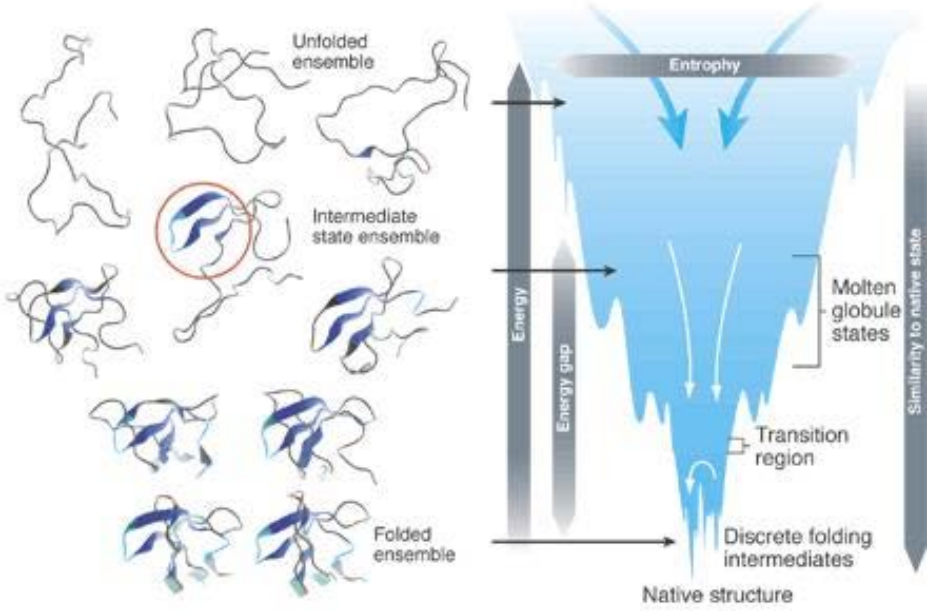
Aging and proteostasis

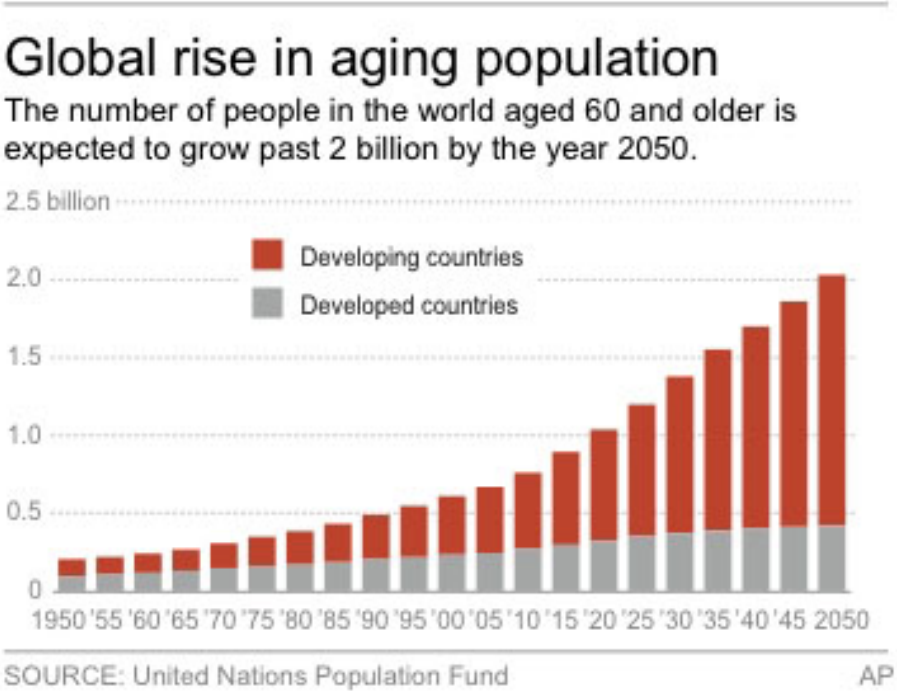


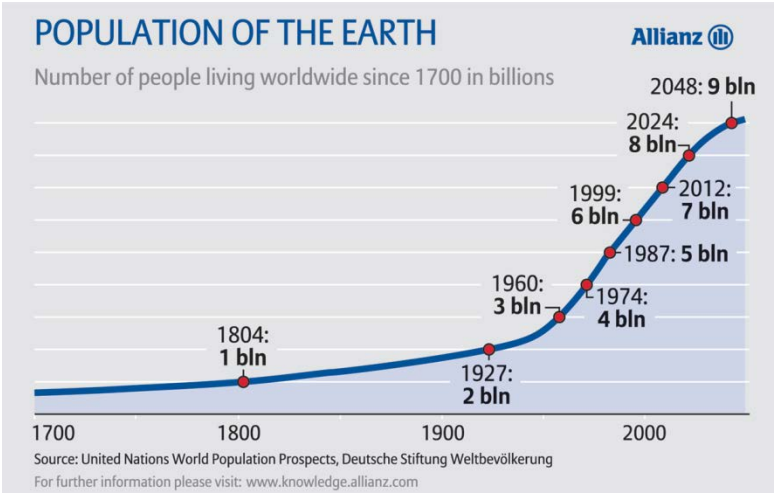
YOUNG SHIK PARK
SCHOOL OF BIOLOGICAL SCIENCES, INJE UNIVERSITY

Jeanne Calment

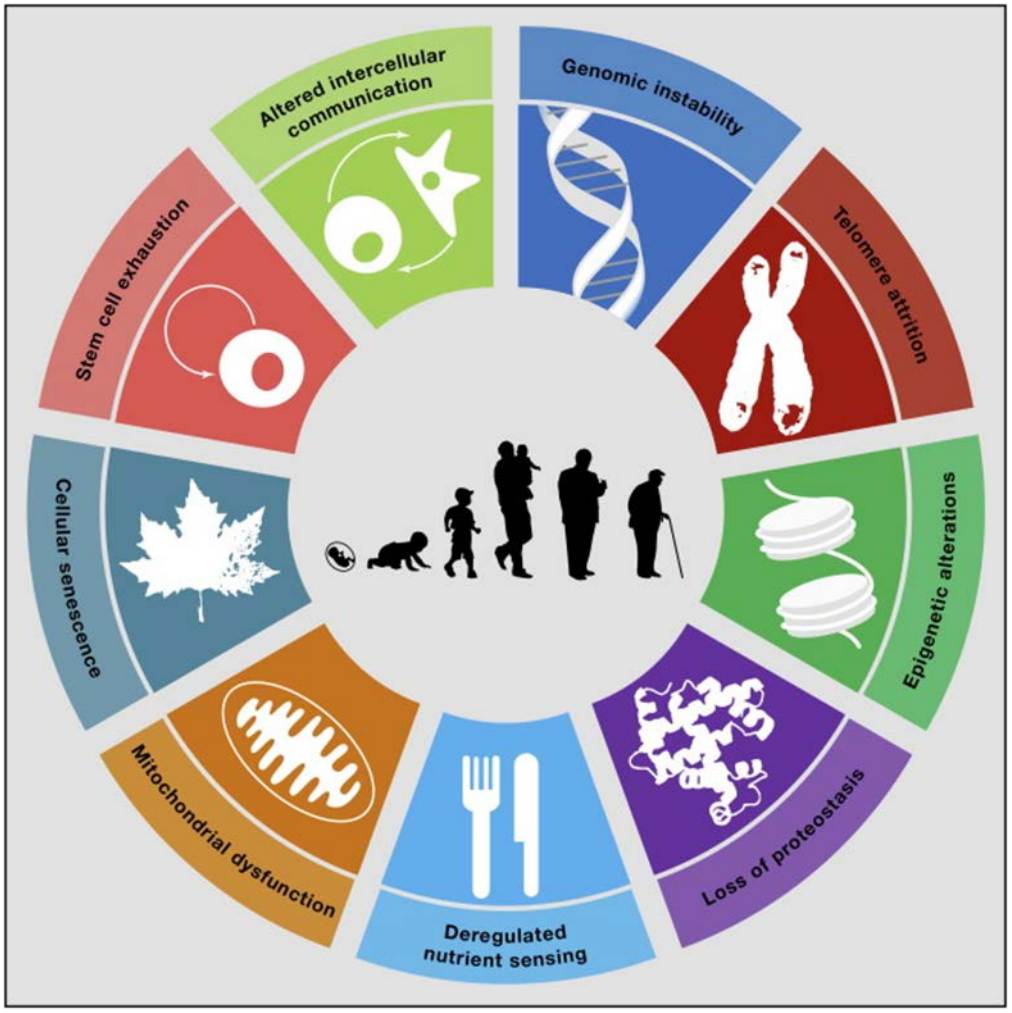
https://en.wikipedia.org/wiki/Jeanne_Calment

Aging population





The Hallmarks of Aging



Cell 153, 1194-1217 (2013)

What is aging?

Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death.

Aging is a complex biological process that has prompted many new theories in regard to its process and origin. (programmed & non-programmed)

Many studies of aging have focused on molecular changes across the lifetime of an organism with the reasonable assumption that a series of progressive events collectively contribute to the aging process.

Proteostasis: protein homeostasis (through recycling)

A term coined in a landmark Science paper in 2008, is a broad concept that is relevant to all fields of study that involve protein synthesis, folding, processing, and turnover (Science 319:916–919, 2008).

The decline of proteostasis with aging

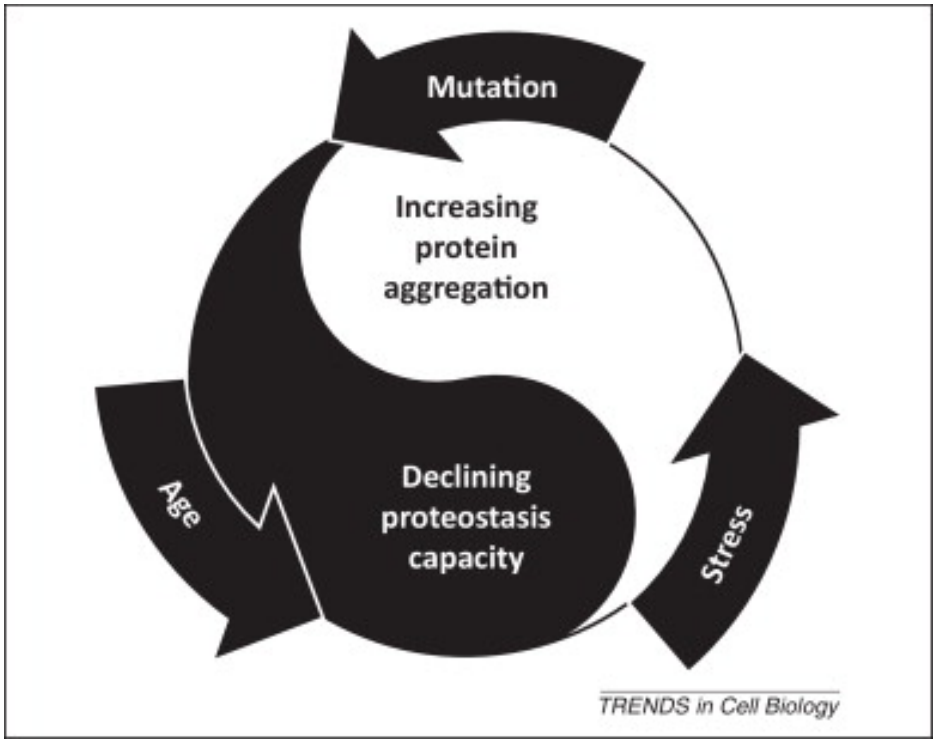
When protein homeostasis degenerates and collapses, cells get old . . . and sick.

Hallmark features of cellular aging include a buildup of proteotoxic stress, mistranslation of nascent polypeptides, and concentration of damaged, misfolded proteins and protein aggregates.

This accumulation of cellular waste is toxic to cells. That's when things start to fall apart.

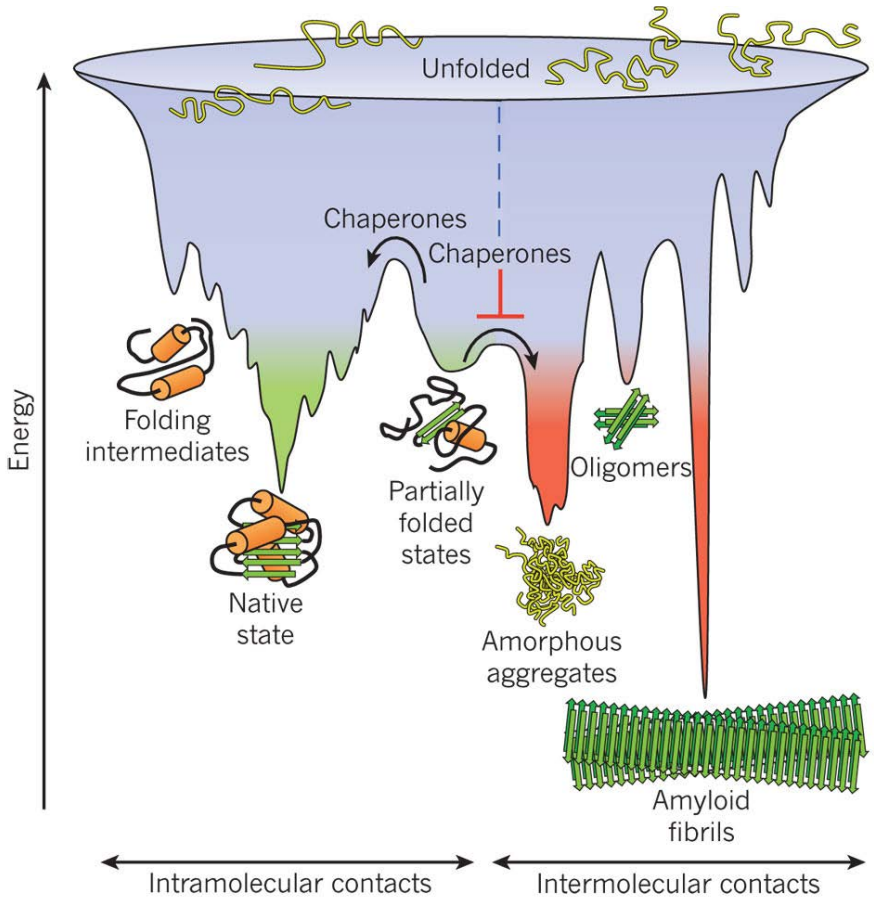
http://irp.nih.gov/catalyst/v21i6/the-sig-beat

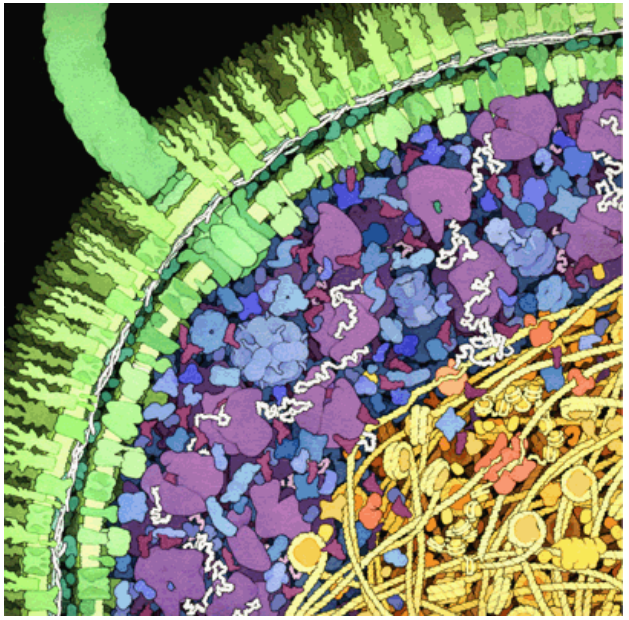
Self-propagating cycle of proteostasis decline in disease and aging



Chronic expression of aberrantly folded protein species caused by disease, aging, or external stress reduces proteostasis capacity by sequestering or otherwise inhibiting proteostasis network (PN) components. This results in misfolding and aggregation of endogenous proteins. These additional misfolded species in turn engage the PN (PN titration model), thereby further reducing the available proteostasis capacity and driving a positive feedback loop that eventually leads to proteostasis collapse. Trends in Cell Biology September 2014, Vol. 24, No. 9

Competing reactions of protein folding and aggregation





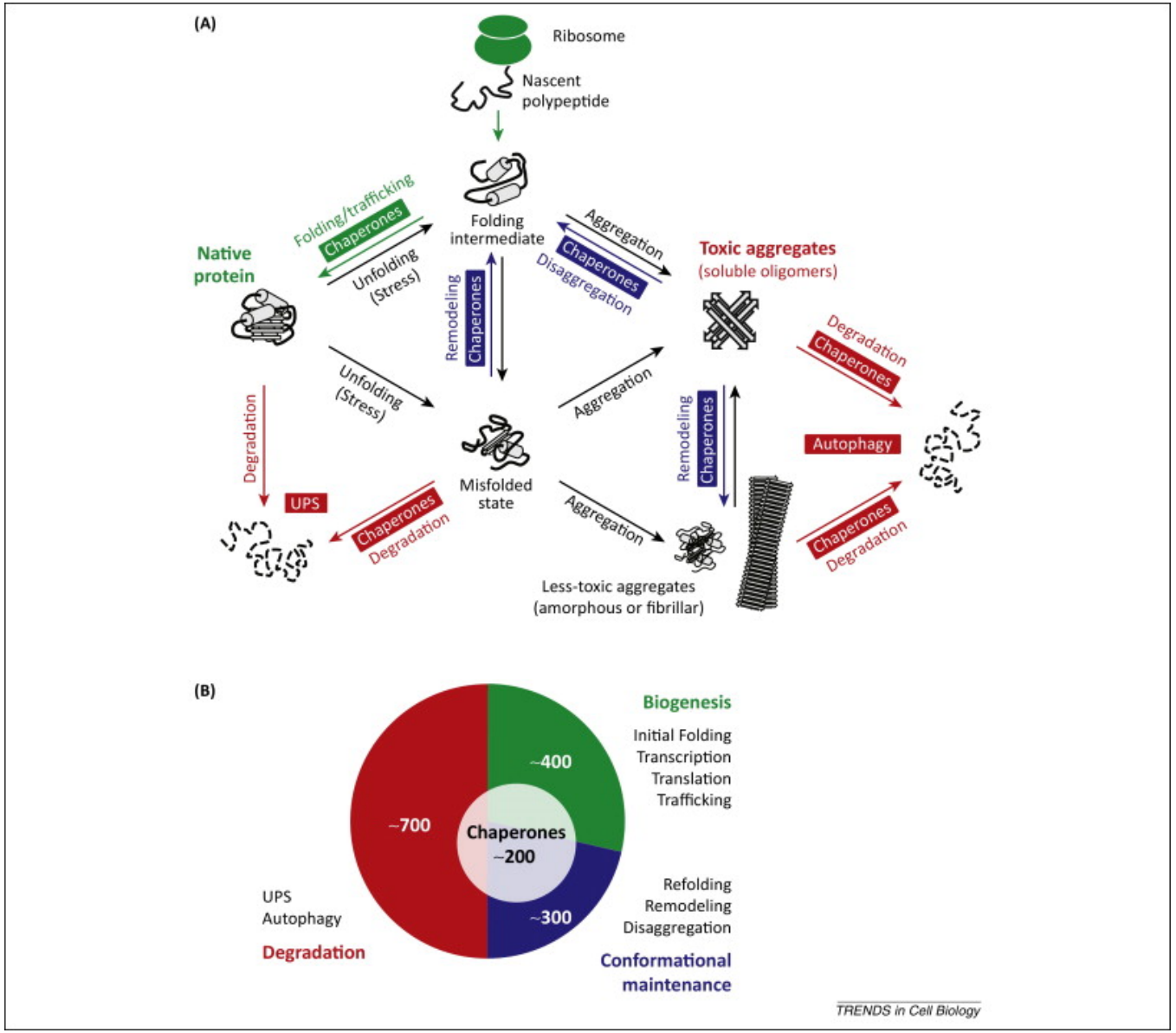
http://www.nature.com/nature/journal/v475/n7356/full/nature10317.html

Proteostasis network

Chaperones

UPS

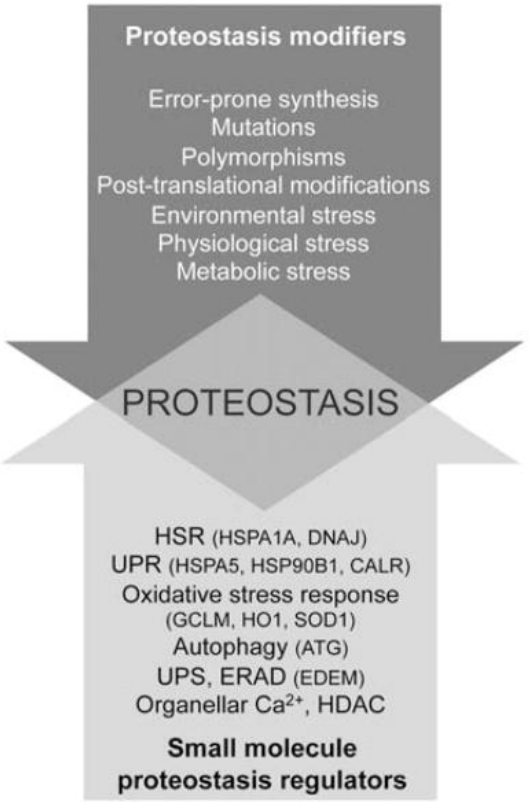
Autophagy



How does impaired proteostasis in one cell or tissue affect the organism?

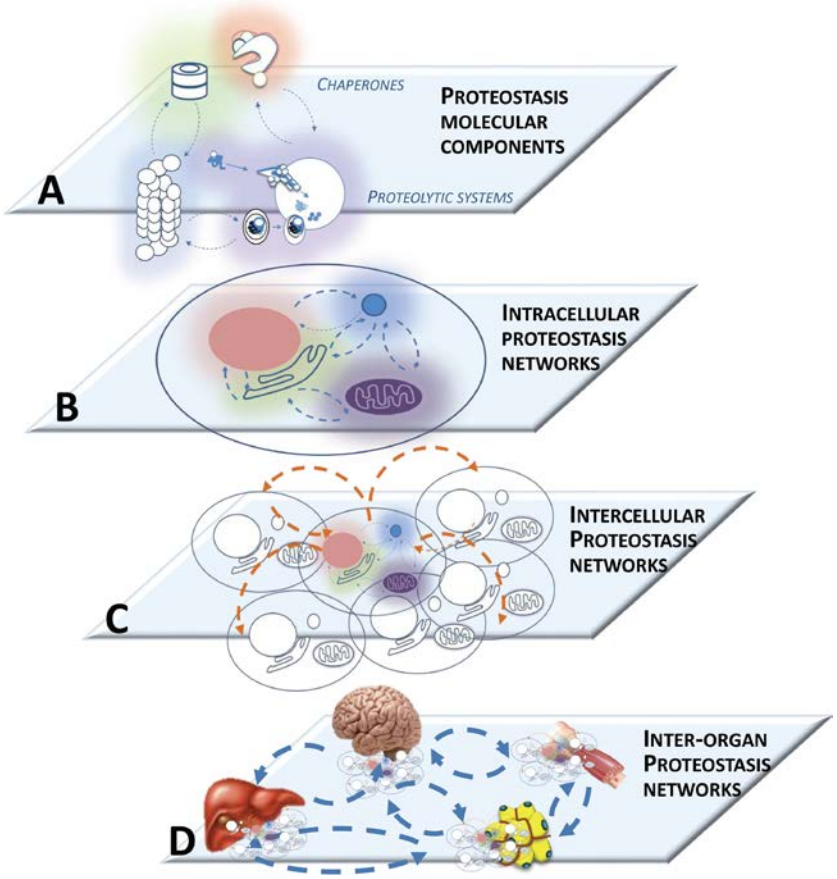
Recent studies show that unlike thought before, stress responses and proteostasis are controlled at the organismal level by inter-tissue communication.

Can impaired proteostasis be a therapeutic target?



Pathways and modifiers influencing proteostasis. Genetic, epigenetic, physiological and environmental stressors affect proteostasis and cause the accumulation of misfunctional proteins. Small molecule modulators of the activities of the proteostasis network pathways (small molecule proteostasis regulators) facilitate chaperone-mediate refolding and/or induce the degradation of misfolded and damaged proteins therefore rebalancing cellular proteostasis. In parenthesis are indicated some of the genes responsible for proteostasis maintenance. Curr Top Med Chem. 2012; 12(22): 2623–2640.

Schematic of the different levels of integration of the proteostasis networks



Intracellular cytosolic organelle

Intercellular organ & tissue

Organism

J Gerontol A Biol Sci Med Sci 2014 June;69(S1):S33–S38

Organismal proteostasis: role of cell-nonautonomous regulation and transcellular chaperone signaling

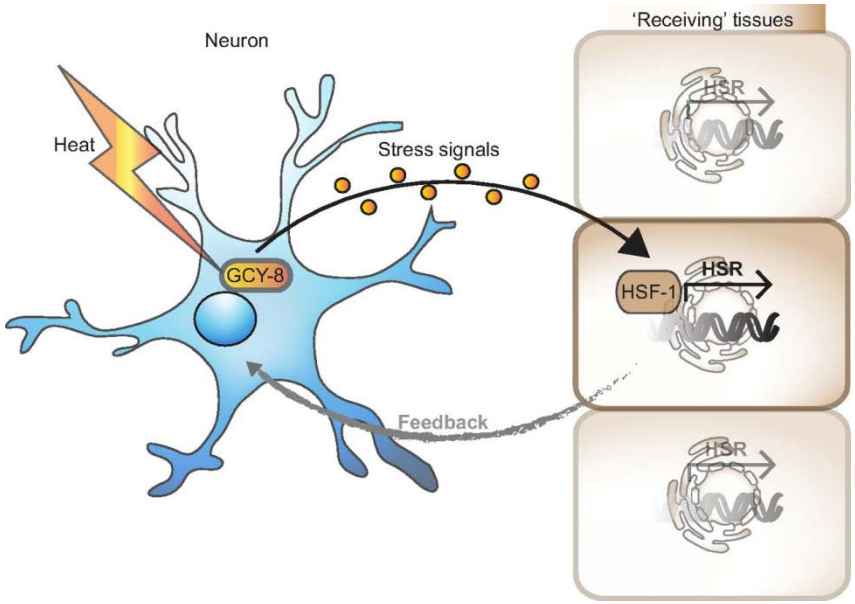
The HSR and other cell stress responses such as the unfolded protein response (UPR) can function autonomously in single-cell eukaryotes and tissue culture cells; however, within the context of a multicellular animal, the PN is regulated by cell-nonautonomous signaling through specific sensory neurons and by the process of transcellular chaperone signaling.
These newly identified forms of stress signaling control the PN between neurons and nonneuronal somatic tissues to achieve balanced tissue expression of chaperones in response to environmental stress and to ensure that metastable aggregation-prone proteins expressed within any single tissue do not generate local proteotoxic risk. Transcellular chaperone signaling leads to the compensatory expression of chaperones in other somatic tissues of the animal, perhaps preventing the spread of proteotoxic damage. Genes Dev. 2014 Jul 15; 28(14): 1533–1543.

Table 1. *Cell-nonautonomous signaling responses*

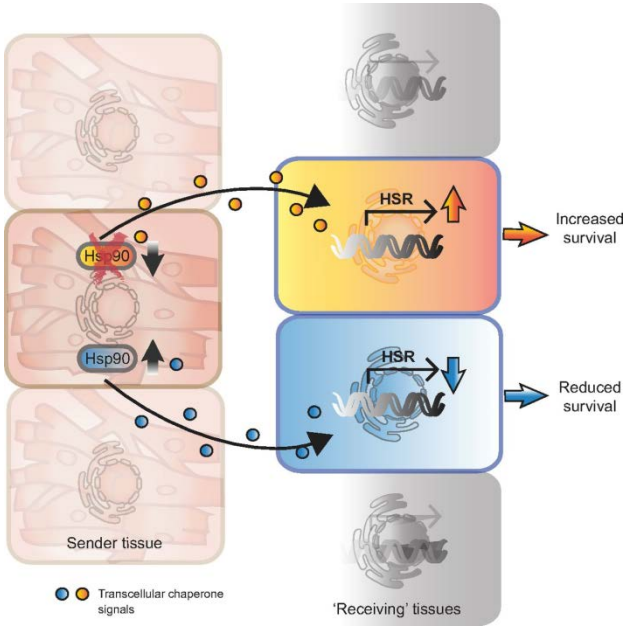
Signaling category	Cell-nonautonomous response	Sender tissue	Signal	References
Neuron to nonneuronal soma	HSR	AFD neuron	AFD-dependent neuropeptide?	Prahlad et al. 2008
	UPR	ASH/ASI neurons	Unknown	Sun et al. 2011
	Noncanonical UPR (innate immunity)	ASH/ASI neurons	Unknown	Sun et al. 2012
	UPR^{mito}	Neurons	Neuronal "mitokine"	Durieux et al. 2011
	UPR^{ER}	Neurons	Neurotransmitter?	Taylor and Dillin 2013
Direct signaling between nonneuronal tissues	Gonadal longevity pathway	Steroidogenic tissues	Dafachronic acid	Antebi et al. 1998; Motola et al. 2006; Yamawaki et al. 2010
	HSR and systemic *hsp* epxression	Intestine, muscle, neurons	Transcellular stress factor (TSF)	van Oosten-Hawle et al. 2013
	FOXO-to-FOXO^{-} signaling	Intestine, muscle, hypodermis	*mdt-15*-dependent lipid signals	Zhang et al. 2013
	Innate immune responses	Gonad, intestine	Secreted immune peptides	Ermolaeva et al. 2013
	Longevity	Gonad to soma	*lipl-4*-dependent fatty acids	Lapierre et al. 2011
	Longevity	Gonad to soma	Oleic acid	Goudeau et al. (2011)
	Longevity	Gonad to soma	*let-7* microRNA family	Shen et al. 2012
	FOXO-to-FOXO	Intestine	IGF-like signals	Murphy et al. 2003
Indirect signaling between nonneuronal tissues (neuronal feedback)	Thermotaxis	Muscle, intestine	Estrogen	Sugi et al. 2011
	Longevity and survival	Gonad, neurons, intestine	*mir-71*	Boulias and Horvitz 2012
	FOXO/4EBP signaling (*D. melanogaster*)	Muscle	IGF?	Demontis and Perrimon 2010
	Mitophagy/ILS (*D. melanogaster*)	Muscle	Secreted IGFBP (impL2)	Owusu-Ansah et al. 2013

Categorization for cell-nonautomonous signaling responses in *C. elegans* (unless otherwise noted).

Cell non-autonomous control of the heat shock response (HSR) by neurons. Organismal control of heat shock transcription factor 1 (HSF-1) transcriptional activity in peripheral tissues by the thermosensory AFD neuron via a guanylyl cyclase GCY-8-dependent signaling cascade. A steroid signalling-dependent feedback loop from peripheral cells reports HSR activity back to the AFD neurons.

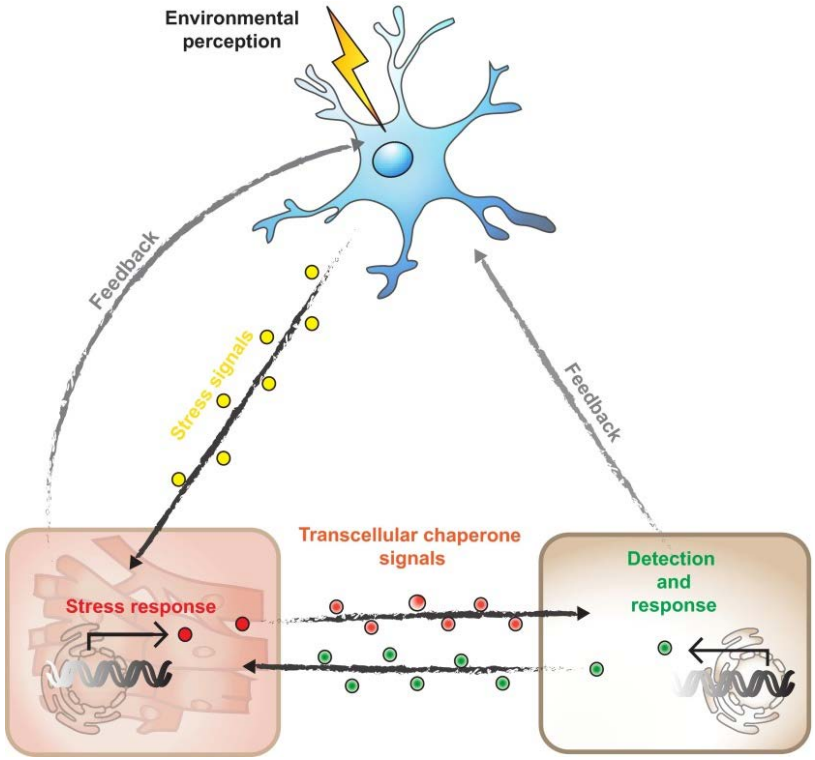


Journal of Experimental Biology 2014 217: 129-136



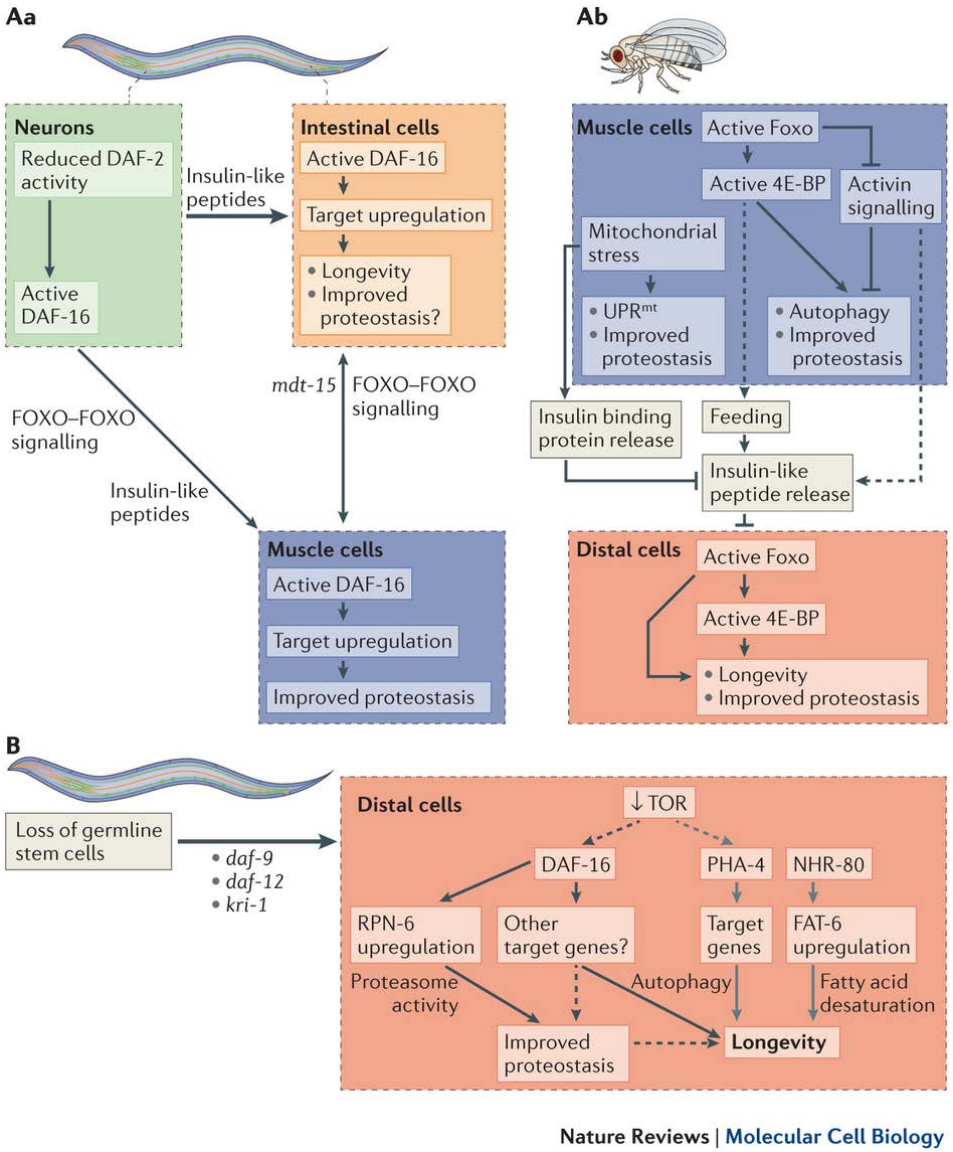
Transcellular chaperone signaling regulates the organismal HSR via tissue-to-tissue crosstalk. An imbalance of proteostasis in a single tissue, through reduced (orange) or elevated (blue) expression of Hsp90, is detected and responded to in a different tissue via transcellular chaperone signaling. Because of the key role of Hsp90 as a negative regulator of the HSF-1-dependent HSR, the HSR is either induced (orange) or repressed (blue) at a cell non-autonomous level, leading to different outcomes for organismal survival during stress conditions.

Cell non-autonomous control of proteostasis via the nervous system and transcellular chaperone signaling.



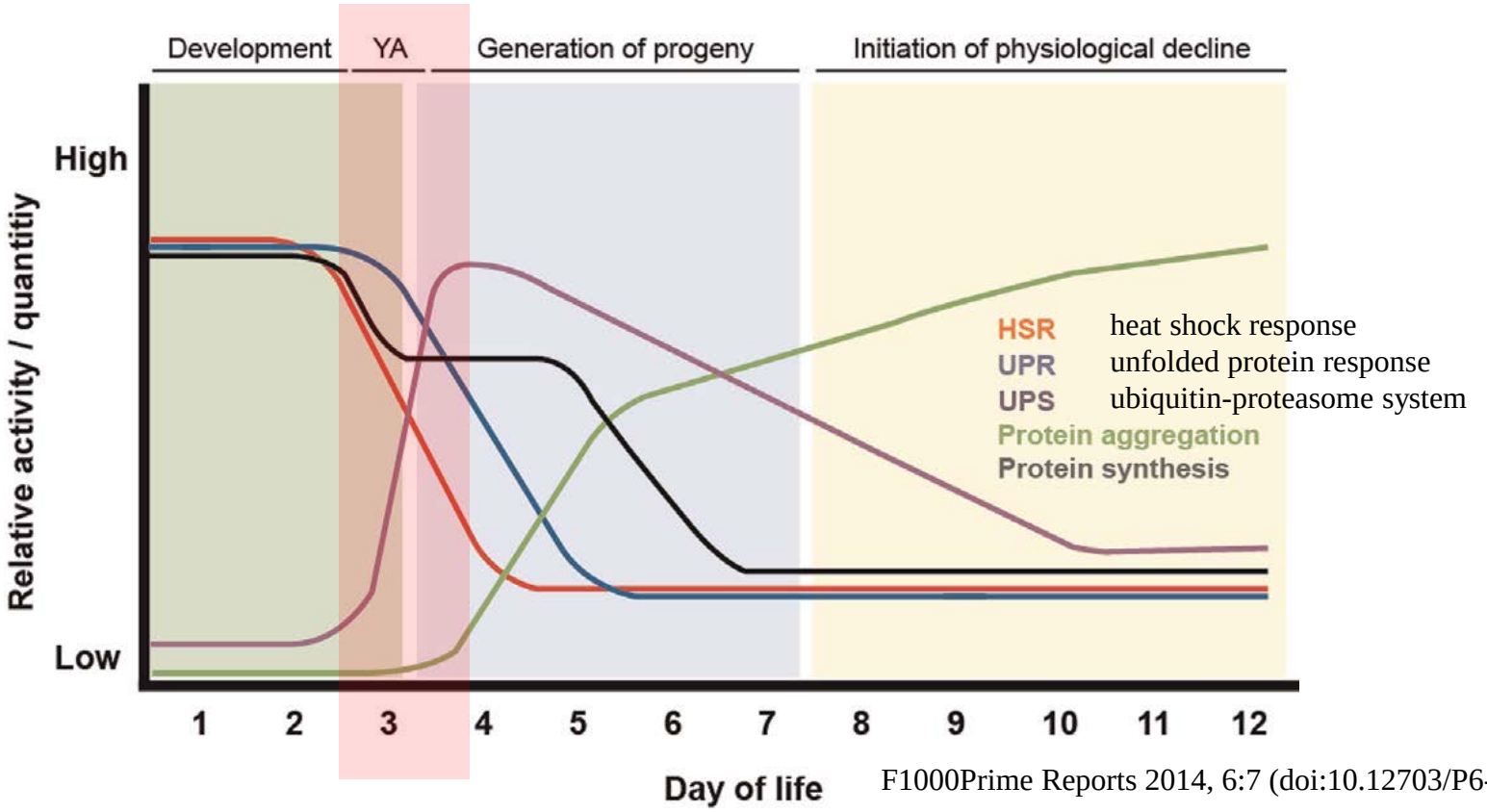
Sensory neurons perceive environmental stimuli and integrate the environmental challenge to fine-tune proteostasis in peripheral tissues. Non-neuronal tissues report altered proteostasis conditions back to the nervous system. At the same time, tissue-to-tissue signals via transcellular chaperone signaling can override the neuronal component to control cell-specific proteostasis. This allows proteostasis crosstalk between somatic cells independent of neural control.

Cell non-autonomous regulation of proteostasis by insulin-like signalling and gonadal signalling



Nature Reviews | Molecular Cell Biology

Temporal relationship between *Caenorhabditis elegans* reproduction, aging, and changes in proteostasis

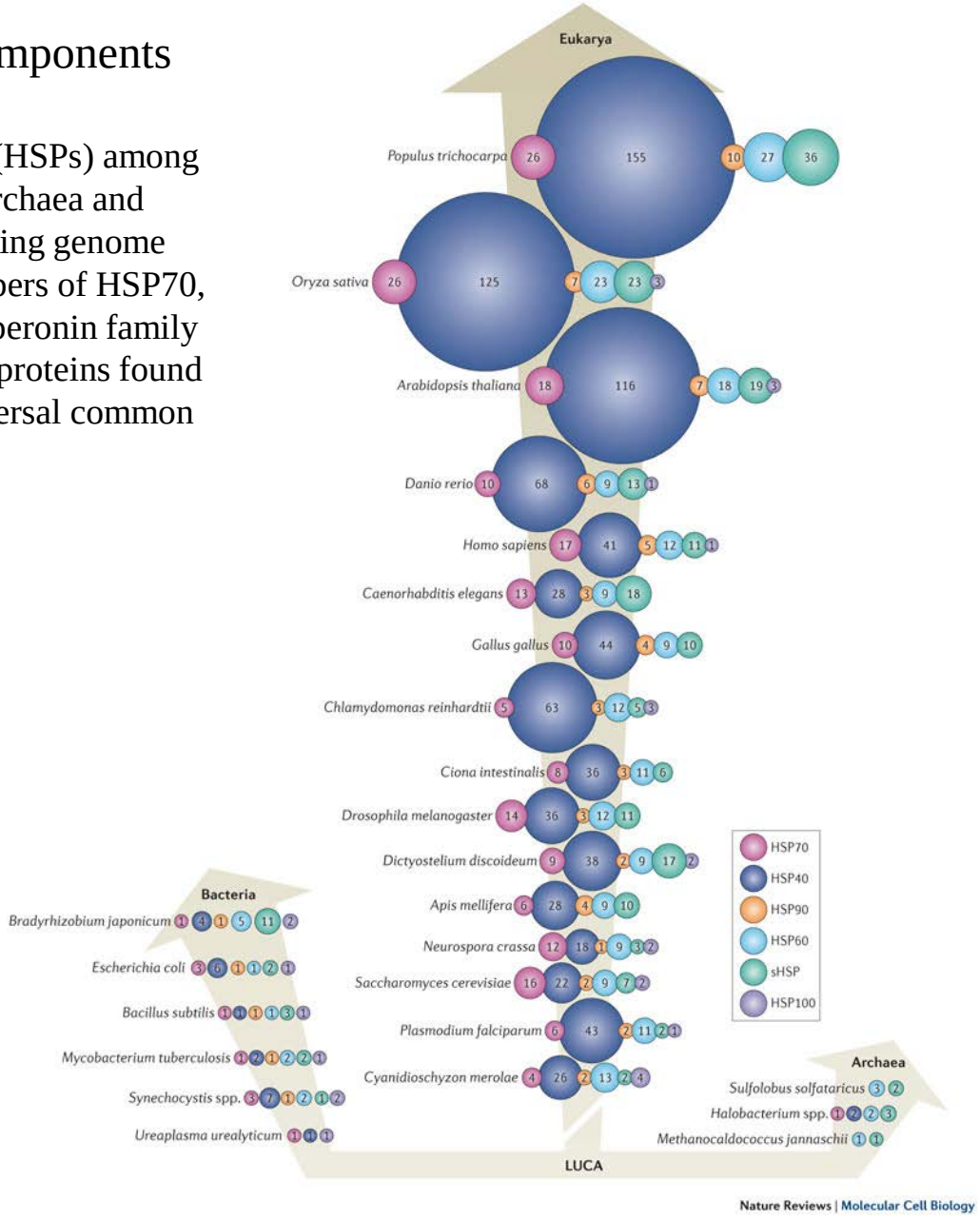


F1000Prime Reports 2014, 6:7 (doi:10.12703/P6-7)

What is the molecular mechanism(s) responsible for PN remodeling and the upstream signals that promote these changes?

Diversity in proteostasis network components

Shown is the evolution of heat shock proteins (HSPs) among the extant three domains of life — Bacteria, Archaea and Eukarya. Vertical arrows correspond to increasing genome size and number of expressed genes. The numbers of HSP70, HSP40 and HSP90 chaperones, of HSP60 chaperonin family subunits, small HSPs (sHSPs) and of HSP100 proteins found in each species are indicated. LUCA, last universal common ancestor.



Pharmacological chaperones: a class of molecules that bind and inhibit the unstable or misfolded proteins.

Protein-misfolding diseases

include conditions where a protein:

- fails to fold correctly (cystic fibrosis, Marfan syndrome, amyotonic lateral sclerosis)
- is not stable enough to perform its normal function (many forms of cancer)
- fails to be correctly trafficked (familial hypercholesterolemia, α1-antitrypsin deficiency)
- forms insoluble aggregates that deposit toxically (neurodegenerative diseases: Alzheimer's, type II diabetes, Parkinson's and many more)

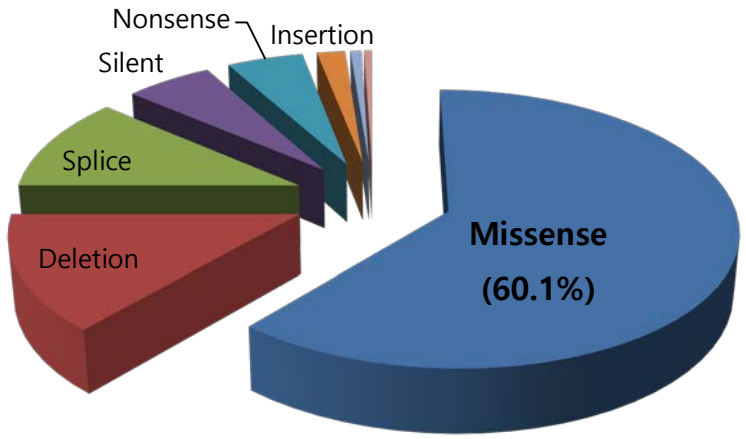
Phenylketonuria (PKU): classified as protein-misfolding disease (PMD)

- An autosomal recessive disorder due to mutations in PAH gene

- >850 genotypes (BioPKU, http://www.biopku.org)

- >2/3 of PAH mutations: missense mutations

- Mainly causing folding defects leading to accelerated proteolytic degradation



PKU phenotype

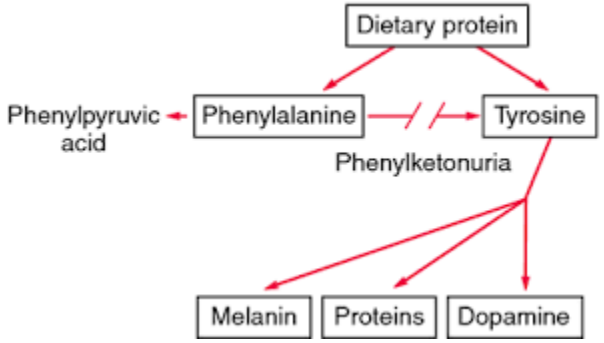
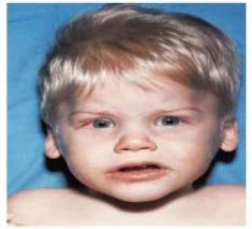


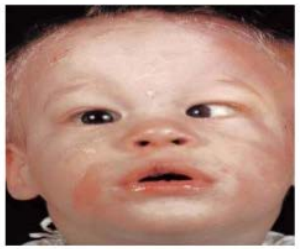
Table 1 - Biochemical classification of hyperphenylalaninemia

Biochemical phenotype	Serum Phe (mg/dL)	Estimated enzyme activity	Treatment
Classical PKU	>20	<1	Yes
Mild PKU	10-20	1-3	Yes
Non-PKU HPA	3,5-10	>3	No

Source: Adapted from Koch & Wenz. Phenylketonuria[11] Phe: phenylalanine; HPA: hyperphenylalaninemia; PKU: phenylketonuria.

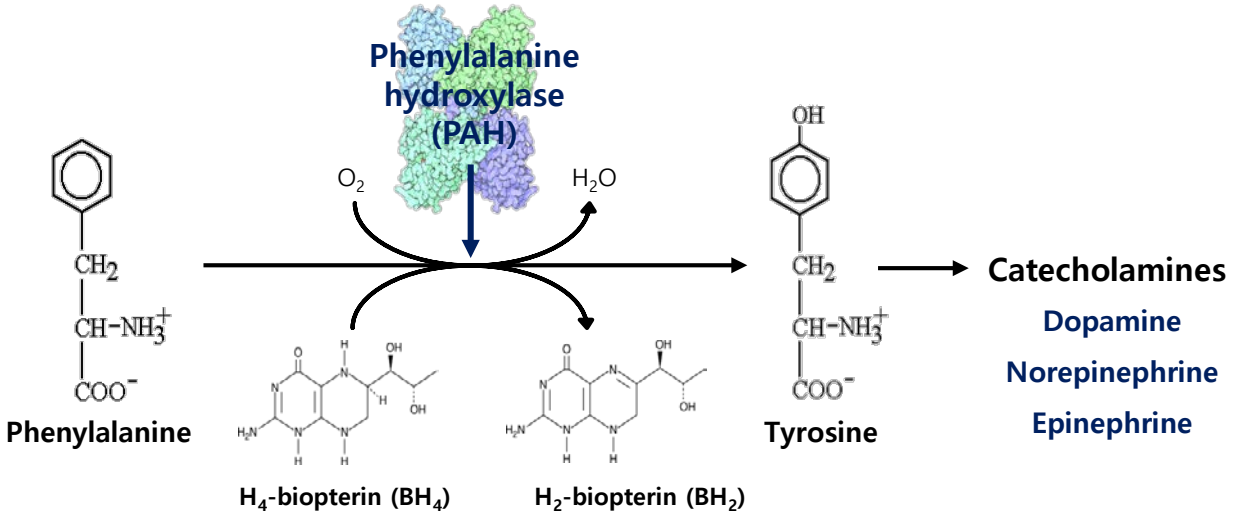
- **Clinical Manifestations of Untreated PKU**
- Eczema.
- Hypopigmentation.
- Seizures.
- Limb spasticity.
- Mousy odor.
- Severe mental retardation.





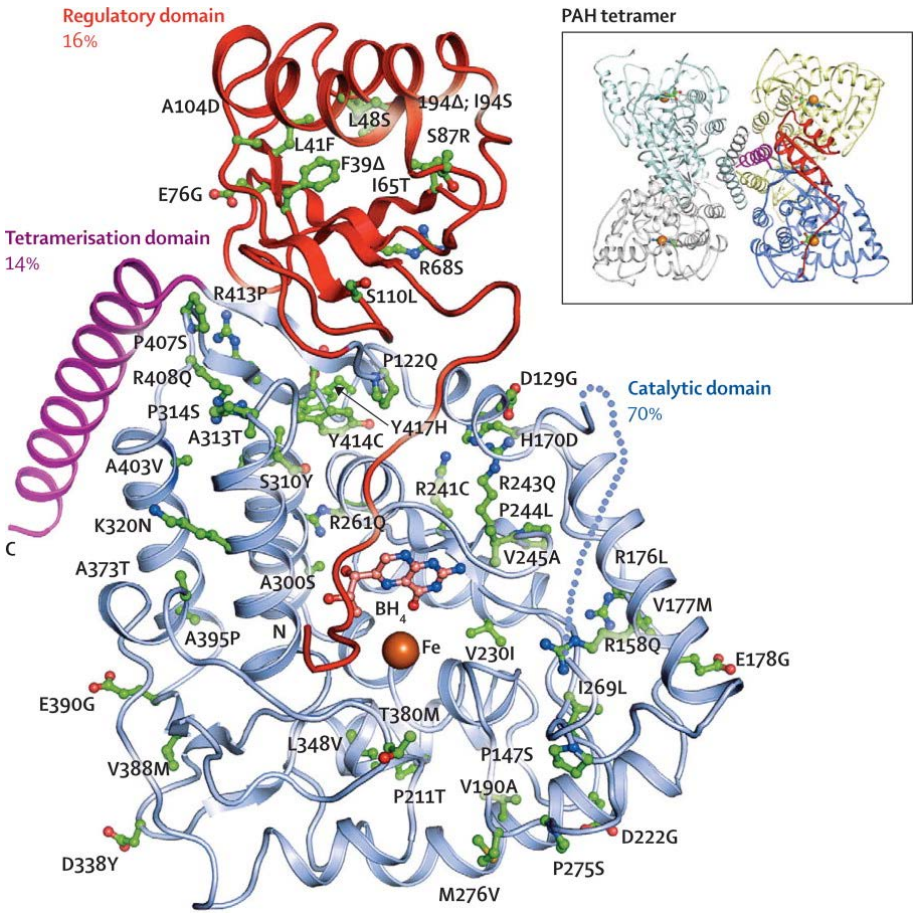
PKU treatment

Diet
Phenylalanine ammonia lyase
Neutral amino acid supplement
Tetrahydrobiopterin (BH4): pharmacological chaperone effect

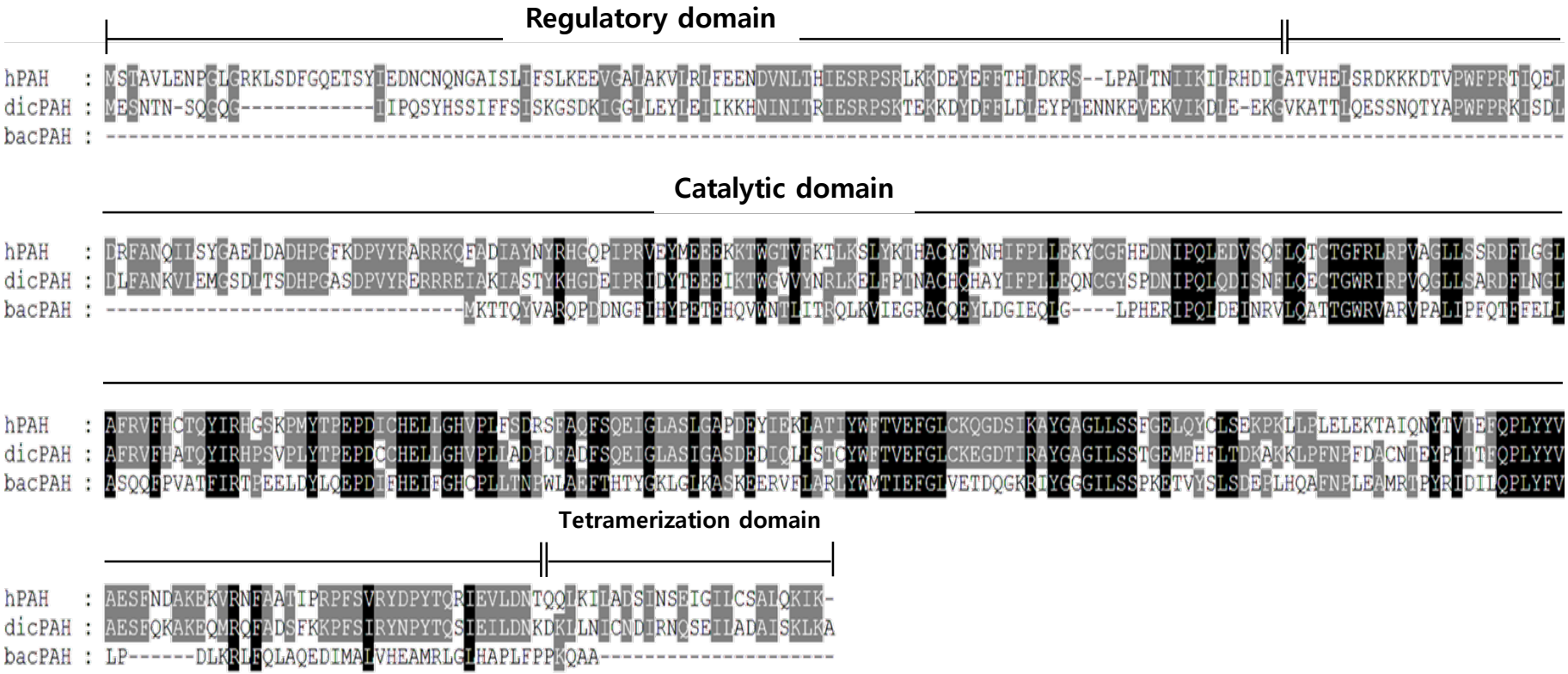


Human PAH mutation study

Genotype-phenotype correlation
In vitro expression and analysis
Computational analysis

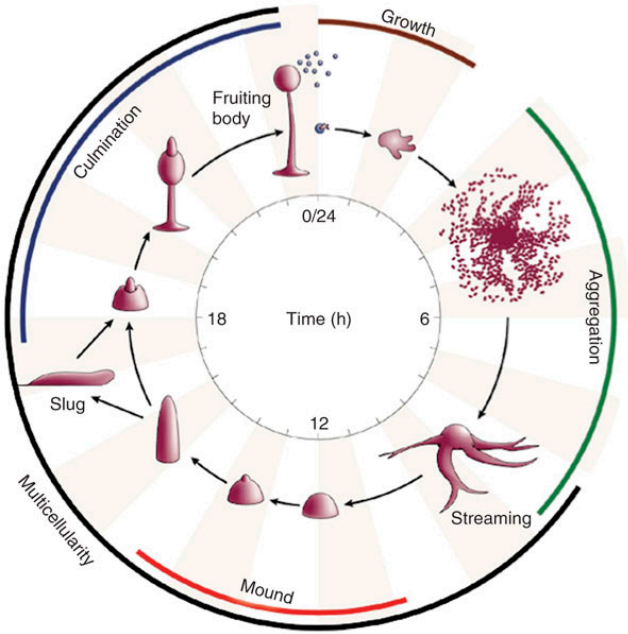


Dictyostelium PAH (dicPAH)



vs. *Dictyostelium*	Regulatory domain	Catalytic domain	Tetramerization domain	Total identity
Human	38%	71%	46%	62.6%

Dictyostelium discoideum Ax2



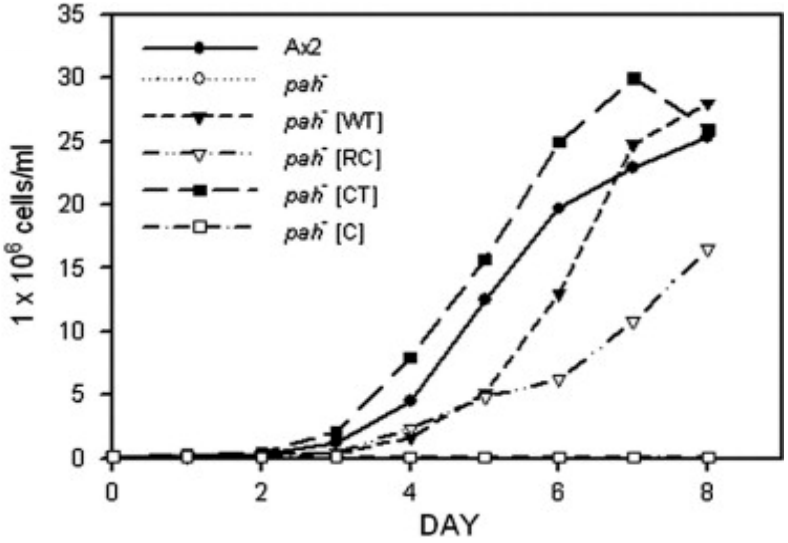
- Short generation time
- Easy genetic manipulation
- PAH homologous to human protein
- Analogy to human BH4 synthesis

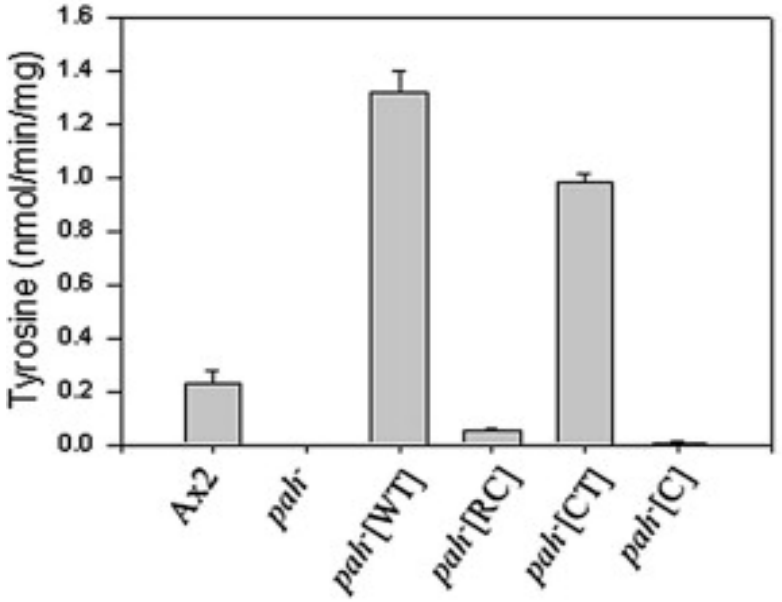
PAH study in *Dictyostelium*

Creation of PAH knockout mutant

The *pah*$^{-}$ strain does not proliferate in FM medium

Rescued by overexpression of dicPAH





Human PAH study in *Dictyostelium*

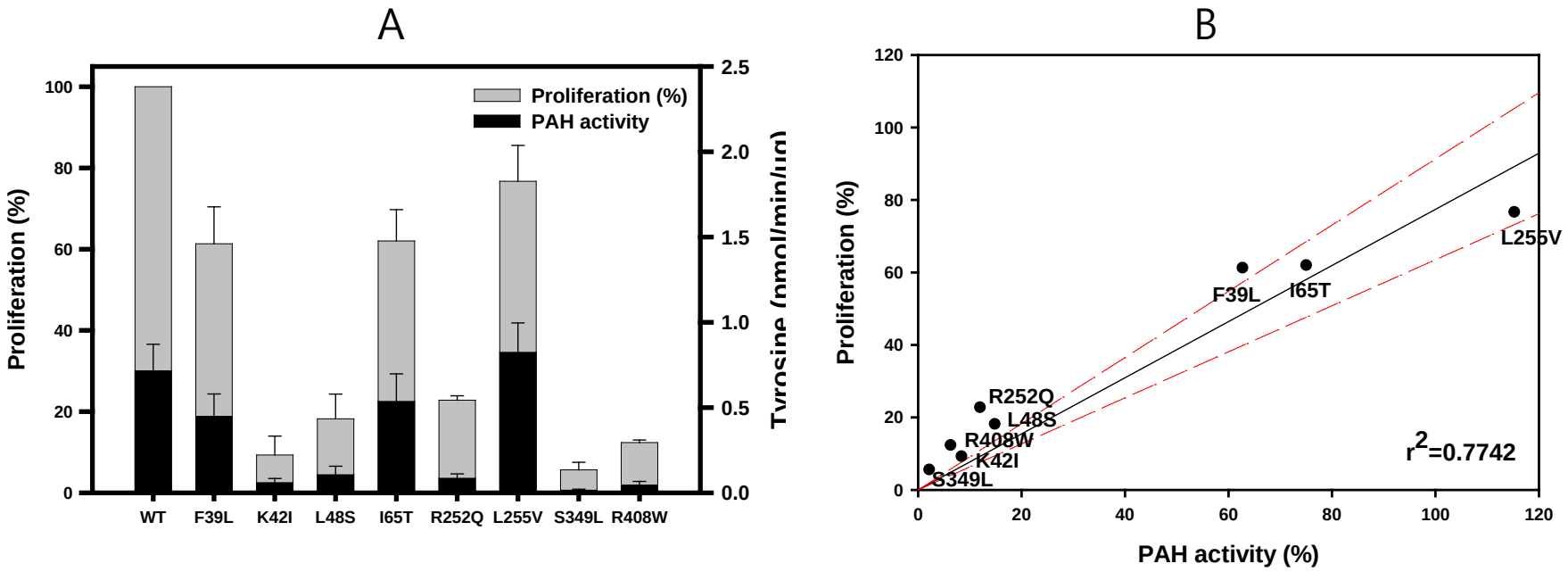
Complementary expression of human PAH in *pah*$^{-}$ strain

8 PKU mutations: F39L, K42I, L48S, I65T, R252Q, L255V, S349L, and R408W

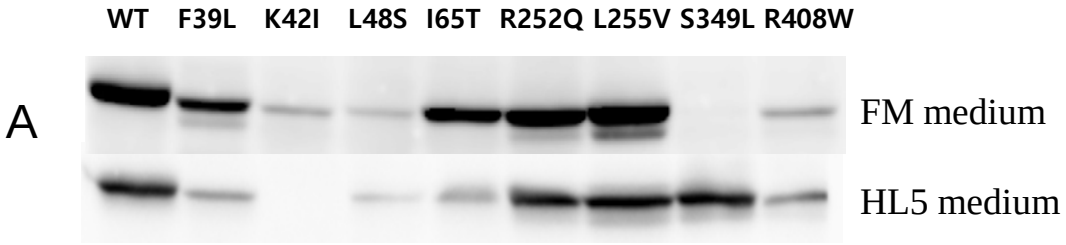
Qualitative analysis (PAH activity)
Quantitative analysis (PAH protein using Ab)

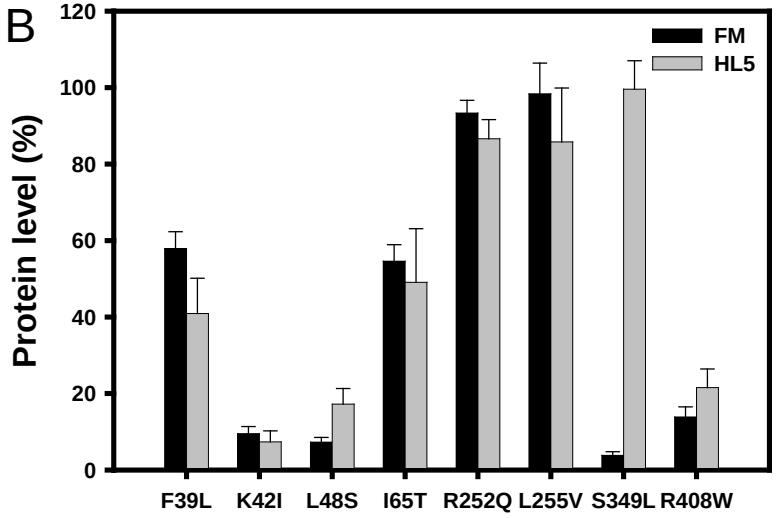
To determine hPAH activity-dependent growth in FM medium
Simple quantitative evaluation of hPAH mutations
Cell-based assay system for pharmacological chaperone screening

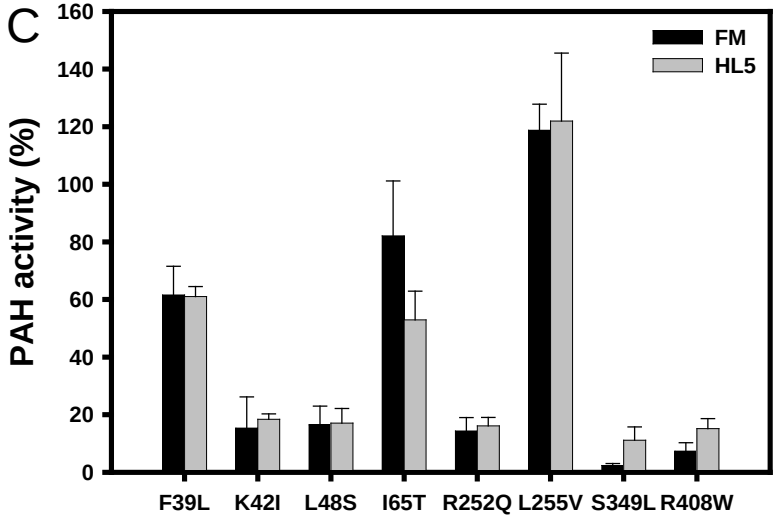
Comparative analysis of growth rate and PAH activity of *pah*$^{-}$ strains transformed with wild type and mutant hPAH cDNAs



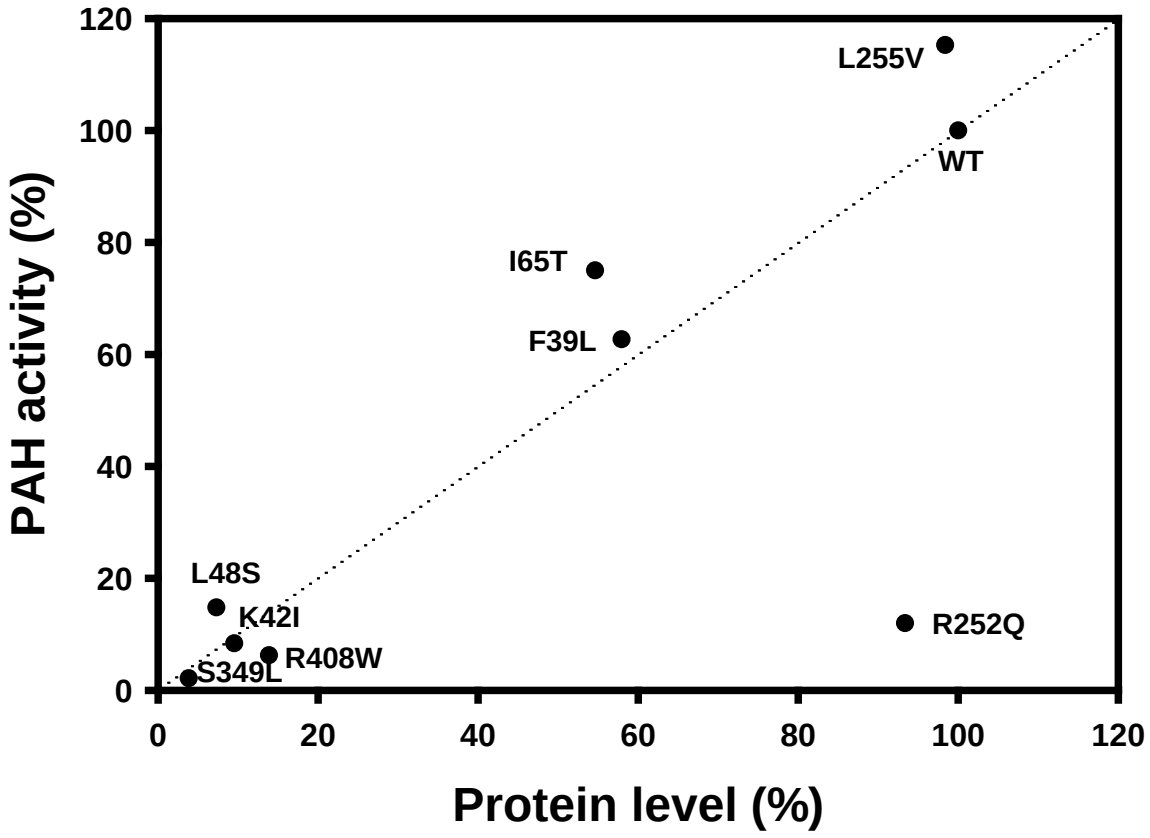
Residual protein levels of hPAHs expressed in *pah*$^{-}$ cells.







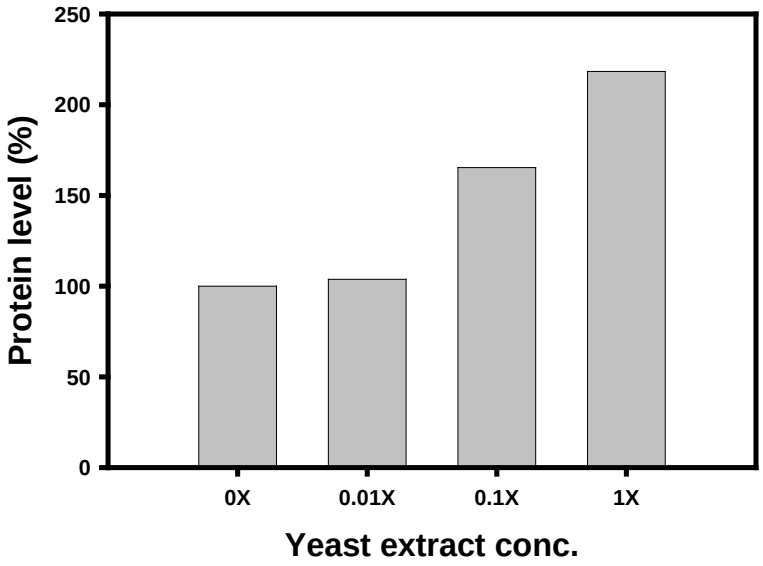
Relationship between PAH activity and protein level of hPAHs expressed in *pah*$^{-}$ strain.



Relative specific activity of mutant hPAHs

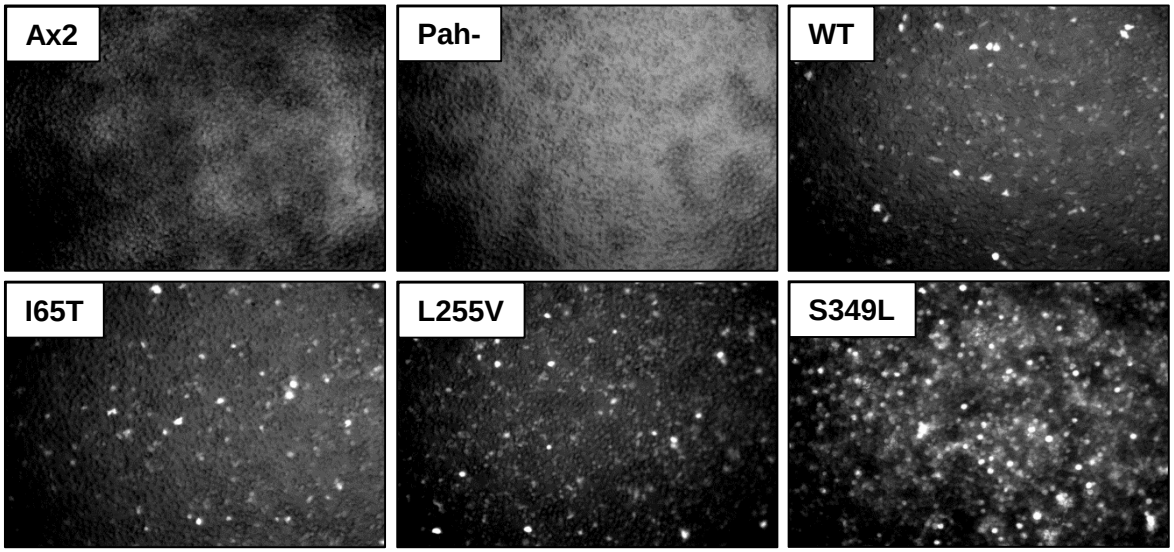
Protein	PAH activity	Protein amount	Specific activity
F39L	0.615±0.100	0.579±0.044	1.062
K42I	0.153±0.108	0.095±0.019	1.611
L48S	0.165±0.065	0.073±0.012	2.260
I65T	0.821±0.191	0.546±0.043	1.504
R252Q	0.143±0.046	0.933±0.033	0.153
L255V	1.187±0.090	0.984±0.080	1.206
S349L	0.023±0.007	0.038±0.010	0.605
R408W	0.073±0.029	0.139±0.026	0.525

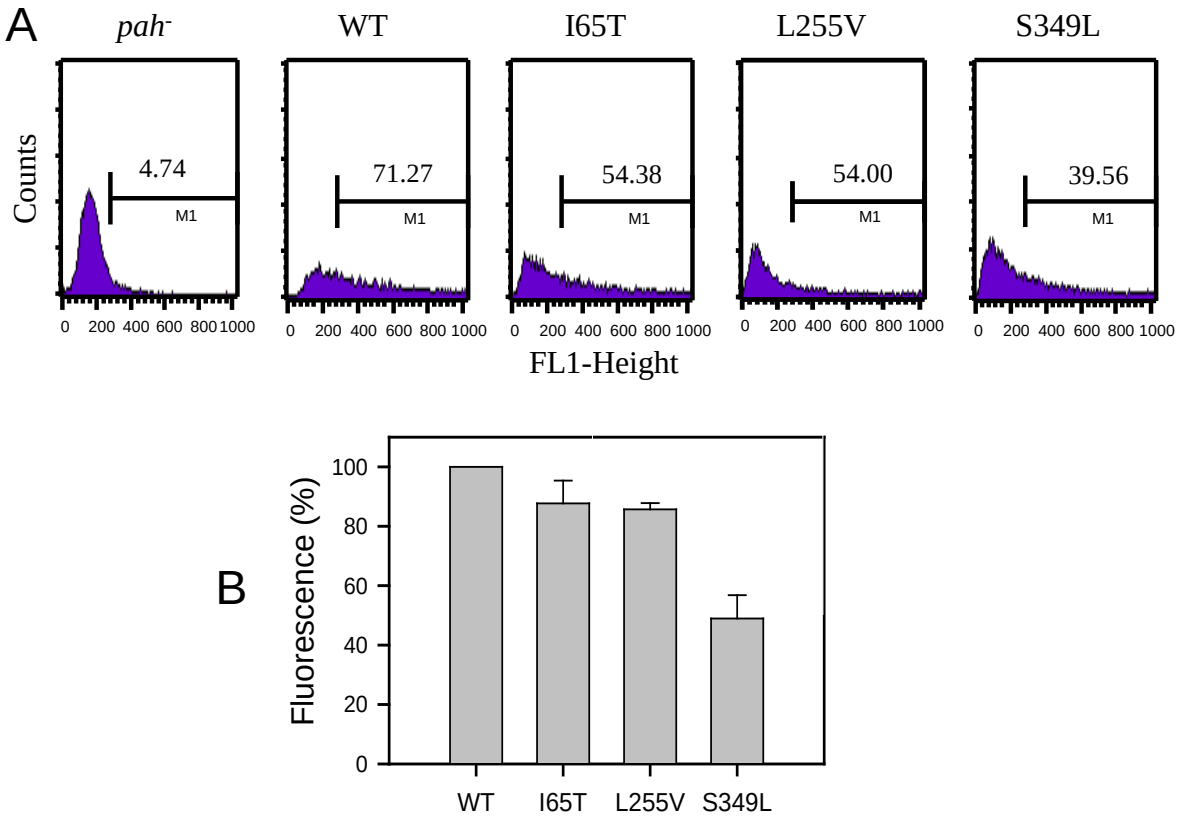
Chaperone effect of yeast extract on S349L hPAH

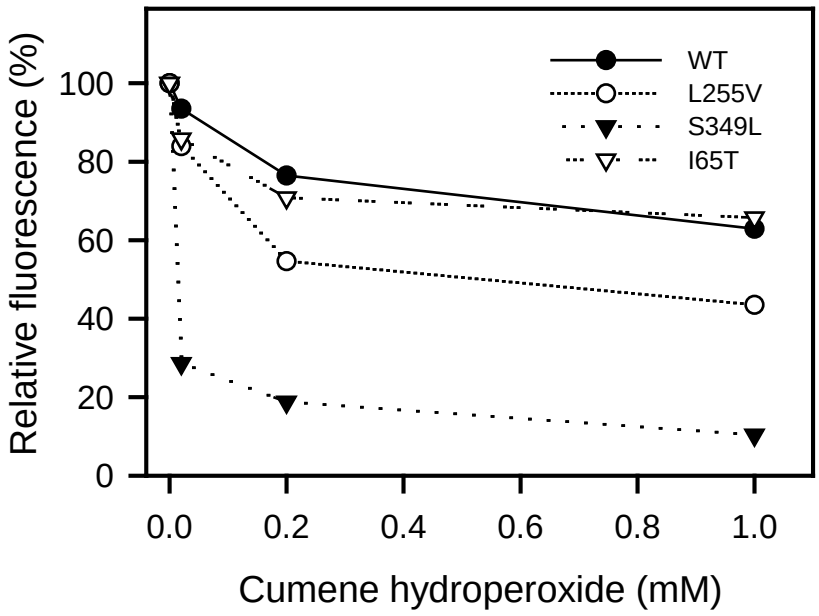


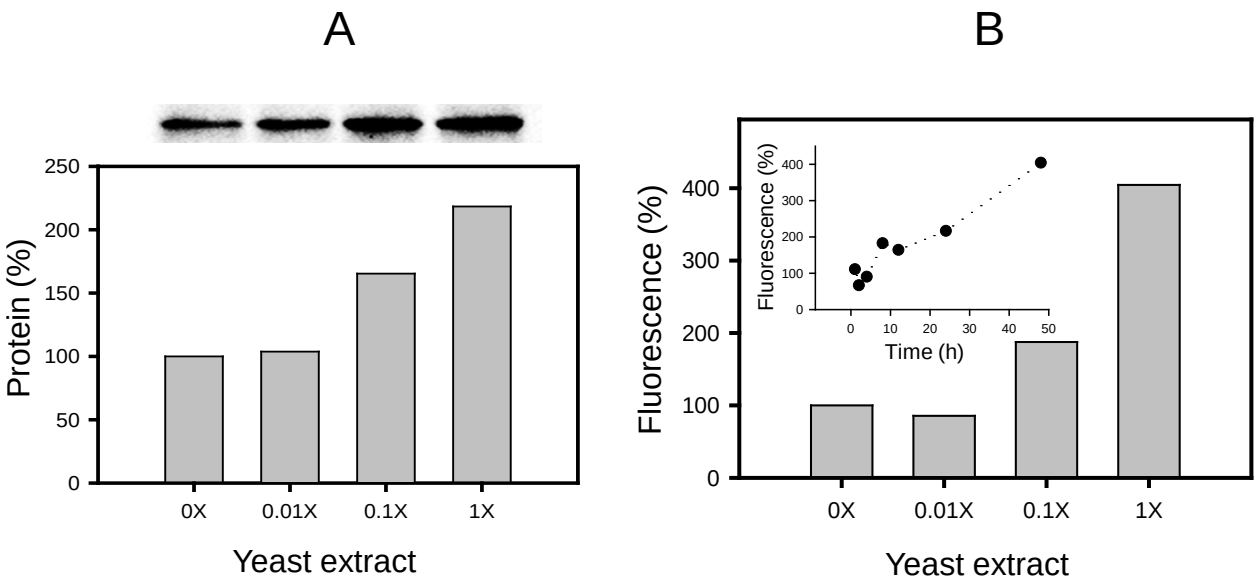
(1X = 5 g per liter)

Quantitative analysis by flow cytometry of green fluorescent protein-tagged human phenylalanine hydroxylase expressed in *Dictyostelium*




A
pah-
WT
I65T
L255V
S349L
Counts
4.74
71.27
54.38
54.00
39.56
M1
0 200 400 600 800 1000
FL1-Height
B
Fluorescence (%)
100
80
60
40
20
0
WT
I65T
L255V
S349L


Relative fluorescence (%)
100
80
60
40
20
0
0.0
0.2
0.4
0.6
0.8
1.0
Cumene hydroperoxide (mM)
WT
L255V
S349L
I65T


A
B
Protein (%)
250
200
150
100
50
0
0X
0.01X
0.1X
1X
Yeast extract
Fluorescence (%)
400
300
200
100
0
Fluorescence (%)
Time (h)
0
10
20
30
40
50
0X
0.01X
0.1X
1X
Yeast extract