



COST CLINIMARK TRAINING SCHOOL
Approaches for Biomarker Discovery and Validation



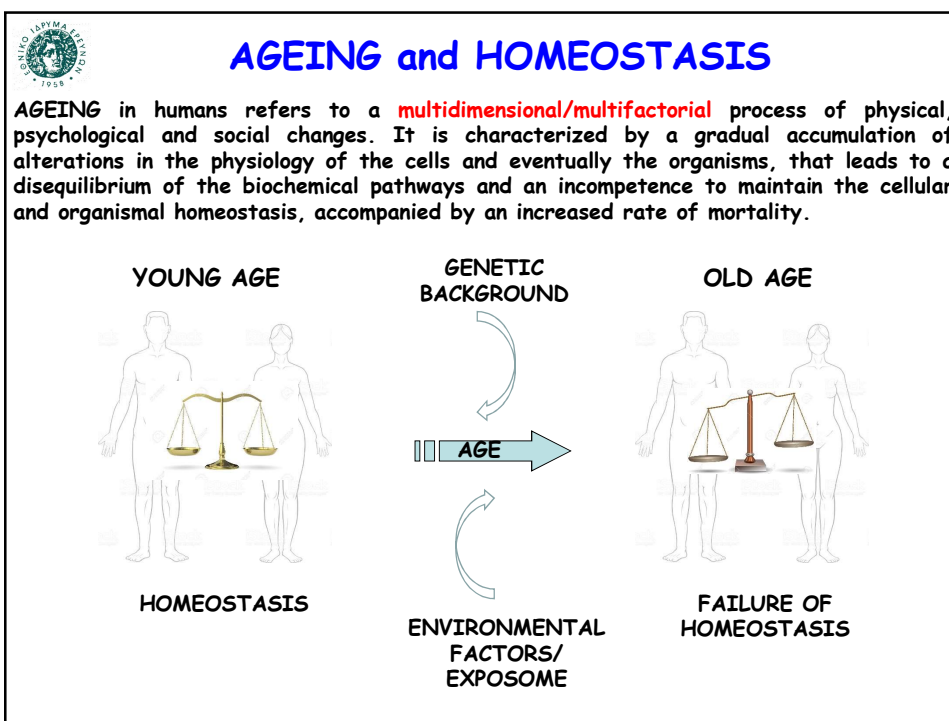
Biomarkers of (healthy) ageing

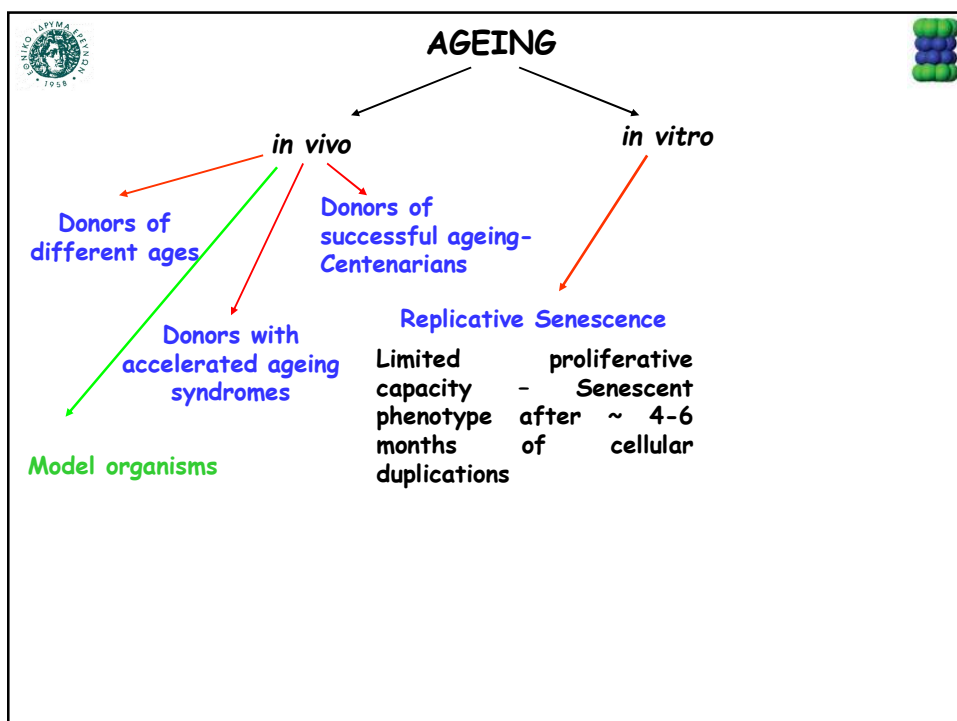
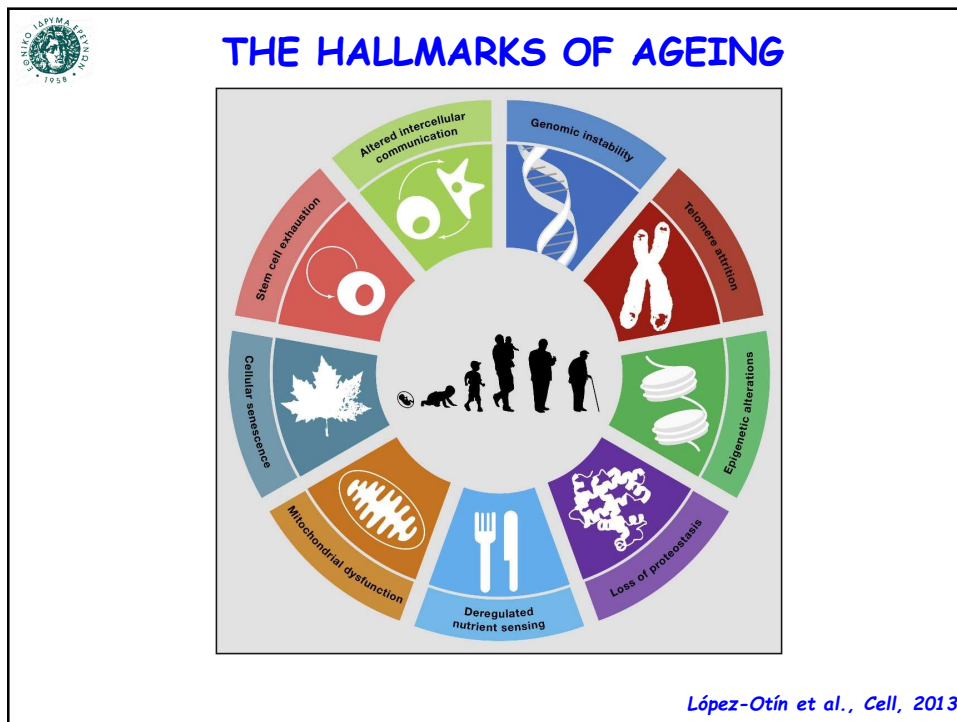
Niki Chondrogianni
 Research Associate Professor
 National Hellenic Research Foundation

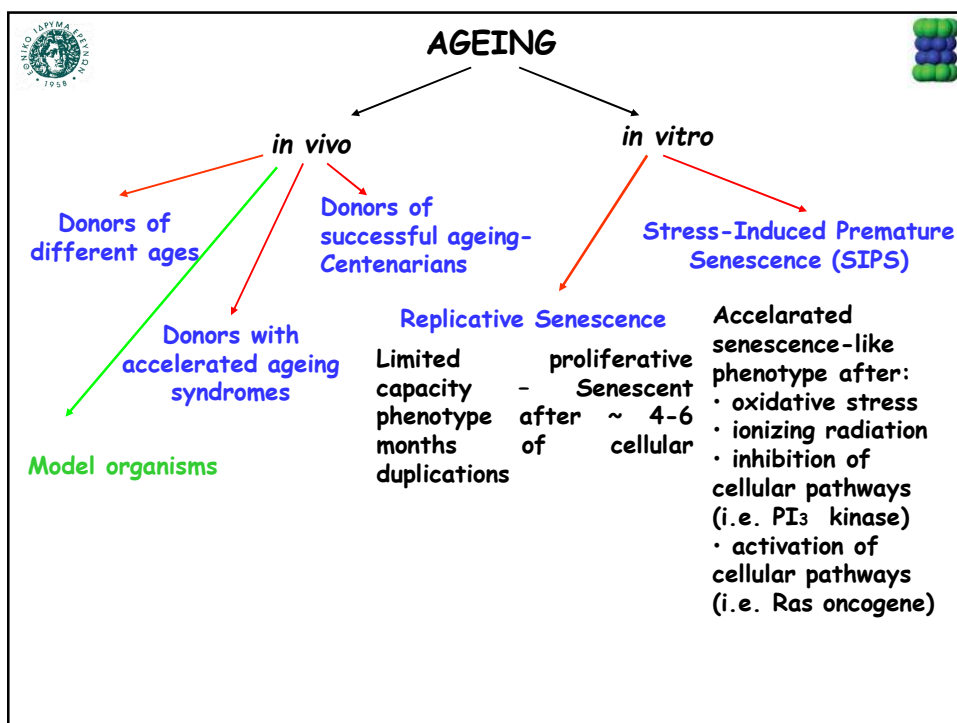
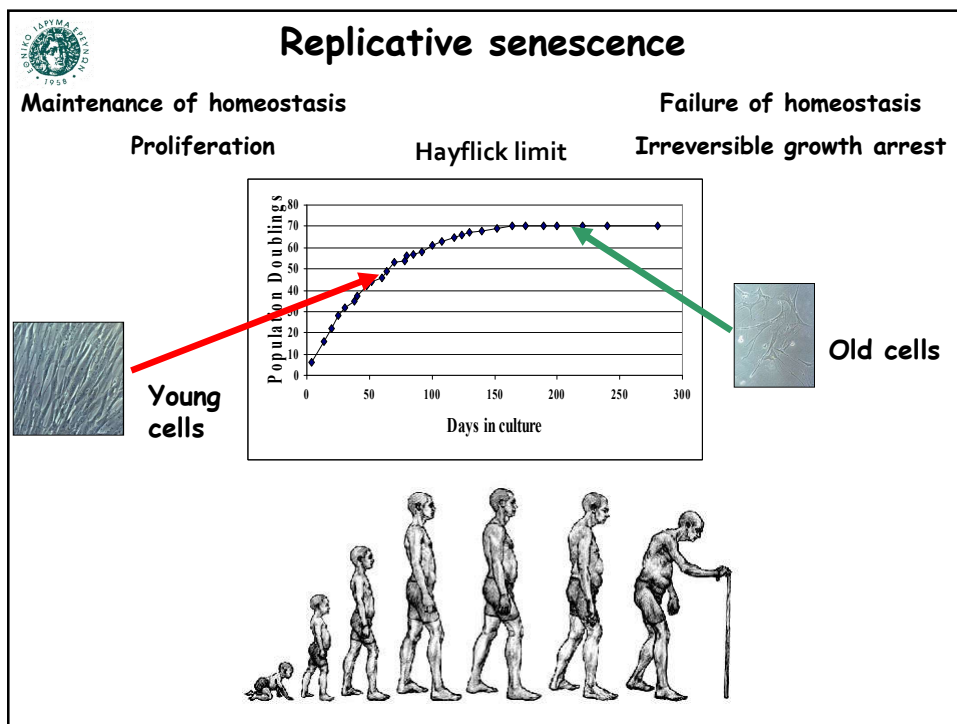


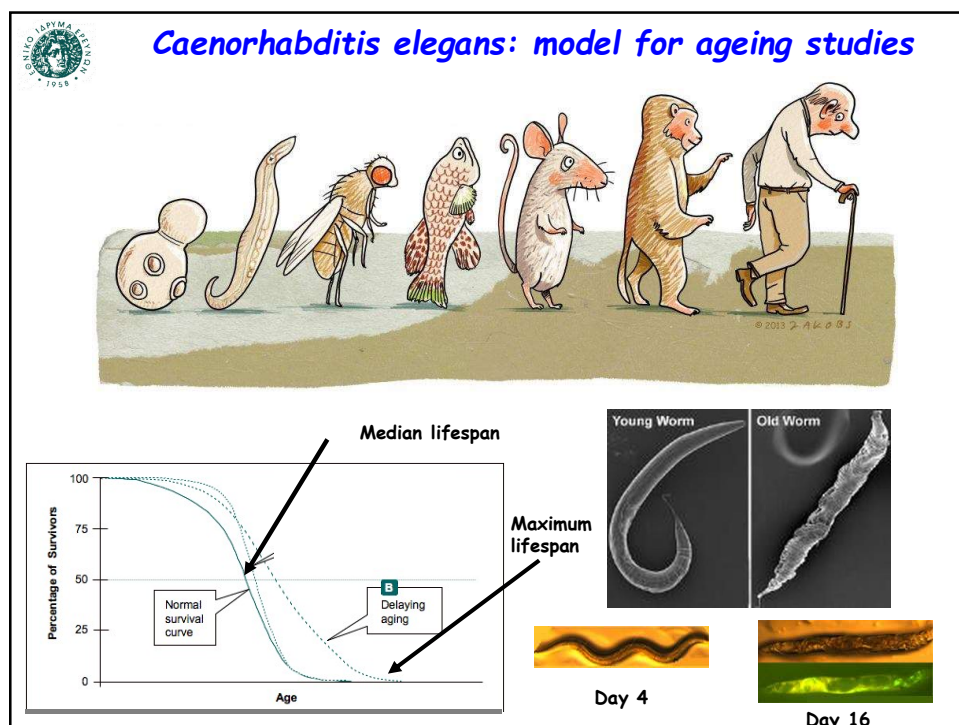
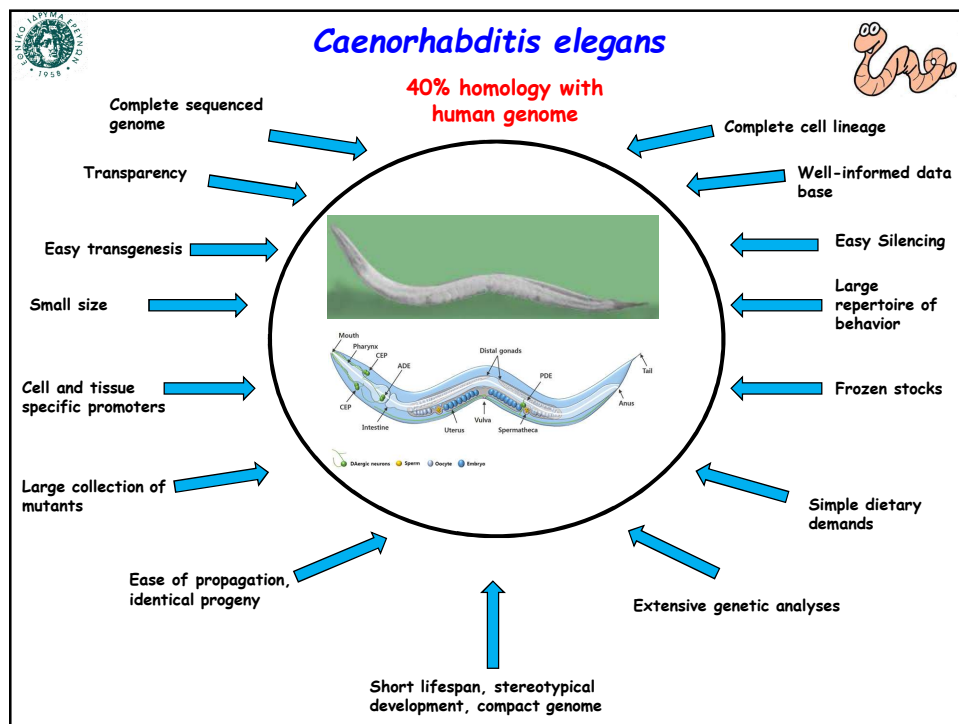












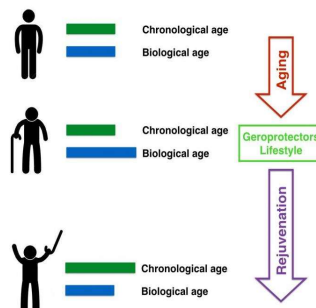


What should the biomarkers of ageing predict?

Biomarkers of ageing are biomarkers that better predict functional capacity at a later age than chronological age.

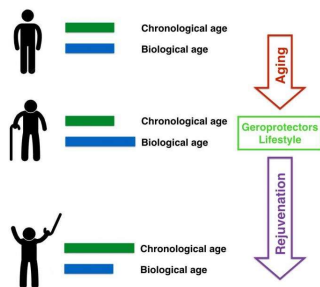
OR

Biomarkers of ageing would give the true "biological age", which may be different from the chronological age.



Why do we need biomarkers of ageing?

- Individuals of the same age may not age at the same rate
- Quantitative biomarkers of ageing are valuable tools to measure physiological age
- Quantitative biomarkers of ageing are valuable tools to assess the extent of 'healthy ageing'
- Quantitative biomarkers of ageing are valuable tools to potentially predict healthspan and life span for an individual





Criteria for an ageing biomarker (as proposed by the American Federation for Aging Research-AFAR)

- (1) it must predict the rate of ageing
- (2) it must monitor a basic process that underlies the ageing process, not the effects of a disease
- (3) it must be able to be tested repeatedly without harming the person
- (4) it must be something that works in humans and in laboratory animals

Biomarkers fulfilling all of the criteria proposed by the AFAR are unlikely to exist



Molecular biomarkers of ageing

Xia et al., F1000Research 2017



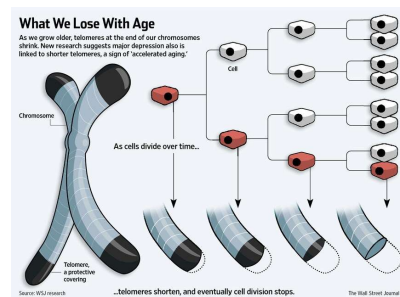
DNA and chromosomes: Telomeres



Telomeres are ribonucleoprotein complexes at the end of chromosomes and become shorter after each replication, as telomerase, the enzyme responsible for its replication, is not regularly expressed in somatic cells.

The **length of telomeres** in leukocytes has been associated with ageing and life span as well as age-related diseases, such as cardiovascular diseases, cancer, and neurological disorders.

Trend with age ↓



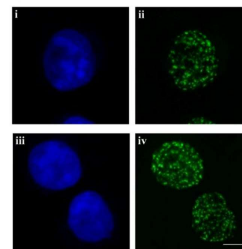
DNA and chromosomes: DNA repair

The link between DNA damage and repair has been implicated in ageing by the accumulation of senescent cells or genomic rearrangements.

Direct demonstration of the link was shown when controlled induction of DNA double-strand breaks in mouse liver induced aging pathologies and gene expression.

Immunohistochemistry of γ -H2A.X is an established quantitative biomarker of ageing because H2A.X is a variant of the H2A protein family, and phosphorylated H2A.X, γ -H2A.X, is an initial and essential component of DNA damage foci and therefore a reliable marker of the extent of DNA damage.

Trend with age ↑





DNA and chromosomes: Epigenetic modifications

Age-related changes in **DNA methylation patterns**, notably as measured by the epigenetic clock, are among the best-studied ageing biomarkers. Analysis of methylation profiles in the blood found that only three CpG sites could predict age with a mean absolute deviation from chronological age of less than 5 years. The association between age and DNA methylation can be extended to age-associated diseases, such as diabetes.

Trend with age: Global hypomethylation and local hypermethylation



RNA and transcriptome: Transcriptome profiles

A recent study used whole-blood gene expression profiles from 14,983 individuals to identify 1,497 genes with age-dependent differential expression and then used them to calculate the 'transcriptomic age' of an individual, suggesting that **transcriptome signatures** can be used to measure ageing.



RNA and transcriptome: Non-coding RNAs

MicroRNAs (miRNAs) are a class of small (21- to 23-nucleotide) non-coding RNAs that, through base-pairing mechanisms, regulate a broad range of biological processes, including metabolism and ageing. Among them, circulating miRNAs can be stable in plasma by residing in exosomes or binding to protein or lipoprotein factors, thus making them easy-to-access biomarkers.

miR-34a was the first observed circulating miRNA with an altered expression pattern during mouse aging, correlated with age-related hearing loss in mice and humans.

miR-21 was defined as an inflammatory biomarker in a study of 365 miRNAs in the plasma of healthy and old humans.

miR-151a-3p, **miR-181a-5p**, and **miR-1248** are reported to be significantly decreased with age in humans and show association with inflammation.

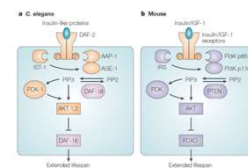
miR-126-3p has been found to be positively correlated with age in 136 healthy subjects from 20 to 90 years of age.



Metabolism: Nutrient sensing

The insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway, which participates in glucose sensing, is the earliest discovered and the most well-known pathway to antagonize longevity. Attenuation of IIS activity extends life span. Such observations led to the potential inclusion of IIS pathway members, such as **growth hormone** and **IGF-1**, as biomarkers of ageing.

Trend with age ↓



The mechanistic target of rapamycin (mTOR) protein senses high amino acid concentrations. Inhibition of mTOR can extend lifespan. **mTOR activity** increases with age in the ovarian surface epithelium of aged human and mouse ovaries, which contributes to pathological changes. **Phosphorylated S6 ribosomal protein (p-S6RP, or pS6)** is a downstream target and also a known marker of active mTOR signaling, which is a potential biomarker of ageing.

Trend with age ↑



Metabolism: Nutrient sensing

5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) and sirtuins sense nutrient scarcity instead of abundance. AMPK detects high AMP levels whereas sirtuins are sensors of high NAD⁺ levels, and both mark low-energy states. The upregulation of **AMPK activity** by metformin, a drug for type II diabetes, could mimic some of the benefits of caloric restriction, and metformin extends life span in male mice.

Trend with age ↓

Sirtuins have the ability to directly link cellular metabolic signaling (reflected by NAD⁺) to protein post-translational modifications through a chemical reaction (deacetylation of lysine). During ageing, NAD⁺ is reduced and sirtuins are downregulated. SIRT1 and SIRT6 are downregulated through passaging. Similarly, levels of SIRT1, SIRT3, and SIRT6 detected by Western blotting showed significant decrease in ovaries of aged mice. In human peripheral blood mononuclear cells, SIRT2 also decreases with age.

Trend with age ↓



Metabolism: Protein metabolism

Protein carbamylation: The nonenzymatic binding of a "carbamoyl" moiety (-CONH₂) to free functional groups of proteins, peptides, and free amino acids, resulting from the interaction between an electrophilic compound (generally isocyanic acid) and a nucleophilic functional group, most of the time an amino group



Trend with age ↑

Advanced glycation end products (AGEs) are a heterogeneous group of bioactive molecules that are formed by non-enzymatic glycation of proteins, lipids, and nucleic acids. Accumulation of AGEs in aged tissues leads to inflammation, apoptosis, obesity, and other age-related disorders.

Trend with age ↑



Metabolism: Lipid metabolism

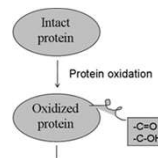
Triglycerides are found to increase with age and thus could be a biomarker of ageing. Studies of serum samples by shotgun lipidomics found that phospho/sphingolipids are putative markers, and biological modulators, of healthy ageing.

Trend with age ↑



Oxidative stress and mitochondria

Biomarkers of oxidative stress have long been regarded as a class of ageing biomarkers. The **products of oxidative damage to proteins** include o-tyrosine, 3-chlorotyrosine, and 3-nitrotyrosine. 8-iso prostaglandin F2a is a biomarker for **phospholipid damage**. 8-hydroxy- 2'-deoxyguanosine and 8-hydroxyguanosine are produced by the **oxidative damage of nucleic acids**.



Trend with age ↑



Oxidative stress and mitochondria

Although free radicals, the source of oxidative stress, are mainly produced in mitochondria, dysfunctional mitochondria can contribute to ageing independently of reactive oxygen species. **Mitochondria function** has been suggested to constitute an ageing biomarker.

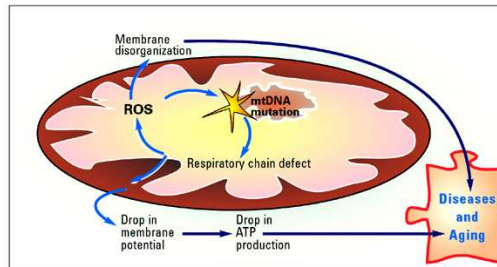


Fig. 6. Possible mechanisms of mitochondrial dysfunction include (1) Mitochondrial DNA (mtDNA) mutation caused by free radical damage; (2) Krebs' cycle decreased efficiency due to inadequate Krebs' cycle intermediates; (3) Respiratory chain defect due to enzyme and substrate alterations; and (4) Membrane disorganization and loss of fluidity. (Rustin, P. et al. "Defective mitochondria, free radicals, cell death—Reality or myth-ochondria," *Mech Age Develop.* 2000-206.)



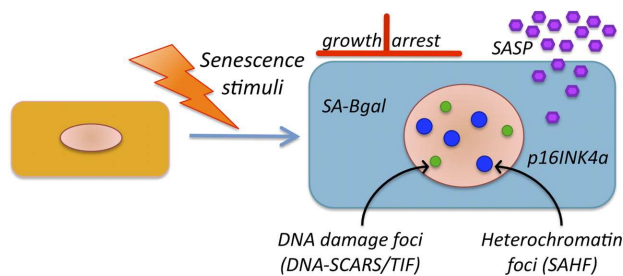
Cell senescence

The gradual accumulation of senescent cells is thought as one of the hallmarks of ageing. Thus, the biomarkers of cell senescence can also be used as markers. The most widely used are:

Senescence-associated β -galactosidase (SA- β -gal) and **p16INK4A**.

Other senescent cell markers include activated and **persistent DNA-damage response**, **telomere shortening** and dysfunction and **senescence-associated secretory phenotype (SASP)**

Trend with age





Cell senescence: SASP

SASP functions in an autocrine/paracrine manner.

Major components of SASP factors: soluble signaling factors including interleukins, chemokines, and growth factors.

Proteins that are associated with the SASP, such as interleukin-6, tumor necrosis factor-alpha, monocyte chemoattractant protein-1, matrix metalloproteinases, and IGF binding proteins, increase in multiple tissues with chronological ageing and occur in conjunction with sterile inflammation.

Catalogs of SASP also include secreted proteases and secreted insoluble proteins/extracellular matrix components.

Trend with age ↑



Phenotypic biomarkers of ageing

Xia et al., F1000Research 2017



Physical function and anthropometry

Walking speed, chair stand, standing balance, grip strength, body mass index, waist circumference, and muscle mass

Trend with age ↓

Trend with age ↑



Facial features

Quantified facial features based on three-dimensional (3D) facial images, such as mouth width, nose width, mouth-nose distance and eye corner droop, are highly associated with age.

Trend with age ↑

mouth width, nose width,
mouth-nose distance

Trend with age ↓

eye corner droop



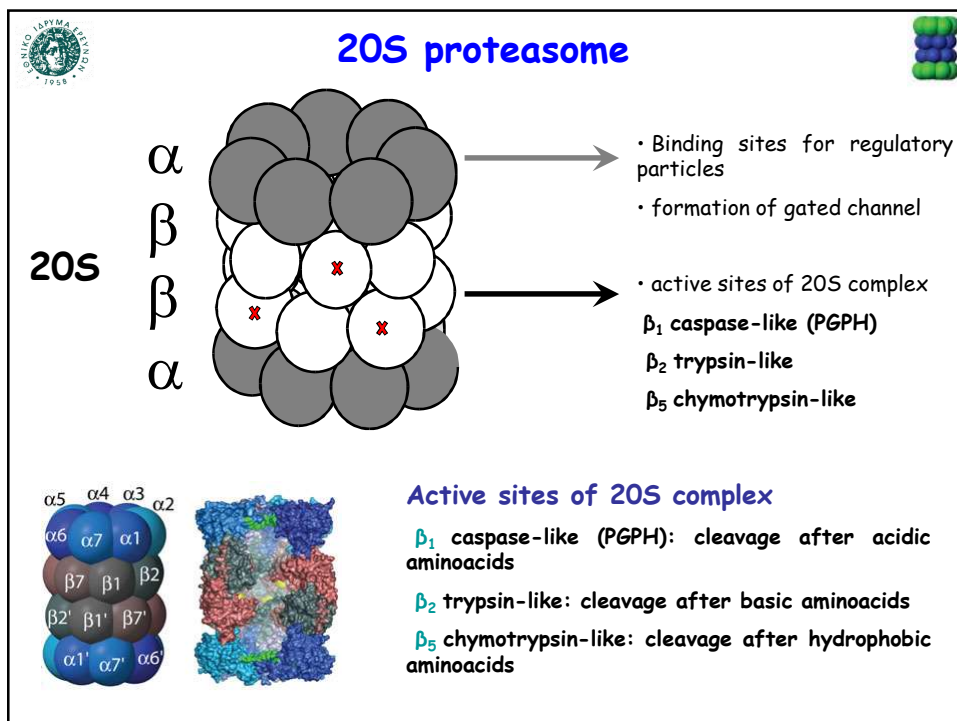
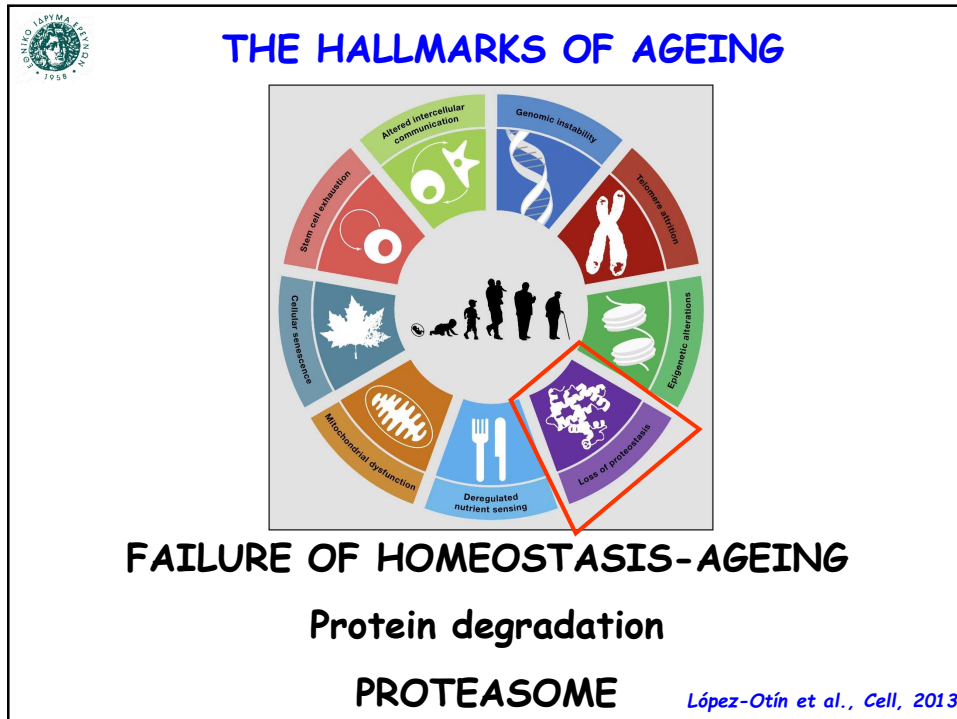
Is there one reliable biomarker of ageing?

- Ageing biomarkers are multilayered and multifaceted (like ageing itself)
- They are not equally useful (despite their involvement in the underlying biological process of ageing)

More to be discussed after Prof. Bürkle's lecture



A paradigm of a potential future biomarker of ageing



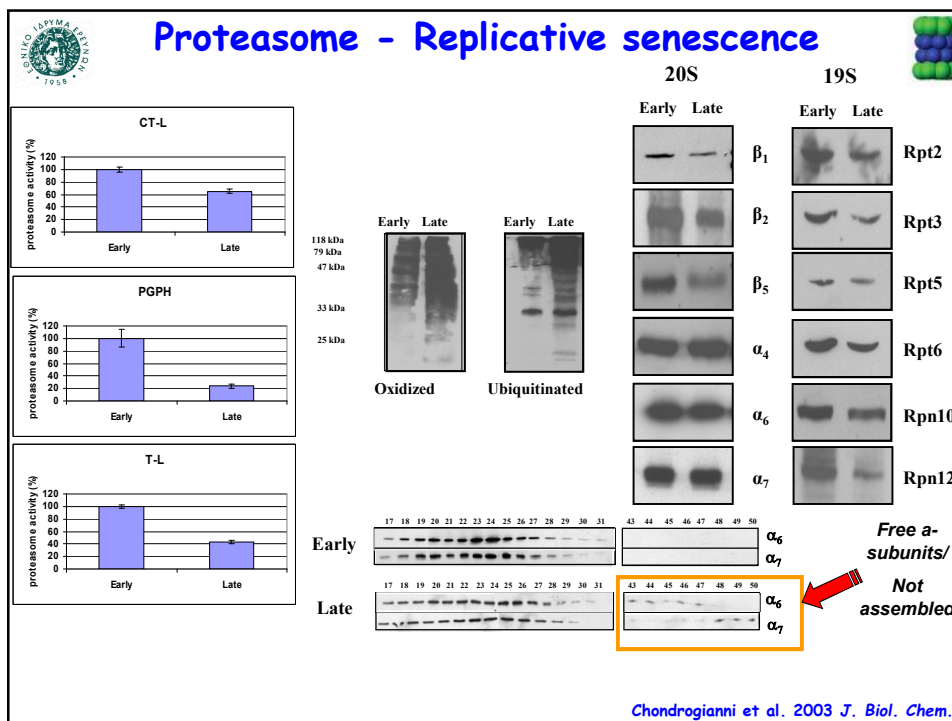
PROTEASOME

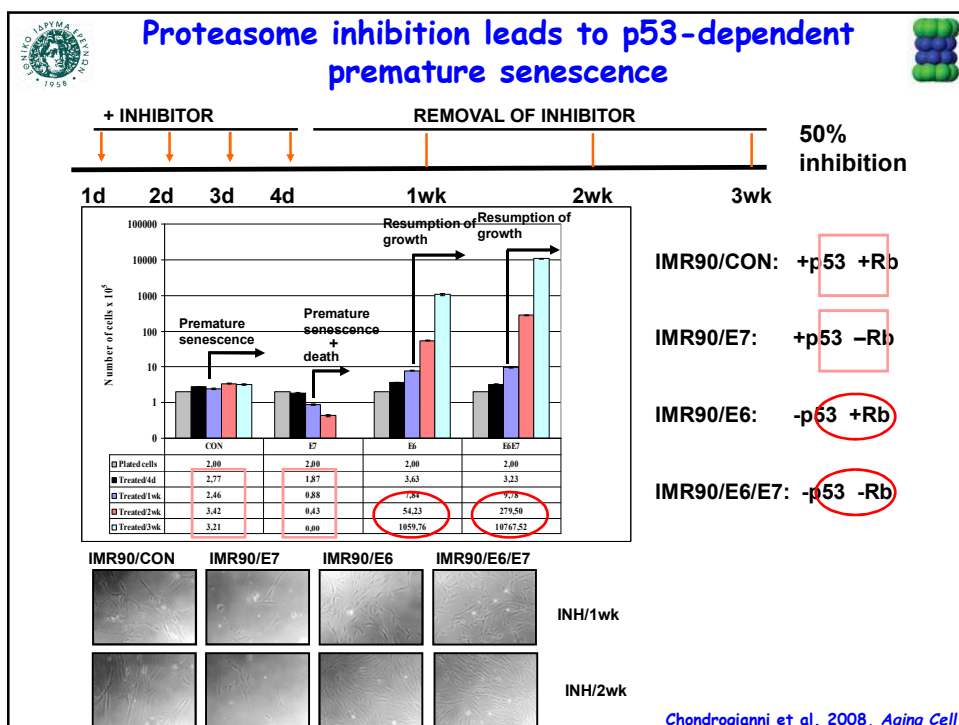
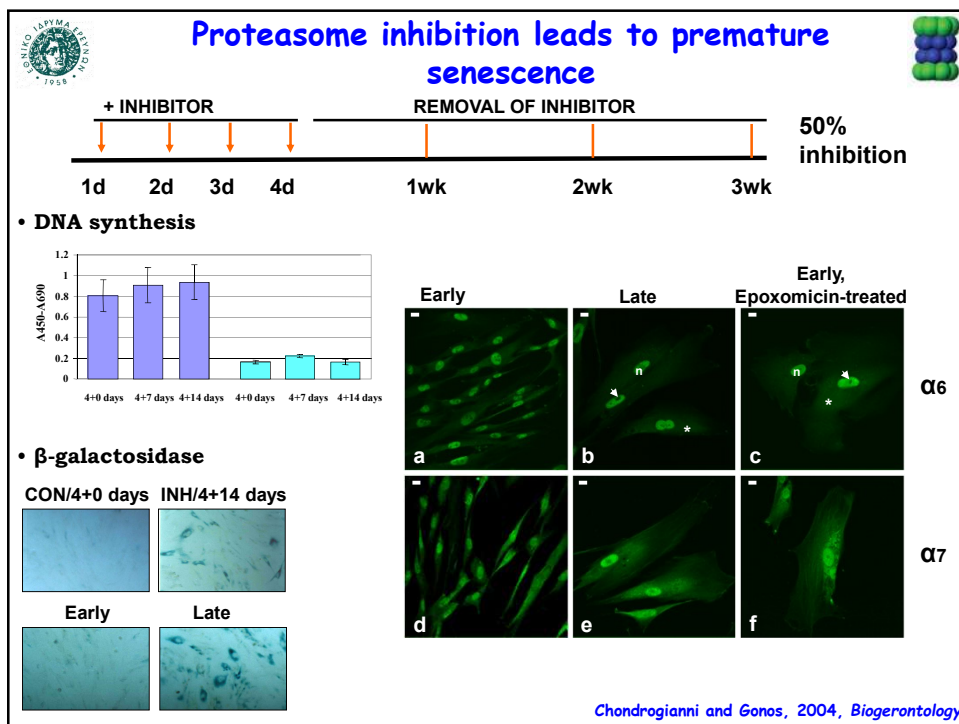
Proteolysis of:

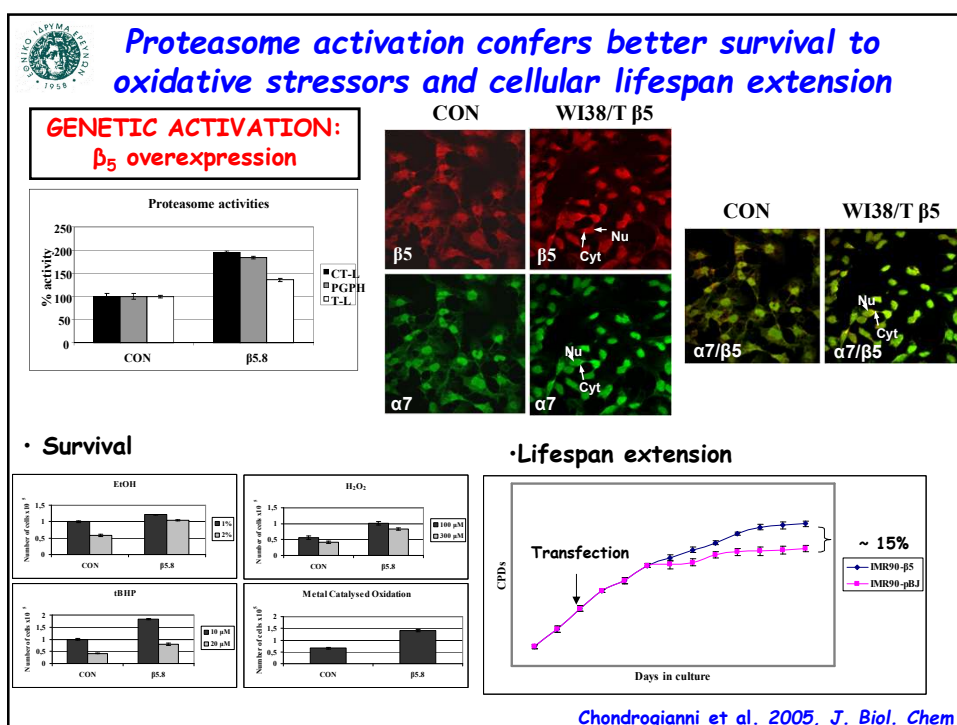
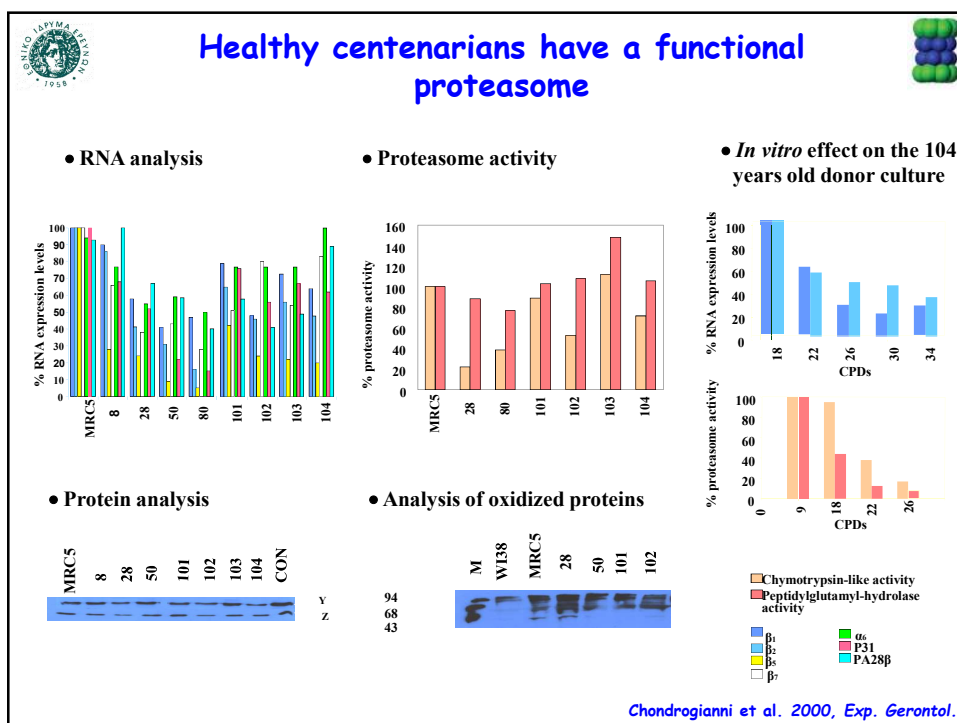
- **normal proteins** (regulation of cellular function)
- **damaged proteins** (cellular detoxification)
- **misfolded proteins** (protein quality control)
- **peptides for antigen presentation** (immune response)

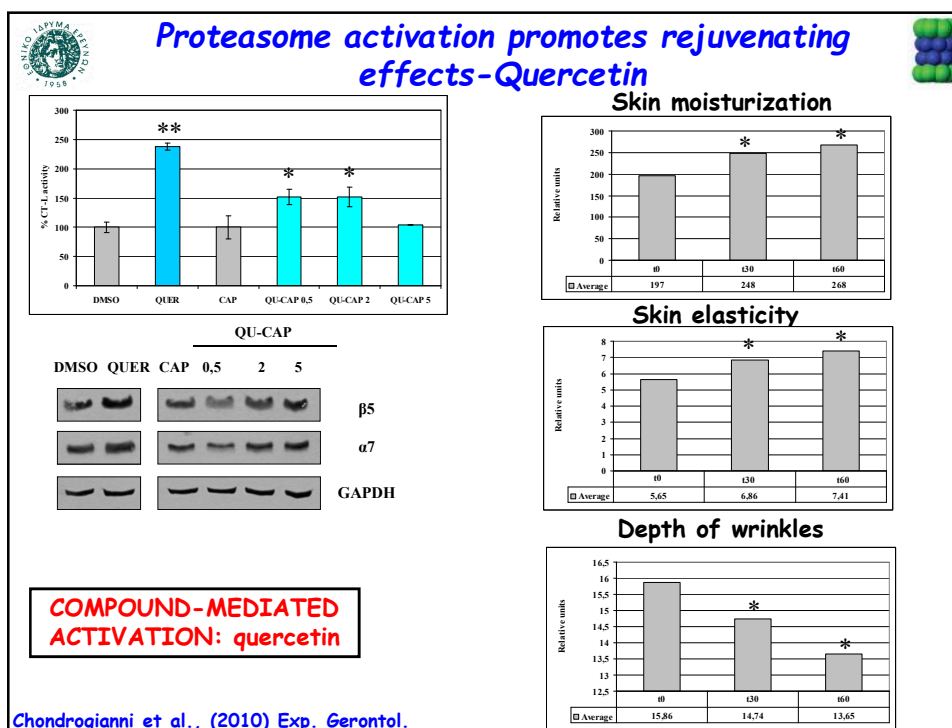
Proteasome implication in age-related diseases

| Neurodegenerative disorders | Pathological features | Proteasome activity |
|---------------------------------|---|---------------------|
| Alzheimer's disease | β -amyloid plaques/tau tangles, neuronal loss | Decreased activity |
| Parkinson's disease | Lewy bodies, neuronal loss | Decreased activity |
| Huntington's disease | Poly-glutamine inclusions, neuronal dysfunction/loss | Decreased activity |
| Polyglutamine disease | Ubiquitinated nuclear inclusions (NI) | Inhibition |
| Amyotrophic lateral neuron loss | SOD1 aggregates, motor sclerosis | Decreased activity |
| Prion Disease | Mutant prion protein | Inhibition |
| Angelman syndrome | Mutation in E6-AP an E3 enzyme | — |
| Lafora disease | Accumulation of starch-like polyglucosans, or Lafora bodies | Decreased activity |









ANTIAGEING PROPERTIES OF QUERCETIN, 18 α -GLYCYRRHETINIC ACID, HEDERAGENIN AND THEIR DERIVATIVES (PATENT NO: 09006147.4-2108)

PROPRIETOR: KORRES S.A. NATURAL PRODUCTS (EUROPE & ASIA)
JOHNSON & JOHNSON CONSUMERS COMPANIES INC (USA)

INVENTORS: E.S. GONOS, I. CHINOI & N. CHONDROGIANNI

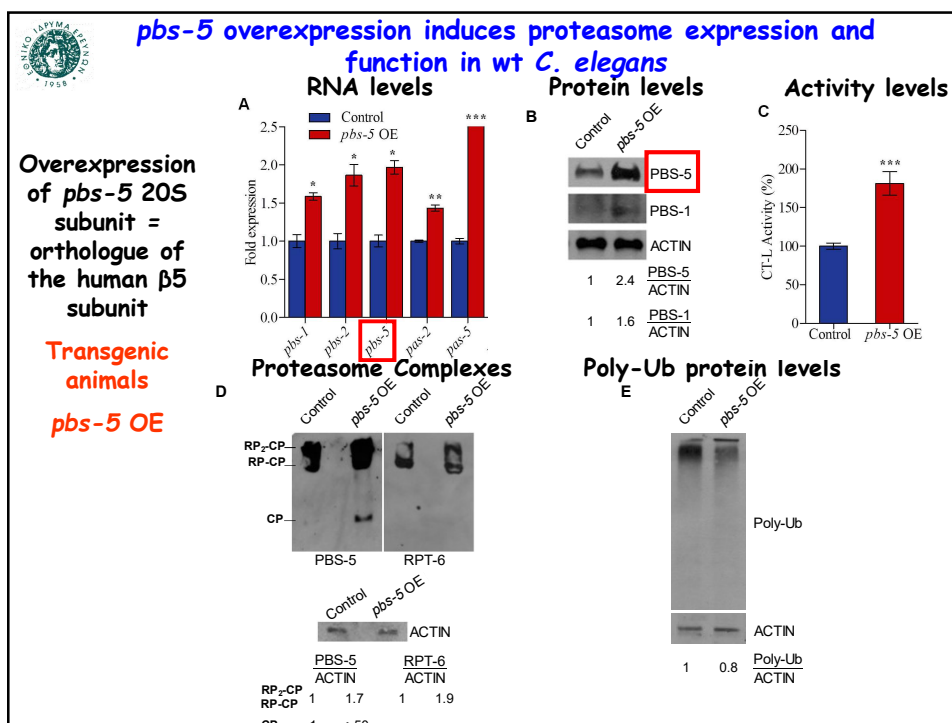
BIO-INSPIRED PROTEASOME ACTIVATORS WITH ANTI-AGEING PROPERTIES (Patent No. GR 20180100094, 07/03/2018, PCT/GR2019/000018)

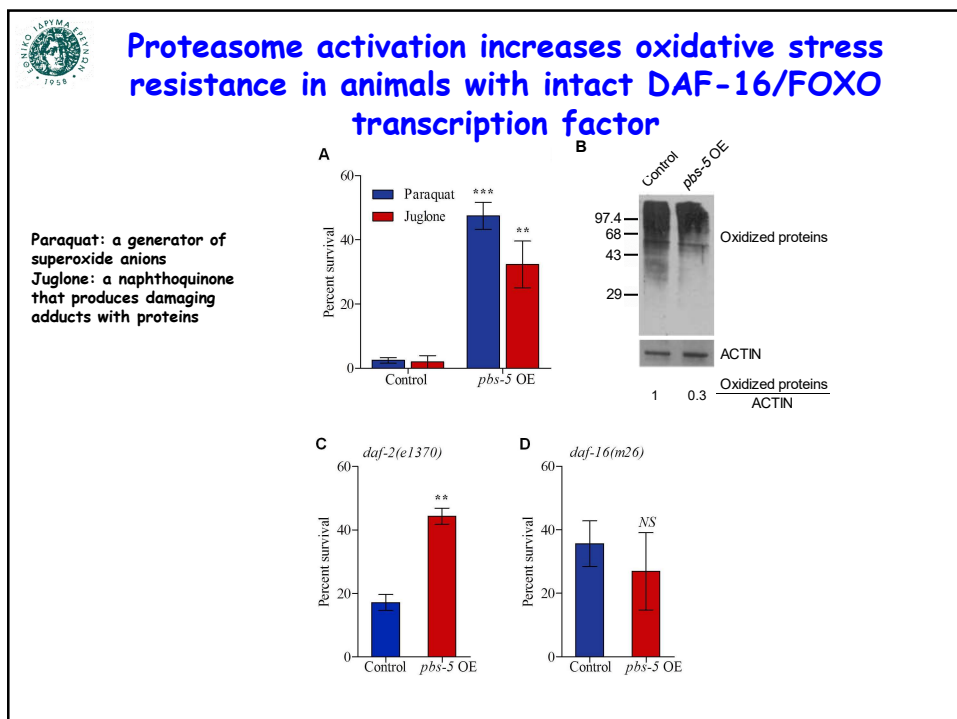
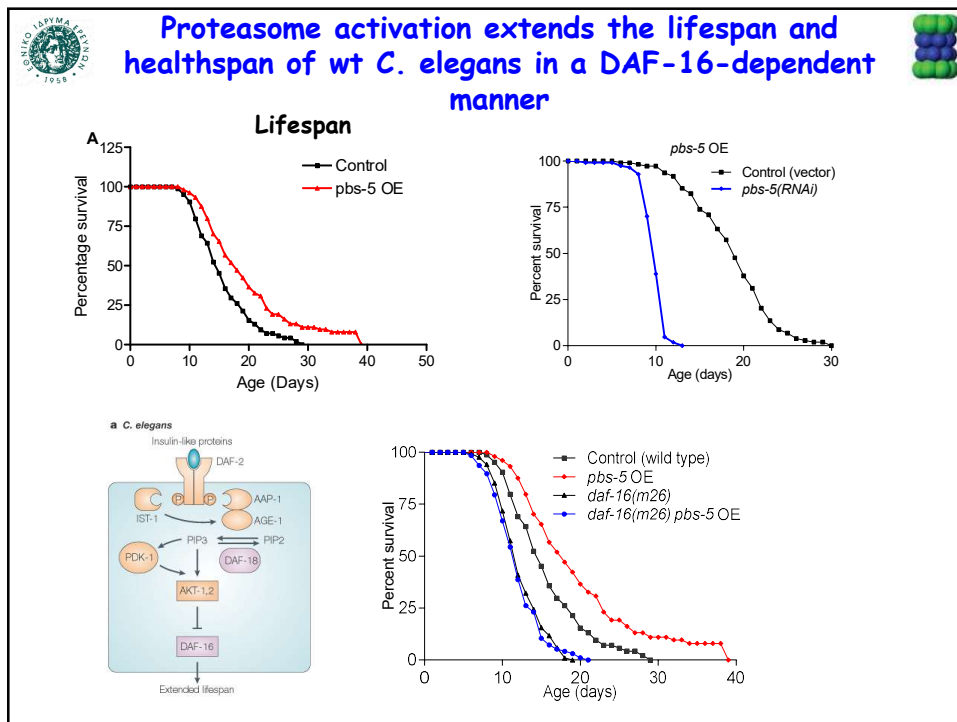
PROPRIETOR: NATIONAL HELLENIC RESEARCH FOUNDATION

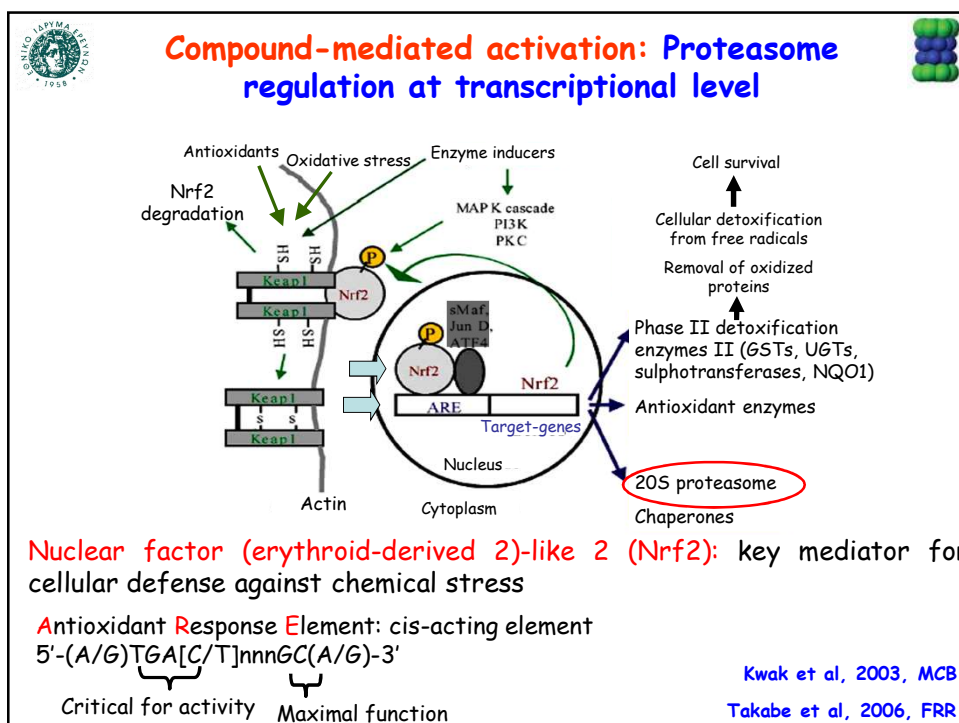
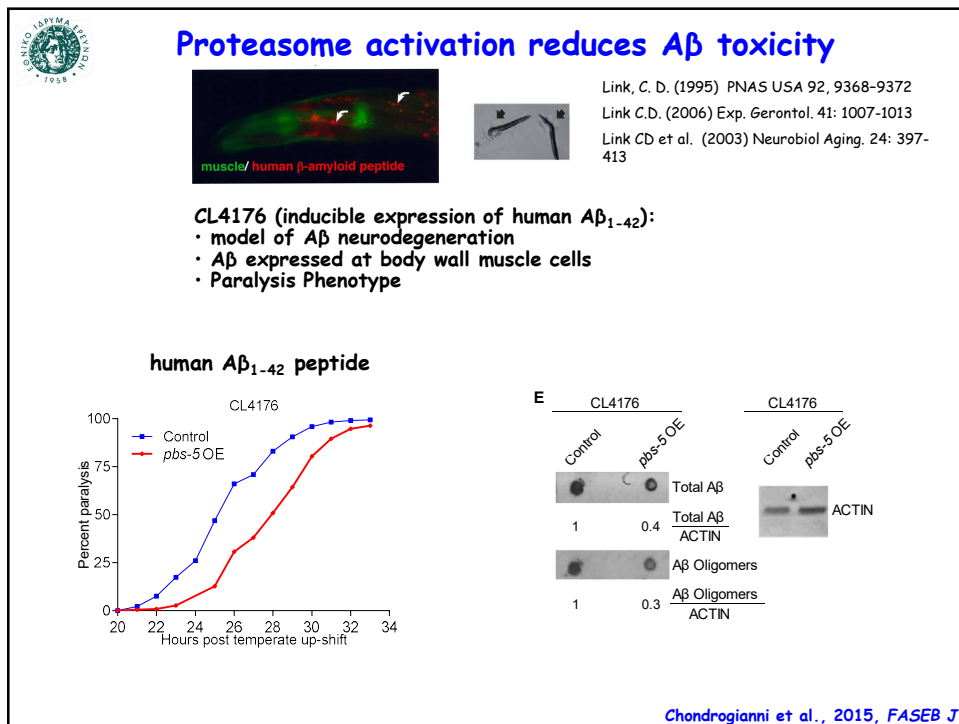
INVENTORS: Koufaki M., Calogeropoulou T., Chondrogianni N., Papahatjis D., Gonos E.S., Fotopoulou T., Prousis K., Chazapi E.

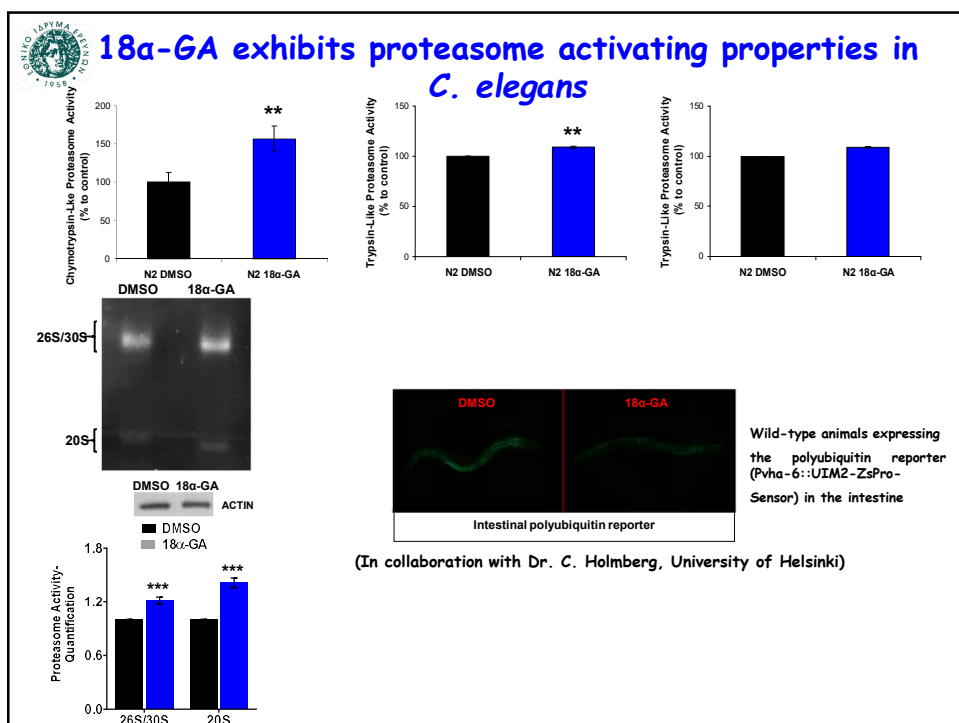
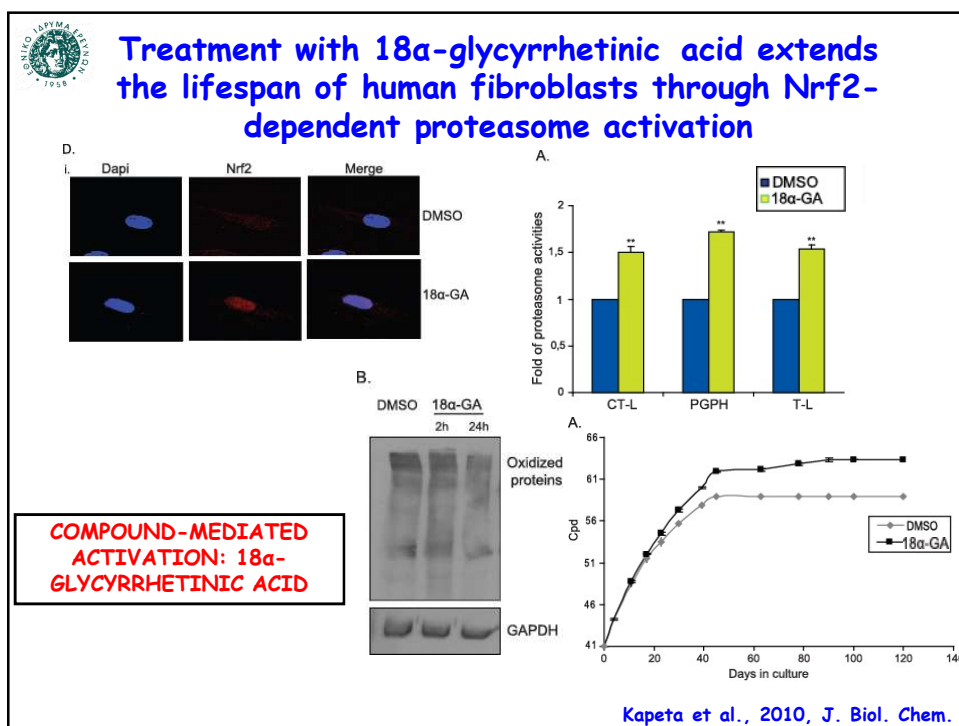
This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH– CREATE – INNOVATE (project code: T1EDK-01610). This work was also supported by the project "STHENOS-b" (MIS 5002398), funded by the Operational Program "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the EU (European Regional Development Fund).

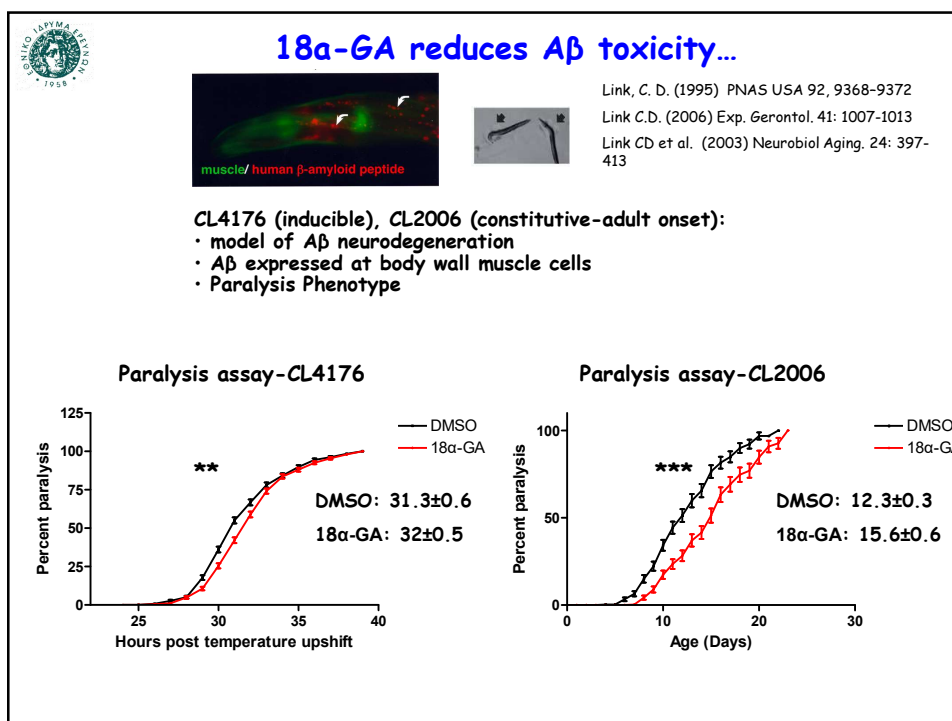
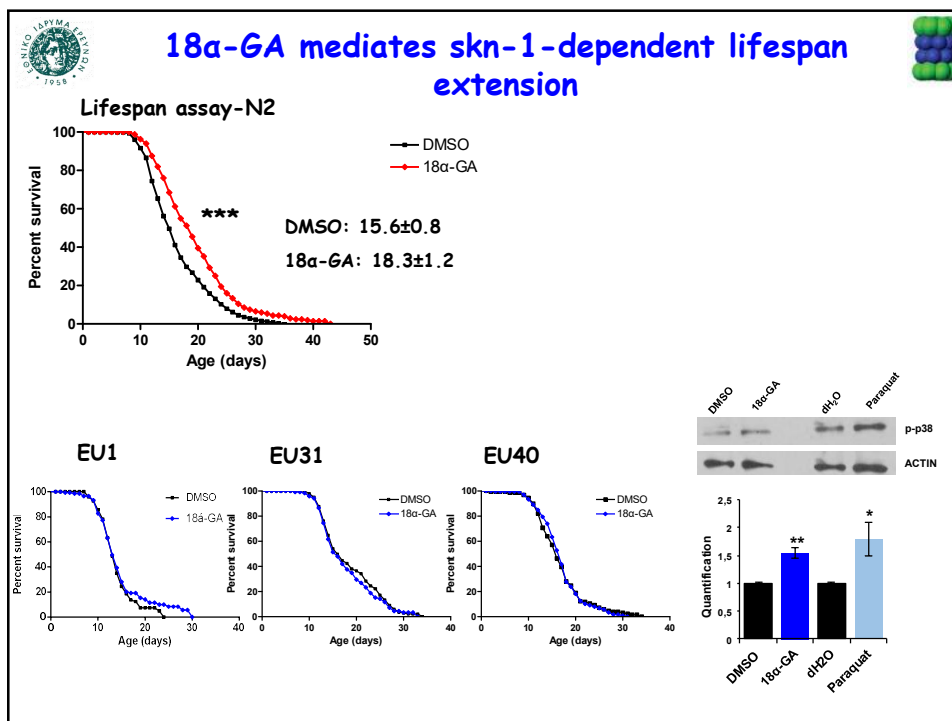
Co-financed by Greece and the European Union

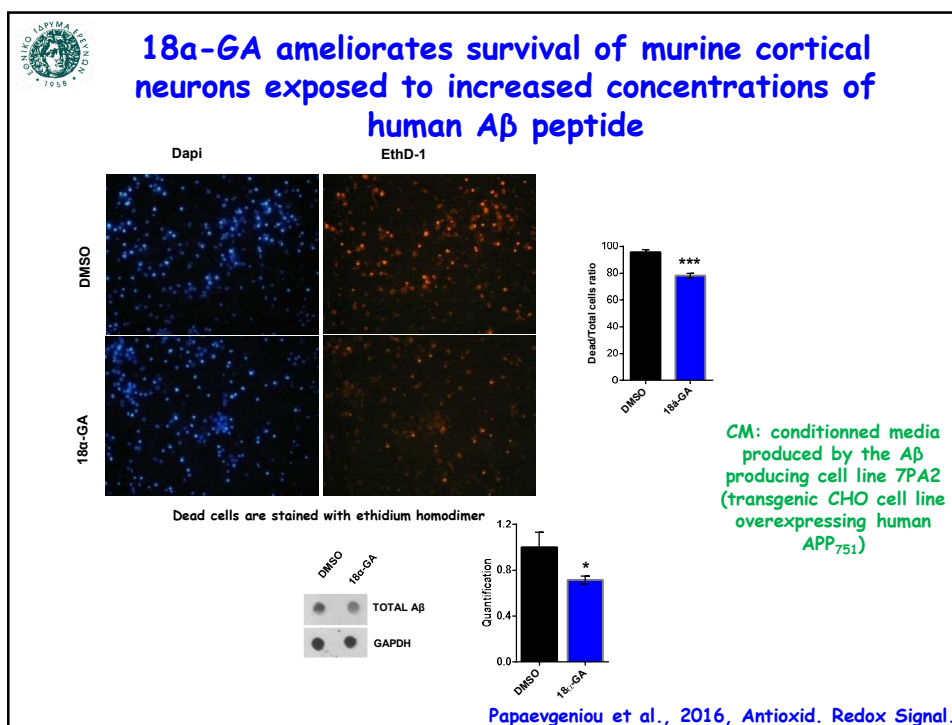
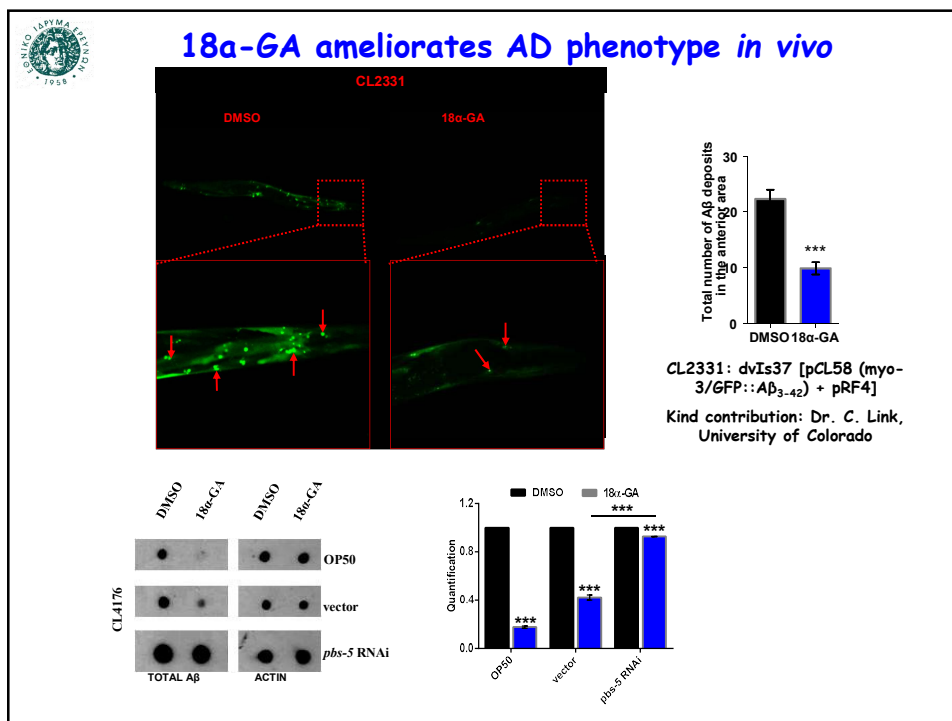














Proteasome is:

- Downregulated with increasing age
- Downregulated during replicative senescence
- Maintained in centenarians

Proteasome activation through genetic means or through treatment with specific compounds is feasible in the multi-cellular level resulting in:

- Lifespan extension
- Healthspan maintenance
- Increased resistance to proteotoxicity

COULD IT BE A POTENTIAL VALUABLE BIOMARKER OF HEALTHY AGEING?



Acknowledgments



National Hellenic Research Foundation

Anna Gioran, PhD
 Nikoletta Papaevgeniou, MSc
 Eleni Panagiotidou, MSc
 Mary Vasilopoulou, MSc
 Foteini Aktypi, BSc
 Apostolos Petsas
 Maria Lefaki, MSc
 Konstantina Filippopoulou, MSc
 Anna Paraskevopoulou, MSc

Mirella Sakellari

Stathis Gonos

**Special thanks to Dr.
 Zoidakis and you for
 your attention**

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 L. Alexopoulos-NTUA
 G. Skretas-NHRF
 C. Holmberg-UH
 I. Fatouros-UTH



Acknowledgments

RESEARCH - CREATE - INNOVATE (project code: T1EDK-00353 and T1EDK-01610)

"Fountain of Youth" by Lucas Cranach the Elder

And in the end,
it's not the Years in your Life that count.
It is the Life in your Years.
-Abraham Lincoln

Thank You!

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