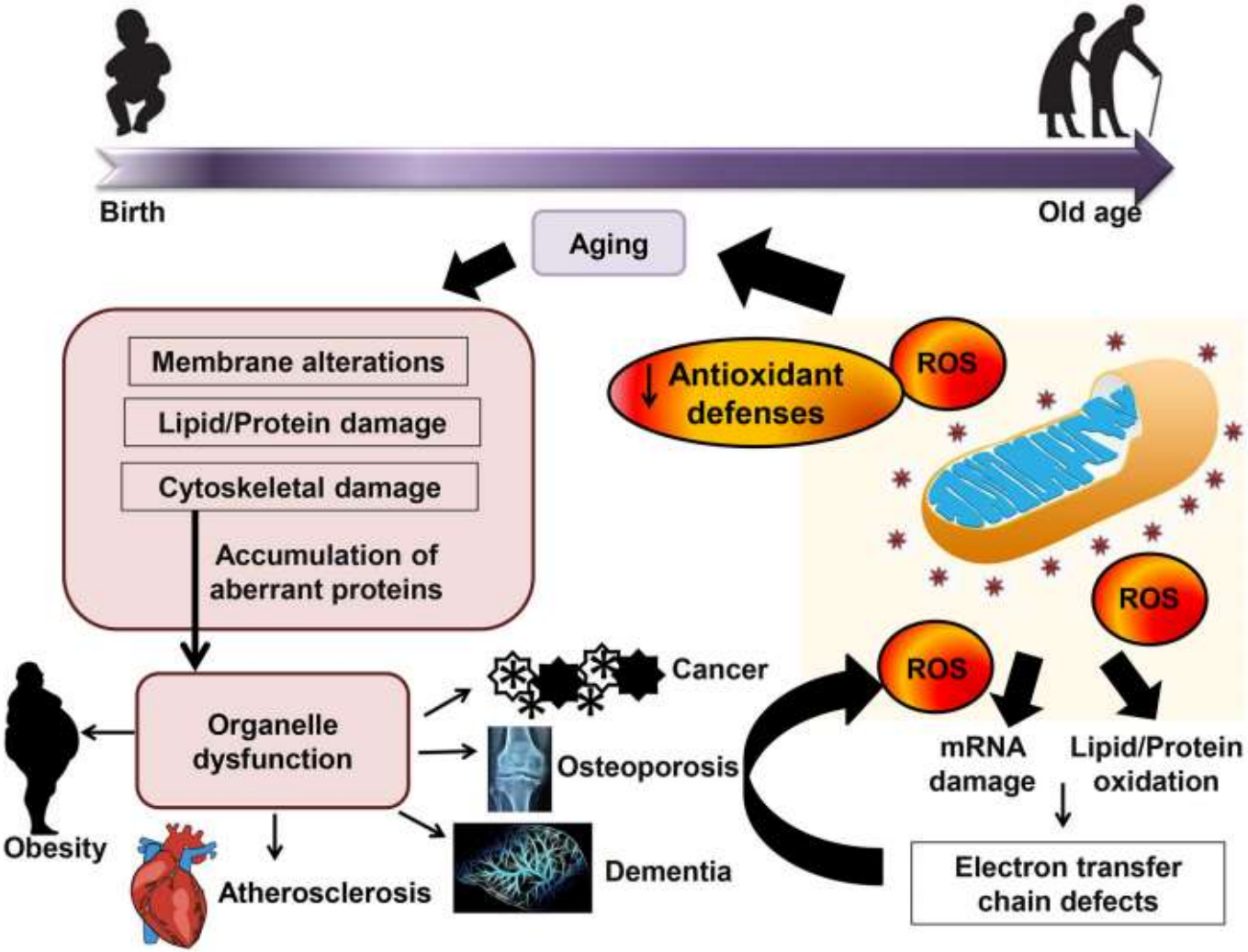
(3) Oxidative stress also directly affects cytoskeletal proteins, causing structural damage and signaling alterations.

(4) On affecting the mitochondria, oxidative stress alters energy production and

(5) on affecting peroxisomes, oxidative stress alters correct metabolic functioning.

(6) Oxidative stress also affects the cellular membrane.

(7) Finally, all of the previously mentioned affections cause an alteration in the transcriptional activity of the cell, leading to an altered gene expression that in turn leads the cell to the aging process or to degenerative disease.



Effect of oxidative stress and the interaction of aging and age-related diseases. Accumulation of reactive oxygen species (ROS) leads to mRNA damage and lipid/protein oxidation and subsequently causes a decrease in mitochondrial function, and ultimately produces more oxidative stress. Mitochondrial function decline and oxidative stress response in aging may subsequently contribute to age-related diseases.

Drosophila with mutations in the *methuselah* gene (named after the Biblical fellow said to have lived 969 years) live 35% longer than wild-type flies. The *methusaleh* mutants have enhanced resistance to paraquat, a poison that works by generating ROS within cells.

These findings not only suggest that aging is under genetic control, but also provide evidence for the role of ROS in the aging process.

In *C. elegans,* too, individuals with mutations that increase the synthesis of ROS-degrading enzymes live much longer than wild-type nematodes.

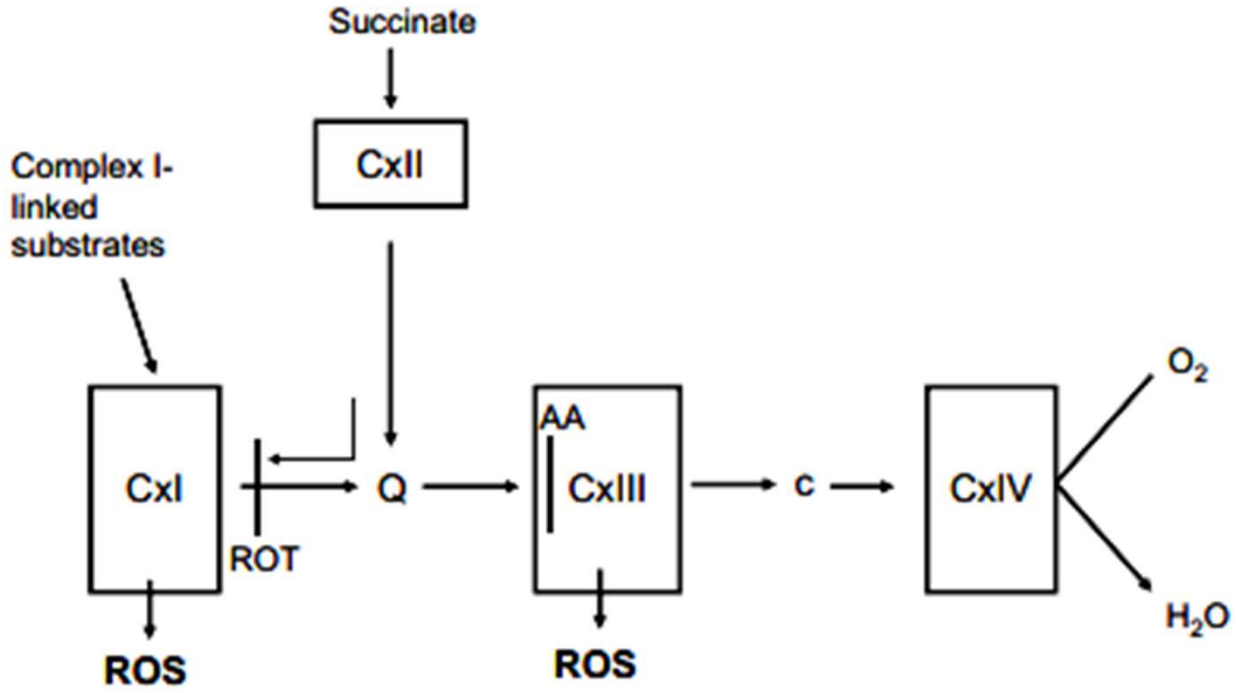
The evidence for ROS involvement in mammalian aging is not as clear.

Mutations in mice that result in the lack of certain ROS-degrading enzymes do not cause premature ageing.

Another type of evidence does suggest that ROS may be important in mammalian aging: aging in mammals can be slowed by caloric restriction. However, caloric restriction can also have other effects, so it is not certain if it works by preventing ROS synthesis.

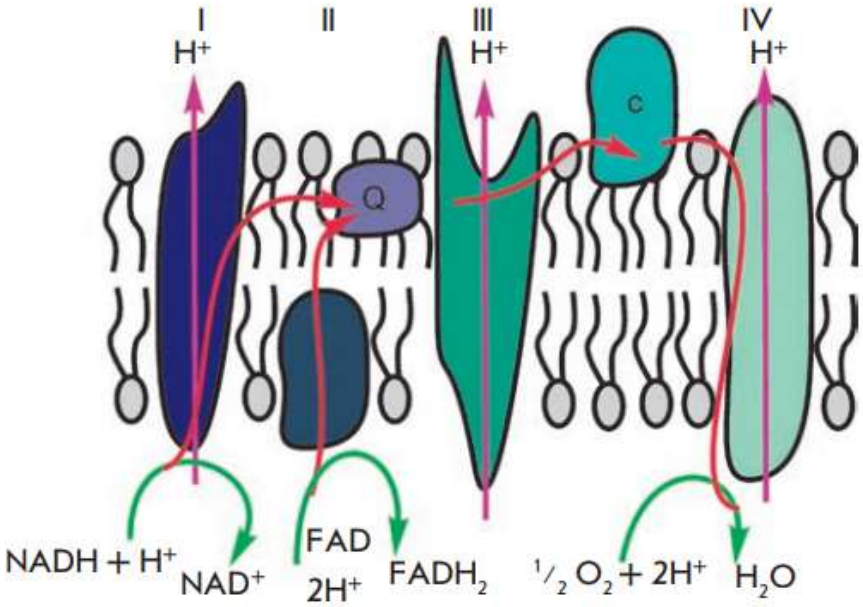
Also, vitamins E and C are both ROS inhibitors, and vitamin E increases the longevity of flies and nematodes when it is added to their diet. However, results in mammals are not as easy to interpret, and there is no clear evidence that ROS inhibitors work as well as in invertebrates .

The Mitochondrial Free Radical Theory of Aging

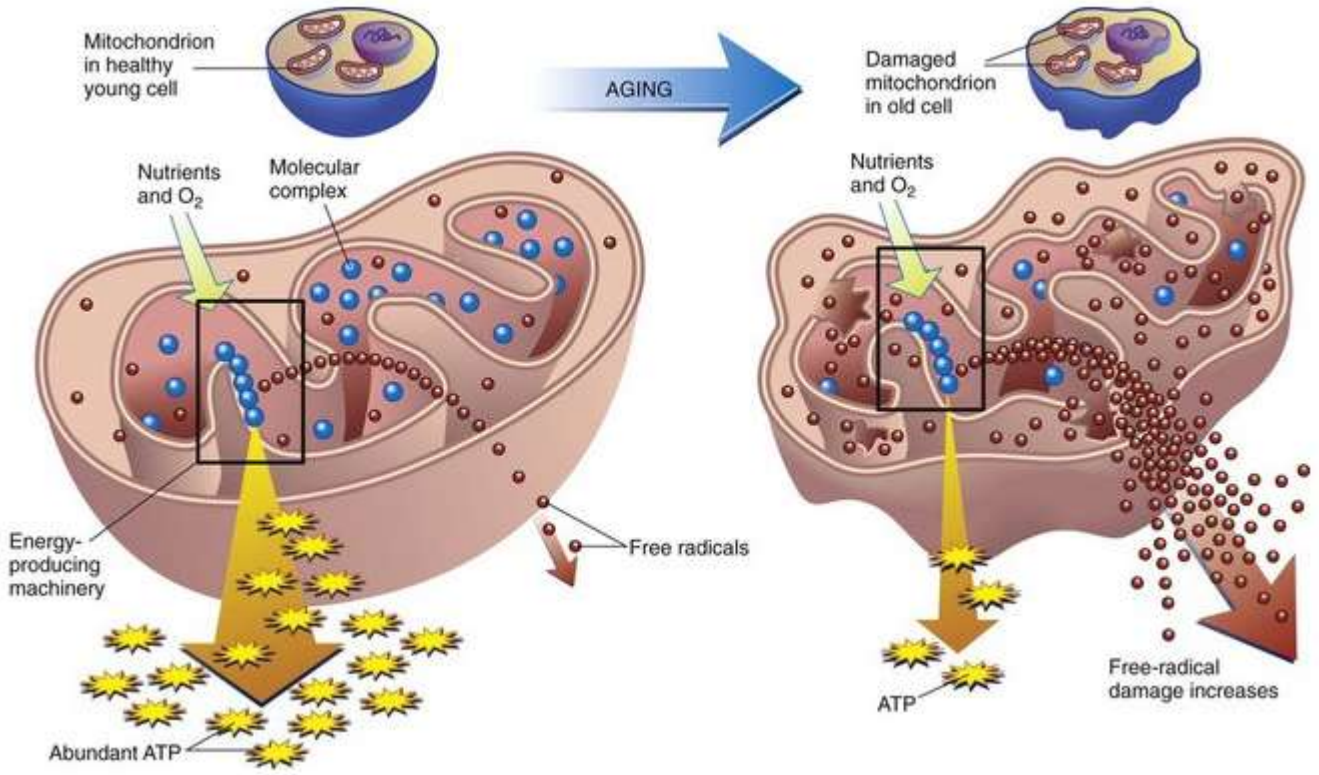


Sites of ROS production at the mitochondrial electron transport chain. The figure shows the four complexes of the respiratory chain (CxI–IV). ROS are mainly produced at CxI and CxIII. Rotenone (ROT) blocks electron transport from ubiquinone (Q) to complex I, thus avoiding complex I ROS production when succinate is used as substrate. AA, antimycin A.

The primary function of mitochondria is respiration, which promotes energy production. Mitochondria break down organic compounds into water and carbon dioxide to release energy in the form of adenosine triphosphate (ATP).
Mitochondrial respiration generates a proton gradient across the inner membrane and a transmembrane potential through respiratory chain complexes (I–IV), enabling electron flow from the reduction equivalents NADH and $FADH_2$ to oxygen. Simultaneously, the energy released in the oxidation of NADH and $FADH_2$ is used to pump H+ ions out of the matrix into the space between the outer and inner membranes. During respiration, oxygen is reduced in several stages, producing a **superoxide radical** (O_2 -) and **hydrogen peroxide**. Most commonly, these molecules, known as **reactive oxygen species (ROS),** remain bound to cytochrome c oxidase until the reduction of oxygen to water is completed. In contrast to the common sequence of oxygen reduction by cytochrome c oxidase, oxygen molecules can occasionally form **superoxide species** by reacting with the reduced components of the electron transport chain. This typically occurs at the level of complexes I and III in the respiratory chain.



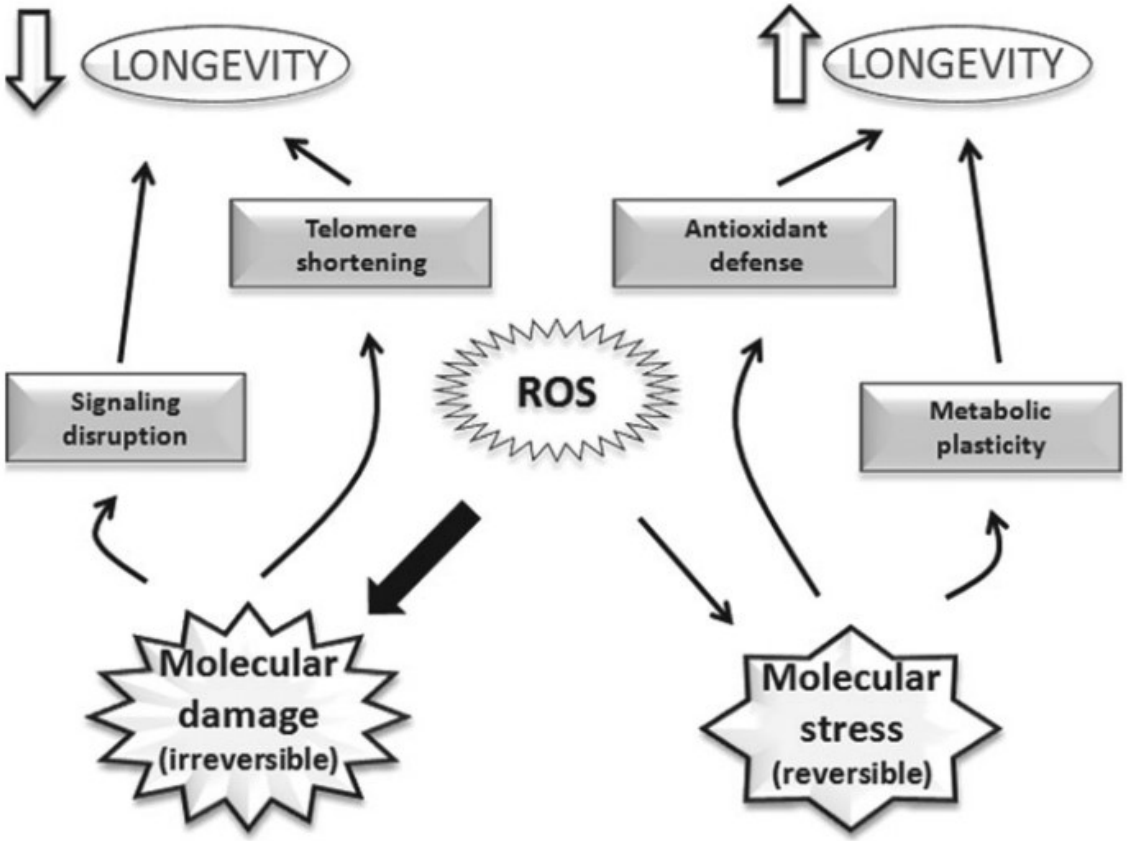
The mitochondrial respiratory chain, illustrating electron transfer from NADH and $FADH_2$ to oxygen.



Mitochondria in young and old cells.ATP, adenosine triphosphate.

Harman proposed that aging and age-associated diseases could be attributed to the deleterious effects of free radicals. Evidence does not support this statement. An interesting modification of the free radical theory of aging has been put forward by Sohal and Orr in 2012.

They propose **"The redox stress theory of aging."** If free radicals cause a stress that cells can cope with, then damage will not occur because antioxidant defenses will overwhelm such stress. Only if the stress is of such magnitude that it deranges cellular signaling mechanisms, age-associated damage will take place.



The double edge sword of free radicals. They have hormetic effects. When radicals cause severe effects on biomolecules it causes damage (i.e., irreversible alterations), whereas when the aggression is mild, a stress is caused and this may have signalling effects, as well as hormetic effects.

Mitochondrial Theory of ageing

The mitochondrial theory of aging was put forward by Denham Harman, an American scientist in 1972, who favoured the idea that the constant accumulation of the damage exerted by free radicals to the mitochondria is the main driving force behind the aging process.

Mitochondria are not only the main source of energy for most eukaryotic cells, but also the main source of free radicals. These reactive molecules can damage all components of a cell such as membranes, proteins and DNA.

The mitochondrial theory of ageing proposes that an accumulation of defective mitochondria is a major contributor to the cellular deterioration that underlies the ageing process.

Mitochondrial genome damage

The mtDNA is much more prone to damage than nDNA, since mtDNA is not protected by histone proteins and it is close to the site of reactive oxygen species (ROS) generation in the mitochondrial membrane. In addition, over all the repair of mtDNA is less efficient than the repair of nDNA.

However, the mtDNA encodes only 37 genes and the relative importance of mtDNA damage for ageing is still controversial and less supported by experimental evidence than damage to nuclear DNA

The mutation rate in mitochondria is 10 to 20 times faster than the nuclear DNA mutation rate. It is thought that mutations in mitochondria could

(1) lead to defects in energy production,
(2) lead to the production of ROS by faulty electron transport
(3) induce apoptosis.

Age-dependent declines in mitochondrial function are seen in many animals, including humans.

There are **"hot spots"** for age-related mutations in the mitochondrial genome, and that mitochondria with these mutations have a higher replication frequency than wild-type mitochondria.

Thus, the mutants are able to outcompete the wild-type mitochondria and eventually dominate the cell and its progeny.

Moreover, the mutations may not only allow more ROS to be made, but may make the mitochondrial DNA more susceptible to ROS-mediated damage.

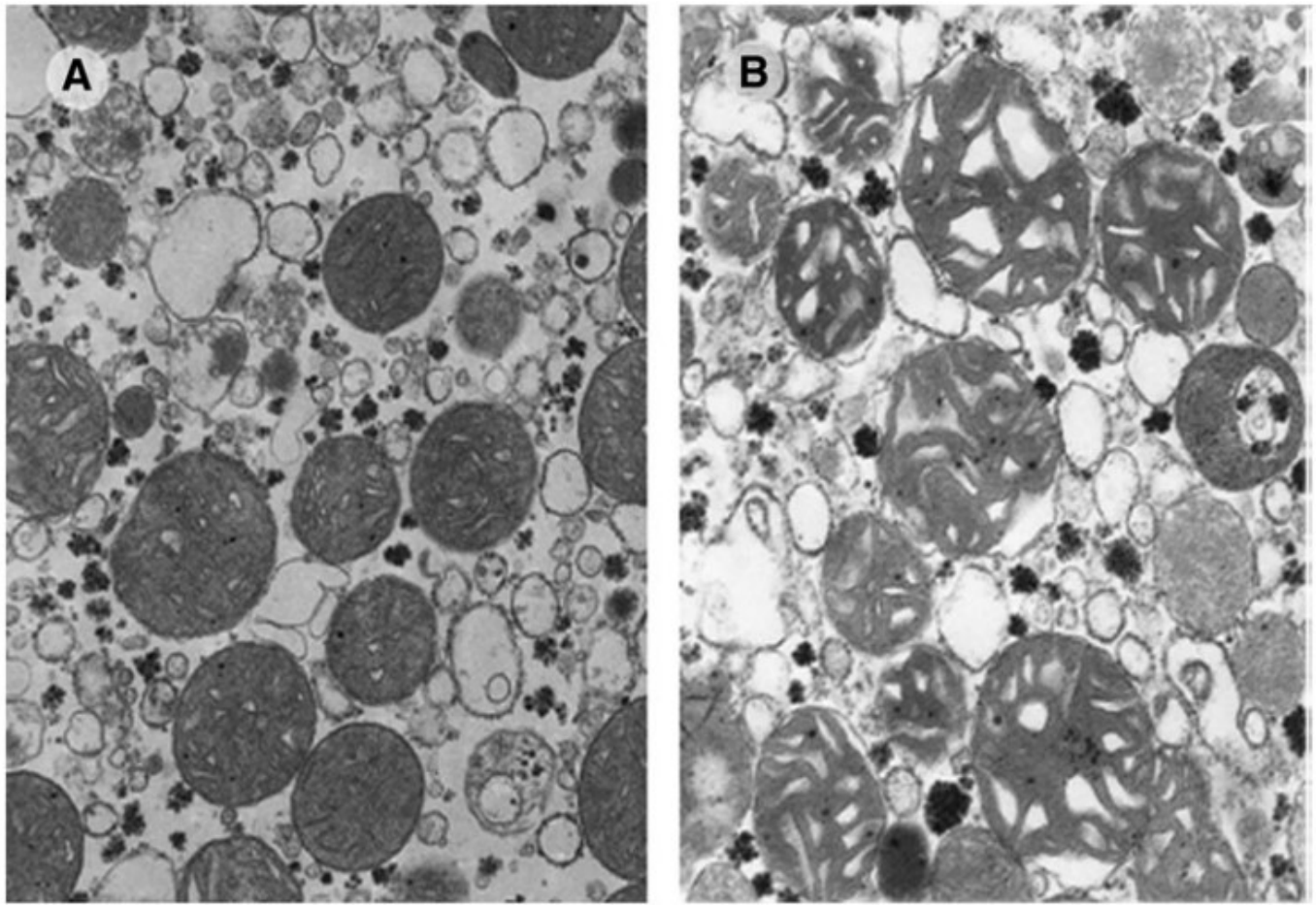
Defective mitochondria theory

Mitochondria are responsible for energy (ATP) production in the cell. These subcellular organelles contain their own genetic material (mtDNA) which is particularly susceptible to violation because of

(i) limited repair capacity, and

(ii) proximity to the major sources of activated oxygen species. Damaged mitochondria are frequently deficient in ATP production but can still replicate because the genes important for mitochondrial reproduction are located not in the mitochondria themselves but in the cell nucleus.

Accumulation of defective mitochondria can thus occur, controlled to some extent by mitochondrial turnover. Because of this vulnerability mitochondrial mutations have been suggested to be a central component in ageing.



Mitochondria from old animals are damaged inside cells. (A) Shows liver mitochondria from young rats and (B) shows liver mitochondria from old rats (original magnification 30,000–1). Mitochondria from old animals show more heterogeneity, bigger size, and disrupted cristae; all indications of histological damage.

Impairment of regulatory pathways during aging

Aging is associated with the dysregulation of regulatory pathways. For example, aging upsets the balance between pro- and anti-inflammatory components, promoting chronic inflammation. An elevated predisposition to inflammatory diseases in early age, as a protective barrier against infection, proves to be detrimental in the elderly.

Aging can also impair other important pathways.
The self-regulatory mechanisms of homeostasis – negative feedback pathways are impaired. One of the essential systems is the hypothalamus-pituitary-adrenal axis. An elevation of the threshold of the hypothalamus to negative feedback signaling accounts for the unfavorable age-related changes in human health; in particular, reproductive decline.

The DNA damage theory of ageing

This theory postulates that the main cause of the functional decline associated with ageing is the accumulation of DNA damage and ensuing cellular alterations and disruption of tissue homeostasis.

DNA damage can dysregulate gene expression and cell function, impair transcription, cause cell cycle arrest and (if the damage is too serious) trigger programmed cell death (apoptosis).

DNA damage can also lead to mutations when the DNA is repaired and/or replicated.

Gene mutation theory

1. mutations can alter normal cellular function
2. Accumulation of mutations lead to malfunction
3. Cells have mechanisms that allow them to repair DNA

Hutchinson-Guilford Progeria, a very rare human genetic disease, accelerates many symptoms of aging including atherosclerotic heart disease. Victims usually die by age 13.

Werner syndrome, another genetic disease, involves acceleration of most symptoms of aging including baldness, hair and skin conditions, heart disease, calcification of blood vessels, some cancers, cataracts, arthritis, diabetes, etc. Victims usually die by age 50.
These conditions suggest aging is centrally controlled such that a single genetic defect could result in proportionally accelerating all of the expressed symptoms. Central control suggests aging-by-design. Non-programmed theories contend that aging is the result of many deficiencies that *independently evolved.*

Summary of major human progeroid syndromes originating in single-gene defects.

Syndrome	Genetic defect and main processes affected	Mean lifespan (years)
Werner	RecQ-like DNA helicase and exonuclease, involved in DNA repair	47
Hutchinson–Guilford	Lamin A, involved in DNA replication, transcription, nuclear organisation	13
Trichothiodystrophy	TFIIH helicase, involved in DNA repair and transcription	10
Cockayne	CSA or CSB gene, involved in DNA repair and transcription	12–20
Ataxia telangiectasia	ATM protein kinase, involved in DNA damage response	20
Rothmund–Thomson	RecQ-like DNA helicase	Normal?
Xeroderma pigmentosum	XPA–XPG genes, involved in DNA repair	Lower than normal?

Telomere shortening

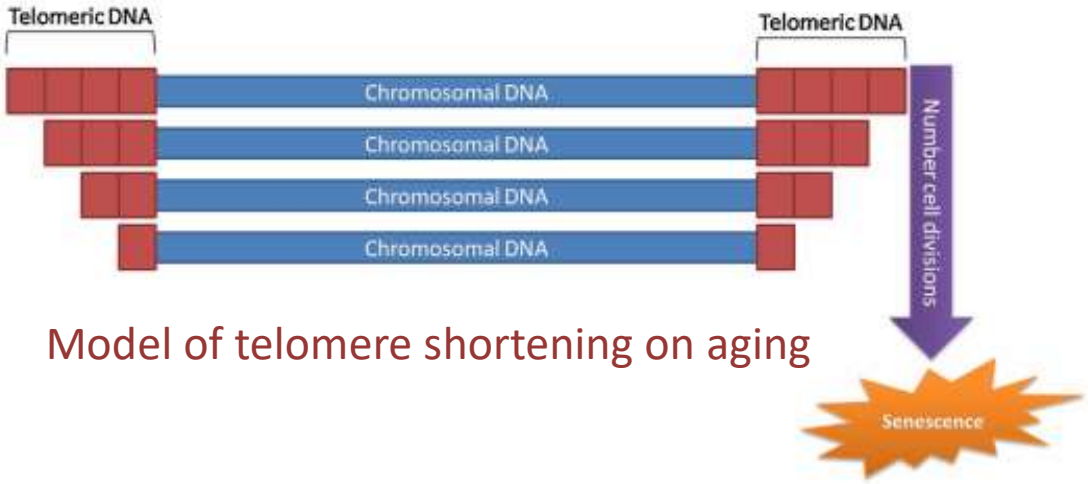
Telomeres are repeated DNA sequences at the ends of chromosomes. They are not replicated by DNA polymerase, and they will shorten at each cell division unless maintained by **telomerase**.

Telomerase adds the telomere onto the chromosome at each cell division. Most mammalian somatic tissues lack telomerase, so it has been proposed (Salk 1982; Harley et al. 1990) that telomere shortening could be a "clock" that eventually prohibits the cells from dividing any more.

When human fibroblasts are cultured, they can divide only a certain number of times, and their telomeres shorten. If these cells are made to express telomerase, they can continue dividing.

However, there is no correlation between telomere length and the life span of an animal (humans have much shorter telomeres than mice), nor is there a correlation between human telomere length and a person's age.

Telomerase-deficient mice do not show profound aging defects, which we would expect if telomerase were the major factor in determining the rate of aging. It has been suggested that telomere-dependent inhibition of cell division might serve primarily as a defense against cancer rather than as a kind of “aging clock.”



Model of telomere shortening on aging

Aberrant proteins and protein turnover

Aberrant proteins accumulate in many tissues during ageing and are implicated in the pathogenesis of age associated diseases.
Aberrant proteins can arise from errors in synthesis, damage (e.g. by radicals), posttranslational modification, misfolding, and denaturation. Protection against the accumulation of aberrant proteins is provided by the proteolytic systems of the cell, including specific enzymatic scavengers.

Error accumulation hypothesis

The alternative hypothesis is that the primary ageing event is stochastic, not determined, only governed by laws of chance. This is called the "error accumulation" hypothesis. It is well known that many types of error appear quite normally in the enormously complex pattern of chemical events in the cell called "metabolism".
The healthy adult cell has wide adaptive capacities and many types of "repair" mechanisms with which to correct these errors before major damage is done.

The action of ionizing and cosmic radiation on the cells causes such errors to occur. These errors have little permanent effect as long as they do not affect the central machinery of protein synthesis and replication in the cell. The group of enzymes responsible for the regulation of protein synthesis and for the replication of the chromosomes is much more critically susceptible to errors than other groups of cellular proteins.

An error introduced into such a central regulatory protein, which for instance can reduce its ability to identify precisely the other molecules it reacts with, will become rapidly magnified many times by what one can call a "vicious circle" mechanism. Once error-containing, such an enzyme will cause more error-containing proteins to be synthesized.

IS SENESCENCE PROGRAMED?

Ageing programme hypothesis

The concept of ageing as a "genetic programme" comes from the detailed studies of the development of the embryo and the growth and differentiation of the organism, which can together be called "morphogenesis", the development of form and structure.

It is of course well-proven that the development of the adult individual from the fertilized egg is directed by the total genetic information, contained in the genes on the chromosomes in each cell nucleus. This "genetic programme" has a very accurate timing rnechanism, which switches genes on and off as required , resulting in a reproducible sequence of events at the molecular, cellular and organ levels.

Although many molecular mechanisms of aging have been studied and are akin to an inevitable accumulation of toxic metabolic waste products or damage caused by them, there have been established theories claiming that aging is programmed.

Most obviously, the average lifespan within a given species is genetically programmed in one way or the other.

Hutchinson-Gilford Progeria Syndrome ("Progeria", or "HGPS") is a rare, fatal genetic condition characterized by an appearance of accelerated aging in children. Its name is derived from the Greek and means "prematurely old." While there are different forms of Progeria, the classic type is Hutchinson-Gilford Progeria Syndrome, which was named after the doctors who first described it in England; in 1886 by Dr. Jonathan Hutchinson and in 1897 by Dr. Hastings Gilford.

HGPS is caused by a mutation in the gene called LMNA (pronounced, lamin - a). The LMNA gene produces the Lamin A protein, which is the structural scaffolding that holds the nucleus of a cell together. Researchers now believe that the defective Lamin A protein makes the nucleus unstable. That cellular instability appears to lead to the process of premature aging in Progeria.

Genetic aging programs

Several genes have been shown to affect aging. In humans, Hutchinson-Gilford progeria syndrome causes children to age rapidly and to die (usually of heart failure) as early as 12 years.

It is caused by a dominant mutant gene, and its symptoms include thin skin with age spots, resorbed bone mass, hair loss, and arteriosclerosis. A similar syndrome is caused by mutations of the *klotho* gene in mice.

Interactions between the components theory of ageing

Free radicals can damage enzymes and thereby destroy their activity as well as provide a source of abnormal proteins which need to be degraded.

Furthermore, radicals can cause damage to mitochondria (proteins, membranes and mtDNA) and impair their functioning.

Translational errors, the incorporation of a wrong amino acid into a growing polypeptide chain, affect all proteins synthesised.

Like radical damage this can lead to an additional load on the proteolytic system via an increase in abnormal proteins.

Furthermore errors in antioxidant enzymes will reduce the level of protection against free radicals, and therefore even if free radicals were not a threat to the stability of young, healthy cells, they would become more problematic in a cell that was experiencing error propagation.

And finally since the majority of mitochondrial proteins is synthesised by cytoplasmic ribosomes translational errors do also affect mitochondria.

Both free radical damage and translational errors provide material that requires scavenging by the specialized enzyme systems that are responsible for the proteolytic removal of aberrant cellular proteins

As human life expectancy increases due to our increased ability to prevent and cure disease, we are still left with a general aging syndrome that is characteristic of our species.

Unless attention is paid to the general aging syndrome, we risk ending up like Tithonios, the miserable wretch of Greek mythology to whom the gods awarded eternal life, but not eternal youth.

[*Greek myth* : Tithonios, the son of Laomedon of Troy who was loved by the goddess Eos.
She asked that he be made be immortal but forgot to ask that he be made eternally young.
When he aged, she turned him into a **grasshopper.]**

The **complexity** of the aging process has led to the realization that an **integrative approach** is necessary to better understand the mechanisms of aging.
In this regard, omics – genomics, transcriptomics, proteomics, lipidomics and metabolomics – can play a pivotal role in the elucidation of the complex, interconnected changes that take place at the different levels of the biological hierarchy during aging.