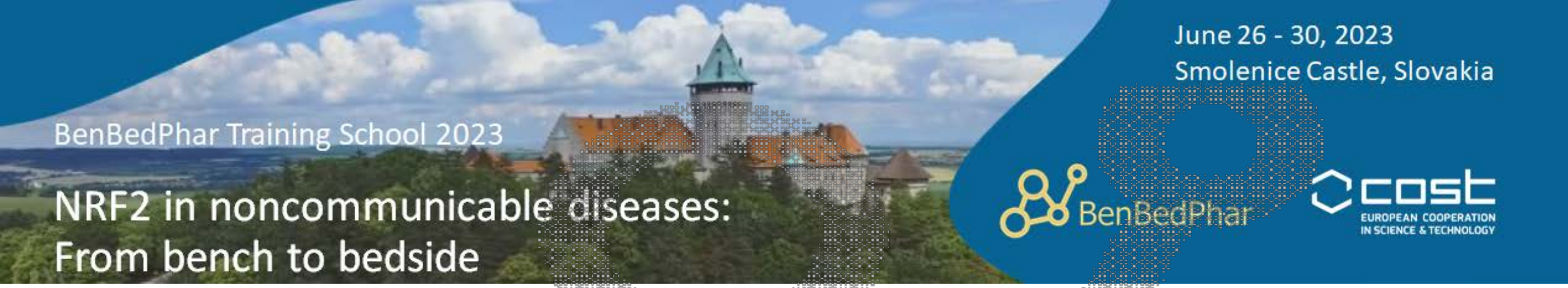


NRF2 in non-mammalian species

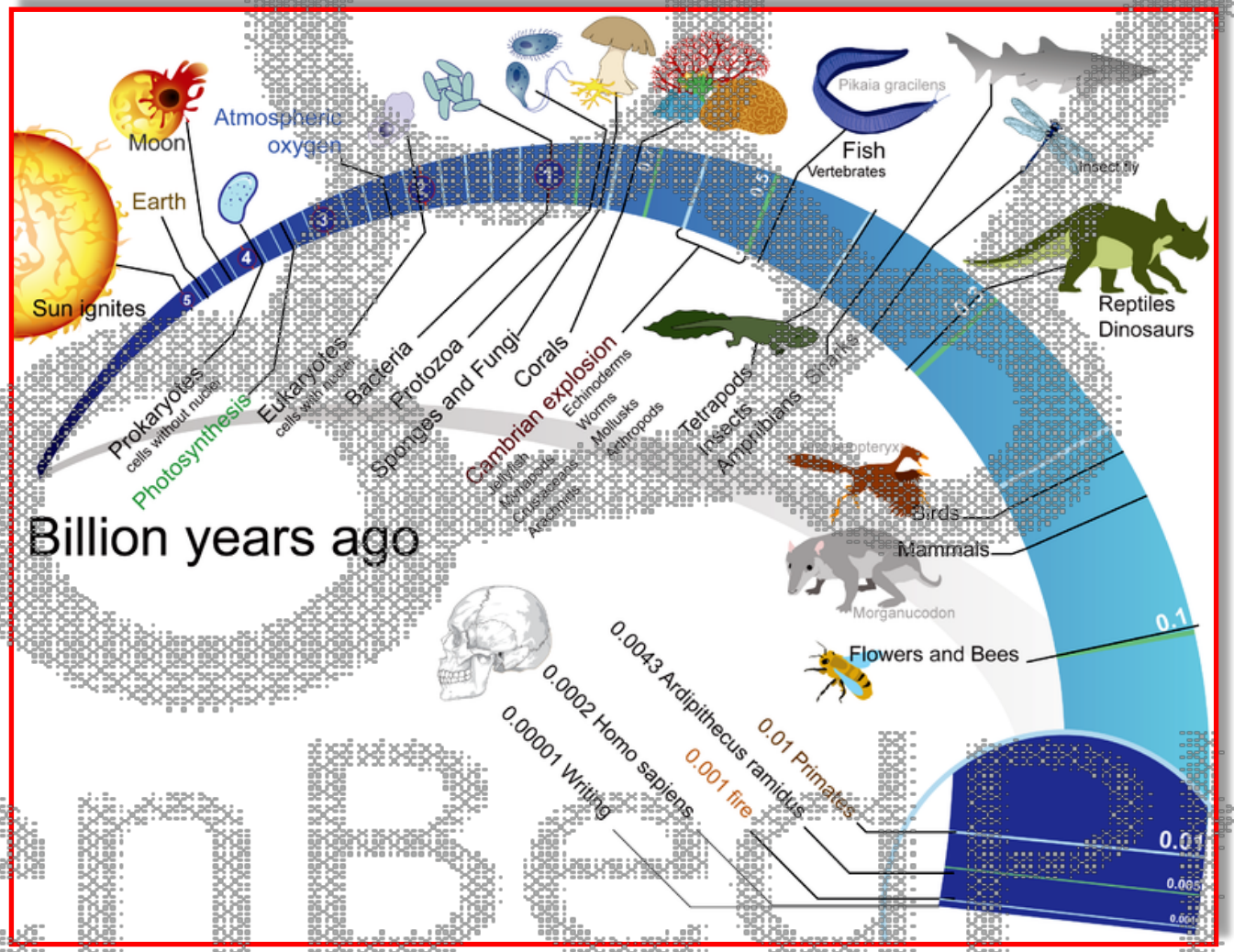
Prof. Ioannis Trougakos
National & Kapodistrian
University of Athens, Greece

Prioritize concepts over experiments

Model organisms (the evolution perspective)

“Nothing in Biology Makes Sense Except in the Light of Evolution”

Theodosius Grygorovych Dobzhansky



Biological Systems

A constant adaptation of the biosphere to all other systems of the planet

The most ancient stress is heat – then it comes **oxidative** (but also reductive) stress…

The five systems of Earth (geosphere, biosphere, cryosphere, hydrosphere and atmosphere) interact to produce the environments we are familiar with.

Non-mammalian model organisms (a stable genome)

Although biological activity in a model organism does not ensure an effect in humans, many drugs, treatments and cures for human diseases are developed in part with the guidance of animal models

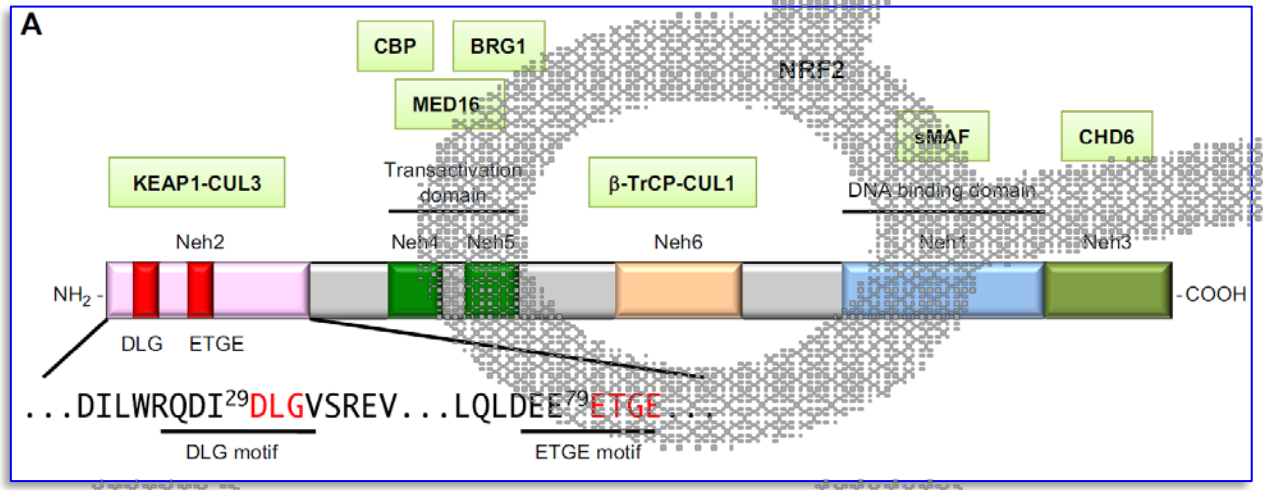
Commonly used non-mammalian model organism:

Yeast (*Saccharomyces cerevisiae*)	Eukaryote - Unicellular
Fly (*Drosophila melanogaster*)	Invertebrates
Nematode worm (*Caenorhabditis elegans*)	
Zebrafish (*Danio rerio*)	Vertebrate

The T4 phage virus, the bacterium *Escherichia coli* and the flowering plant *Arabidopsis thaliana*, *will not be discussed* – mammalian model organisms include guinea pigs (*Cavia porcellus*), the mouse (*Mus musculus*), etc.

NRF2 across evolution

All animal species must cope with oxidative stress **generated by their own metabolism**. They were also forced to evolve detoxifying systems in case of accidental encounters **with toxic chemicals in the environment**. **Animals could not have prospered without the anti-stress mechanisms evolved by their ancestral species**



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

Non-mammalian animals have anti-stress systems analogous to the mammalian KEAP1-NRF2 system

Vertebrates have 4 Nrf genes—Nrf1, Nrf2, Nrf3 and NF-E2—while lower animals (ascidians, sea urchin, octopus, fly and Hydra) seem to have only 1 Nrf gene locus, implying the diversification of this protein family in vertebrate evolution.

Of the diversified Nrf proteins, only vertebrate Nrf2 has all six Neh domains

NRF2 across evolution

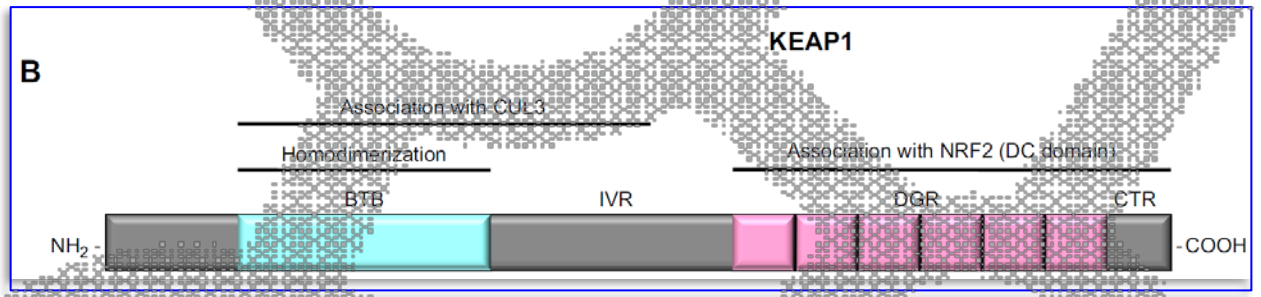
		ER binding	Neh2 DLG	Neh2 length	Neh2 ETGE	Neh4	Neh5	Neh6 DSGIS	Neh6 DSAPGS	Neh1 CNC	Neh1 Basic	Neh1 Zip	Neh3 VFLVPK
Nrf2	*Mm* Nrf2	×	◎	47 aa	◎	◎	◎	◎	◎	◎	◎	◎	◎
	Gg Nrf2	×	◎	47 aa	◎	◎	◎	◎	○	◎	◎	◎	◎
	Xt Nrf2	×	◎	47 aa	◎	○	◎	○	△	◎	◎	◎	◎
*	*Dr* Nrf2a	×	◎	47 aa	◎	◎	◎	○	△	◎	◎	◎	◎
*	*Dr* Nrf2b	×	◎	45 aa	◎	×	○	△	△	◎	◎	◎	○
Nrf1	*Mm* Nrf1	◎	◎	47 aa	◎	×	○	○	×	◎	◎	◎	○
	Gg Nrf1	◎	◎	47 aa	◎	△	○	○	×	◎	◎	◎	○
	Xt Nrf1	◎	◎	39 aa	◎	△	○	○	×	◎	◎	◎	×
*	*Dr* Nrf1a	◎	×		×	○	○	○	×	◎	◎	◎	×
*	*Dr* Nrf1b	×	◎	45 aa	◎	×	○	△	×	◎	◎	◎	○
Nrf3	*Mm* Nrf3	◎	×		×	△	×	○	×	◎	◎	◎	○
	Xt Nrf3	◎	×		×	×	×	○	×	◎	◎	◎	×
*	*Dr* Nrf3	◎	×		×	○	×	○	×	◎	◎	◎	×
Nfe2	*Mm* Nfe2	×	×		×	×	○	○	×	◎	◎	◎	○
	Xt Nfe2	×	×		×	×	△	○	×	◎	◎	◎	×
*	*Dr* Nfe2	×	×		×	×	○	○	×	◎	◎	◎	×
Invertebrate Nrf	*Ci* Nrf	○	◎	34 aa	◎	○	△	○	×	◎	◎	◎	△
	Sp Nrf	○	○	36 aa	◎	○	○	△	×	◎	◎	◎	△
	Ob Nrf	○	◎	59 aa	◎	×	×	△	×	◎	◎	◎	◎
	Dm CncC	○	◎	100 aa	◎	×	×	△	×	◎	◎	◎	○
	Dm CncI	×	◎	100 aa	◎	×	×	△	×	◎	◎	◎	○
	Hm Nrf	○	×		×	×	×	○	×	◎	◎	◎	○
Skn-1	*Ce* Skn-1a	○	×		×	×	×	×	×	◎	◎	×	×
	Ce Skn-1c	×	×		×	×	×	×	×	◎	◎	×	×

Fuse and Kobayashi (2017). Molecules 22, 436.

A summary of the domain structure of the Nrf/Cnc transcription factors.

Conservation of the Neh domains was evaluated as follows: ◎, highly conserved; ○, relatively conserved; △, partially conserved; ×, not conserved. Specific motifs were described as "highly conserved" only when the sequences were identical to mouse Nrf2. The amino acid lengths between DLG and ETGE motifs are also shown.

KEAP1 across evolution



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

Keap1 is composed of three domains, broad complex-tramtrack-bric a brac (BTB), intervening region (IVR) and double glycine repeat (DGR) domains, all of which are important for the inhibition of Nrf2 activity. **N-terminal BTB is the essential region for the formation of the homodimer of Keap1**. Without this dimerization, Keap1 is unable to ubiquitinate Nrf2, and Ser^{104} in this domain is reported to be necessary for dimer formation. **Of note, this serine, including the surrounding amino acids, is highly conserved in both vertebrates and invertebrates**.

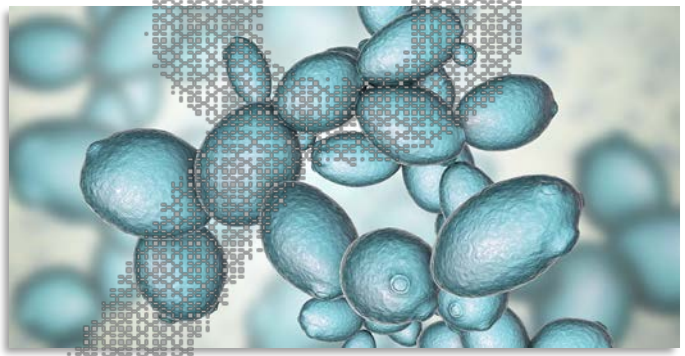
KEAP1 across evolution

	Cysteine (mouse)	23	38	77	151	171	196	226	241	249	257	273	288	297	319	368	395	406	434	489	513	518	583	613	622	624
Vertebrate Keap1	*Mm* Keap1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	Gg Keap1	×	○	○	○	○	○	○	○	○	○	○	○	○	×	○	○	○	○	×	○	○	○	○	○	○
	Ac Keap1	×	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	×	○	○	○	○	○	○	○
	Xt Keap1	×	○	○	○	○	○	○	○	○	○	○	○	○	×	○	○	○	○	○	○	×	○	○	×	×
	Lc Keap1	×	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	×	○	○	○	○	×	×
Actinopterygii Keap1b	*Dr* Keap1b *	×	○	○	○	○	○	○	○	○	×	○	×	×	×	○	○	○	○	○	○	×	○	○	○	○
	Ol Keap1b	×	○	○	○	○	○	○	○	○	×	○	×	○	○	○	○	○	○	○	○	×	○	○	△	×
	Tn Keap1b	×	○	○	○	○	○	○	○	○	×	○	×	○	○	○	○	○	○	○	○	×	○	○	×	×
Vertebrate Keap1a	*Xt* Keap1a	×	○	○	○	○	○	○	○	○	×	×	○	○	×	○	×	×	×	○	×	×	○	○	×	○
	Lc Keap1a	×	×	○	○	○	○	○	○	○	×	×	○	○	×	○	×	×	×	○	×	×	○	○	×	×
	Dr Keap1a *	×	×	○	○	○	○	○	○	○	×	×	○	○	×	○	○	×	×	×	△	×	○	○	×	×
	Ol Keap1a	×	×	○	○	○	○	○	○	○	×	×	○	○	×	○	○	×	×	×	△	×	○	○	×	×
	Tn Keap1a	×	×	○	○	○	○	○	○	○	×	×	○	○	×	○	○	×	×	×	△	×	○	○	×	×
Invertebrate Keap1	Ci Keap1	×	○	○	○	○	○	○	○	△	×	○	○	○	×	○	△	×	×	×	×	×	×	○	×	×
	Sp Keap1	×	×	○	△	○	×	×	○	○	×	×	○	○	×	×	×	×	○	○	×	×	○	○	×	×
	Ob Keap1	×	×	○	△	○	○	○	○	×	×	○	○	○	○	○	×	○	×	○	×	×	○	○	×	×
	Dm Keap1	×	×	×	△	○	○	×	×	×	×	○	○	○	×	×	×	○	×	○	×	×	×	△	×	×

A summary of the cysteine residues of Keap1. The conservation of each cysteine is indicated as follows ○: conserved; △: not conserved but cysteine exists within three amino acids; ×: not conserved. Sensor cysteines are shaded in red, and cysteine residues conserved among Kelch family proteins in mice are shaded in orange.

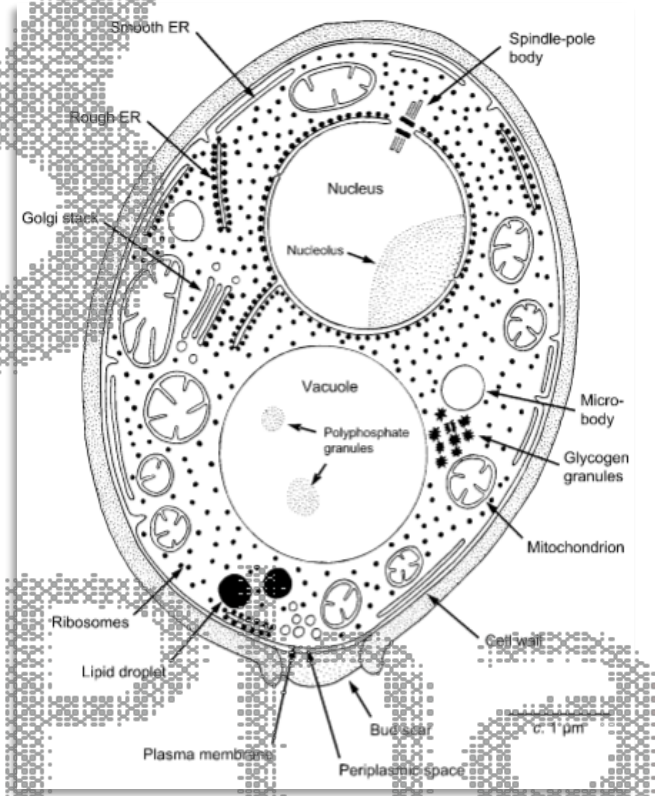
Fuse and Kobayashi (2017). Molecules 22, 436.

Yeast (Saccharomyces cerevisiae)

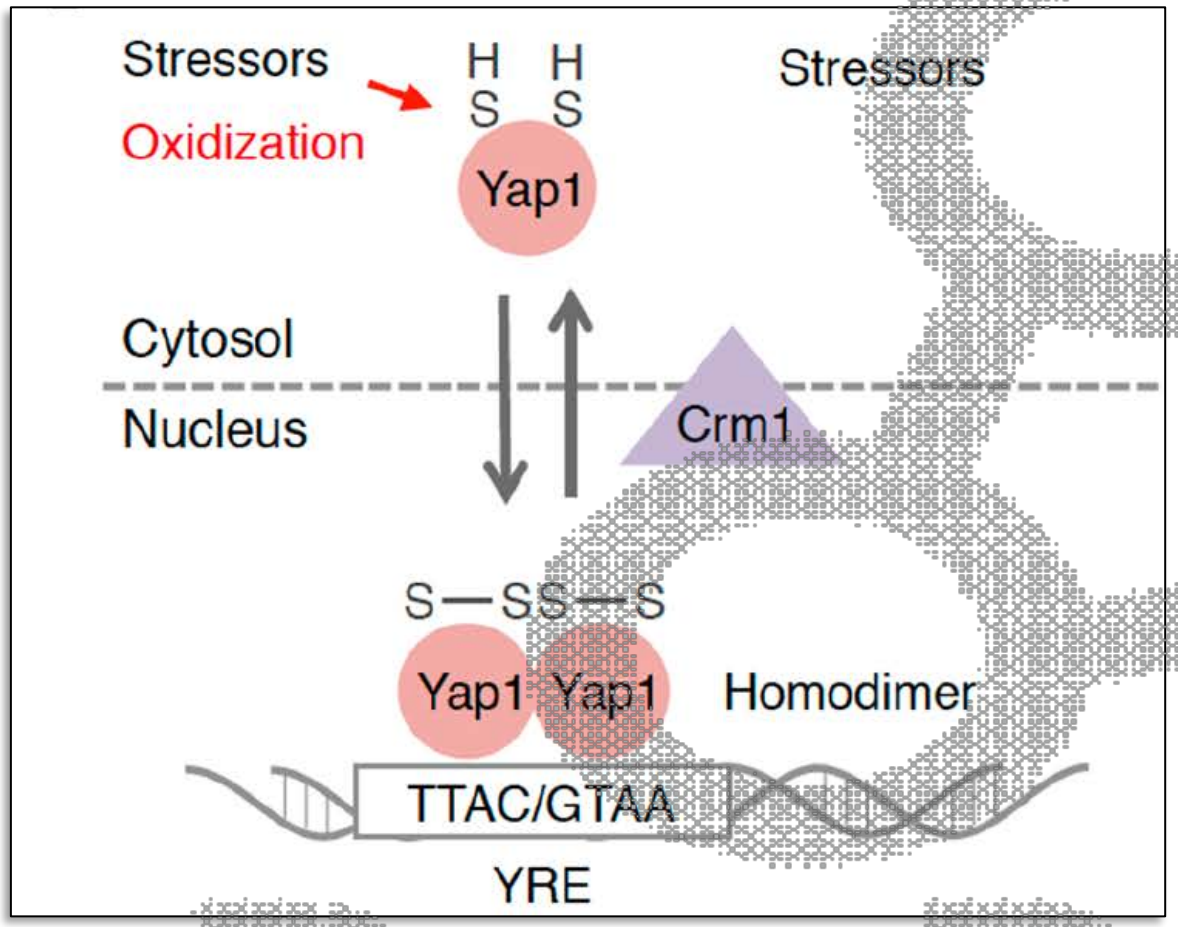


Saccharomyces cerevisiae (brewer's yeast or baker's yeast) is a species of yeast (single-celled fungus microorganisms). It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model bacterium. S. cerevisiae cells are round to ovoid, 5–10 µm in diameter. It reproduces by budding.

Many proteins important in human biology were first discovered by studying their homologs in yeast; these proteins include *cell cycle proteins*, *signaling proteins*, and *protein-processing enzymes*.



Yeast (NRF2)



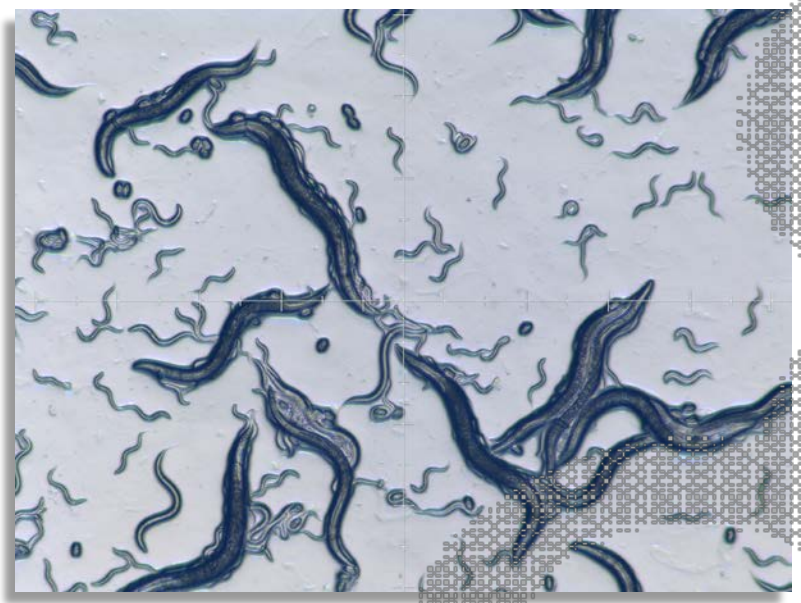
Fuse and Kobayashi (2017). Molecules 22: 436.

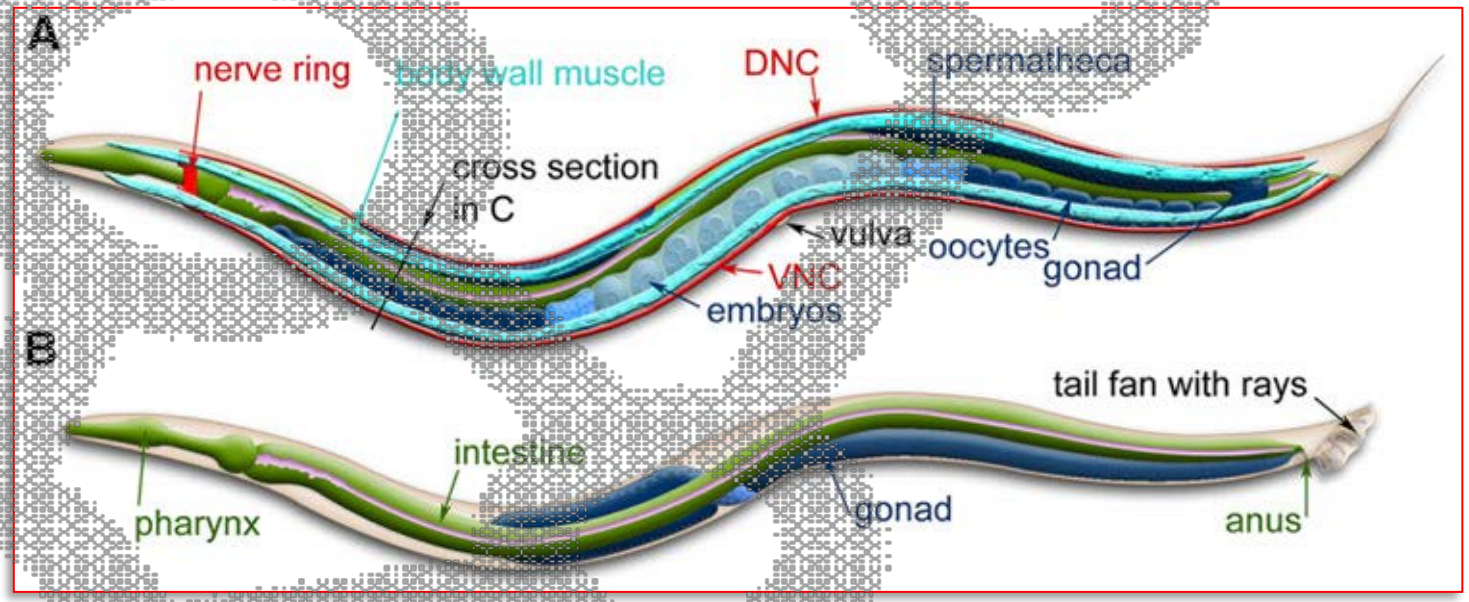
Regulatory mechanisms of the transcription factor-based oxidative stress response in eukaryotes. The activation mechanism of Yap1 in *S. cerevisiae*

Yap in *Saccharomyces cerevisiae* - Yap family proteins are a well-studied group of transcription factors that confer protection against oxidative and chemical stress. The Yap family consists of eight paralogs of basic leucine zipper (bZip)-type transcription factors, Yap1-8. **Of these members, Yap1 is the major isoform that confers protection against oxidative stress**. Unlike Nrf2 in mammals, **Yap1 forms homodimers** that bind to specific sequences of DNA, **Yap response element** (YRE), and activate the transcription of target genes.

The stress-sensing system is also unique: Yap1 has cysteine residues in its C-terminus that function as sensors. Under unstressed (reduced) conditions, Yap1 localizes in the cytosol by the action of exportin chromosomal maintenance 1 (Crm1), and the transcriptional activation is inhibited. When exposed to oxidative stress, the cysteine residues of Yap1, however, are oxidized with the assistance of **glutathione peroxidase 3** (Gpx3), a thiol peroxidase, and an intramolecular disulfide bond is formed. In this structure, **Crm1 cannot approach the nuclear export signal (NES) region of Yap1**, resulting in the nuclear retention of Yap1 and its target gene activation. **Regarding this unique activation mechanism, the Yap1 system might stem from a different evolutionary origin from the Keap1-Nrf2 system.**

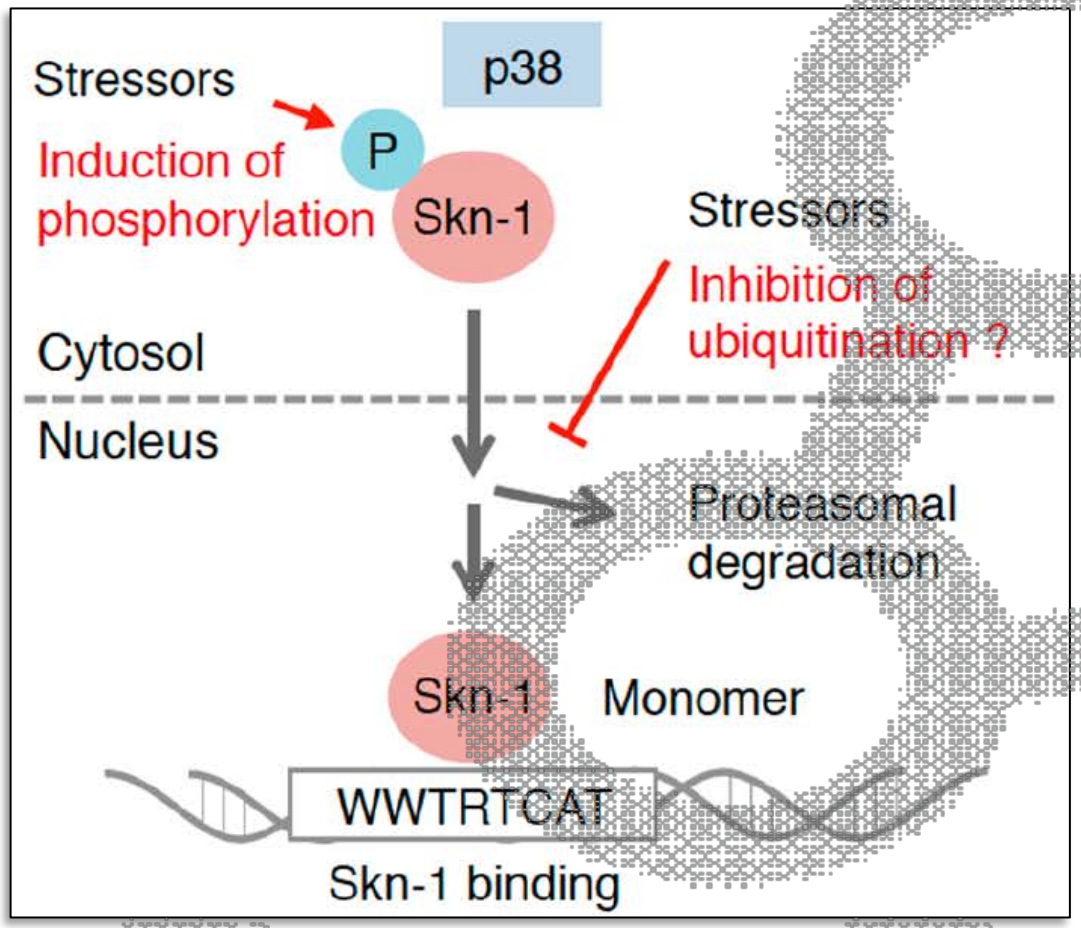
Nematode worm (Caenorhabditis elegans)





C. elegans is unsegmented, vermiform, and bilaterally symmetrical. It has a cuticle, four main epidermal cords, and a fluid-filled pseudocoelom**. It also has some of the same organ systems as larger animals**. **Like all nematodes, they have neither a circulatory nor a respiratory system. The four bands of muscles that run the length of the body are connected to a neural system that allows the muscles to move**. In relation to **lipid metabolism**, ***C. elegans* does not have any specialized adipose tissues, a pancreas, a liver, or even blood to deliver nutrients compared to mammals**. *C. elegans* neurons contain dendrites which extend from the cell to receive neurotransmitters, and a process that extends to the nerve ring (the "brain") for a synaptic connection between neurons.

Nematode worm (NRF2)

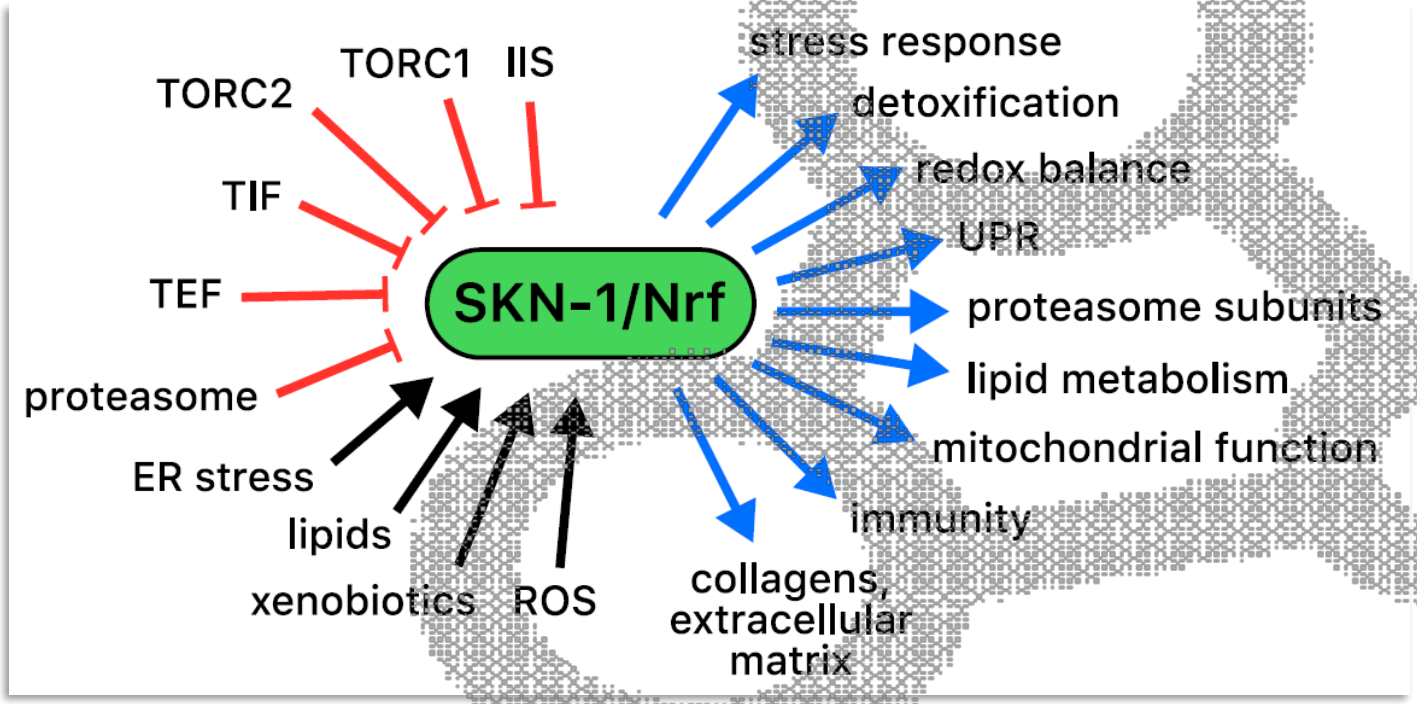


Fuse and Kobayashi (2017). Molecules 22, 436.

Regulatory mechanisms of the transcription factor-based oxidative stress response in eukaryotes. The activation mechanism of Skn-1 system in *C. elegans*.

Skn-1 (discovered as a protein being important for pharynx development) in *Caenorhabditis elegans*. Skn-1 has a similar inducible defense function to Nrf2, namely protection against chemical stresses. Uniquely, Skn-1 **binds to specific DNA sequences as a monomer due to the loss of leucine zipper domain, which is important for dimerization with small Mafs in mammals**. Although the transcriptional activity of Skn-1 seems to be regulated at the protein level**, C. elegans does not have an authentic ortholog of Keap1**. Instead, in *C. elegans*, **the WD40 repeat protein-23 (WDR-23)/damaged DNA binding protein 1 (DDB1) complex is involved in the ubiquitination of Skn-1 under basal conditions. It remains unclear how Skn-1 escapes this negative regulation in stressed situations. Phosphorylation-based regulation has also been described in the Skn-1 system, and three kinases—AKT, PMK-1 (p38) and GSK-3—were shown to target the serine residues on Skn-1. Of these kinases, phosphorylation by PMK-1 activates Skn-1, while the others negatively regulate the Skn-1 function.**

Nematode worm (NRF2)

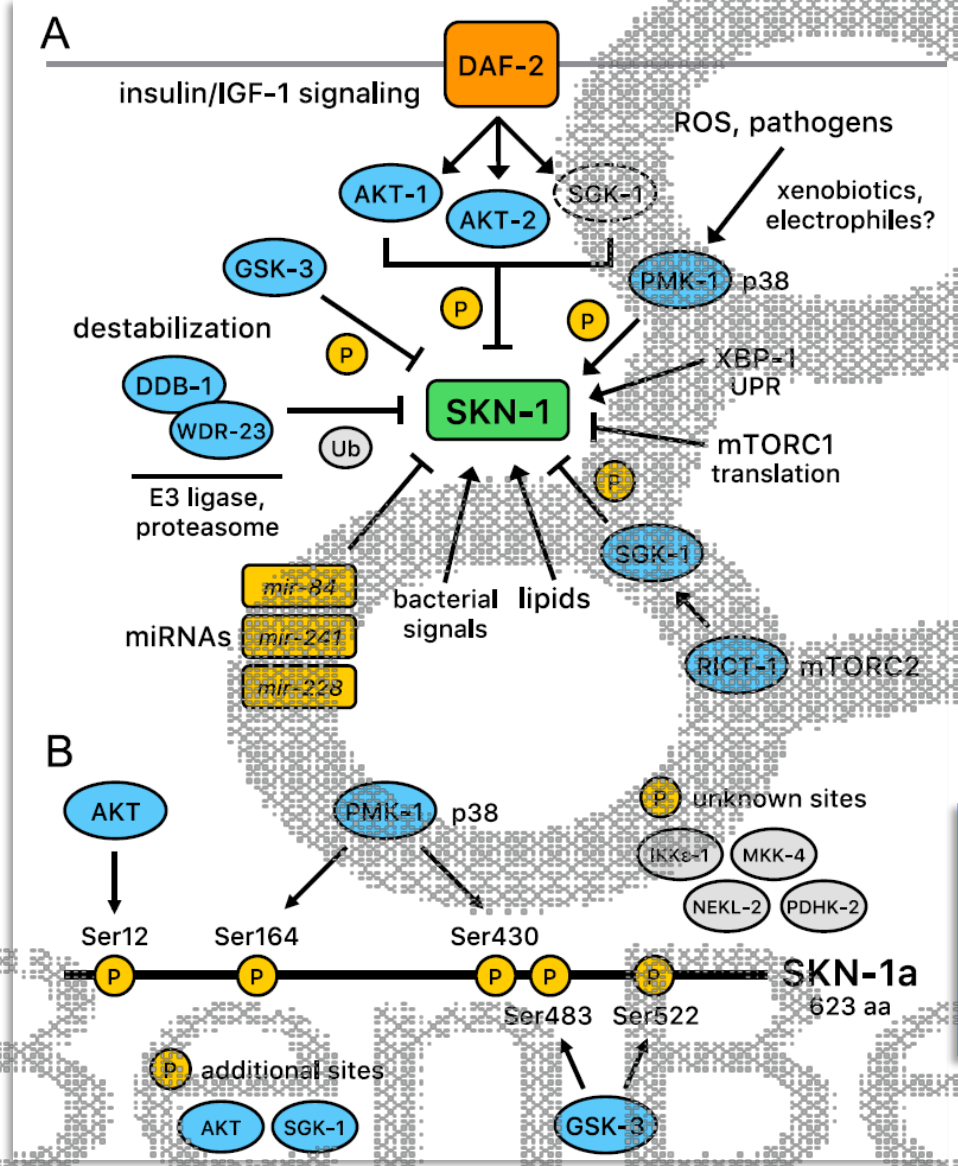


T Keith Blackwell et al. (2015). Free Radic Biol Med. 88, 290-301.

Complexity of SKN-1 functions. SKN-1 directly or indirectly controls genes involved in a wide variety of biological processes (blue arrows), overlapping subsets of which may be up-regulated by different stresses.

Although the regulatory mechanism differs from that of the mammal Nrf2 system, the target genes of Skn-1 are similar to those of mammalian Nrf2. The gene expressions of phase I, II and III detoxifying enzymes, antioxidant proteins and proteasome subunits are regulated in a Skn-1-dependent manner. This implies that Keap1-Nrf2/CncC and Skn-1 stem from the same ancestral system, while in ***C. elegans***, a unique regulatory mechanism was evolved.

Nematode worm (NRF2)



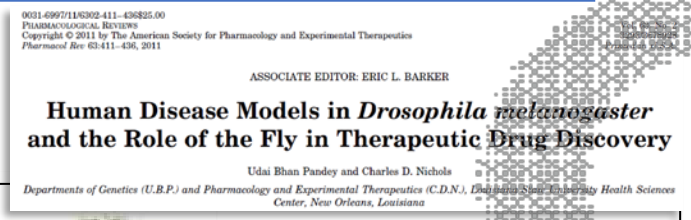
(**A**) **Mechanisms that regulate SKN-1.** (**B**) Post-translational regulation of SKN-1 **through phosphorylation**. AKT, PMK- 1/p38 and GSK-3 have been found to regulate SKN-1 directly at the positions indicated. SGK-1 also phosphorylates and inhibits SKN-1. Of these kinases, **PMK-1/p38 activates SKN-1 and the other kinases are inhibitory**. The kinases shown as white ovals also inhibit SKN-1, but it is unknown whether they act directly or indirectly. Numbering is according to positions within SKN-1a isoform.

Mammalian Gsk-3 and p38 are also known to be involved in Nrf2 regulation; therefore, this phosphorylation-based regulation may be conserved from an ancestral system.

SKN-1 and longevity

Blackwell et al. (2015). Free Radic Biol Med. 88, 290-301.

The fly (Drosophila melanogaster)


0031-6997/11/6302-411–436$25.00
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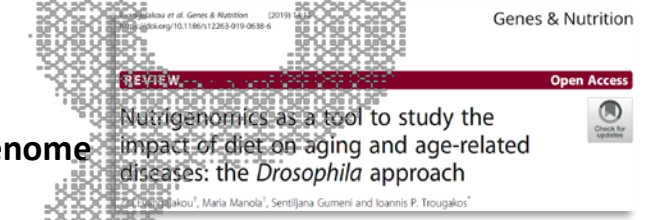
Pharmacol Rev 63:411–436, 2011

ASSOCIATE EDITOR: ERIC L. BARKER

Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery

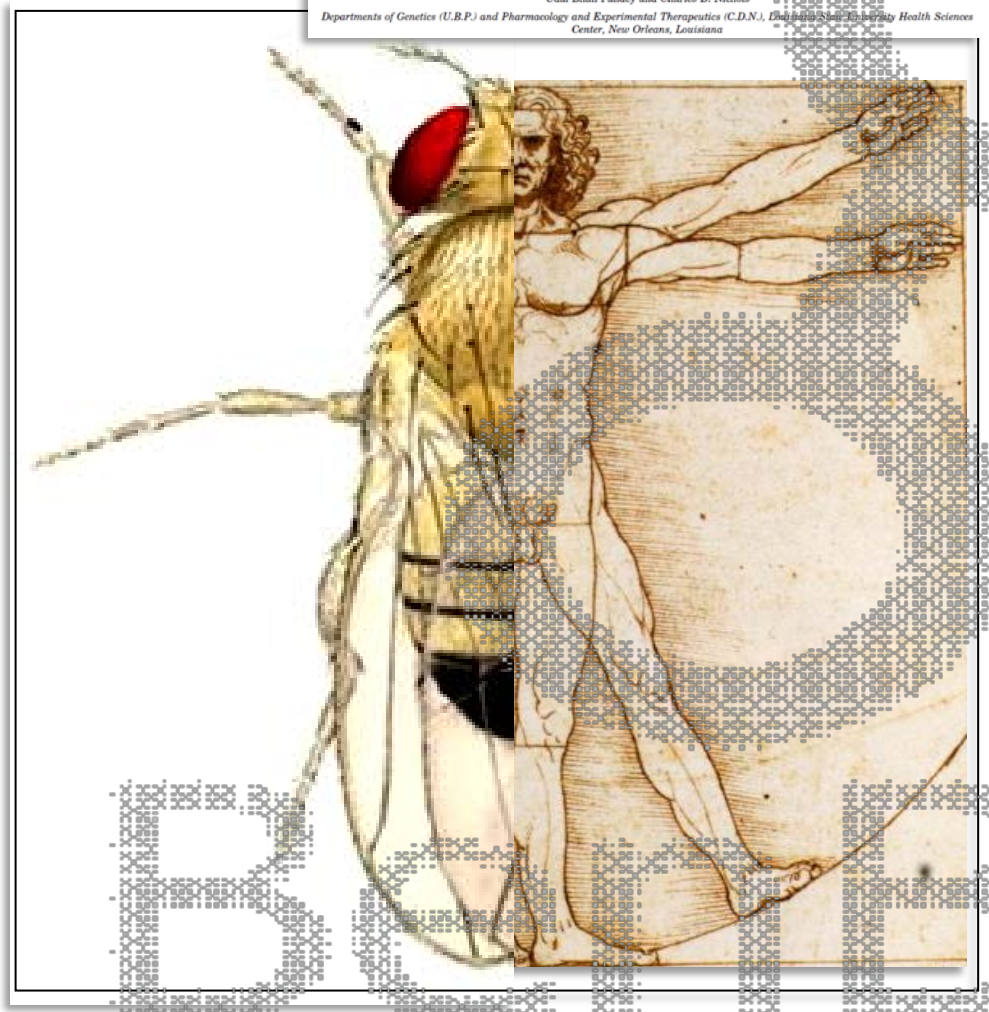
Udai Bhan Pandey and Charles D. Nichols

Departments of Genetics (U.B.P.) and Pharmacology and Experimental Therapeutics (C.D.N.), Louisiana State University Health Sciences Center, New Orleans, Louisiana


Genes & Nutrition

REVIEW Open Access

Nutrigenomics as a tool to study the impact of diet on aging and age-related diseases: the *Drosophila* approach



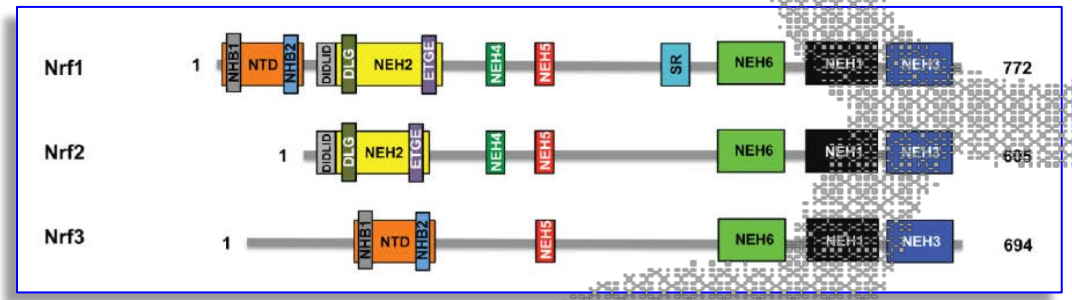
- Completely sequenced and annotated genome
- Encodes for ~14,000 genes
- ~ 75% of disease-related genes in humans have functional orthologs in the fly
- Overall identity at the nucleotide level or protein sequence between fly and mammal is usually approximately 40% between homologs; however, in conserved functional domains, it can be 80 to 90% or higher
- Very rapid life cycle
- Multiple model organisms (embryo, the larva, the pupa, and the adult)
- The adult fly is a very sophisticated and complex organism not unlike higher organisms. The adult fly has structures that perform the equivalent functions of the mammalian heart, lung, kidney, gut, and reproductive tract.
- The brain of the adult fly is quite remarkable. More than 100,000 neurons form discreet circuits and neuropil that mediate complex behaviors, including circadian rhythms, sleep, learning and memory, courtship, feeding, aggression, grooming, and flight navigation.
- The response of flies to many drugs that act within the CNS is similar to the effects observed in mammalian systems
- Advanced “high tech” genetics – molecular “tools”
- **Minimal genetic redundancy**

Although there are many differences between flies and humans, the degree of conserved biology and physiology position *D. melanogaster* as an extremely valuable tool in the drug discovery process.

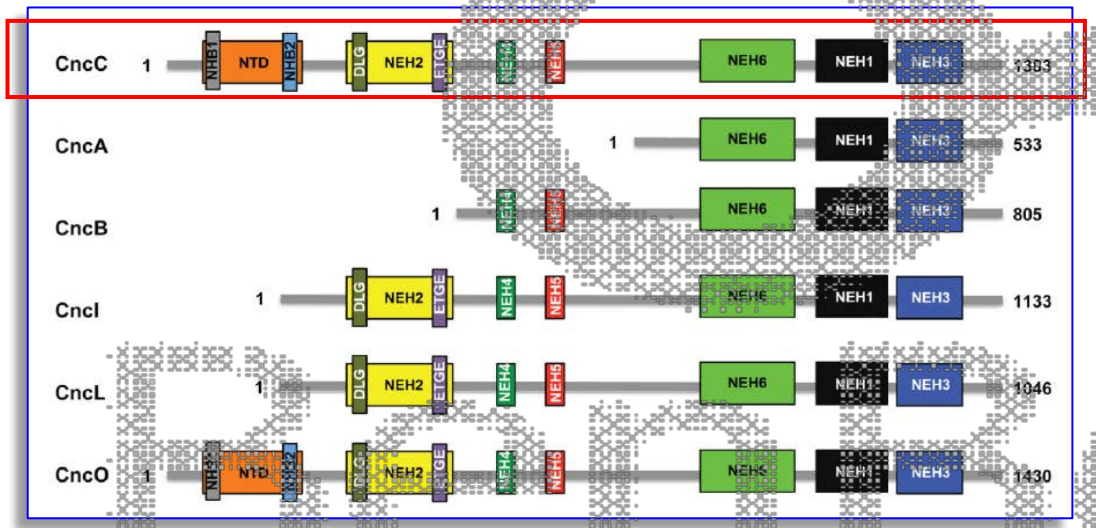
Drosophila (NRF2)

A representation of Cap'n'collar transcription factors found in humans and *Drosophila*

Human Nrf family



Drosophila Cnc splice variants



Sykiotis and Bohmann (2010). Sci Signal. 3(112), re3.
Pitoniak and Bohmann (2015). Free Radic. Biol Med. 88, 302-313.

The fly homolog of Nrf2 was discovered as an important protein in the development of the cranial portion (labral and mandibular structure) of larvae. Because of its unique expression pattern, this gene was named **Cap'n'collar** (CNC). Despite highly conserved amino acid sequences with Nrf2, the anti-stress function of Cnc was not described until the discovery of the transcript variant, **CncC, which contains N-terminal domains homologous to Nrf2**. CncC was demonstrated to have an anti-stress function in adult flies. In addition, the activity of CncC was regulated at the protein level by the direct interaction with **Keap1**, and heterodimerization with *Drosophila* small Maf protein, **Maf-S**, was also demonstrated. **The target genes of fly CncC are similar to those of Nrf2 in mammals**. Phase I and II enzymes, antioxidant proteins and proteasome subunits are shown to be under the regulation of CncC. **These analogies suggest that the Keap1-CncC system in *Drosophila* evolved from a common ancestral system with the mammalian Keap1-Nrf2 system.**

Drosophila (NRF2)

The Nrf2/Keap1 pathway is constitutively active in DDT-resistant *Drosophila*

Nrf2/Keap1 activation leads to widespread overexpression of detoxification genes

One or more factors on the third chromosome are sufficient for Nrf2/Keap1 activation

Constitutive activation of Nrf2 may contribute to acquired insecticide resistance

Misra et al. (20113). Genes Dev. 25, 1796-806.
Misra et al. (2013). Insect Biochem Mol Biol. 43, 1116-24.

The Nrf2/Keap1 pathway contributes to the widespread overexpression of detoxification genes in insecticide-resistant strains and raises the possibility that inhibitors of this pathway could provide effective synergists for insect population control.

Alterations in Organismal Physiology, Impaired Stress Resistance, and Accelerated Aging in *Drosophila* Flies Adapted to Multigenerational Proteome Instability

Maria S. Manola, Eleni N. Tsakiri, and Ioannis P. Trougakos

Oxidative Medicine and Cellular Longevity
Volume 2019, Article ID 7823285, 14 pages

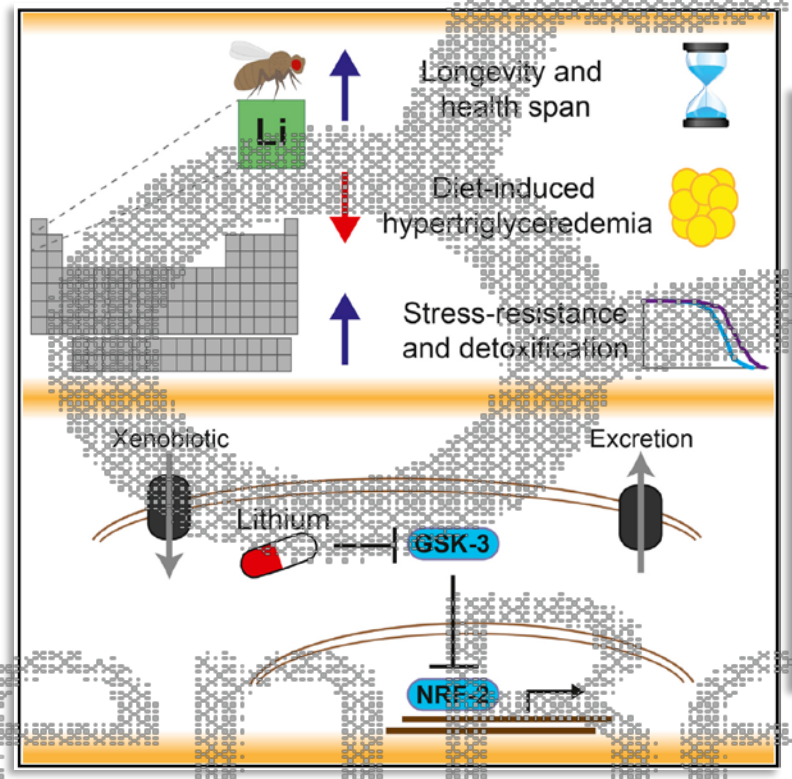
Overall, persistent **proteotoxic stress** triggers a **highly conserved adaptive metabolic response mediated by the IIS pathway**, which **reallocates resources from growth and longevity to somatic preservation and stress tolerance**. Yet, these trade-off adaptations occur at the cost of accelerated aging and/or reduced tolerance to additional stress, illustrating **the limited buffering capacity of survival pathways**.

Drosophila (NRF2)

Cell Reports

Lithium Promotes Longevity through GSK3/NRF2-Dependent Hormesis

Castillo-Quan et al., 2016, Cell Reports *15*, 638–650



Highlights

- Lithium extends Drosophila lifespan independent of sex and genetic background
- Lithium reduces triglycerides and confers stress-resistance
- Genetic or pharmacological inhibition of GSK-3 activates NRF-2
- NRF-2 activation is required for the longevity effects of lithium

Drosophila (NRF2)

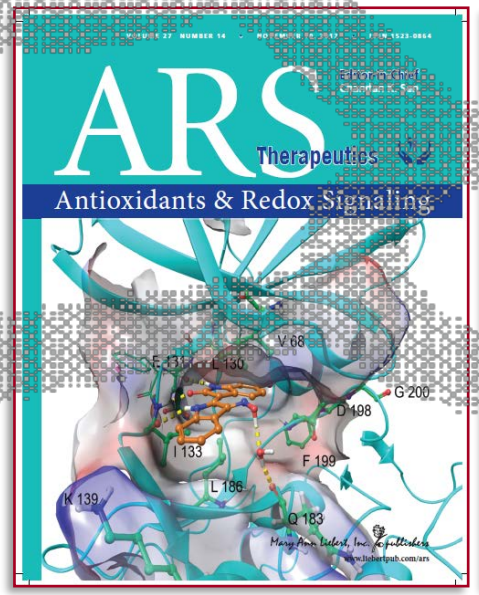
ANTIOXIDANTS & REDOX SIGNALING
Volume 00, Number 00, 2017

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]



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Published online: 15 September 2017

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

Aimilia D. Sklirou[1], Nicolas Gaboriaud-Kolar[2], Issidora Papassideri[3], Alexios-Leandros Skaltsounis[2] & Ioannis P. Trougakos[1]

BenBedPhar

Drosophila (NRF2)

Redox Regulation by Keap1 and Nrf2 Controls Intestinal Stem Cell Proliferation in *Drosophila*

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DOI 10.1016/j.stem.2010.12.006

Cell Stem Cell *8*, 188–199, February 4, 2011

Article

Cell Reports

The Nrf2-Keap1 pathway is activated by steroid hormone signaling to govern neuronal remodeling

Chew et al., 2021, Cell Reports 36, 109466

JCB Journal of Cell Biology

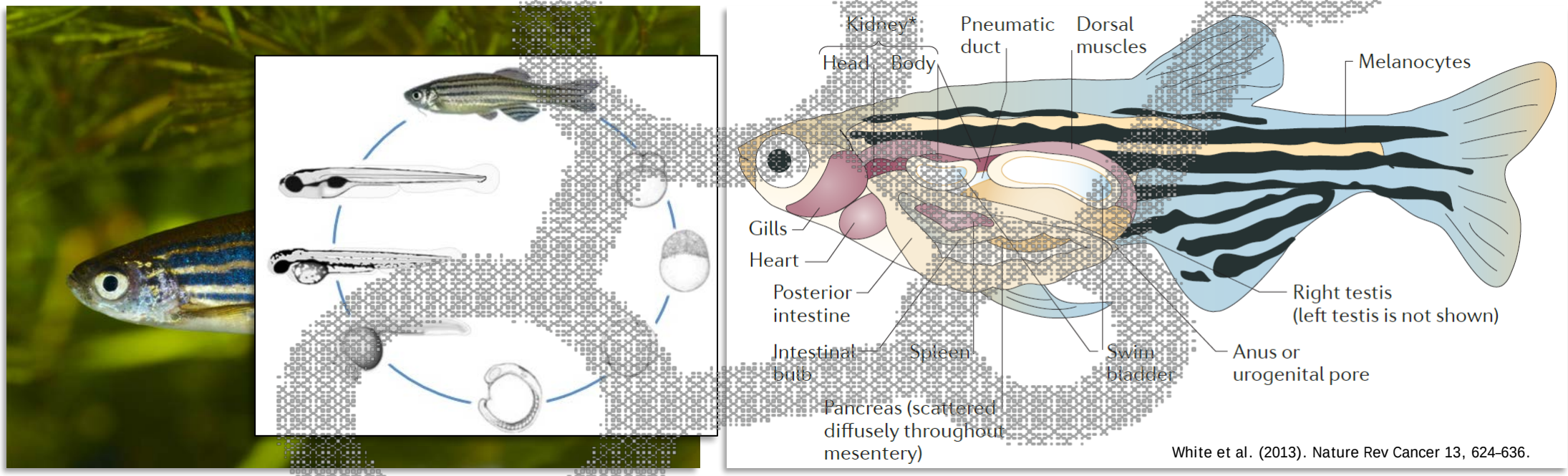
REPORT

A PI3K-calcium-Nox axis primes leukocyte Nrf2 to boost immune resilience and limit collateral damage

Giuliana D. Clemente[1] and Helen Weavers[1]

J. Cell Biol. 2023 Vol. 222 No. 6 e202203062

Zebrafish (Danio rerio)



Zebrafish anatomy. An adult zebrafish is shown with the anatomical structures labelled. Zebrafish share most of their organs with mammalian counterparts, including the brain, heart, liver, spleen, pancreas, gallbladder, intestines, kidney, testis and ovaries. *The kidney is also the site of haematopoiesis in zebrafish.

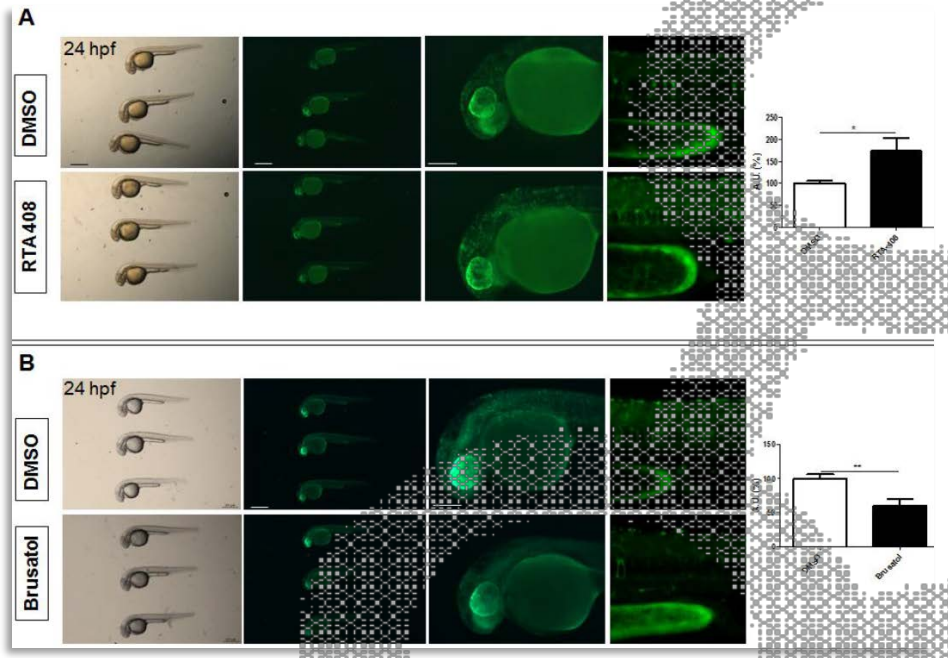
Zebrafish (NRF2)

- **In zebrafish (*Danio rerio*) the Keap1-Nrf2 system is highly conserved with that of the mammalian system.**
- Zebrafish Nrf2 and Keap1 were first cloned in 2002 and found to be structurally similar proteins to their mammalian counterparts. Dimeric partners of zebrafish Nrf2 have also been identified and revealed to **have conserved small Mafs, MafG (co-ortholog MafG1 and MafG2) and MafK**, **along with fish-specific MafT** (a possible ortholog of mammalian MafF).
- All of these homologs can function as binding partners of zebrafish Nrf2. The function of the upstream ARE sequence was shown to be necessary for the Nrf2-dependent induction of a gene encoding phase II enzyme, gstp1.
- The defense function against xenobiotics and oxidative stress was also demonstrated *in vivo* using Nrf2 mutant zebrafish strain. **Nrf2 target genes are also conserved in zebrafish.** Proteins involved in the detoxification pathway, antioxidant proteins, proteasome subunits and pentose phosphate pathway enzymes are also regulated by the Nrf2 system in zebrafish.

Vertebrates have an evolutionarily conserved Keap1-Nrf2 system

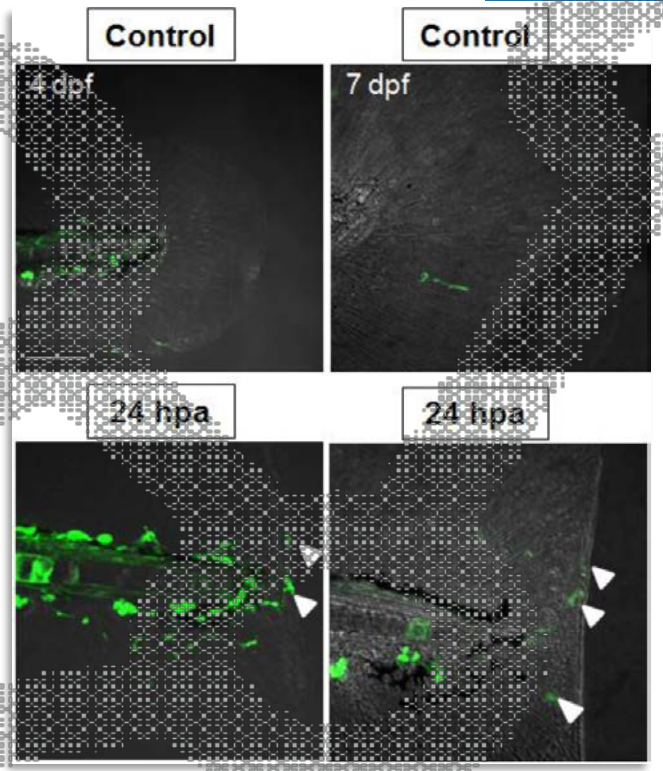
☛ In Zebrafish two Keap molecules: Keap1A (strong CUL3 binding) and Keap1B (weak CUL3 binding)

Zebrafish (NRF2 reporter)



Badenetti et al. (2023). Int J Mol Sci. 24, 6804.

Pharmacological validation of Nrf2/ARE reporter fish. (**A**) Whole-mount bright field and fluorescence microscopy acquisition of a 24 hpf transgenic larva treated with DMSO and **the Nrf2 pathway agonist, RTA-408**, for 16 h. (**B**) Whole-mount bright field and fluorescence microscopy acquisition of a 24 hpf transgenic larva treated with DMSO and the **Nrf2 pathway antagonist, Brusatol**, for 16 h.



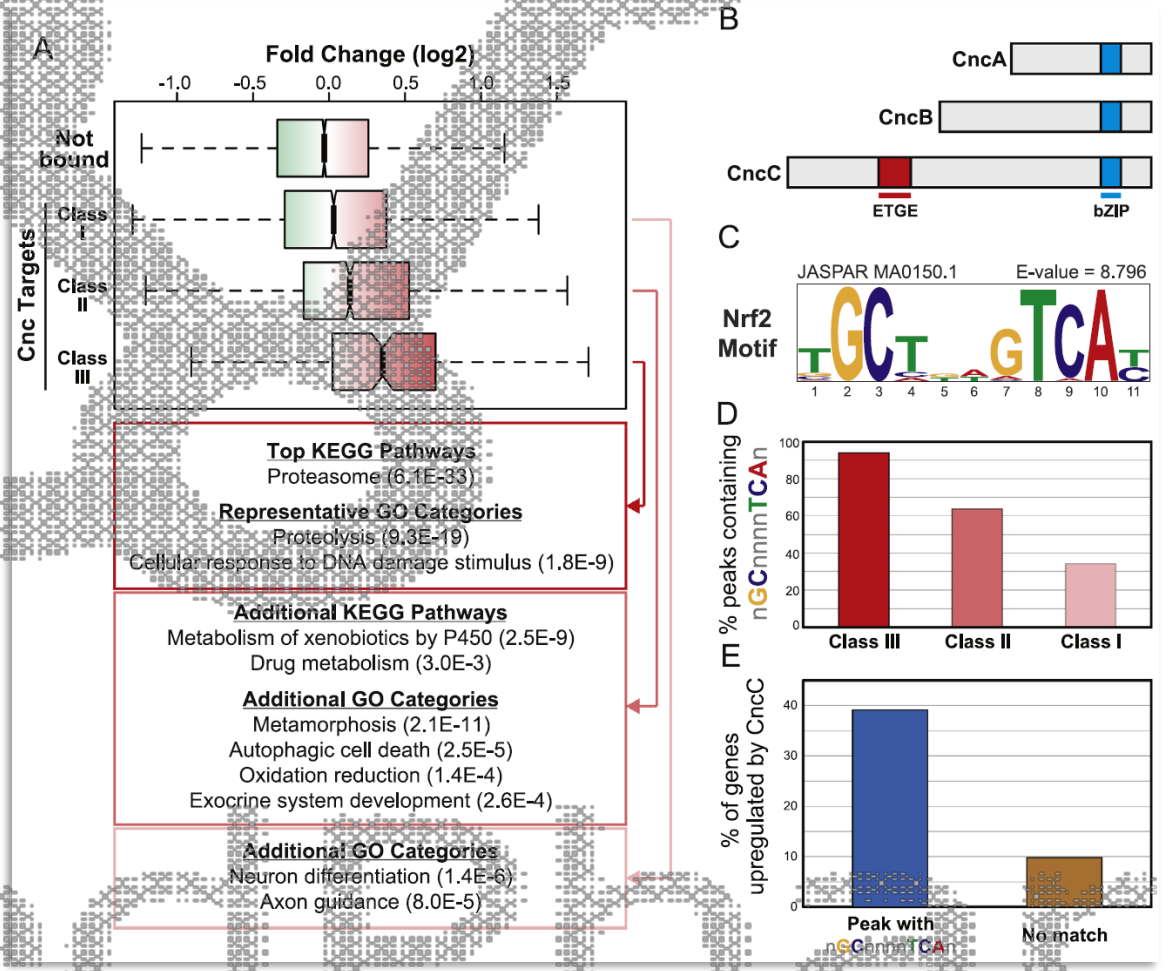
Badenetti et al. (2023). Int J Mol Sci. 24, 6804.

Nrf2/ARE pathway activity increases in regenerating tail fins. Representative Z-stack projection of a confocal fluorescence microscopy acquisition of unamputated and amputated tail fin of representative 4 dpf and 7 dpf Nrf2/ARE transgenic fish after at 24 h post amputation (hpa), showing fluorescent cells migrating along the stump (white arrowheads). Scale bar: 100 m.

NRF2 across evolution

Beyond antioxidant genes in the ancient NRF2 regulatory network

Genome-wide ChIP and gene expression data were used to identify direct Nrf2 target genes in *Drosophila* and humans. These data allowed the construction of the deeply conserved ancient Nrf2 regulatory network-target genes that are conserved from *Drosophila* to human. The ancient network consists of canonical **antioxidant genes**, as well as genes related to **proteasomal pathways and metabolism and a number of less expected genes**. We have also used enhancer reporter assays and electrophoretic mobility-shift assays to confirm Nrf2-mediated regulation of ARE activity at a number of these novel target genes. Interestingly, the ancient network also highlights a **prominent negative feedback loop**; this, combined with the finding that Nrf2-mediated regulatory output is tightly linked to the quality of the ARE it is targeting, suggests **that precise regulation of nuclear Nrf2 concentration is necessary to achieve proper quantitative regulation of distinct gene sets.**



Lacher et al. (2015). Free Radic Biol Med. 88, 452-465.

NRF2 across evolution

SCIENCE ADVANCES | RESEARCH ARTICLE

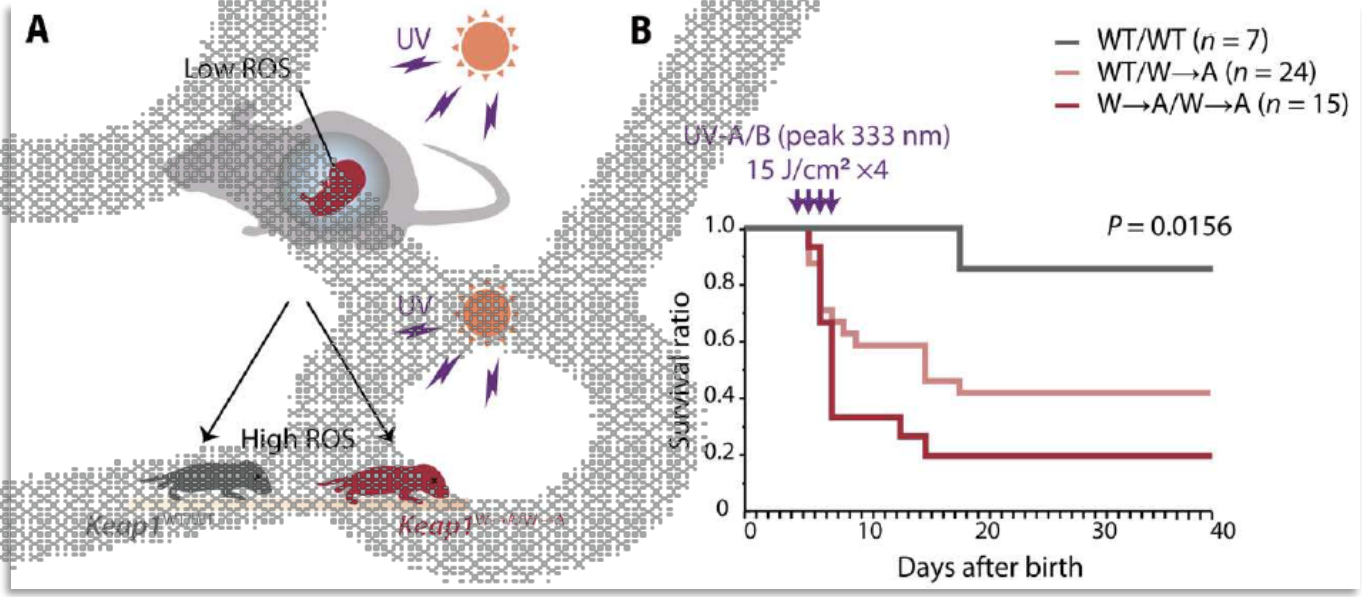
EVOLUTIONARY BIOLOGY

Molecular evolution of Keap1 was essential for adaptation of vertebrates to terrestrial life

Kanae Yumimoto[1], Shigeaki Sugiyama[1], Saori Motomura[1], Daisuke Takahashi[2†], Keiichi I. Nakayama[1*]

Yumimoto *et al.*, *Sci. Adv.* **9**, eadg2379 (2023) 19 May 2023

Keap1A (strong CUL3 binding) was subsequently lost in reptiles and their descendants, and Keap1B (weak CUL3 binding) evolved into mammalian Keap1. It was found that replacement of C3IR of mammalian Keap1 with the corresponding region of zebrafish Keap1A attenuated Nrf2 activity in cells subjected to stress, and most mouse neonates harboring this mutation were found to die on exposure to sunlight-level UV radiation. **The molecular evolution of Keap1 was essential for adaptation of organisms to the higher ROS levels associated with a terrestrial environment.**



(**A**) The mouse fetus is protected from oxidative stress in the womb but is exposed to high ROS levels after birth as a result of the high ambient oxygen concentration and UV in sunlight. (**B**) Kaplan-Meier survival curves for WT, *Keap1*$^{W/W\rightarrow A}$, and *Keap1*$^{W\rightarrow A/W\rightarrow A}$ mice exposed to UV-A/B radiation.

Conclusive remarks

- The **appearance of O_2 in earth** transformed the atmosphere for reductive to oxidative.
- ROS **posed a risk** for the transition of vertebrates from aquatic to terrestrial life (a key event in evolution) since O_2 concentrations were higher on land than in the sea
- Moreover, the **development of aerobic respiration**, which **produces energy far more efficiently than does anaerobic respiration**, is thought to be an adaptation that allowed organisms to migrate to and thrive on land.

The evolution of defense mechanisms (like the NRF2/KEAP1 sensor system) against ROS has been necessary as organisms moved onto land