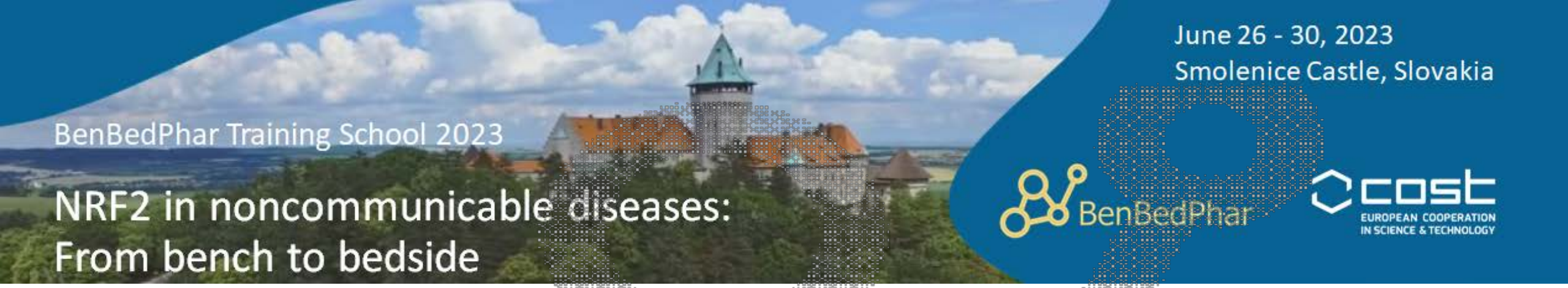


NRF2 in ageing

Prof. Ioannis Trougakos
National & Kapodistrian
University of Athens, Greece

BenBedPhar

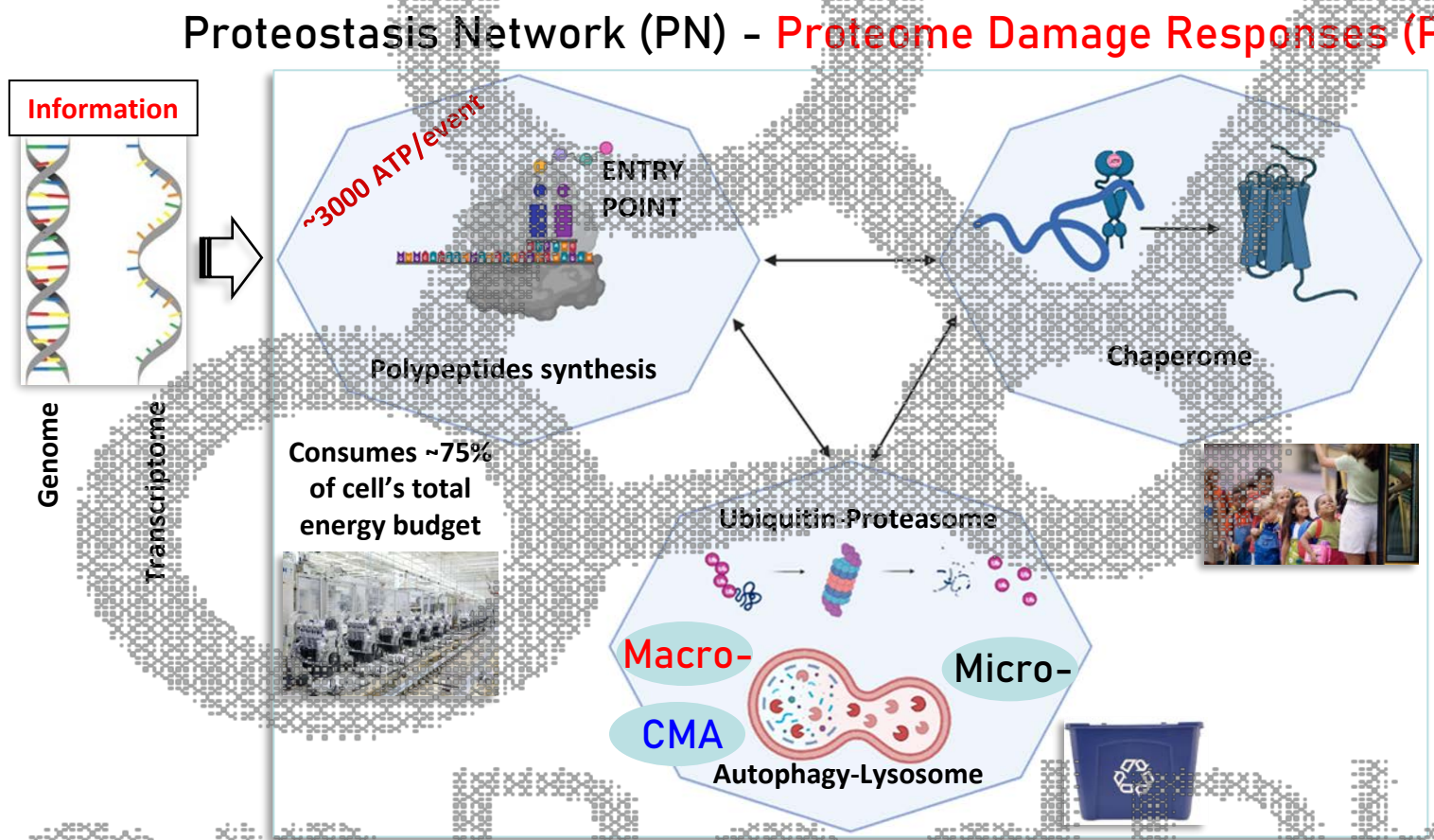
Structure of the tutorial

1. *Basic principles of biological systems*
2. *The need for sensors (e.g., the Nrf2/Keap1 system)*
3. *The unnatural process of ageing*
4. *The Nrf2/Keap1 pathway during ageing*
5. *The Nrf2/Keap1 pathway in age-related diseases*

For this lecture: **Prioritize concepts** over experiments

The world of molecular machines

The function module (largely unmapped)

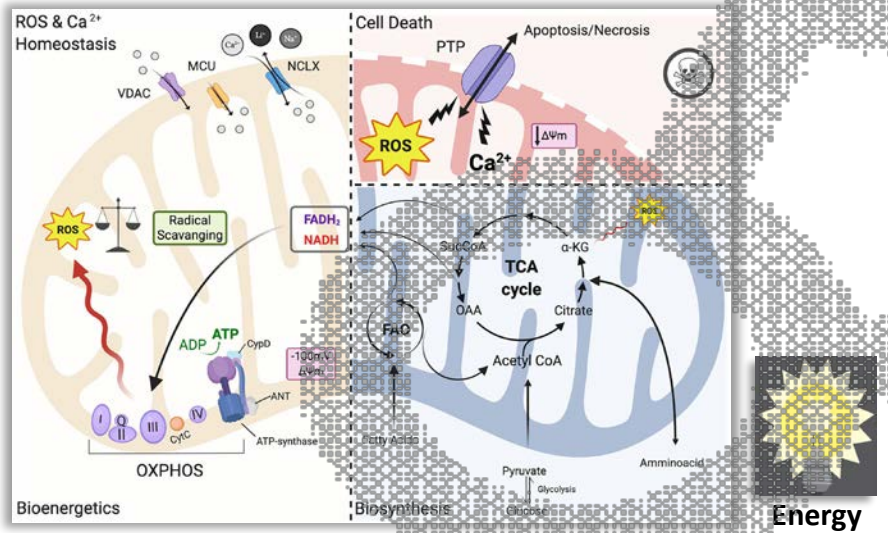


Niforou et al. (2014). Redox Biol. 30, 323-332
Gumeni and Trougakos (2016). Oxid Med Cell Longev. 4587691
Gumeni et al. (2017). Int J Mol Sci. 18(10)
Sklirou A. et al. (2018) Cancer Lett. 413, 110-121
Tsakiri and Trougakos (2019). Int Rev Cell Mol Biol. 314, 171-237

(adult, max fitness)

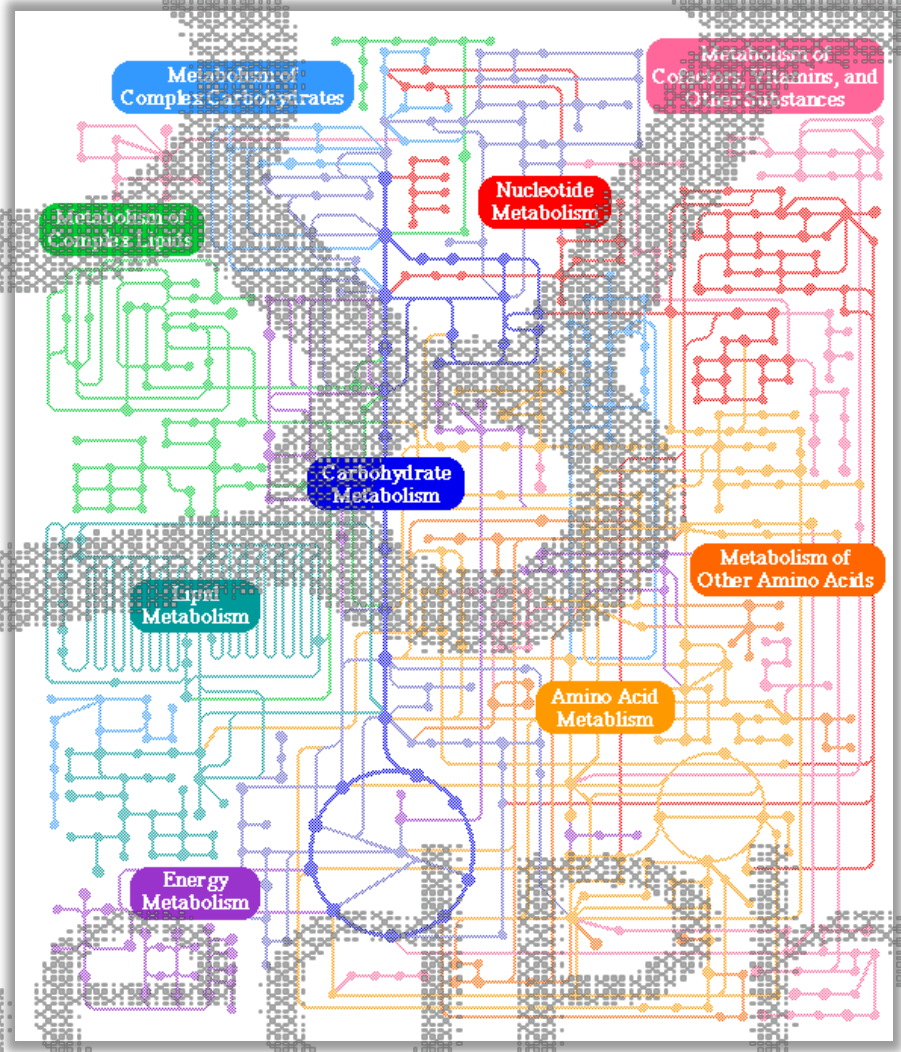
Energy, metabolism

The need to maintain machines functionality (support "life")



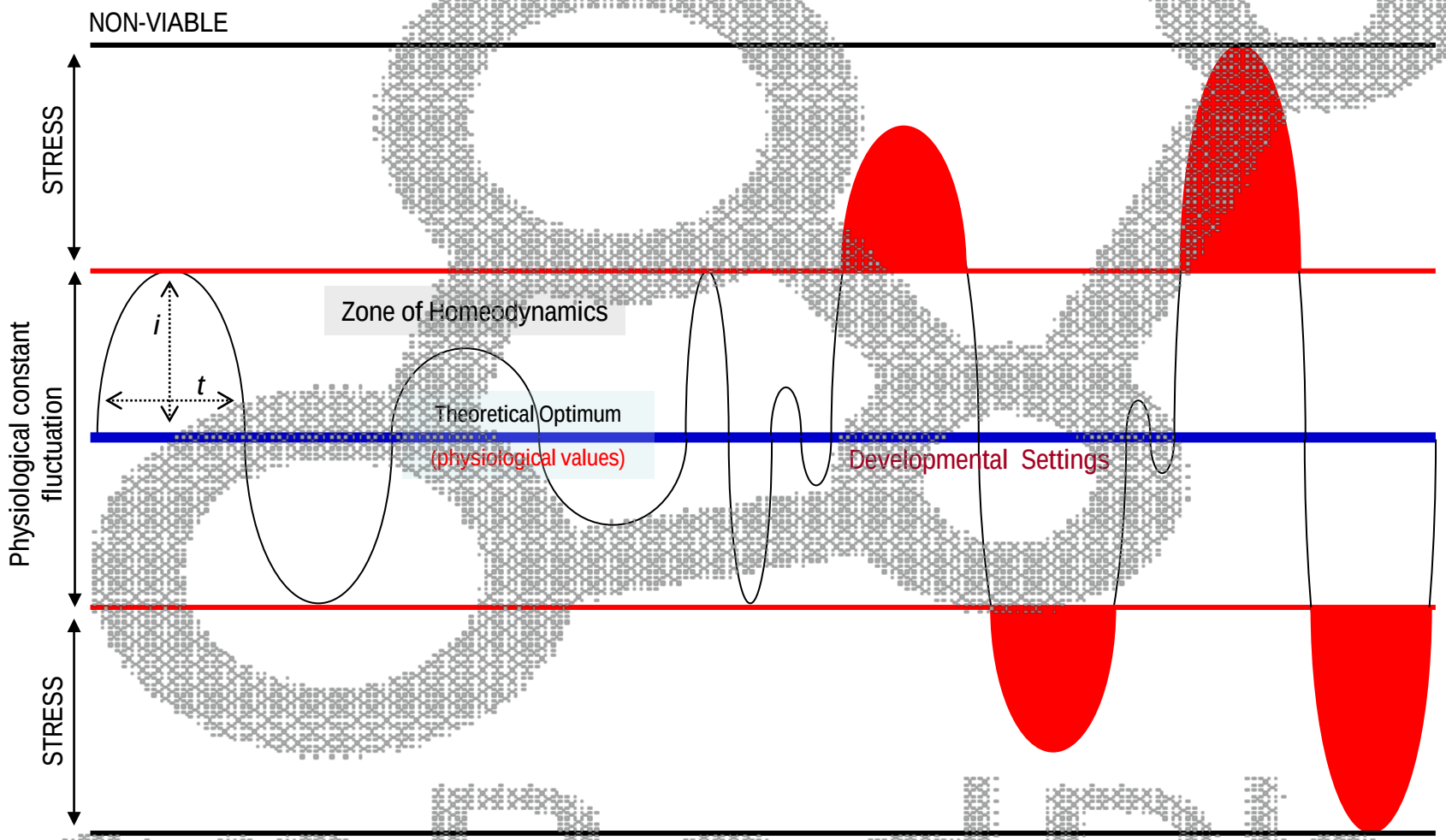
Ramaccini et al. (2021). Front Cell Dev Biol. 12, 624216

Energy



(adult, max fitness)

“Stress” - a reaction to demands of life



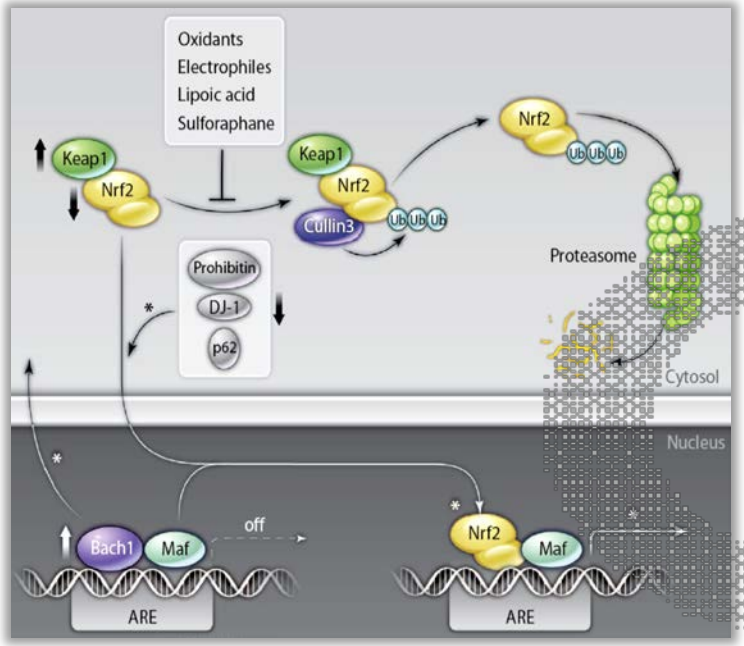
Stress is a normal psychological and physical reaction to the demands of life. In a medical or biological context stress is a physical, mental, or emotional factor that causes bodily or mental tension. **Stressors can be external (from the environment, psychological, lifestyle, or social situations) or internal (diet, illness, or from a medical procedure).**

(adult, max fitness)

Trougakos (2019) Aging 11, 5289-5291

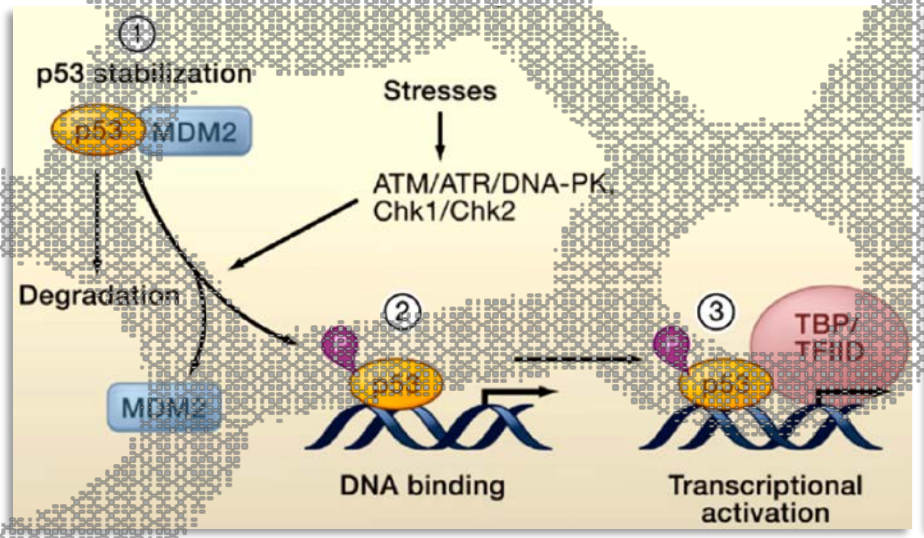
Sensors (short-lived proteins)

The need to maintain design (also known as "physiological values")

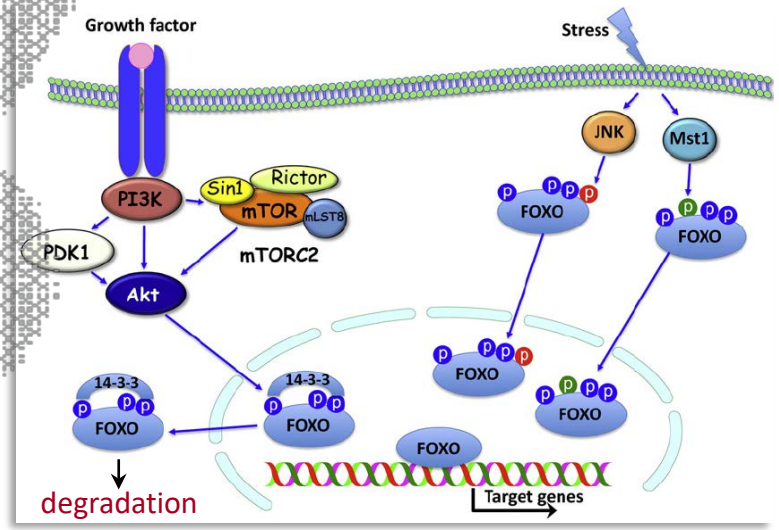


Sykiotis GP, Bohmann D. (2010). Sci Signal. 3, re3

Sensors



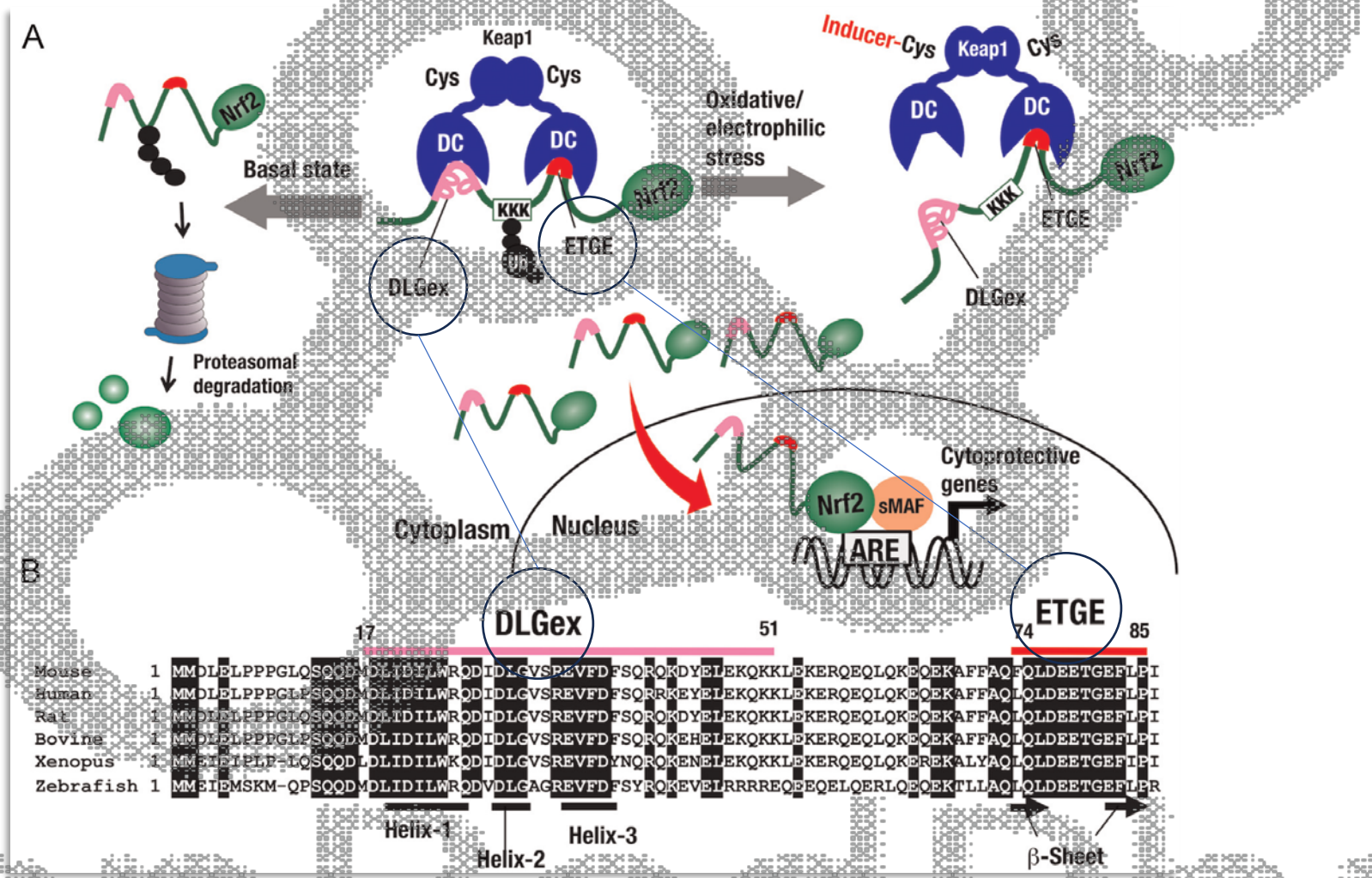
Kruse and Gu (2009). Cell 137, 609-622



Hay (2011). Biochim Biophys Acta 1813, 1965-1970

(adult, max fitness)

NRF2/KEAP1 (sensing stress)

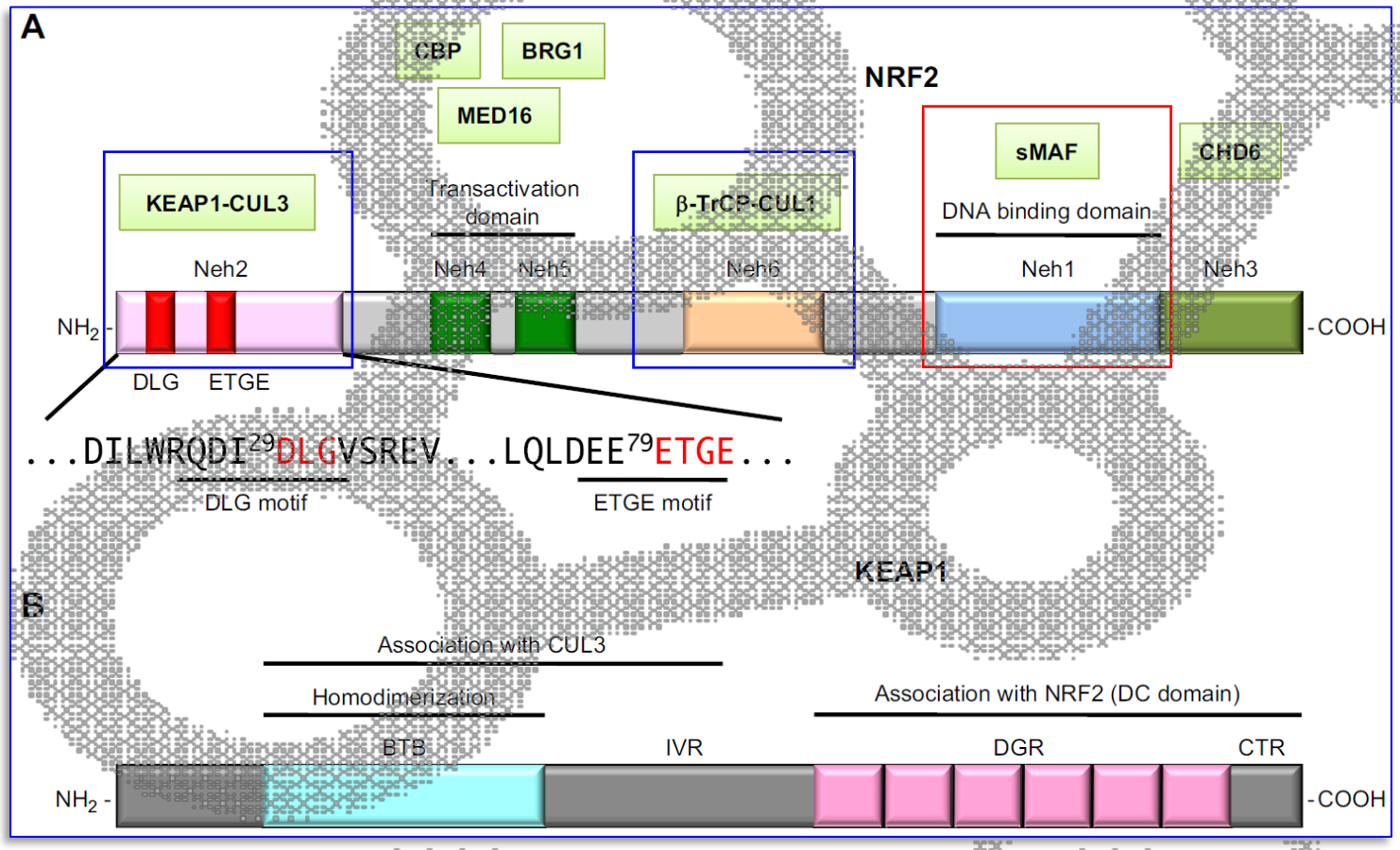


Suzuki and Yamamoto (2015). Free Rad Biol Med. 88, 9-100.

The rapid inducibility of a response based on a derepression mechanism is an important feature of the KEAP1-NRF2 system

(adult, max fitness)

NRF2/KEAP1 (functional domains)



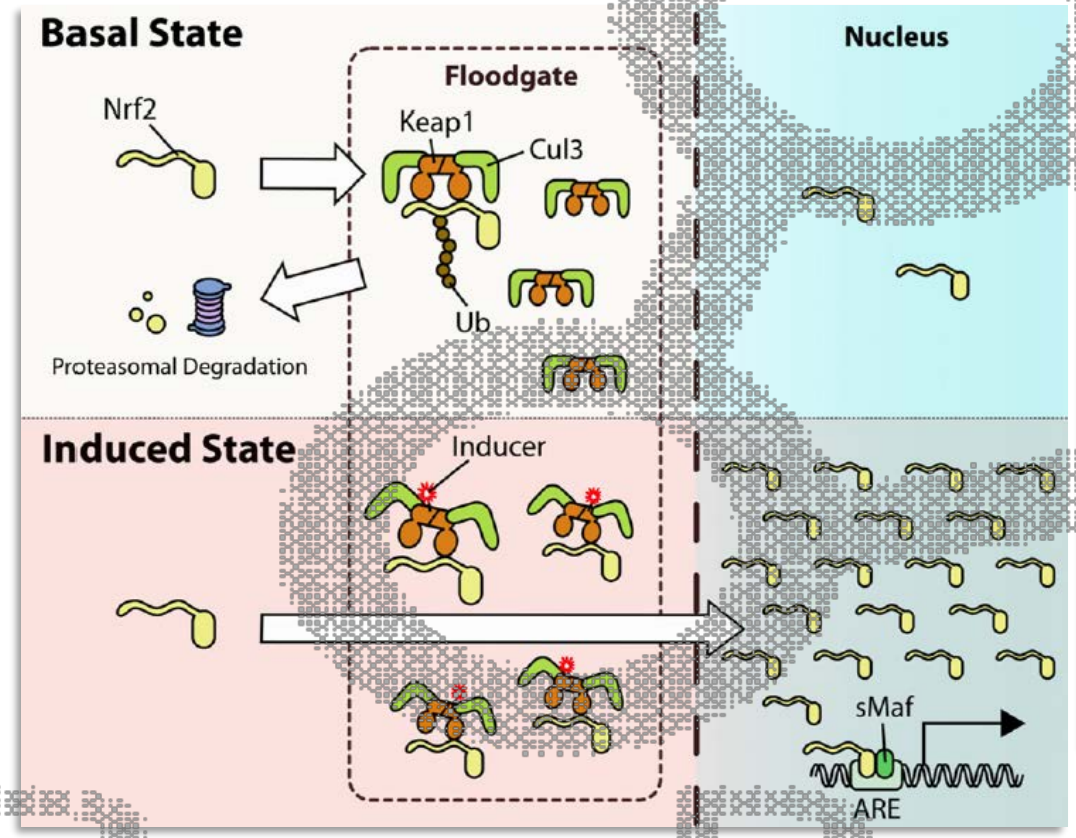
Yamamoto et al., (2018). Physiol Rev. 98, 1169-1203.

Domain structures of NRF2 (*A*) and KEAP1 (*B*). NRF2-interacting molecules are shown in green boxes and placed above their interacting domains.

(adult, max fitness)

NRF2 (amount - localization)

Absolute Amounts and Status of the Nrf2-Keap1-Cul3 Complex within Cells



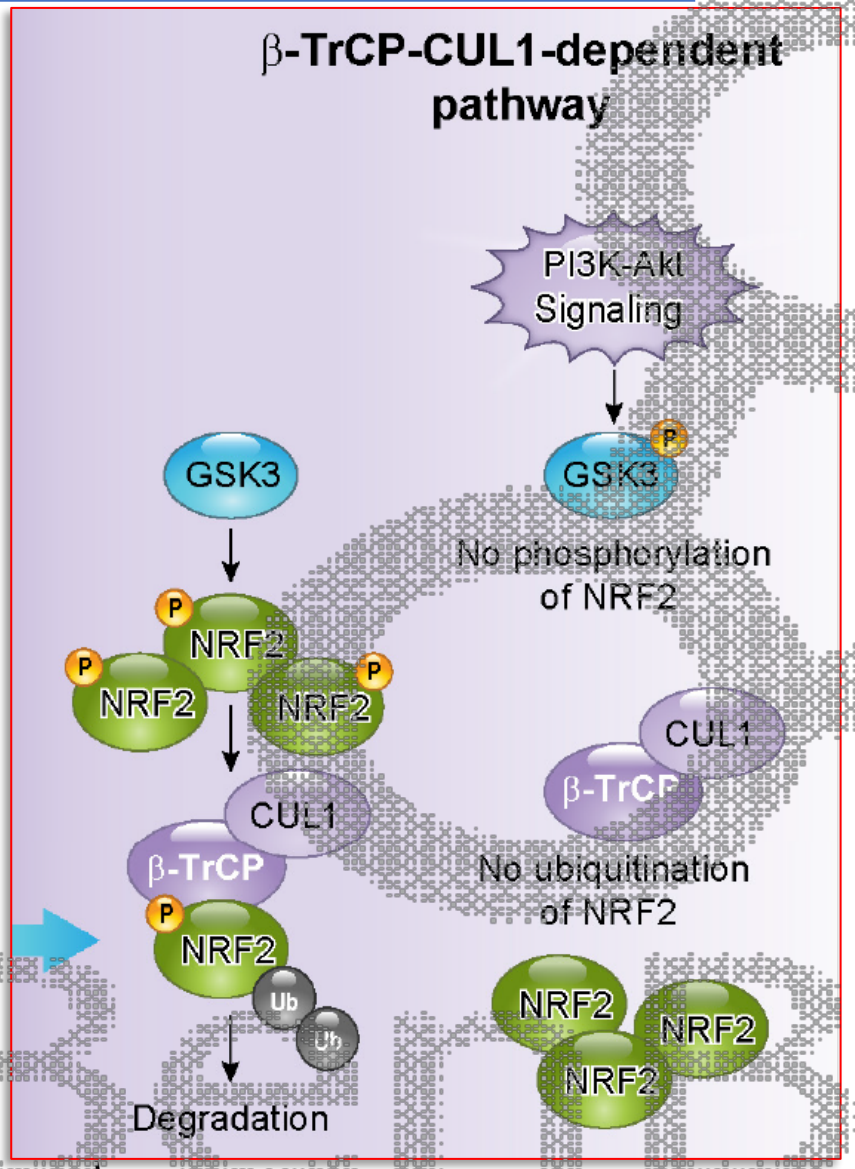
In the **basal state**, the amount of Nrf2 was maintained at lower levels than those of Keap1 and Cul3 proteins, whereas the **electrophilic agent diethylmaleate** dramatically increased Nrf2 to a level greater than that of Keap1 and Cul3, resulting in the accumulation of Nrf2 in the nucleus. In contrast, Keap1 and Cul3 did not display any changes in their abundance, subcellular localization, or interaction in response to electrophilic stimuli.

Iso et al. (2016). Mol Cell Biol 36, 3100-3112.

Distinct "populations" in the cytosol, the ER, mitochondria and nucleus

(adult, max fitness)

NRF2 (links to Growth Factors signaling)



In addition to the Keap1-dependent degradation, Nrf2 is also regulated in a Keap1-independent manner. **Glycogen synthase kinase-3 (GSK-3)** inhibits Nrf2 activity by direct phosphorylation. Phosphorylated Nrf2 then interacts with-transducing repeat-containing protein (-TrCP), a substrate receptor for ubiquitin ligase complex, and is ubiquitinated.

The physiological context in which this phosphorylation pathway is modulated is unclear; it is found that that the inhibited GSK-3 pathway in cancer cells activates Nrf2 and confers drug resistance by upregulating anti-stress genes.

(adult, max fitness)

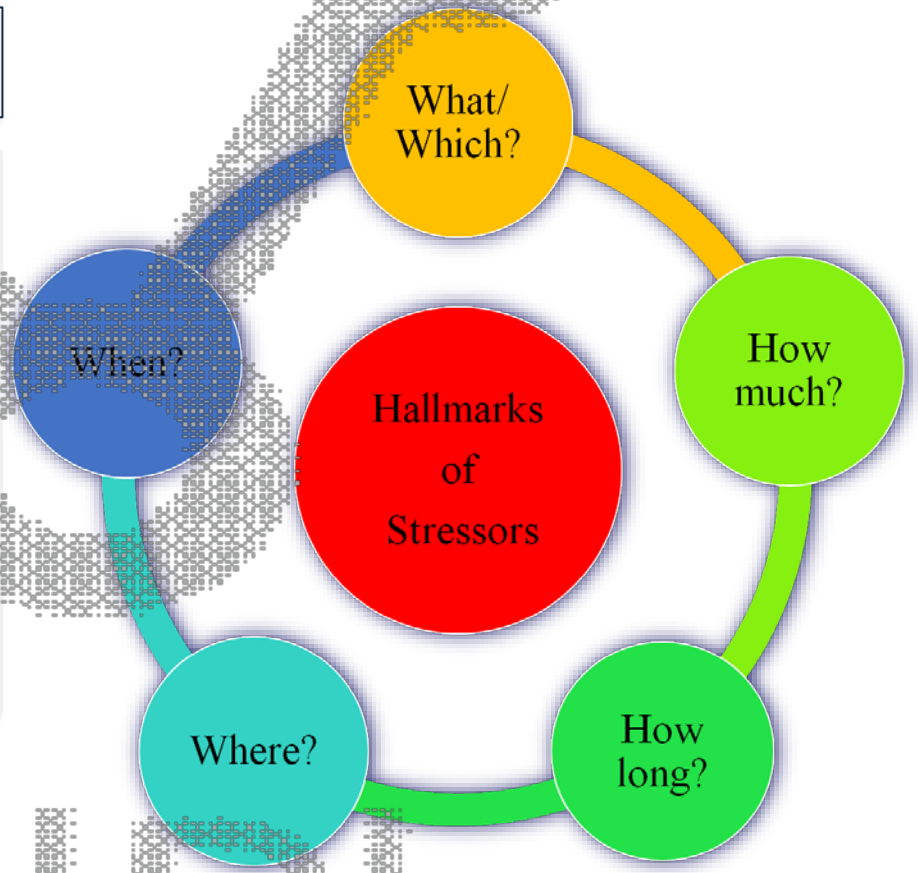
Yamamoto et al., (2018). Physiol Rev. 98, 1169-1203.

NRF2/KEAP1 (a multifunctional sensor)

Beyond detoxification (phase II, III) enzymes

The pleiotropic effects of the Nrf2/Keap1 sensor system: Regulation of a wide breadth of (among others) DDR, UPR, UPP, ALP, mitochondrial, inflammatory, metabolic, (endogenous signals) and antioxidant (exogenous/endogenous signals) genes

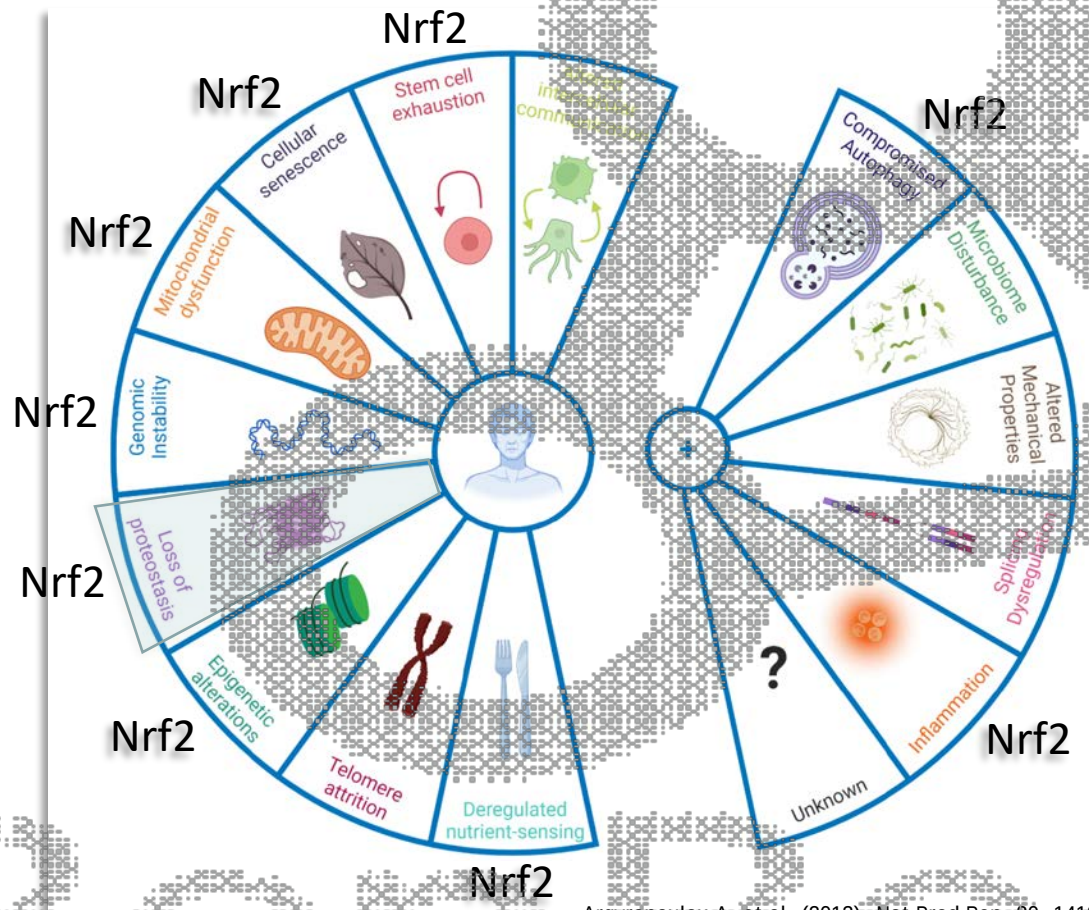
An array of micro-RNA genes are also regulated by Nrf2



Gorgoulis et al. (2018) J Pathol 246, 12-40.

(adult, max fitness)

PN modules collapse during ageing



The proteostasis network and its decline in ageing

- Mutations
- External stresses (e.g. elevated temperature, heavy metals or reactive oxygen species)
- Translation inefficiency (for example, limited tRNA availability)
- Translation errors
- Defective mRNA

Hipp et al. (2019). Nat Rev Mol Cell Biol. 20, 421-435.

Argyropoulou A. et al. (2013). Nat Prod Rep. 30, 1412-37
López-Otín C. et al. (2013). Cell. 153. 1194-217
Schmauck-Medina et al. (2022). Aging (Albany NY). 14, 6829-6839

NRF2 functionality during ageing

Aging Cell (2013) **12**, pp554–562 Doi: 10.1111/acel.12078

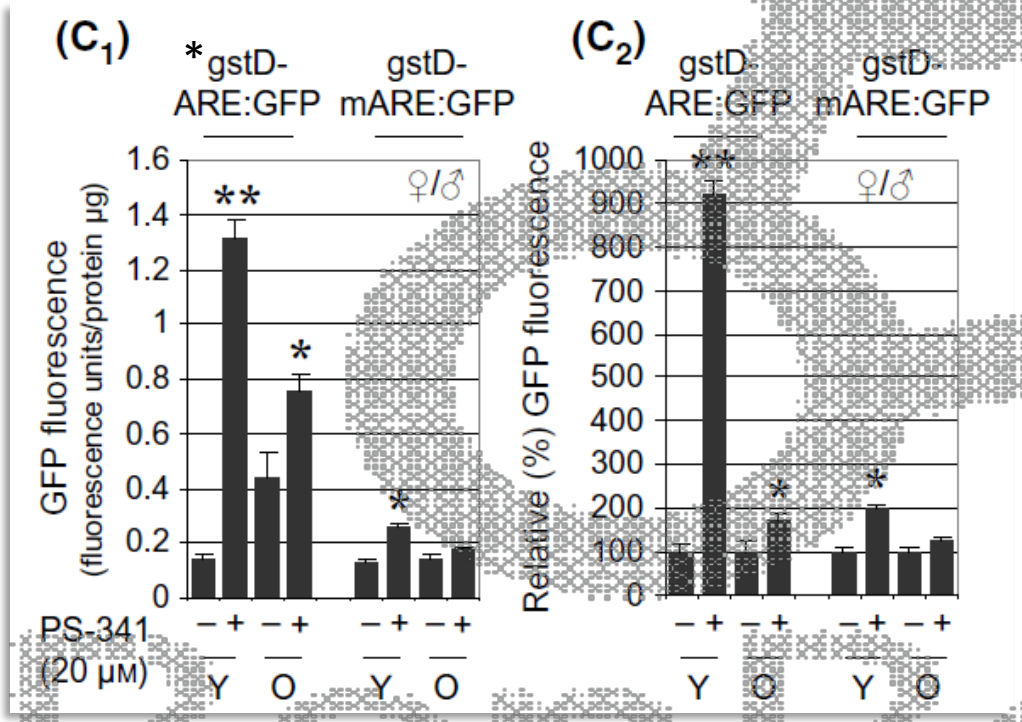
Declining signal dependence of Nrf2-MafS-regulated gene expression correlates with aging phenotypes

Mohammed Mahidur Rahman,[1,3] Gerasimos P. Sykiotis,[1,4] Mayuko Nishimura,[2,5] Rolf Bodmer[2] and Dirk Bohmann[1]

Aging Cell (2013) 12, pp802–813

Proteasome dysfunction in *Drosophila* signals to an Nrf2-dependent regulatory circuit aiming to restore proteostasis and prevent premature aging

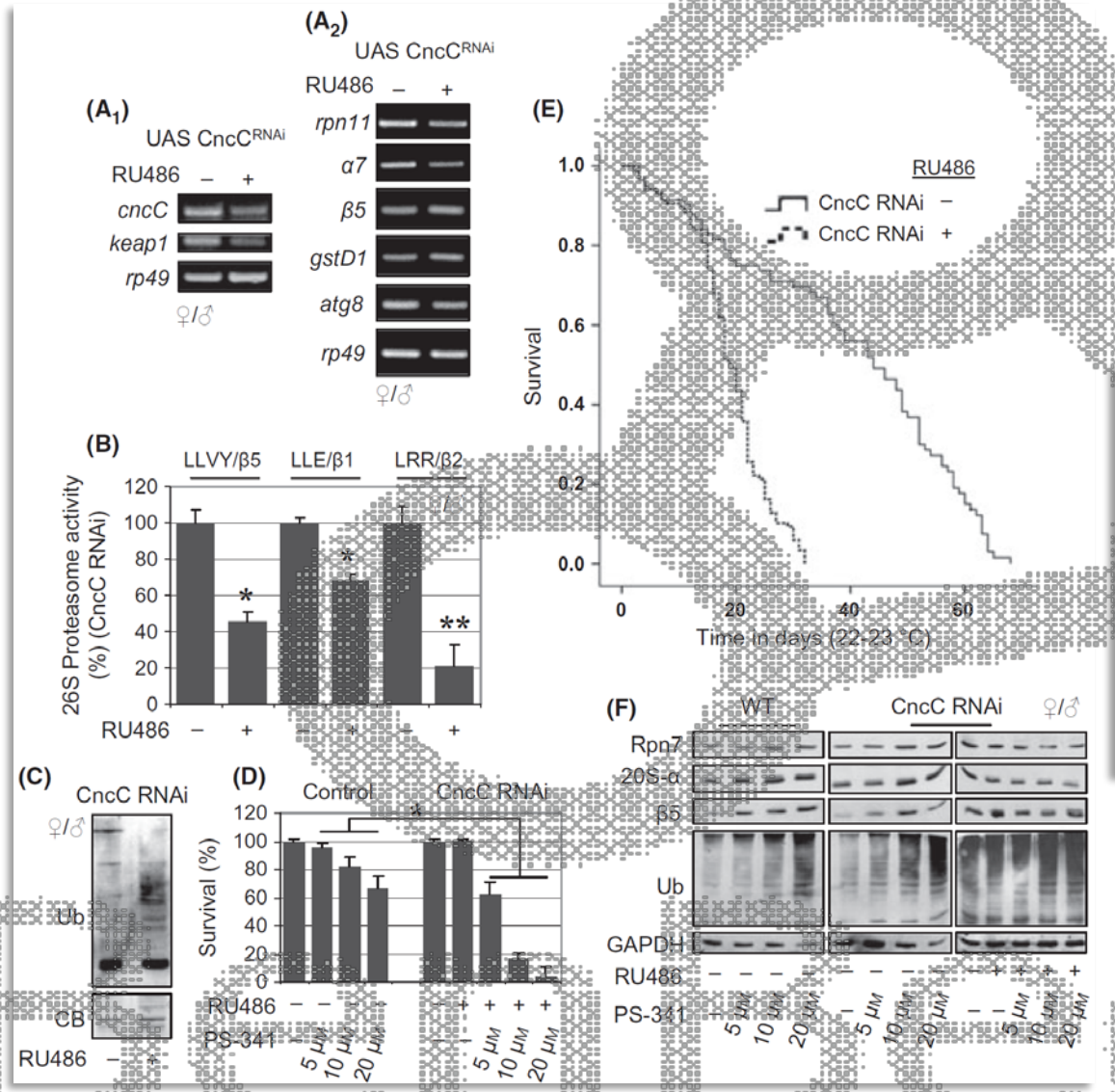
Eleni N. Tsakiri,[1] Gerasimos P. Sykiotis,[2] Issidora S. Papassideri,[1] Evangelos Terpos,[3] Meletios A. Dimopoulos,[3] Vassilis G. Gorgoulis,[4,5] Dirk Bohmann[6] and Ioannis P. Trougakos[1]



Proteasome loss of function triggers activation of antioxidant response elements (AREs) in young *Drosophila* somatic tissues. (C) GFP-related fluorescence levels per somatic tissue protein μg (C_1) or (%) (C_2) following PS-341-mediated proteasome inhibition in young (Y) or old (O) transgenic gstD-ARE:GFP or gstD-mARE:GFP flies. In all cases, flies were exposed to the indicated concentrations of PS-341 (Bortezomib, proteasome inhibitor) for 4 days.

* *gstD*: Glutathione S transferase D

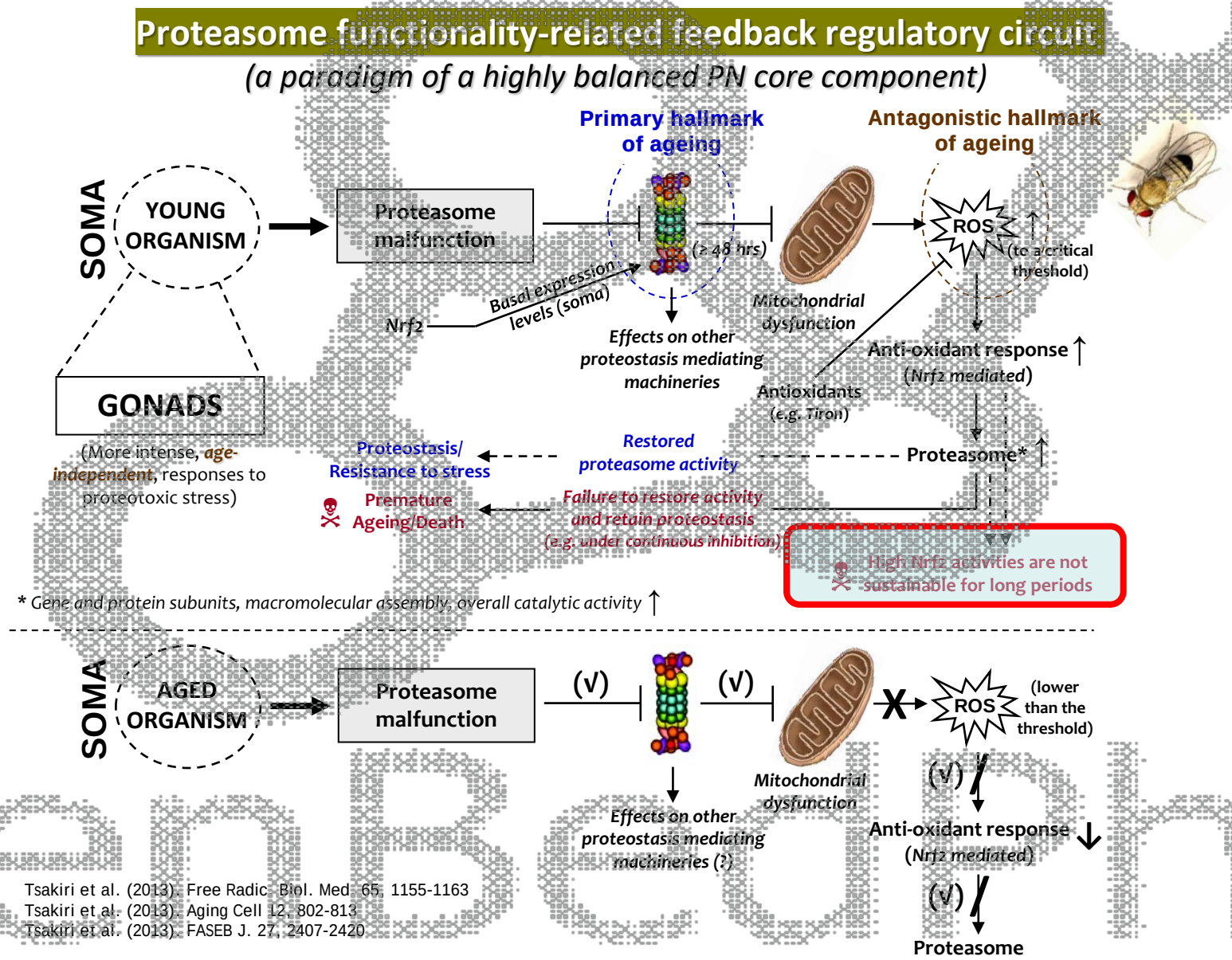
NRF2 as ageing modulator



RNAi-mediated Nrf2 knockdown in enclosed flies suppresses proteasome activities, disrupts proteostasis, and decreases resistance to proteotoxic stress; it also reduces flies' lifespan and abolishes proteasome components upregulation after proteasome loss of function.

Tsakiri et al. (2013). Aging Cell 12, 802-813.

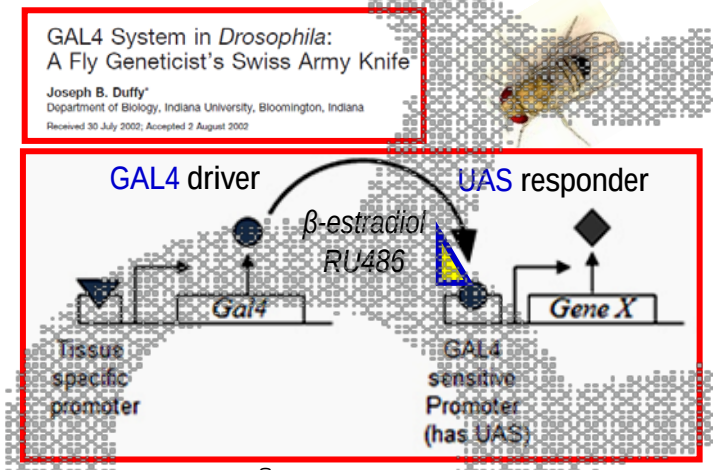
NRF2 as ageing modulator

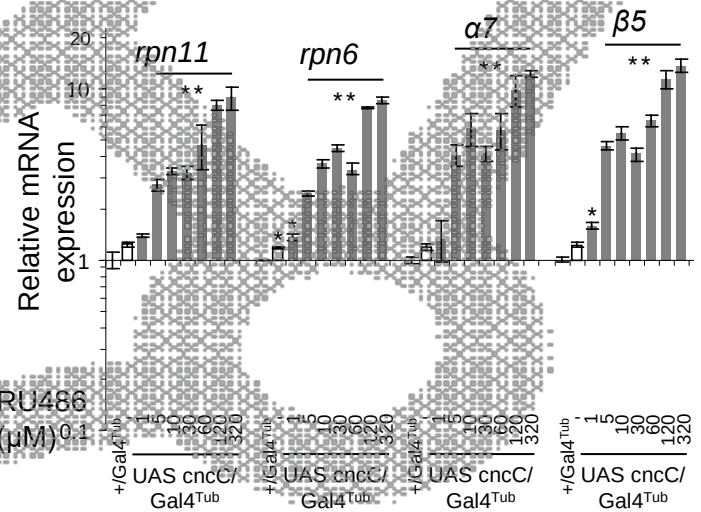


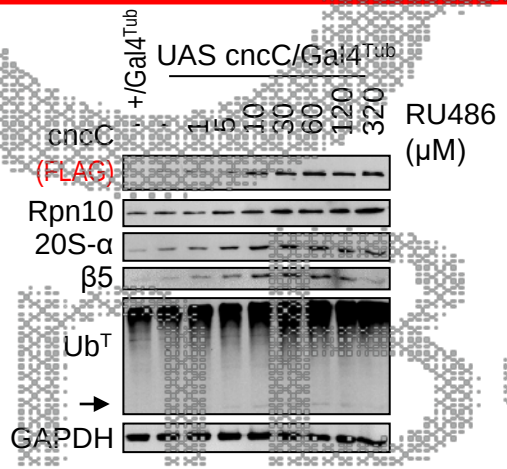
Tsakiri et al. (2013). Free Radic. Biol. Med. 65, 1155-1163
Tsakiri et al. (2013). Aging Cell 12, 802-813
Tsakiri et al. (2013). FASEB J. 27, 2407-2420

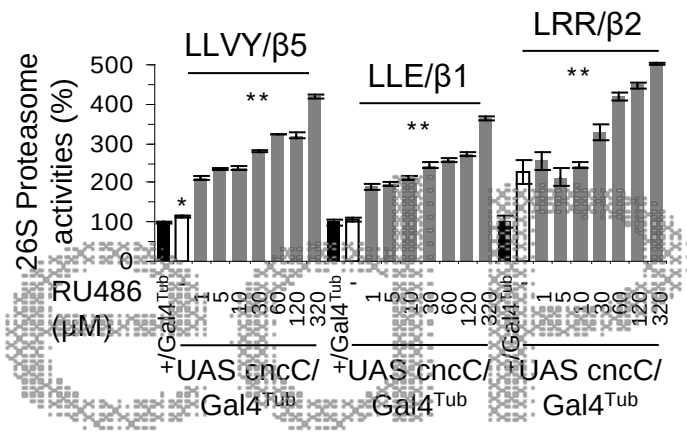
NRF2 as ageing modulator

Ubiquitous OE of the *nrf2* transgene resulted (*at doses higher than 10 μM of RU486*) in larval growth retardation, reduction of locomotion and lethality during late larval/early pupal stages



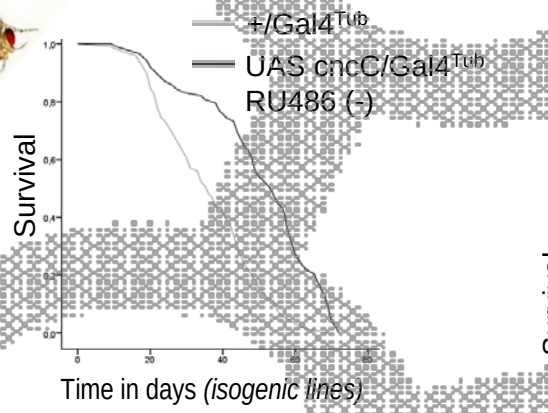


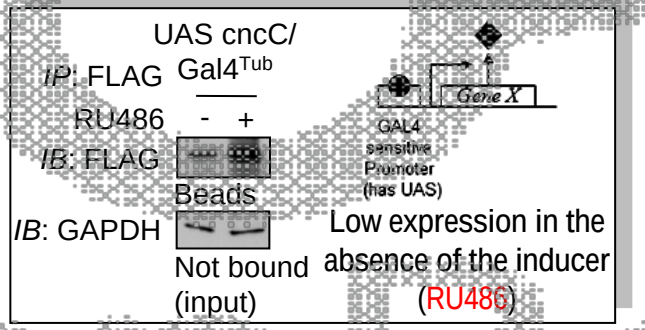


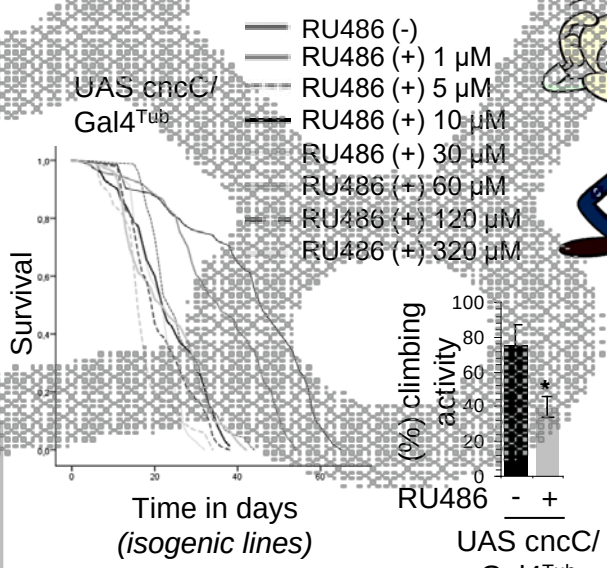


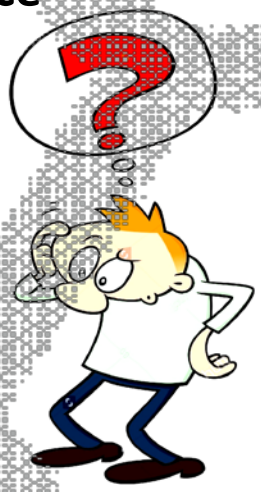
NRF2 as ageing modulator

The Nrf2 paradox: Nrf2 overexpressing flies experience lifespan shortening; while at the same time, the organism is in a state of maximum stress tolerance





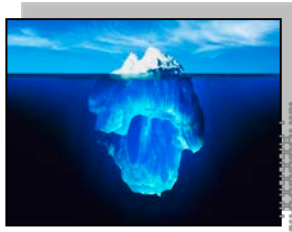


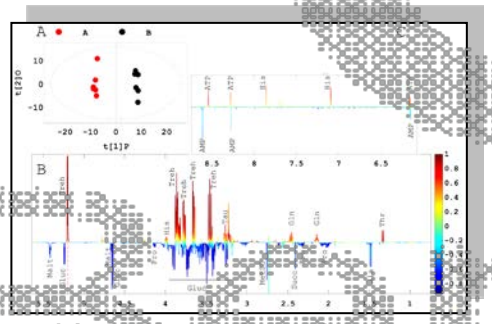


Long term OE of cytoprotective Nrf2 is toxic

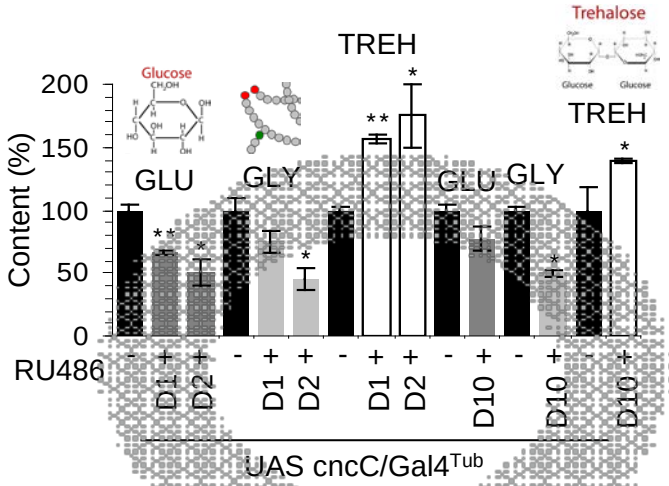
The activation level of anti-stress effectors which maximizes healthspan/lifespan in physiological contexts is likely *considerably lower* than that which maximizes protection against toxic doses of stressors

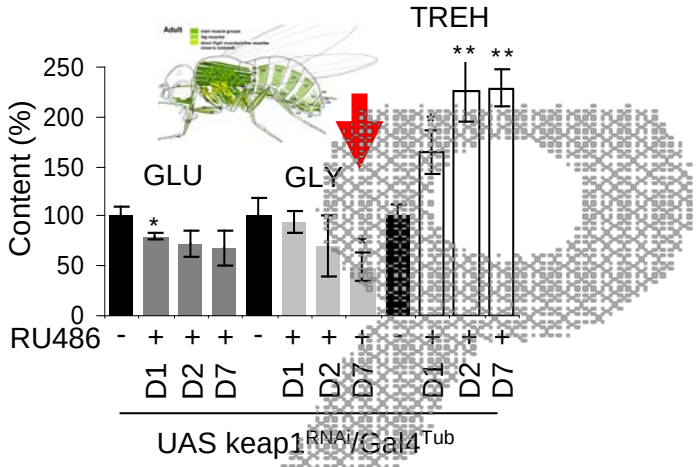
Prolonged Nrf2 activation resulted in gradual decrease of GLU and GLY content in flies' tissues; it also progressively increased levels of TREH (*hyperglycemia*), and caused *extensive lipolysis* in the fat body

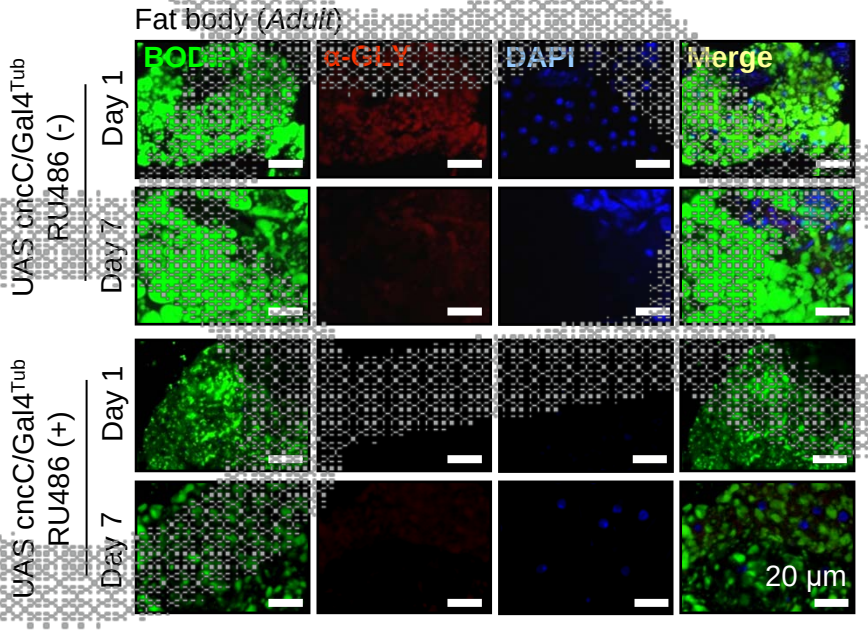


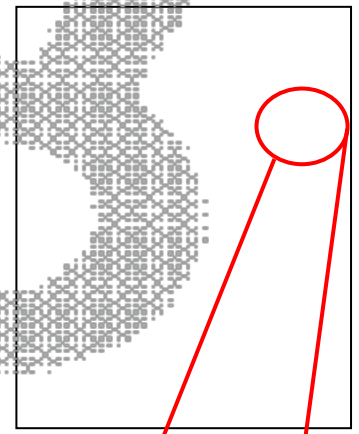


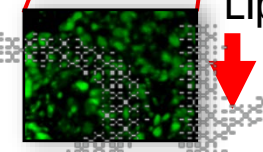
Unbiased NMR-based metabonomics analyses extended these observations

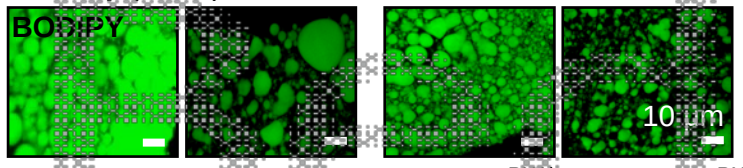








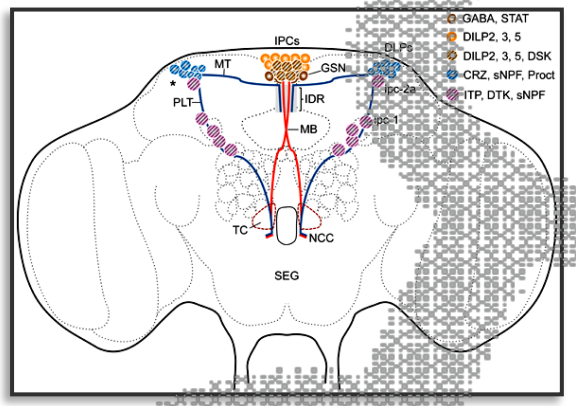


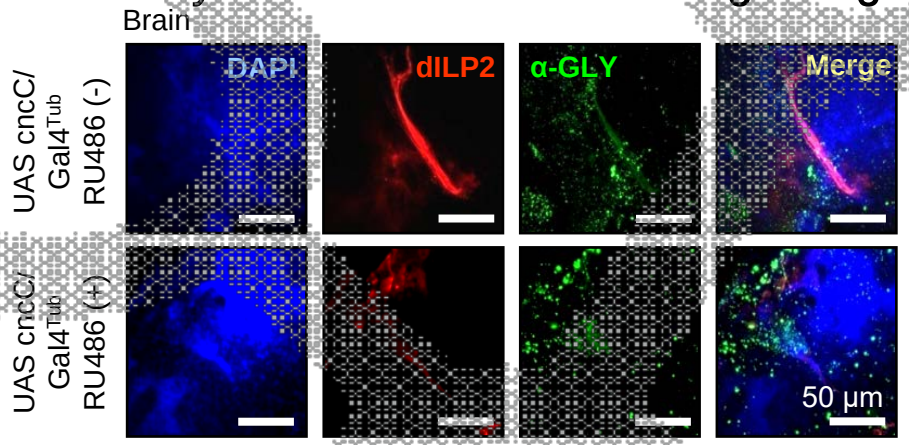


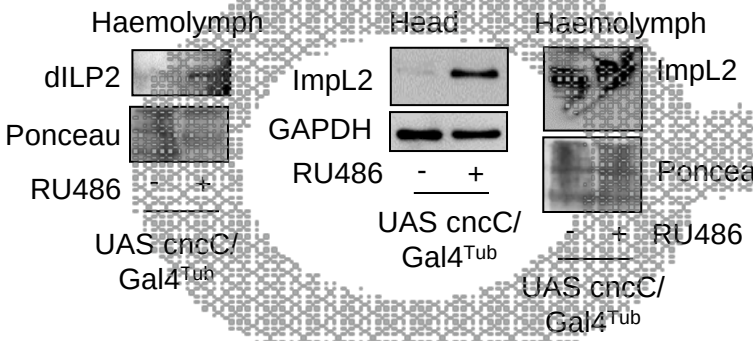
"Starvation in the midst of plenty" a hallmark of Type I diabetes

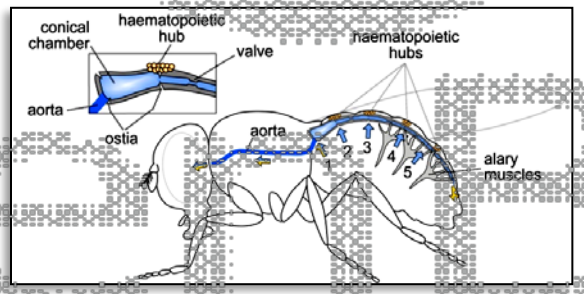
NRF2 as ageing modulator

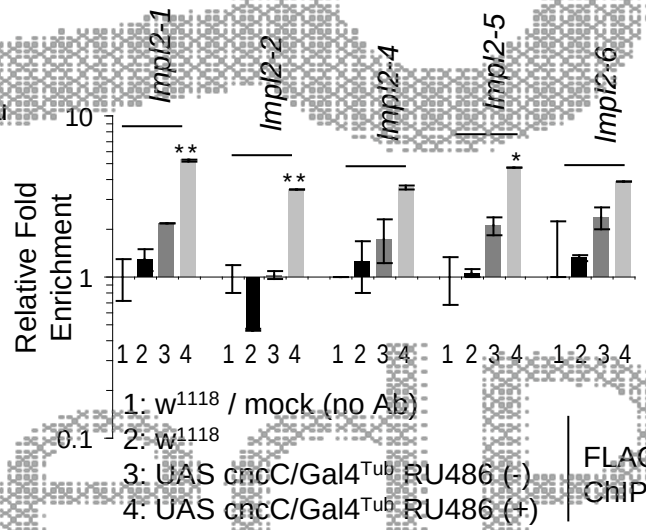
Nrf2 is a negative regulator of the systemic Insulin/IGF-like signaling









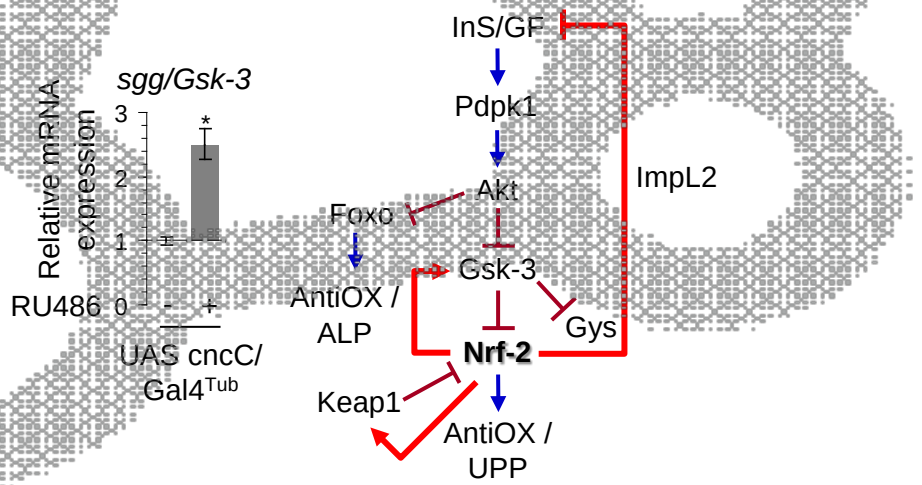


Chromatin IP

Tsakiri et al. (2019). Autophagy 15, 1757-1773.
Tsakiri et al. (2019). Aging Cell 18, e12845.

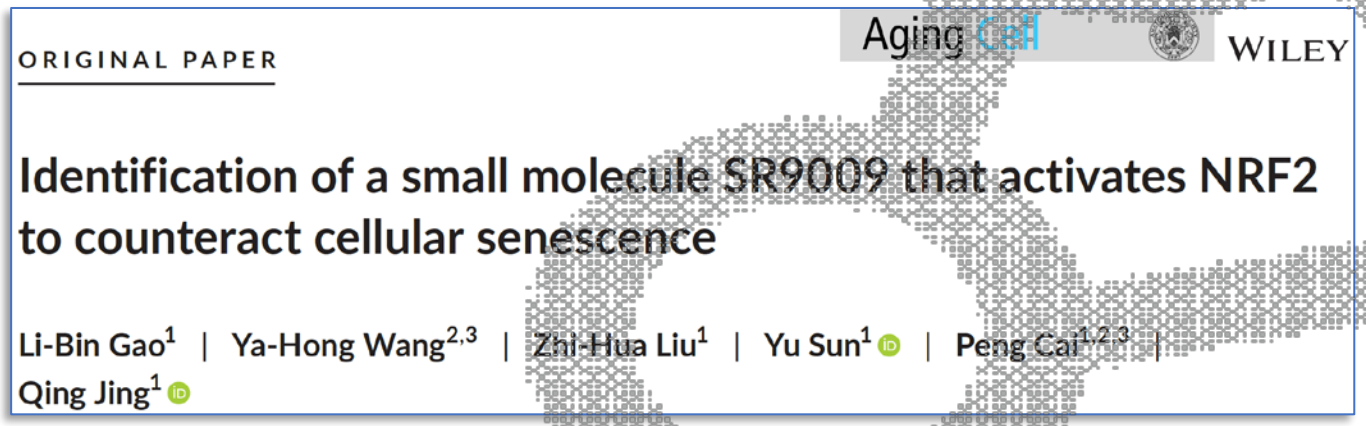
NRF2 as ageing modulator

Nrf2 OE (apart from *keap1*, *ImpL2*) also upregulated its other inhibitor, namely *sgg/gsk-3* (Shaggy, the fly ortholog of mammalian Gsk-3). This autoregulatory network motif indicates that as Nrf2 network evolved in higher metazoans one of its major functions is to limit its own activity



Hyperactivation of Nrf2 increases stress tolerance at the cost of aging acceleration due to metabolic deregulation

NRF2 as cellular senescence modulator



ORIGINAL PAPER

Aging Cell WILEY

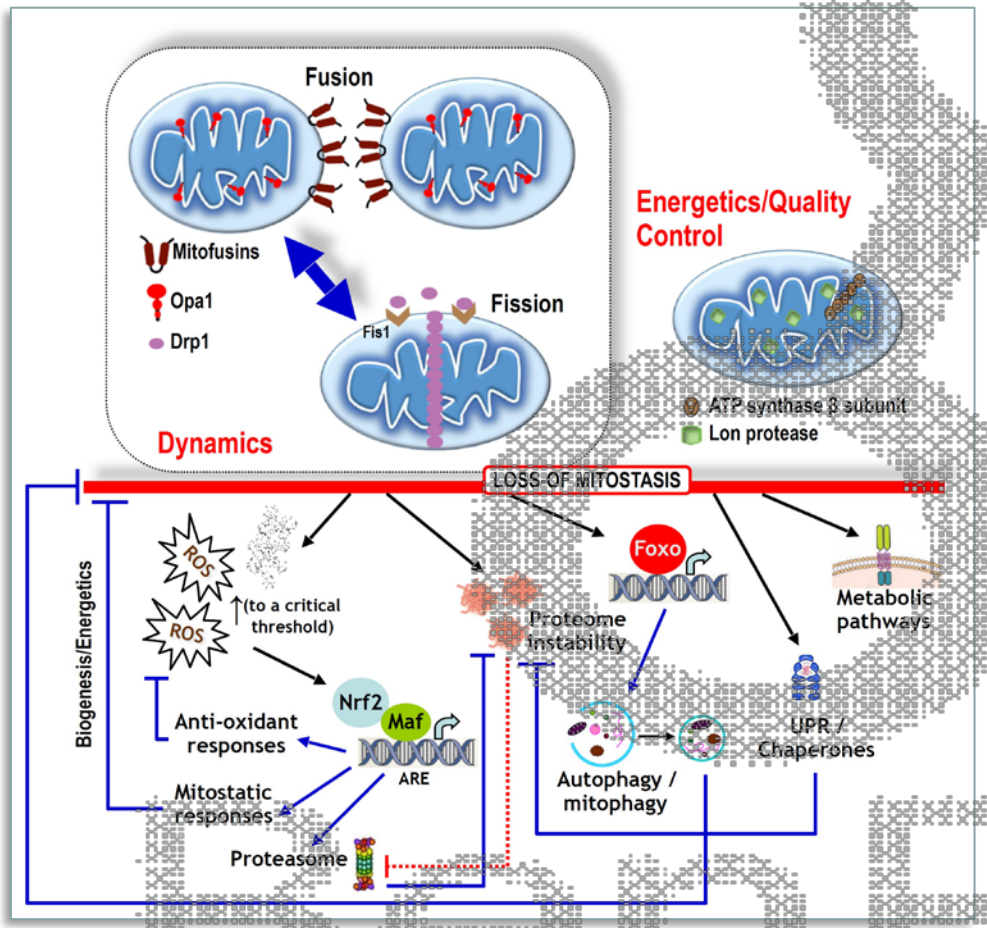
Identification of a small molecule SR9009 that activates NRF2 to counteract cellular senescence

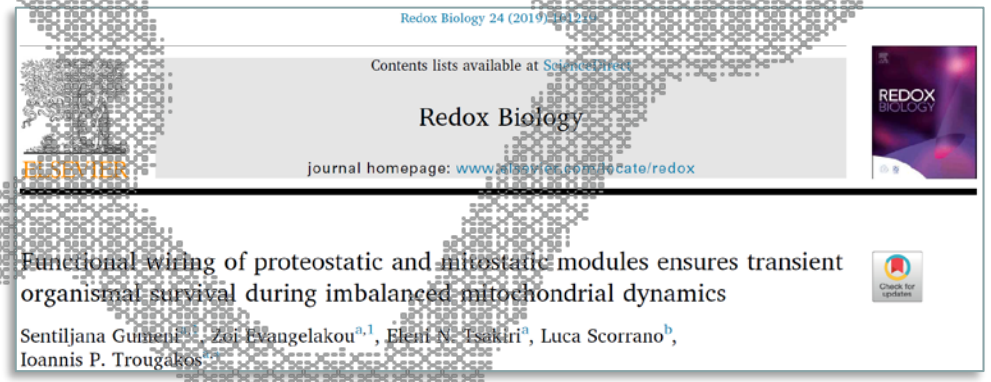
Li-Bin Gao[1] | Ya-Hong Wang[2,3] | Zhi-Hua Liu[1] | Yu Sun[1] | Peng Cai[1,2,3] | Qing Jing[1]

Aging Cell. 2021;20:e13483.

The senescence-associated secretory phenotype (SASP) is a striking characteristic of senescence. Accumulation of SASP factors causes a pro-inflammatory response linked to chronic disease. Suppressing senescence and SASP represents a strategy to prevent or control senescence-associated diseases. Here, we identified **a small molecule SR9009 as a potent SASP suppressor in therapy-induced senescence (TIS) and oncogene-induced senescence (OIS).** The mechanism studies revealed that SR9009 inhibits the SASP and full DNA damage response (DDR) activation **through the activation of the NRF2 pathway,** thereby decreasing the ROS level by regulating the expression of antioxidant enzymes. **SR9009 effectively prevents cellular senescence and suppresses the SASP in the livers of both radiation-induced and oncogene-induced senescence mouse models**, leading to alleviation of immune cell infiltration.

NRF2 mediated proteostasis – mitostasis crosstalk




Redox Biology 24 (2019) 101219

Contents lists available at ScienceDirect

Redox Biology

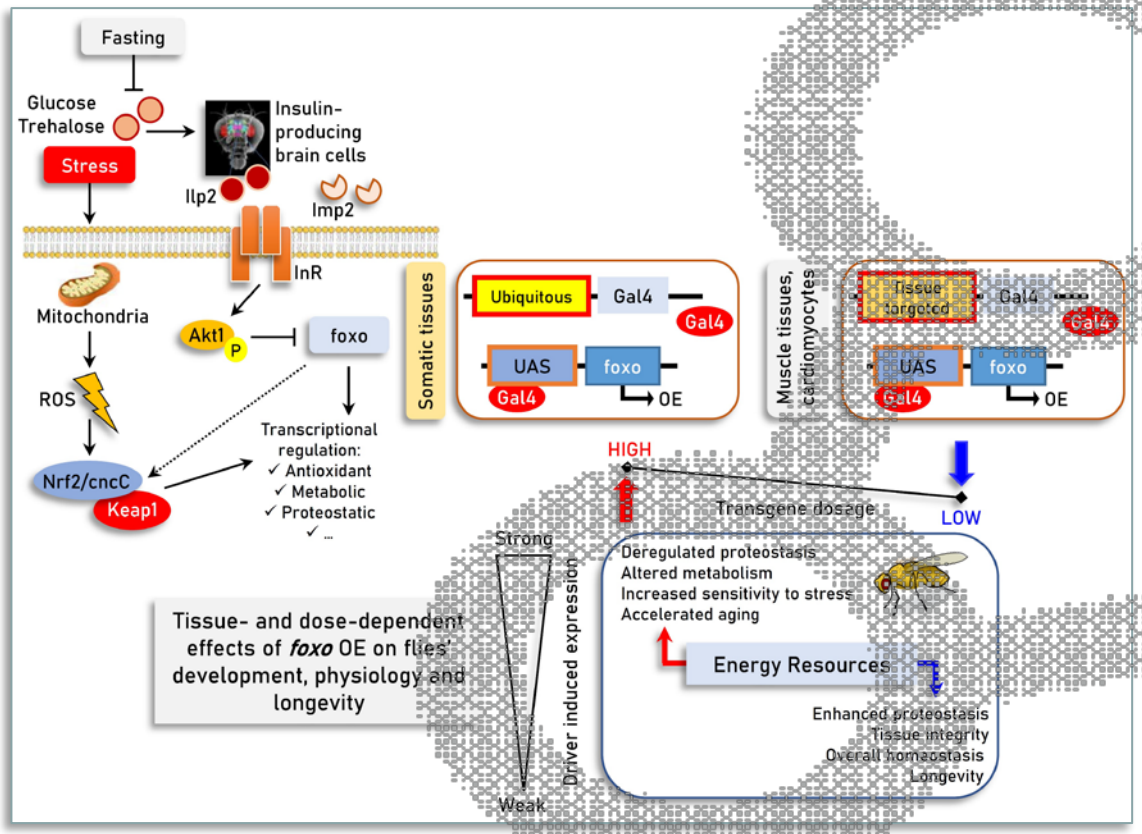
journal homepage: www.elsevier.com/locate/redox

Functional wiring of proteostatic and mitostatic modules ensures transient organismal survival during imbalanced mitochondrial dynamics

Sentiljana Gumeni[a,2], Zoi Evangelakou[a,1], Eleni N. Tsakiri[a], Luca Scorrano[b], Ioannis P. Trougakos[a,*]

Reduced mitochondrial fusion rates in *Drosophila* caused developmental lethality or if induced in the adult accelerated aging. Imbalanced mitochondrial dynamics were tolerable for various periods in young flies, where they caused oxidative stress and proteome instability **that mobilized Nrf2 and foxo to upregulate cytoprotective antioxidant/proteostatic modules**. Consistently, proteasome inhibition or Nrf2, foxo knock down in young flies exaggerated perturbed mitochondrial dynamics toxicity. Neither Nrf2 overexpression (with concomitant proteasome activation) nor Atg8a upregulation suppressed the deregulated mitochondrial dynamics toxicity, which **was mildly mitigated by antioxidants**.

NRF2 – FOXO functional crosstalk



cells Cells 2021, 10, 3577. https://doi.org/10.3390/cells10123577 MDPI

Article

Differential Dose- and Tissue-Dependent Effects of foxo on Aging, Metabolic and Proteostatic Pathways

Maria S. Manola, Sentiljana Gumeni and Ioannis P. Trougakos *

Ubiquitous foxo overexpression (OE) accelerated aging, induced the early onset of age-related phenotypes, increased sensitivity to thermal stress, and deregulated metabolic and proteostatic pathways; these phenotypes were more intense in transgenic flies expressing high levels of foxo. **Interestingly, there is a defined dosage of foxo OE in muscles and cardiomyocytes that shifts energy resources into longevity pathways and thus ameliorates not only tissue but also organismal age-related defects**.

Foxo OE stimulates in an Nrf2/cncC dependent-manner, counteracting proteostatic pathways, e.g., the ubiquitin-proteasome pathway, which is central in ameliorating the aberrant foxo OE-mediated toxicity.

PN modules collapse (normal ageing)

Viability of metazoans largely depends on their capacity to mount anti-stress responses, as well as on their ability to regulate metabolic processes in order to produce energetic molecules. **At the whole organism level, these responses require extremely complicated coregulation and wiring of cell autonomous and non-autonomous mechanisms.**

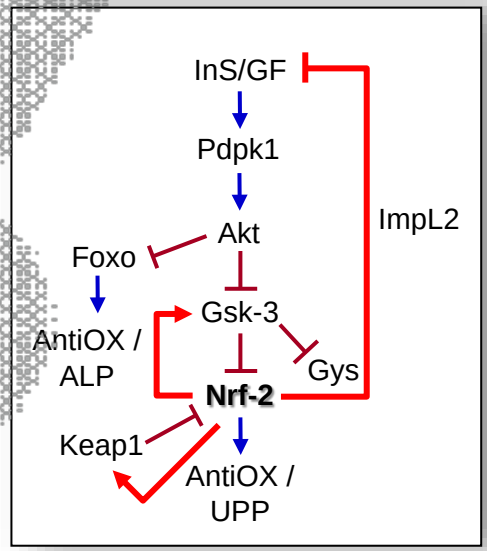
Thus, **loss of proteostasis is likely the driving force in aging and in most (if not all) age-related diseases**, since the decline of a core PN component (e.g., proteasome or the Nrf2/Keap1 pathway) (or suppression of signaling competence during physiological aging) saturates the PN, resulting in the collapse of genome stability, proteostasis and the mitochondrial functionality boundary below a threshold that sustains cell viability.

Tsakiri et al. (2013). Free Radic Biol. Med. 65, 1155-1163
Tsakiri et al. (2013). Aging Cell 12, 802-813
Tsakiri et al. (2013). FASEB J. 27, 2407-2420
Tsakiri et al. (2019) Aging Cell 18, e12845
Tsakiri et al. (2019) Autophagy 15, 1757-1773
Gumeni et al. (2019) Redox Biol 24, 101219
Papanagnou et al. (2022). Aging Cell 21, e13715

Sustained stress signaling (even with no damage) accelerates ageing

Even in the absence of biomolecular damage, **persistent stress signaling** triggers a highly conserved adaptive metabolic response which reallocates resources from growth and longevity to somatic preservation and stress tolerance. This notion provides a **reasonable explanation of why most (if not all) cytoprotective stress sensors (e.g., Nrf2, Foxo, p53, etc.) are short-lived proteins, and it also explains the build-in negative feedback loops (shown here for Nrf2); the low basal levels of these proteins, and why their suppressors were favored by evolution**.

Thus, the critical issues of **correct dosage of stress sensors activators and of their interactions with disease-related pathways** remain critical to avoid clinical trial failures.

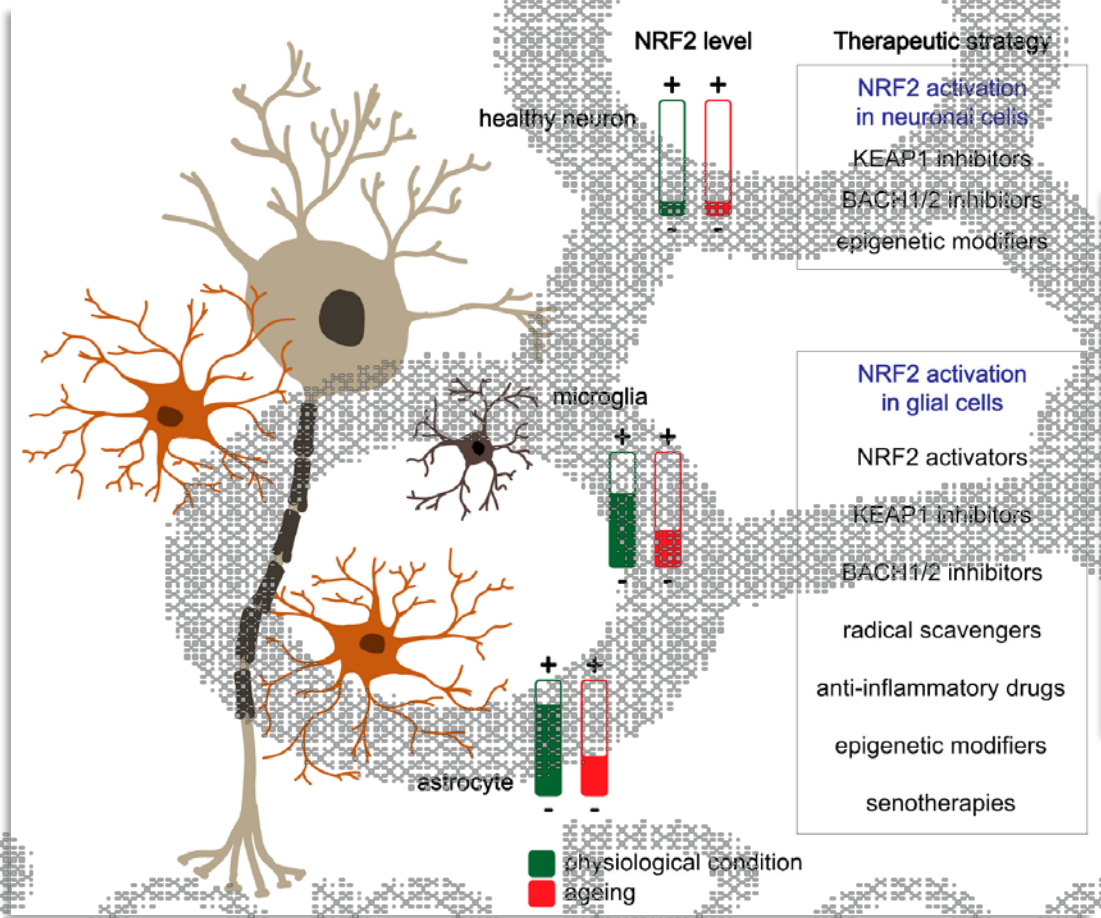


Tsakiri et al. (2019). Autophagy 15, 1757-1773.
Tsakiri et al. (2019). Aging Cell 18, e12845.
Gumeni et al., (2023). *submitted*

NRF2 in age-related diseases

Recent advances have revealed that the Nrf2/Keap1 system is related to a number of **age-related human diseases**, such as **neurodegenerative diseases, cancer, diabetes mellitus, etc.**; thus, many researchers are investigating potential medical applications

NRF2 in (neuro)-degenerative diseases



Heurtaux et al. (2022). Antioxidants (Basel) 11, 1426.

NRF2-signalling-based therapeutic strategies in brain dysfunctions. Targeting NRF2 pathway in brain diseases appears as a promising therapeutic strategy. Different options exist depending on the context e.g., ageing or neurodegenerative diseases.

NRF2 in (neuro)-degenerative diseases

Neurobiology of Aging 105 (2021) 132–142

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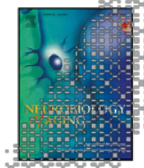
Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging.org



Amyloid toxicity in a *Drosophila* Alzheimer's model is ameliorated by autophagy activation

Eleni N. Tsakiri*, Sentiljana Gumeni#, Maria S. Manola, Ioannis P. Trougakos*

CDDpress

www.nature.com/cddis

ARTICLE OPEN

Nrf2 activation induces mitophagy and reverses Parkin/Pink1 knock down-mediated neuronal and muscle degeneration phenotypes

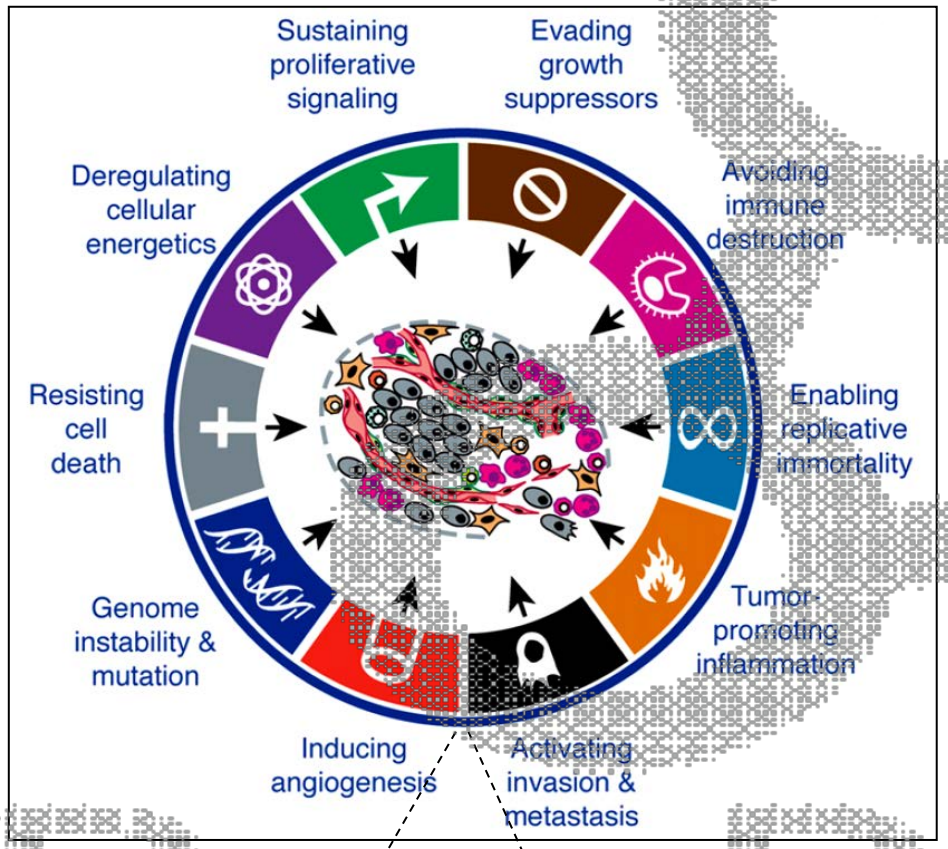
Sentiljana Gumeni[1], Eleni-Dimitra Papanagnou[1], Maria S. Manola[1] and Ioannis P. Trougakos[1]✉

Cell Death and Disease (2021)12:671

SPRINGER NATURE

NRF2 in cancer

The hallmarks of Cancer



From: Hanahan and Weinberg (2011). Cell 144, 646-674

Secondary hallmarks:

Oxidative - proteotoxic stress

From: Negrini et al. (2010). Nat Rev Mol Cell Biol. 11, 220-228

NRF2 and the Hallmarks of Cancer

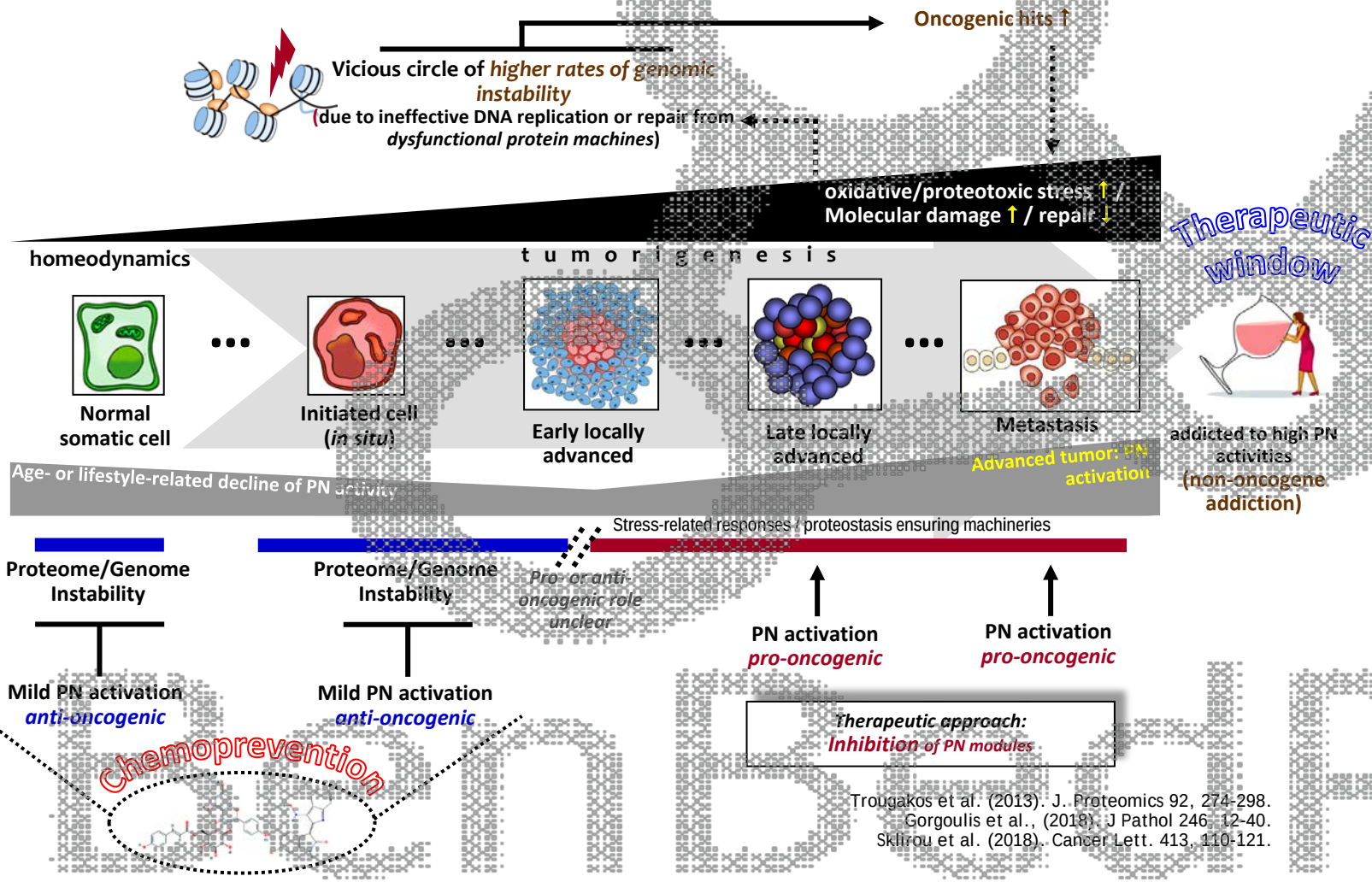
Montserrat Rojo de la Vega,[1] Eli Chapman,[1] and Donna D. Zhang[1,2,*]

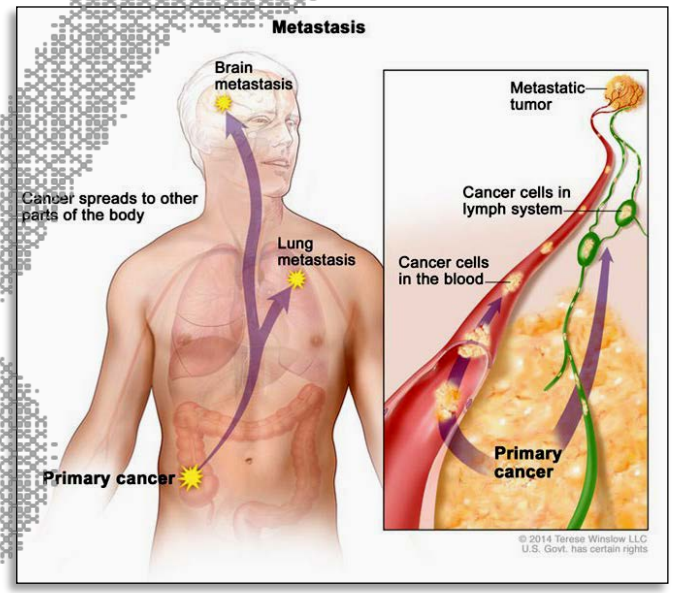
Cancer Cell 34, July 9, 2018

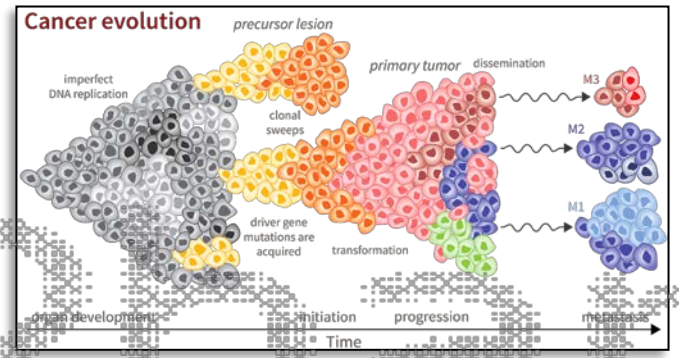
NRF2 has direct and indirect roles that promote or block the emergence of the hallmarks of cancer.

NRF2 in cancer

Impact of *age-* or *lifestyle-related* proteome instability to oncogenesis and potential therapeutic strategies

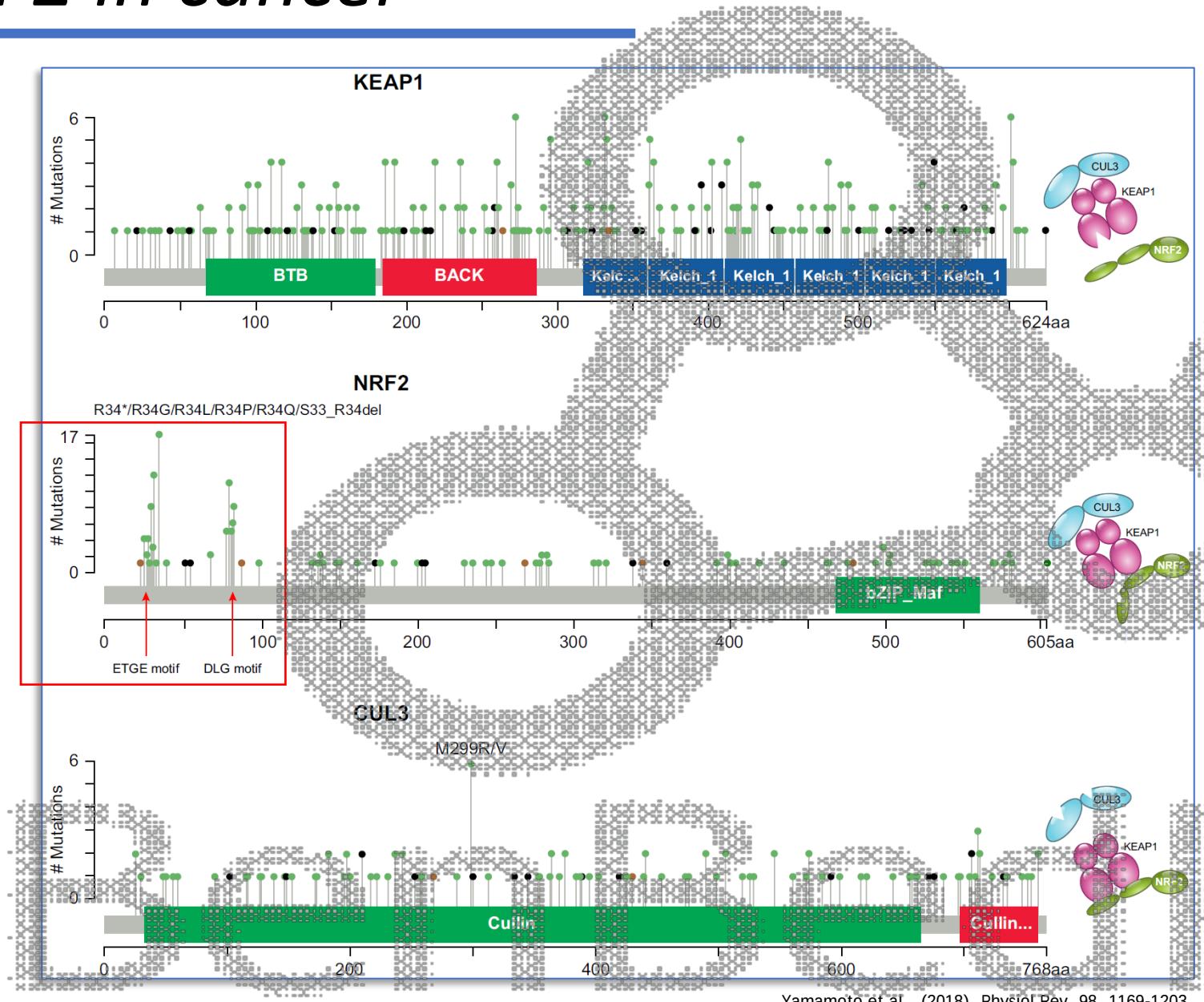






Trougakos et al. (2013). J. Proteomics 92, 274-298.
Gorgoulis et al., (2018). J Pathol 246, 12-40.
Sklirou et al. (2018). Cancer Lett. 413, 110-121.

NRF2 in cancer

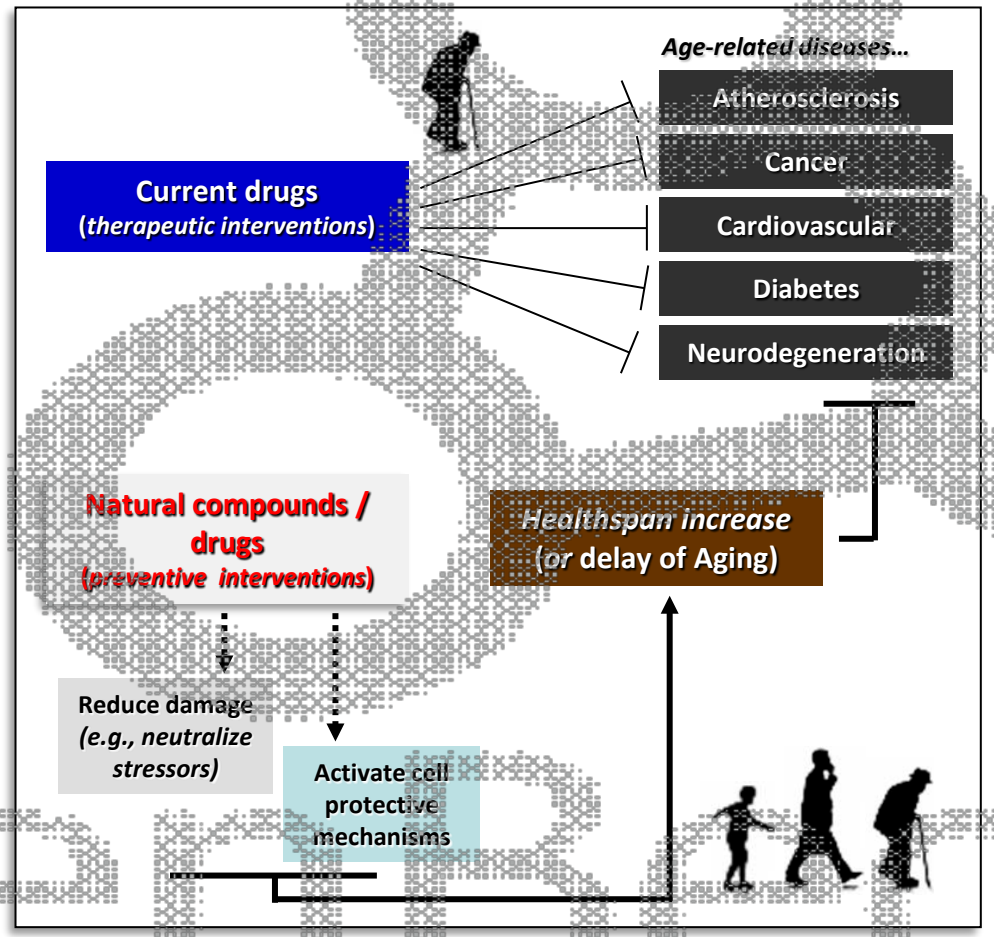


Somatic mutations found in *KEAP1*, *NRF2*, and *CUL3* genes in cancer. *KEAP1* and *CUL3* mutations are distributed in a wide range over the coding region, whereas ***NRF2* mutations are mostly clustered in the ETGE and DLG motifs in Neh2** (Keap1-Cul3 interaction).

Yamamoto et al., (2018). Physiol Rev. 98, 1169-1203.

Can we decelerate the clock?

Anti-aging interventions as a systemic approach to also tackle age-related diseases



Natural products (*extracts or pure compounds*) exert a broad range of biological activities, and therefore, they constitute the ultimate inventory of seeking novel structures capable of diverse and sometimes extraordinary anti-aging effects

Argyropoulou et al. (2013). Nat Prod Rep. 30: 1412-37

A feasible approach

Preserving transcriptional stress responses as an anti-aging strategy

Yang Cheng[1] | Andrew Pitoniak[1] | Julia Wang[2] | Dirk Bohmann[1]

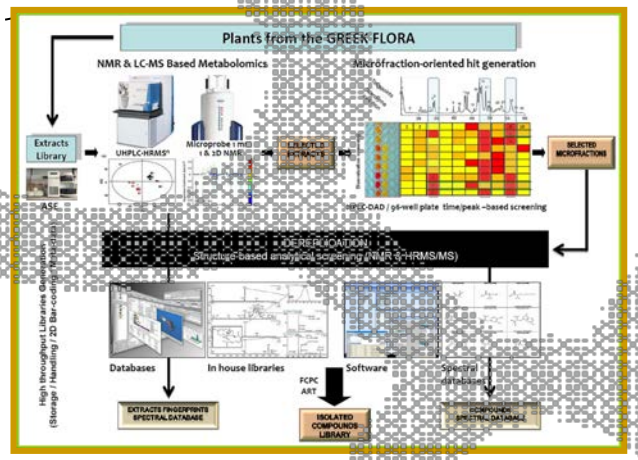
Aging Cell. 2021;20:e13297.

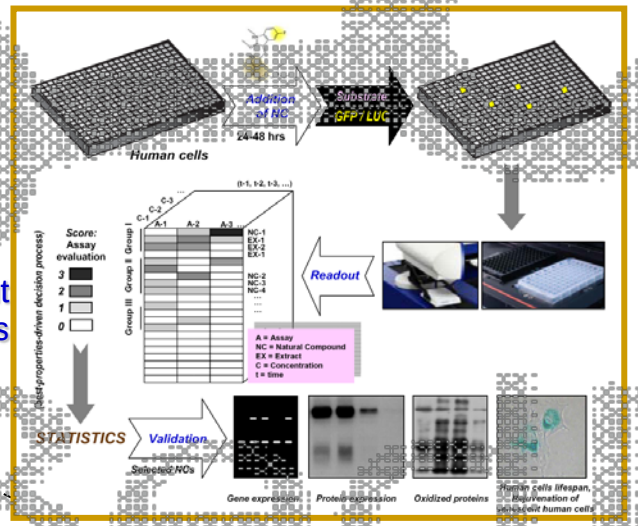
The progressively increasing frailty, morbidity and mortality of aging organisms coincides with, and may be causally related to, their waning ability to adapt to environmental perturbations. Transcriptional responses to challenges, such as oxidative stress or pathogens, diminish with age. **This effect is manifest in the declining function of the stress responsive transcription factor Nrf2. Protective gene expression programs that are controlled by the *Drosophila* Nrf2 homolog, CncC, support homeostasis and longevity. Age-associated chromatin changes make these genes inaccessible to CncC binding and render them inert to signal-dependent transcriptional activation in old animals.**

Overexpression of the CncC dimerization partner Maf-S counteracts this degenerative effect and preserves organism fitness. **Maf-S overexpression prevents loss of chromatin accessibility and maintains gene responsiveness.** Moreover, the same outcome, along with an extension of lifespan, can be achieved by inducing CncC target gene expression **pharmacologically throughout adult life.**

A feasible approach

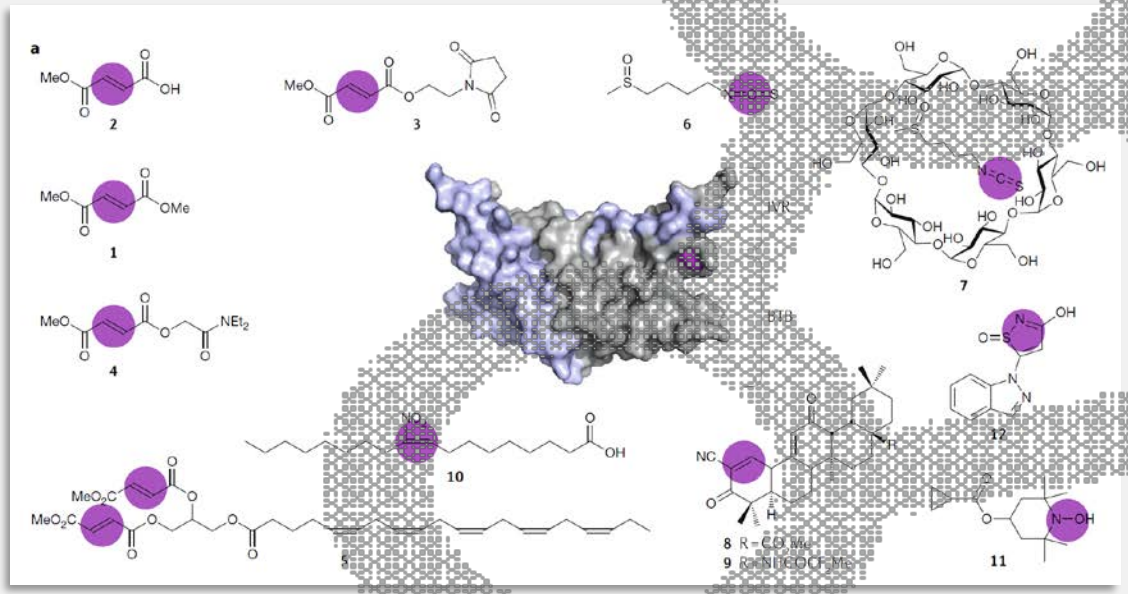
High throughput
Small Molecules
Discovery
Platform





When it comes to Nrf2 and, despite the severe side-effects of Nrf2 overactivation, none of these is sufficient reason to discredit the Nrf2 pathway as a drug target, e.g., for anti-aging purposes; evidence comes from the fact that humans have been safely ingesting Nrf2 activators in their diet for millennia and from the increased healthspan associated with mild Nrf2 activation.

A feasible approach



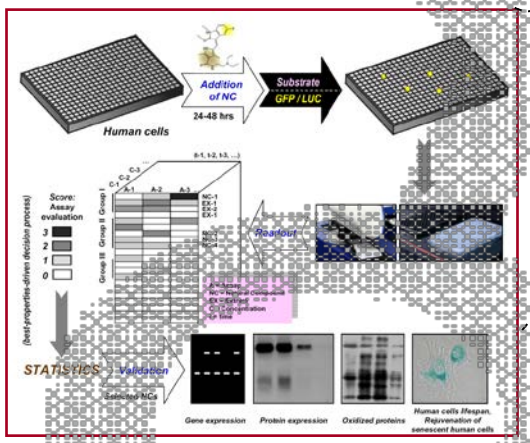
Cuadrado et al. (2019). Nat Rev Drug Discov 18, 295-317.

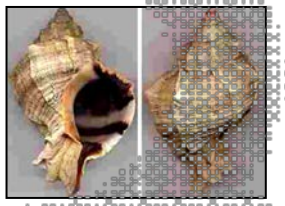
Crystal structure of KeAP1 domains. Crystal structure of the KEAP1 BTB domain (Protein Data Bank identifier: 5NLB; residues 49–180); the protein is shown as a dimer in a surface representation (left- hand side blue; right- hand side grey) with the visible C151 shown in purple. **The electrophilic motif of selected NRF2 activators 1–12 is indicated by a purple circle**.

BenBedPhar

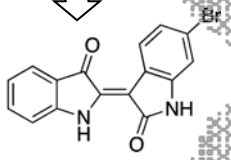
A feasible approach

The indirubin derivative 6-Bromoindirubin-3'-oxime (6BIO) activates proteostatic modules, reprograms cellular bioenergetics pathways and exerts *in vivo* anti-aging effects



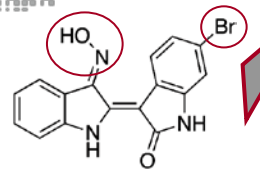


Hexaplex trunculus (Muricidae)

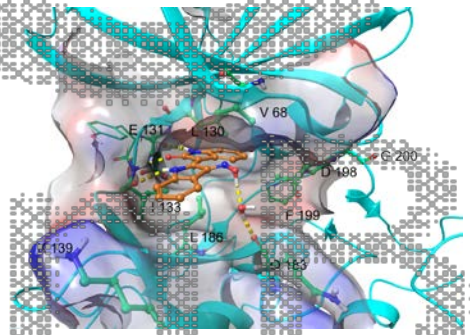


6-bromoindirubin

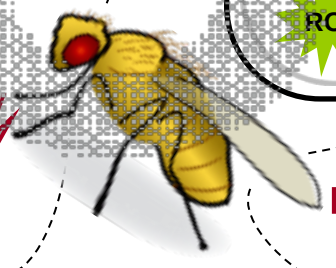
Hemi-Synthetic modification

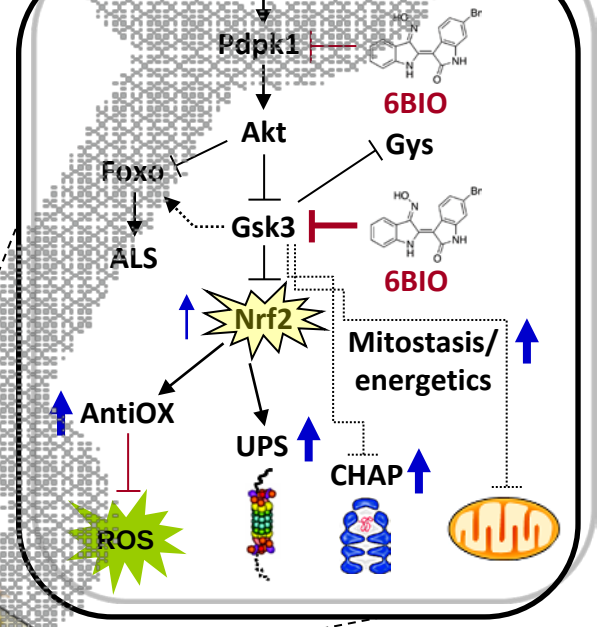


6-bromoindirubin-3'-oxime (6BIO)

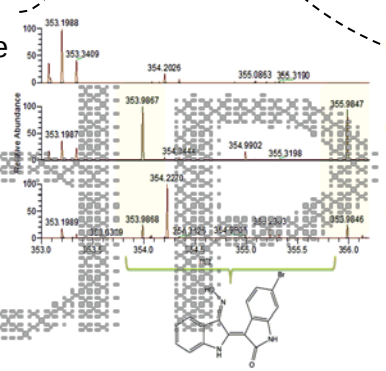


Dietary





- Locomotor activity
- Resistance to stressors
- Glucose/Lipids
- Healthspan/Lifespan



BenBedPhar

A feasible approach

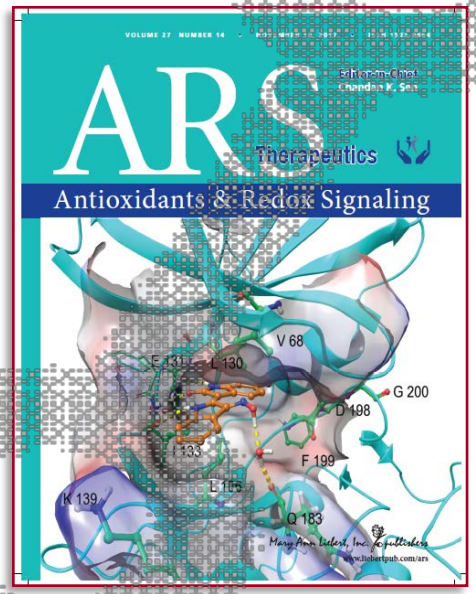
ANTIOXIDANTS & REDOX SIGNALING
Volume 00, Number 00, 2017

DOI: 10.1089/ars.2016.6910

ORIGINAL RESEARCH COMMUNICATION

The Indirubin Derivative 6-Bromoindirubin-3′-Oxime Activates Proteostatic Modules, Reprograms Cellular Bioenergetic Pathways, and Exerts Antiaging Effects

Eleni N. Tsakiri,[1] Nicolas Gaboriaud-Kolar,[2] Kalliopi K. Iliaki,[1] Job Tchoumtchoua,[2] Eleni-Dimitra Papanagnou,[1] Sofia Chatzigeorgiou,[1] Konstantinos D. Tallas,[1] Emmanuel Mikros,[3] Maria Halabalaki,[2] Alexios-Leandros Skaltsounis,[2] and Ioannis P. Trougakos[1]



SCIENTIFIC REPORTS

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6-bromo-indirubin-3′-oxime (6BIO), a Glycogen synthase kinase-3β inhibitor, activates cytoprotective cellular modules and suppresses cellular senescence-mediated biomolecular damage in human fibroblasts

Received: 29 December 2016
Accepted: 25 August 2017
Published Online: 15 September 2017

Aimilia D. Sklirou[1], Nicolas Gaboriaud-Kolar[2], Isidora Papassideri[1], Alexios-Leandros Skaltsounis[2] & Ioannis P. Trougakos[1]

Thank you