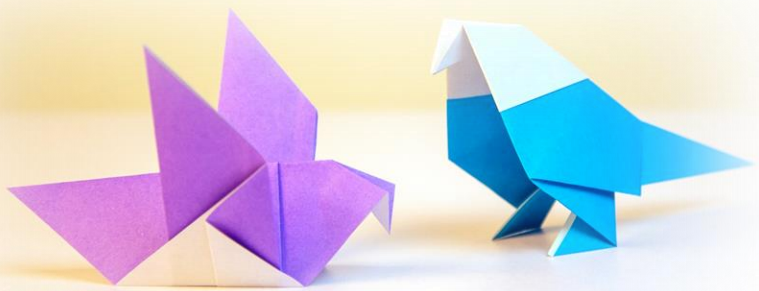
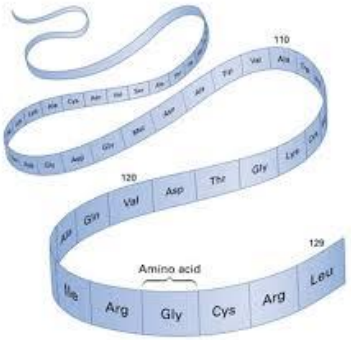
MUNI
MED

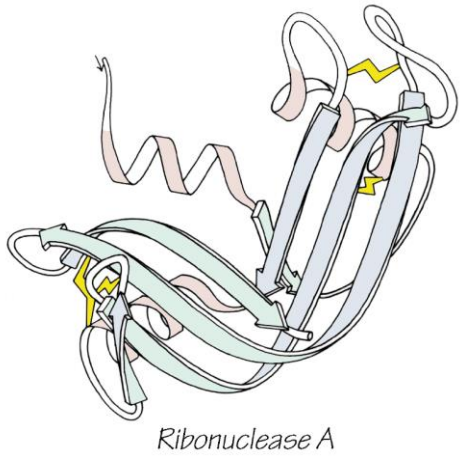


Stress response and protein folding

Petr Müller

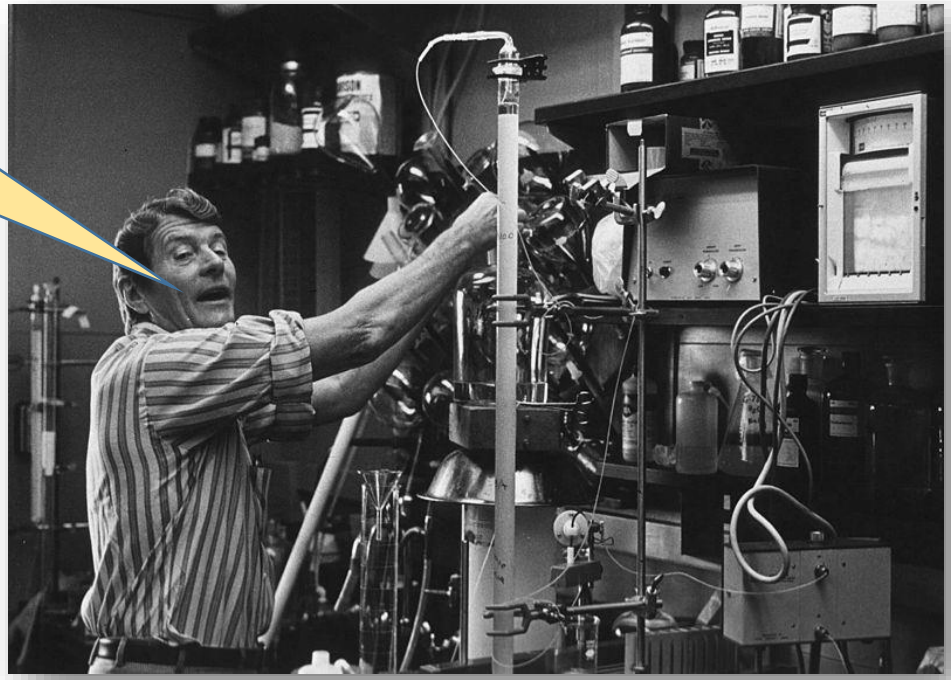
At the environmental conditions (temperature, solvent concentration and composition, etc.) at which folding occurs, the native structure is a unique, stable and kinetically accessible minimum of the free energy





Ribonuclease A

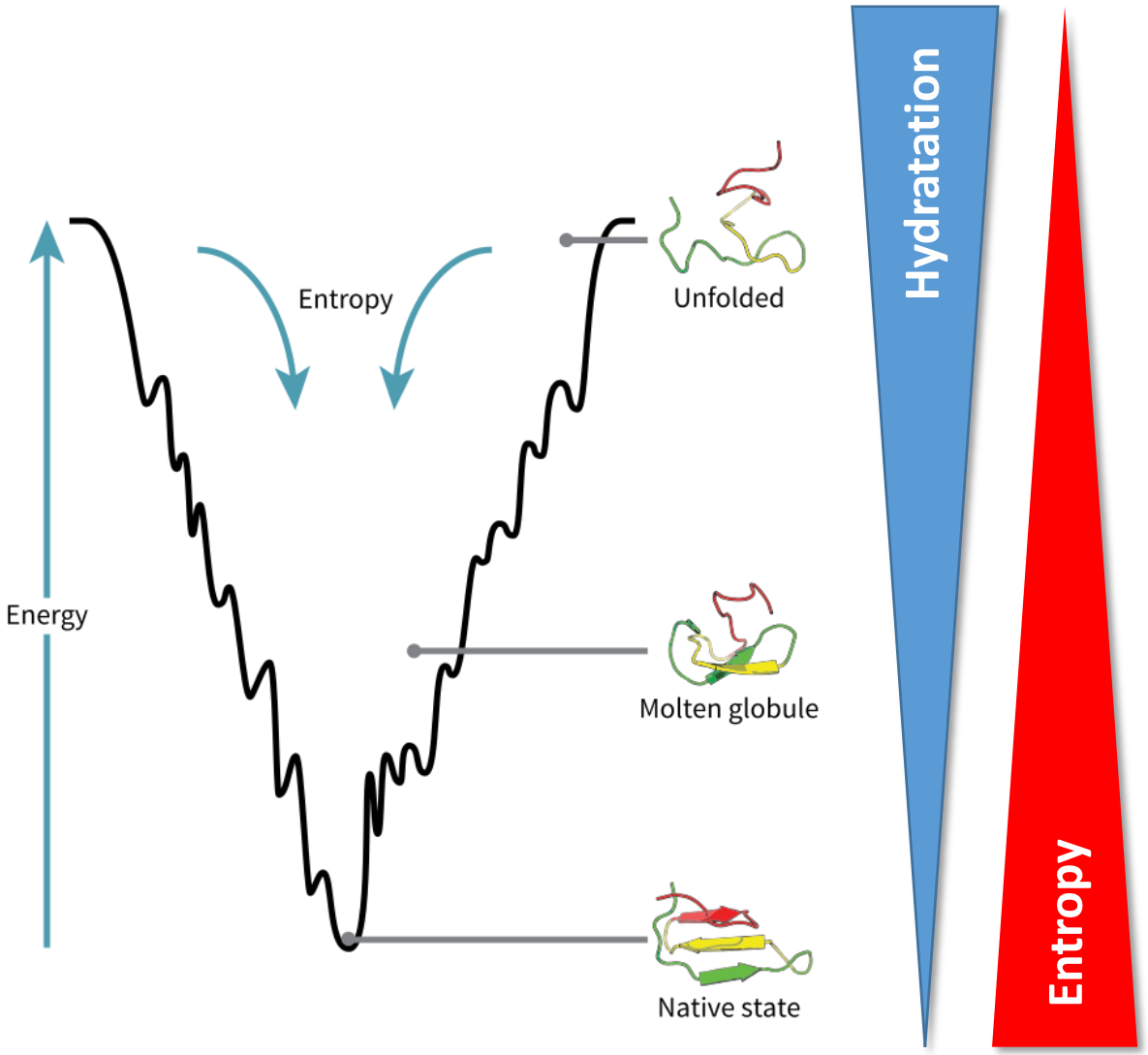
The native structure is determined only by the protein's amino acid sequence

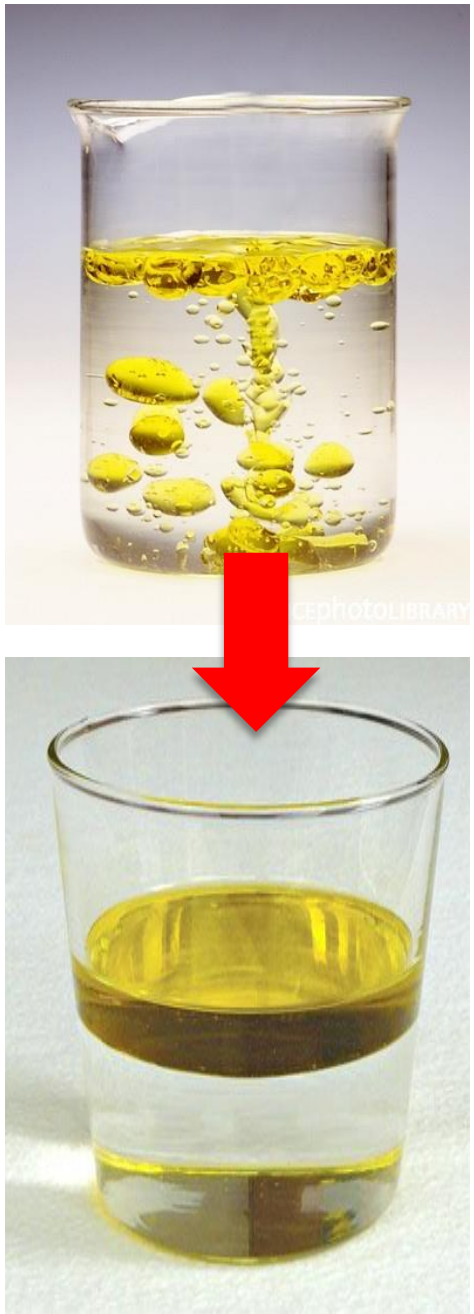


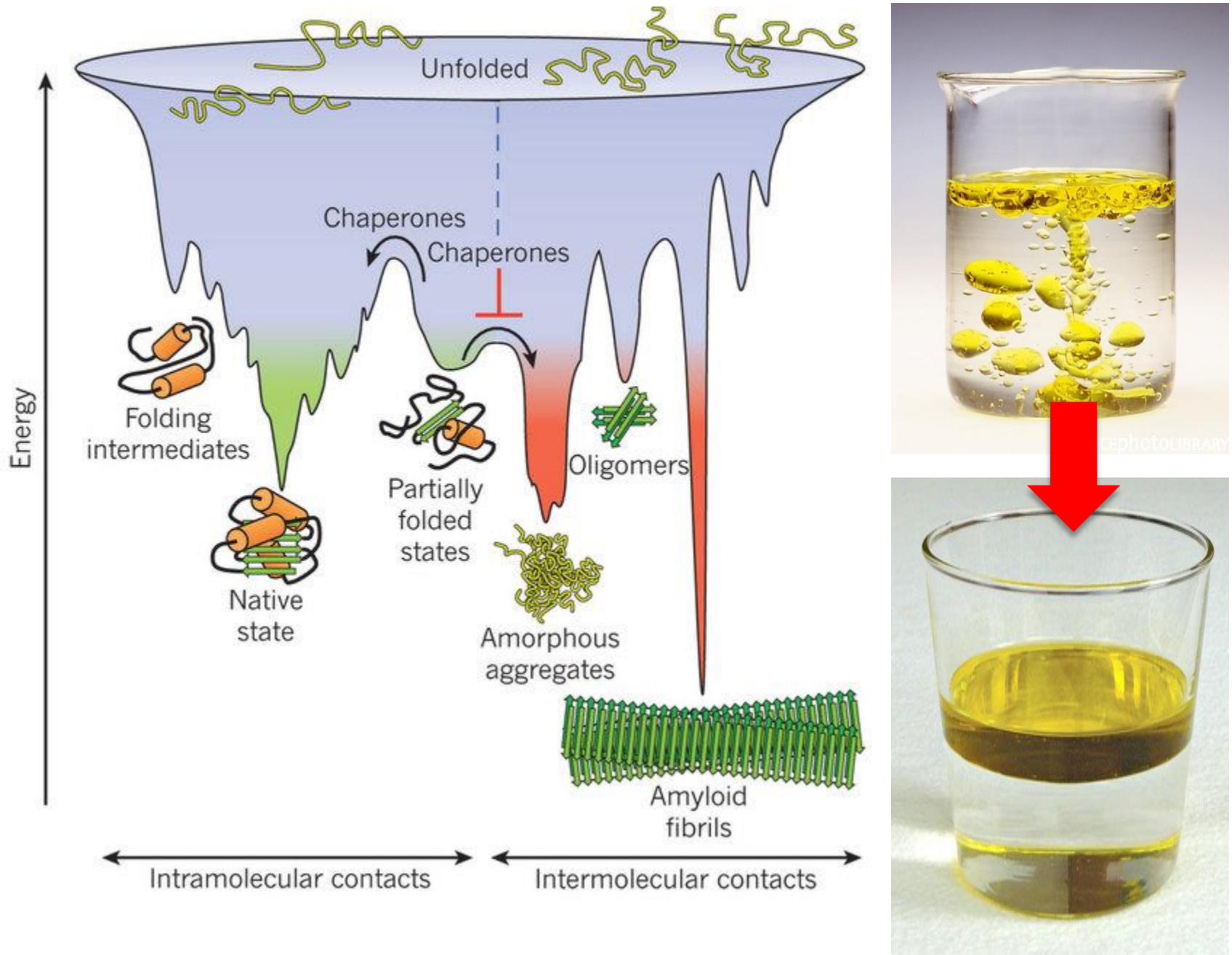
Christian Boehmer Anfinsen, Jr.
(March 26, 1916 – May 14, 1995)

Nobel Prize in Chemistry (1972)

Folding is entropy driven process

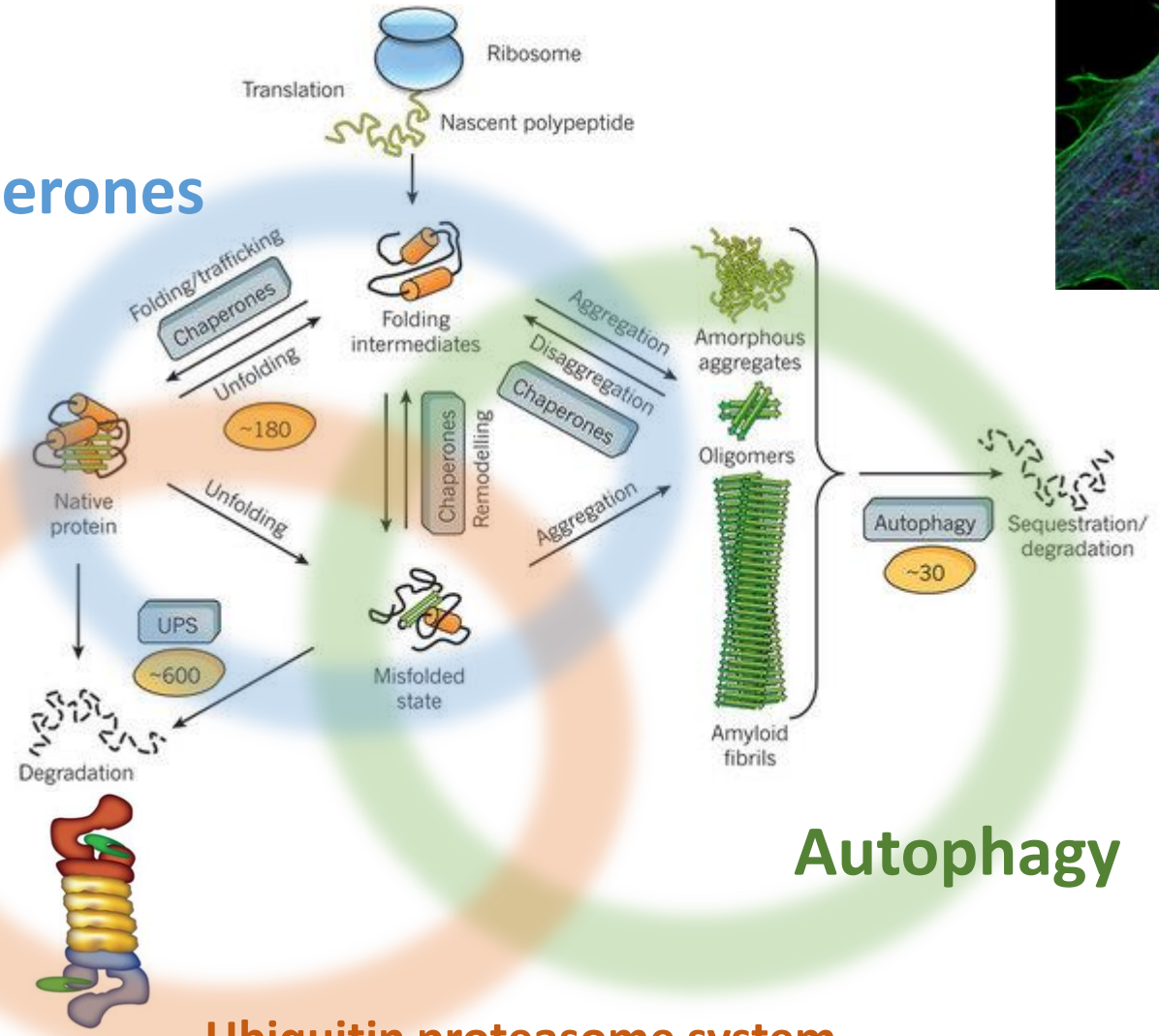





Unfolded
Chaperones
Chaperones
Folding intermediates
Native state
Partially folded states
Oligomers
Amorphous aggregates
Amyloid fibrils
Energy
Intramolecular contacts
Intermolecular contacts

Protein homeostasis / proteostasis

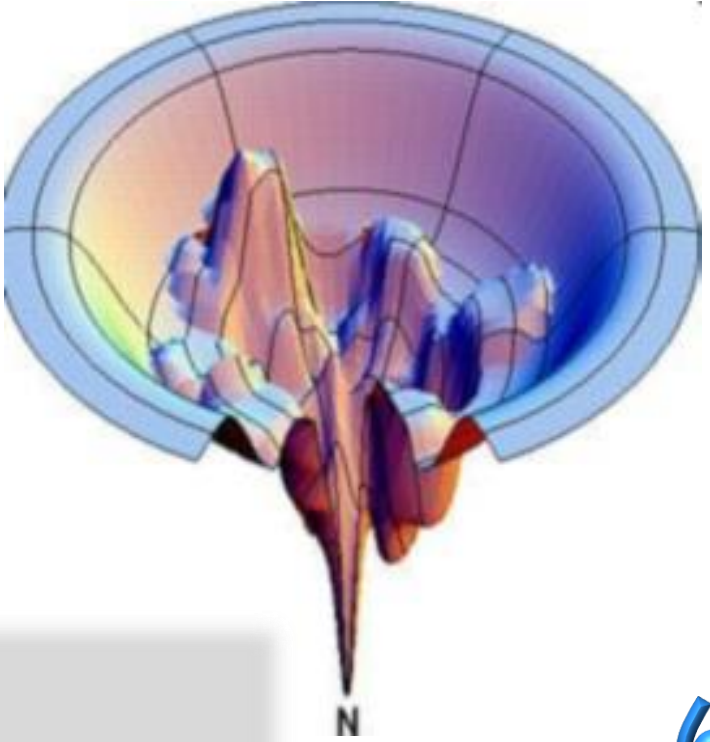
Chaperones



Autophagy

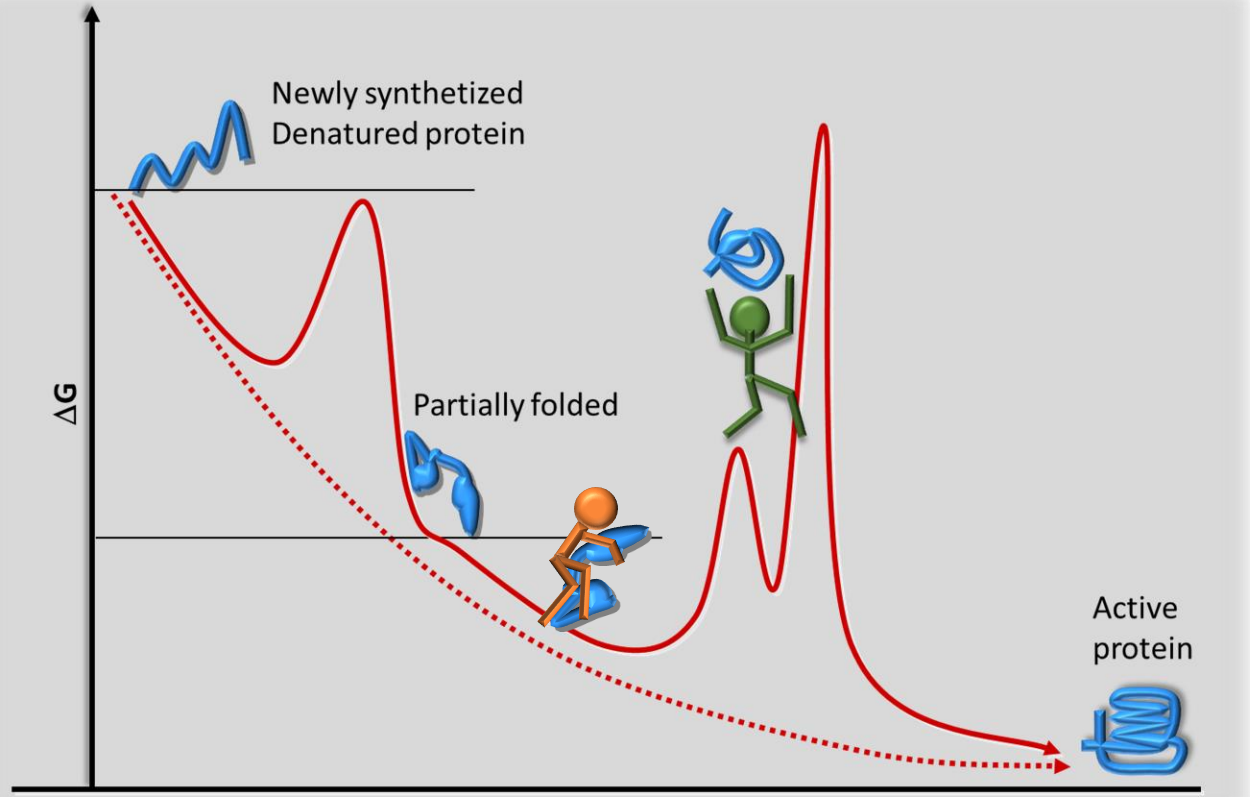
Ubiquitin proteasome system

Stress proteins / Chaperones



Holdases bind folding intermediates to prevent their aggregation

Crystalins, p23, Hsp40…

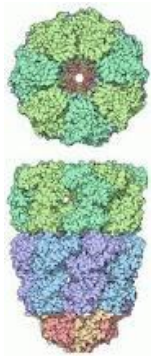


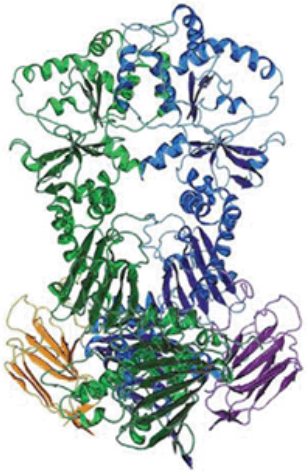
Foldases are chaperones that accompany other proteins to help them to overcome the energy barriers during folding to native conformation
(ATP dependent)

Hsp70, Hsp90, GroEL…

Stress proteins

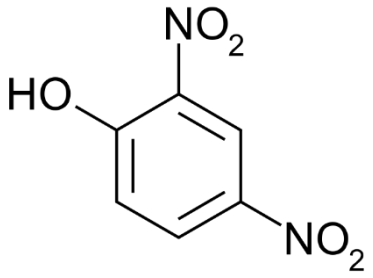
Proteins induced by increased temperature, mainly represented by chaperones



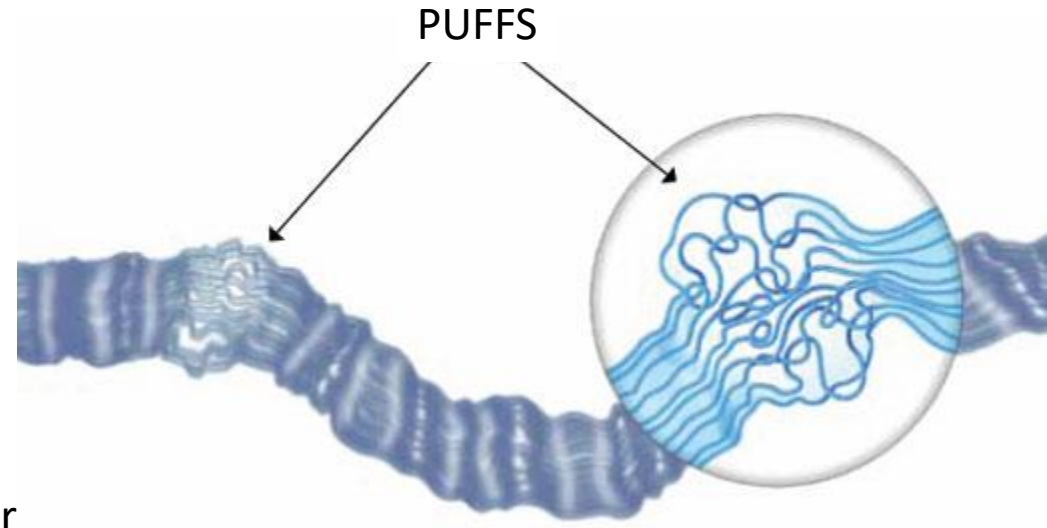


Hsp90
Hsp70, DnaK
Hsp60, GroEL
Hsp40 DnaJ
Hsp27, Crystalins,
Hsp10,chaperonins, GroES

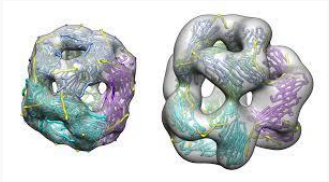
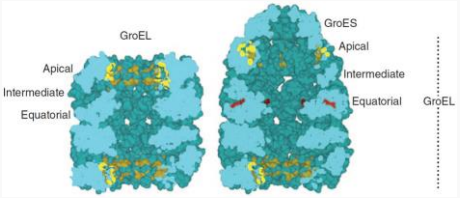
Teplota



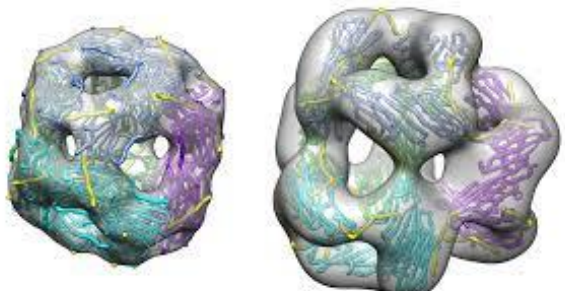
Dinitrophenol-mitochondrialní uncoupler

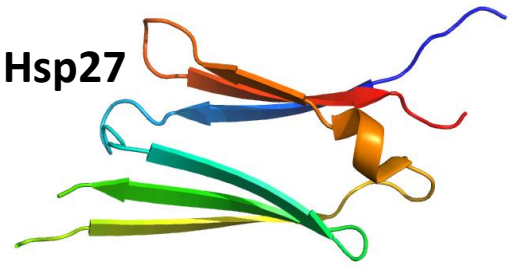


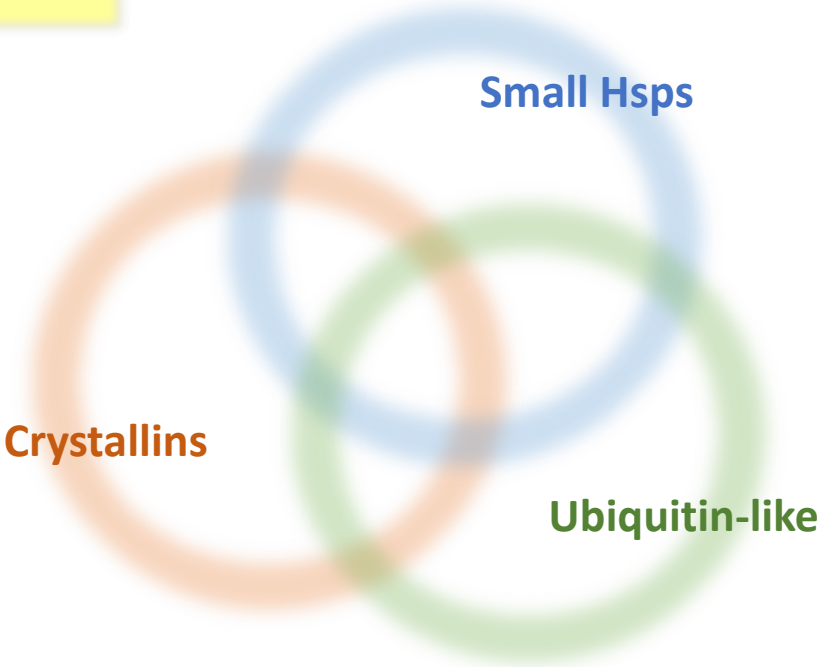
1962 by Ferruccio Ritossa

Approximate molecular weight(kDa)	Prokaryotic proteins	Eukaryotic proteins	Function
10 kDa	GroES	Hsp10	
20-30 kDa	GrpE	The HspB group of Hsp. Eleven members in mammals including Hsp27, HSPB6 or HspB1[28]	
40 kDa	DnaJ	Hsp40	Co-factor of Hsp70
60 kDa	GroEL, 60kDa antigen	Hsp60 	Involved in protein folding after its post-translational import to the mitochondrion/chloroplast
70 kDa	DnaK	The HspA group of Hsp including Hsp71,Hsp70, Hsp72, Grp78 (BiP), Hsx70 found only in primates	Protein folding and unfolding, provides thermotolerance to cell on exposure to heat stress. Also prevents protein folding during post-translational import into the mitochondria/chloroplast.
90 kDa	HtpG, C62.5	The HspC group of Hsp including Hsp90, Grp94	Maintenance of steroid receptors and transcription factors
100 kDa	ClpB, ClpA, ClpX	Hsp104, Hsp110	Tolerance of extreme temperature

HspB group/ small chaperones



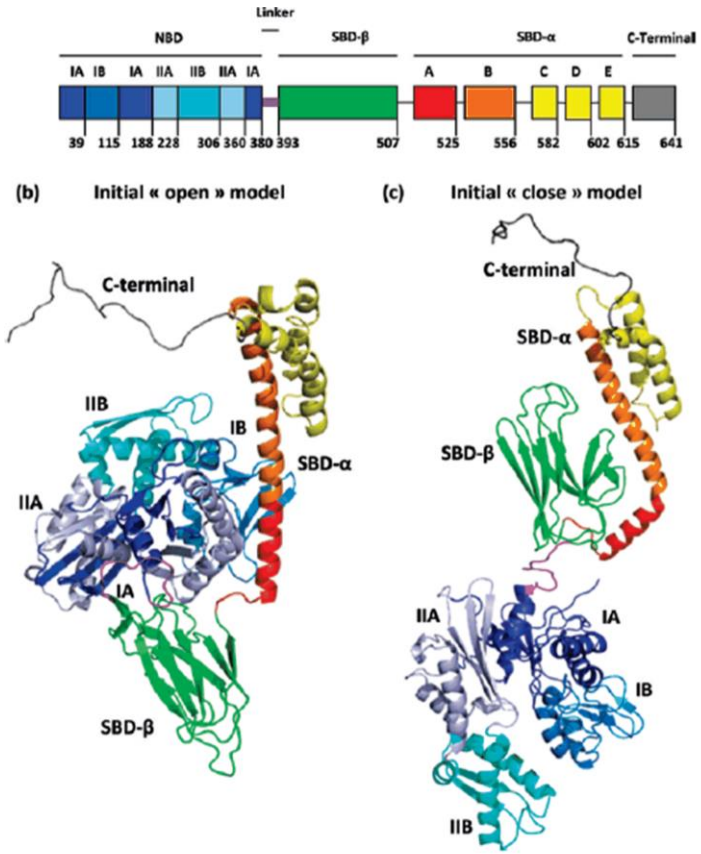




Prevent aggregation
Thermotolerance

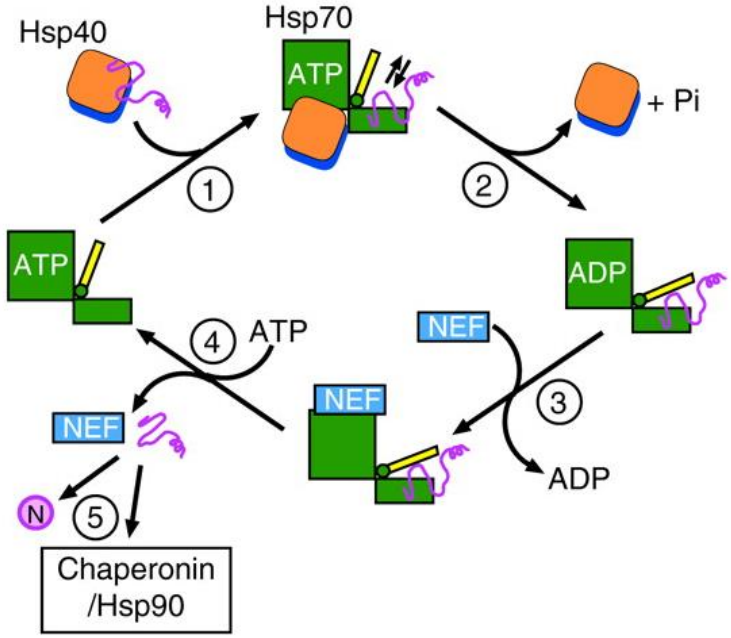
	Gene name	Protein name	Old names	Human gene ID	Mouse ortholog ID
1	*HSPB1*	HSPB1	CMT2F; HMN2B; HSP27; HSP28; HSP25; HS.76067; DKFZp586P1322	3315	15507
2	*HSPB2*	HSPB2	MKBP; HSP27; Hs.78846; LOH11CR1K; MGC133245	3316	69253
3	*HSPB3*	HSPB3	HSPL27	8988	56534
4	*HSPB4*[a]	HSPB4	crystallin alpha A; CRYAA, CRYA1	1409	12954
5	*HSPB5*[a]	HSPB5	crystallin alpha B, CRYAB; CRYA2	1410	12955
6	*HSPB6*	HSPB6	HSP20; FLJ32389	126393	243912
7	*HSPB7*	HSPB7	cvHSP; FLJ32733; DKFZp779D0968	27129	29818
8	*HSPB8*	HSPB8	H11; HMN2; CMT2L; DHMN2; E2IG1; HMN2A; HSP22	26353	80888
9	*HSPB9*	HSPB9	FLJ27437	94086	75482
10	*HSPB10*[a]	HSPB10	ODF1; ODF; RT7; ODF2; ODFP; SODF; ODF27; ODFPG; ODFPGA; ODFPGB; MGC129928; MGC129929	4956	18285
11	*HSPB11*	HSPB11	HSP16.2; C1orf41; PP25	51668	72938

Hsp70 (DnaK, Grp78,..) chaperone machinery



ATP

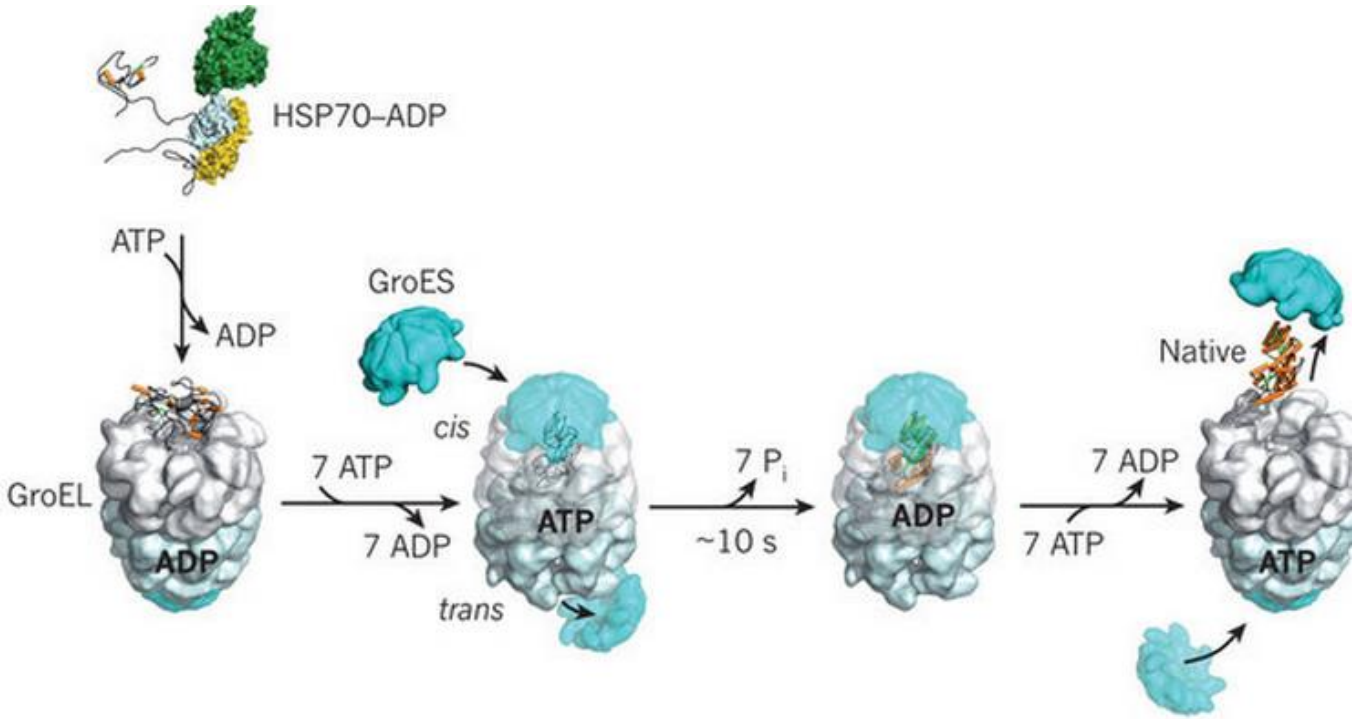
ADP



BAG NEF-Nucleotide exchange factor

Hsp40 DnaJ J-proteins

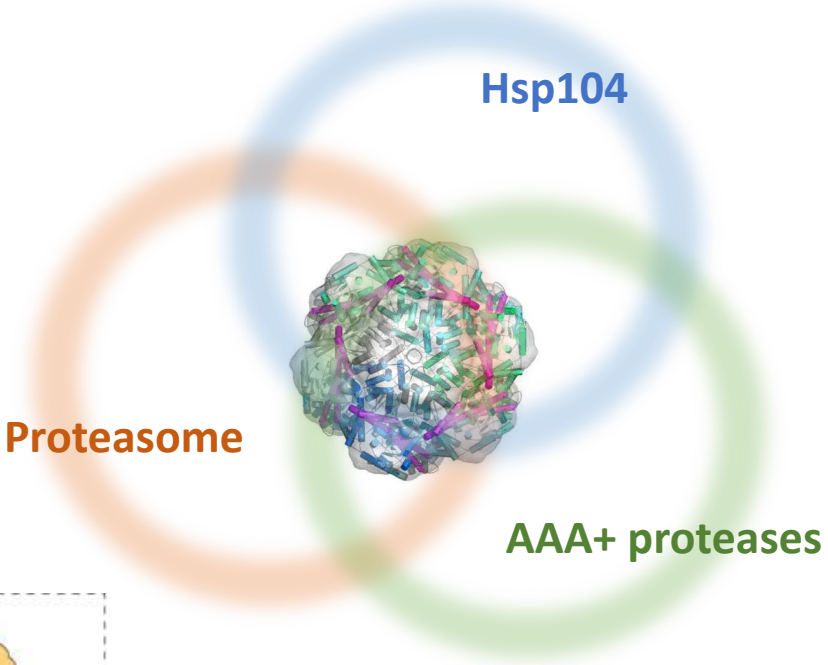
Chaperonins
(GroEL-GroES, Hsp60, CCT-TRiC)

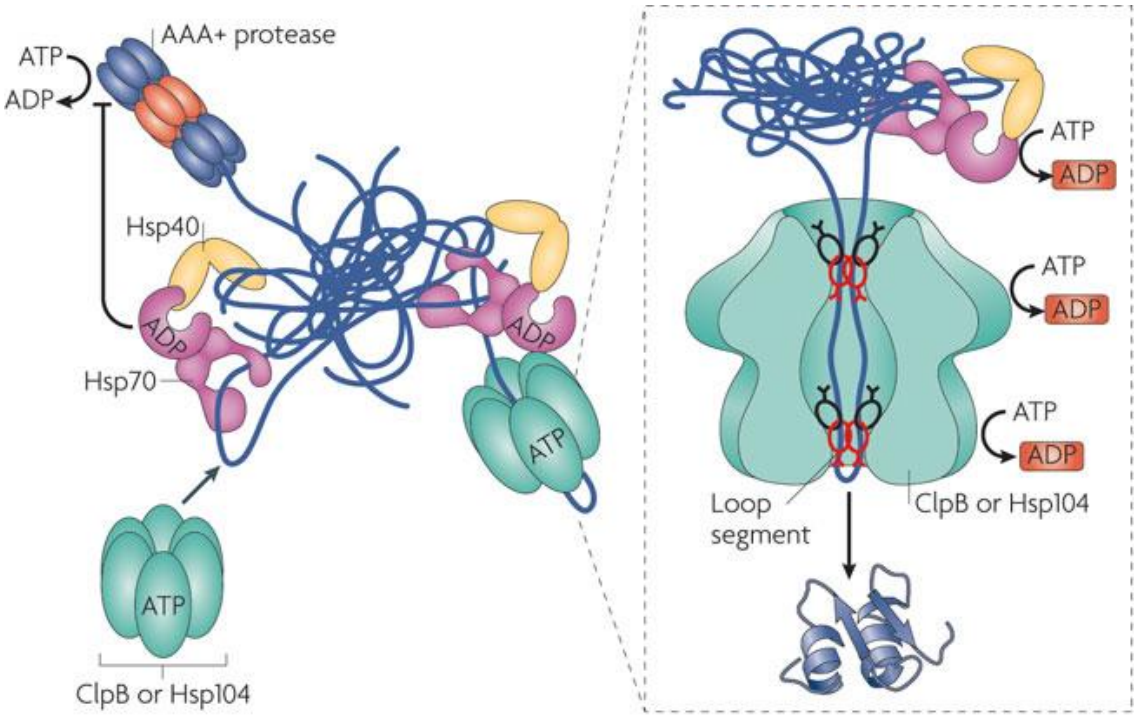


Folding of cytoskeletal proteins (tubulin)
Protein transport

Hsp104 (ClpB, ClpX,..)

Thermotolerance
Aggregate refolding
Prion folding (yeast Psi+/-)





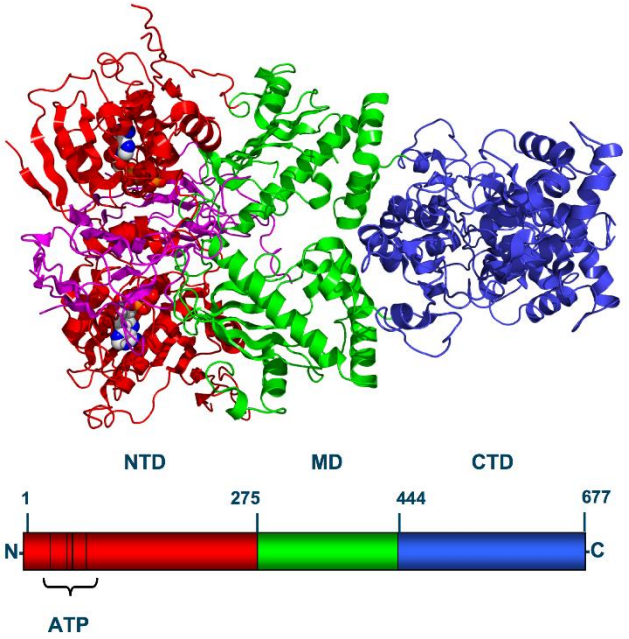
Nature Reviews | Molecular Cell Biology

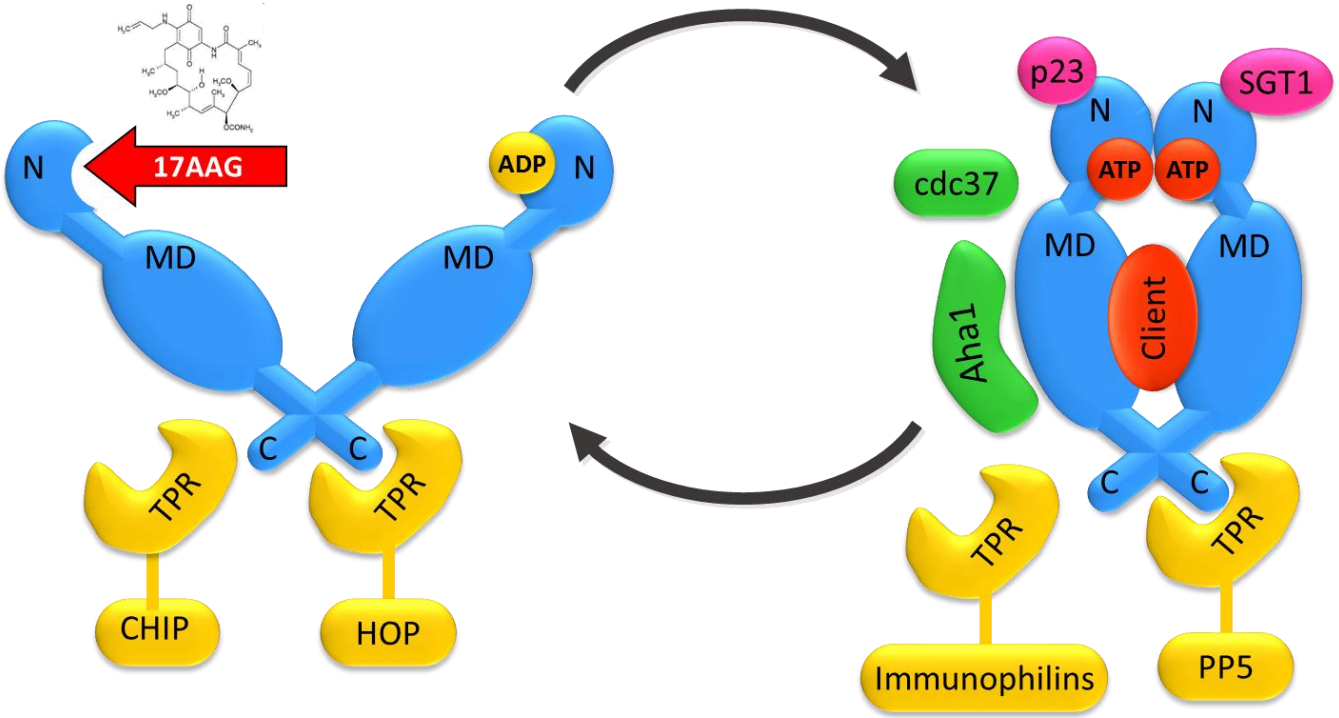
AAA+ ATPases

Converts ATP to "mechanical" energy (molecular motors)

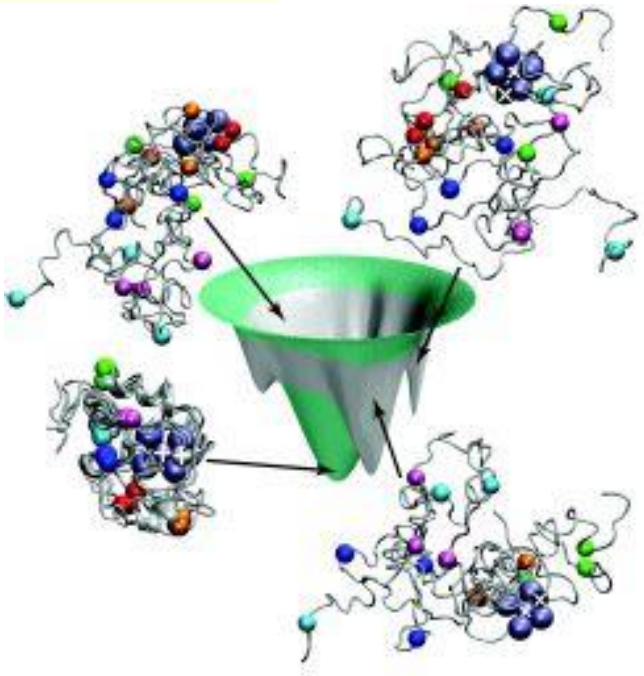
Hsp90
chaperone machinery

- Conserved from procarytes to mammals
- ATPase aktivity (like gyrase)
- Mitochndrial, ER, cytoplasmic
- Redundant isoformes





Stress proteins/ Chaperones/Hsp90



Hsp90 as a capacitor for morphological evolution

Suzanne L. Rutherford*† & Susan Lindquist*
*Howard Hughes Medical Institute, University of Chicago, 5841 South Maryland Avenue MC1028, Chicago, Illinois 60637, USA

NATURE | VOL 396 | 26 NOVEMBER 1998 | www.nature.com

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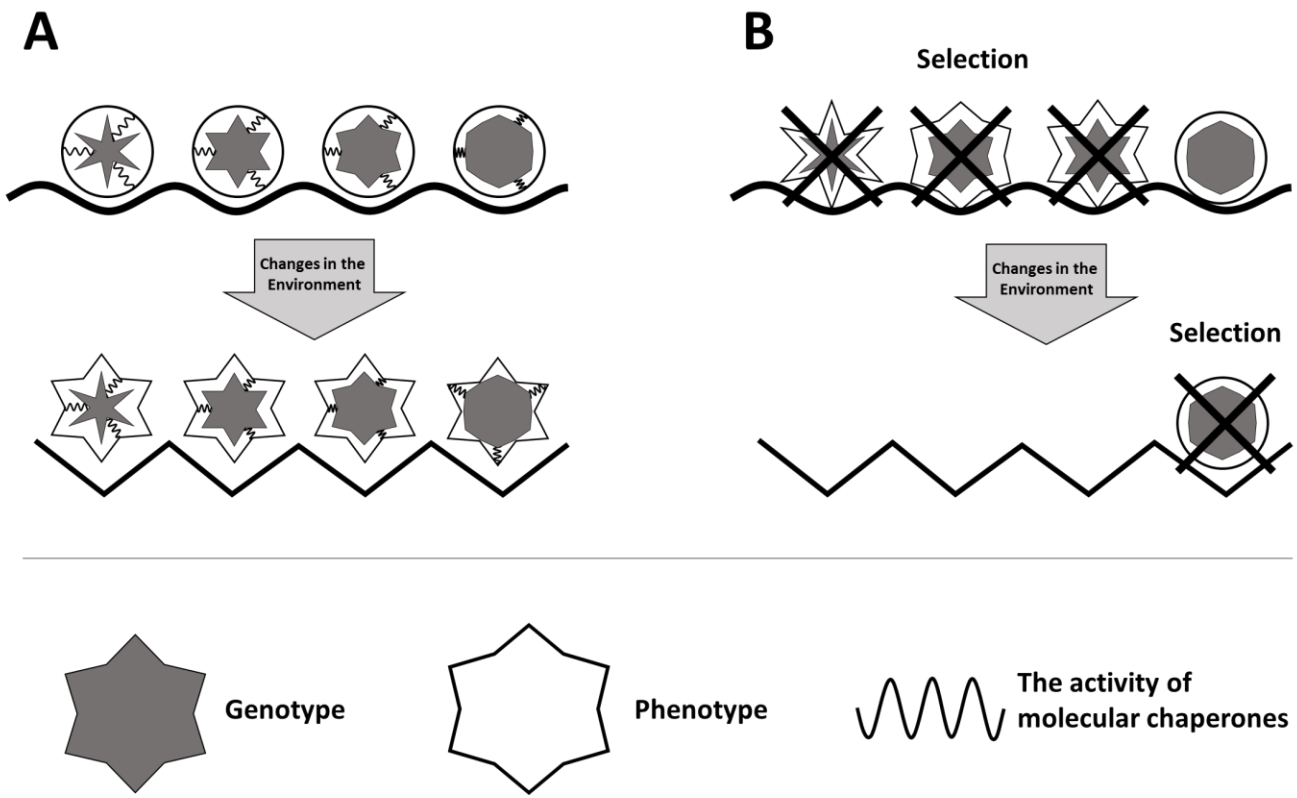
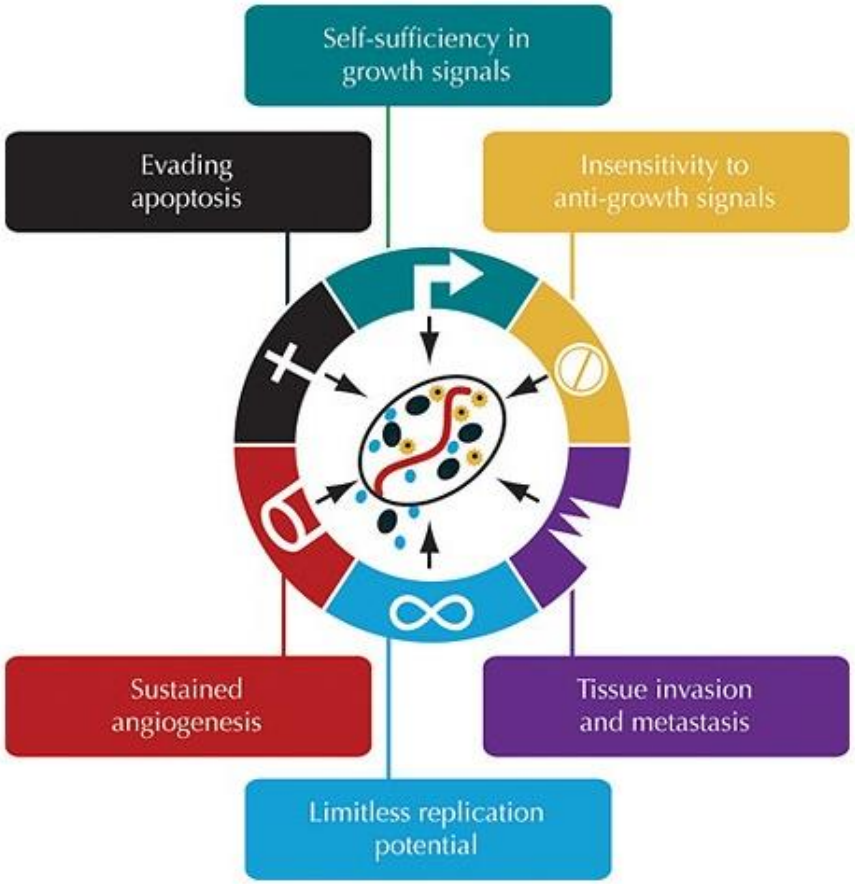
Supplementum ad Acta Musei Moraviae *XCVIII, 2013*

CHAPERONES AND EVOLUTION

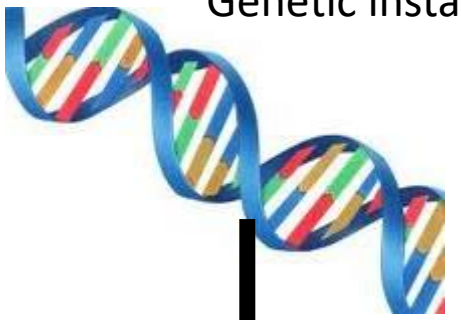
FILIP TRCKA, BORIVOJ VOJTESEK, PETR MULLER
Regional Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute,
Zluty kopec 7, 656 53 Brno



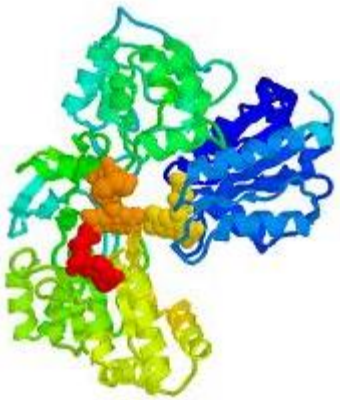
The tumor cells demand high quality and amount of protein



Genetic instability

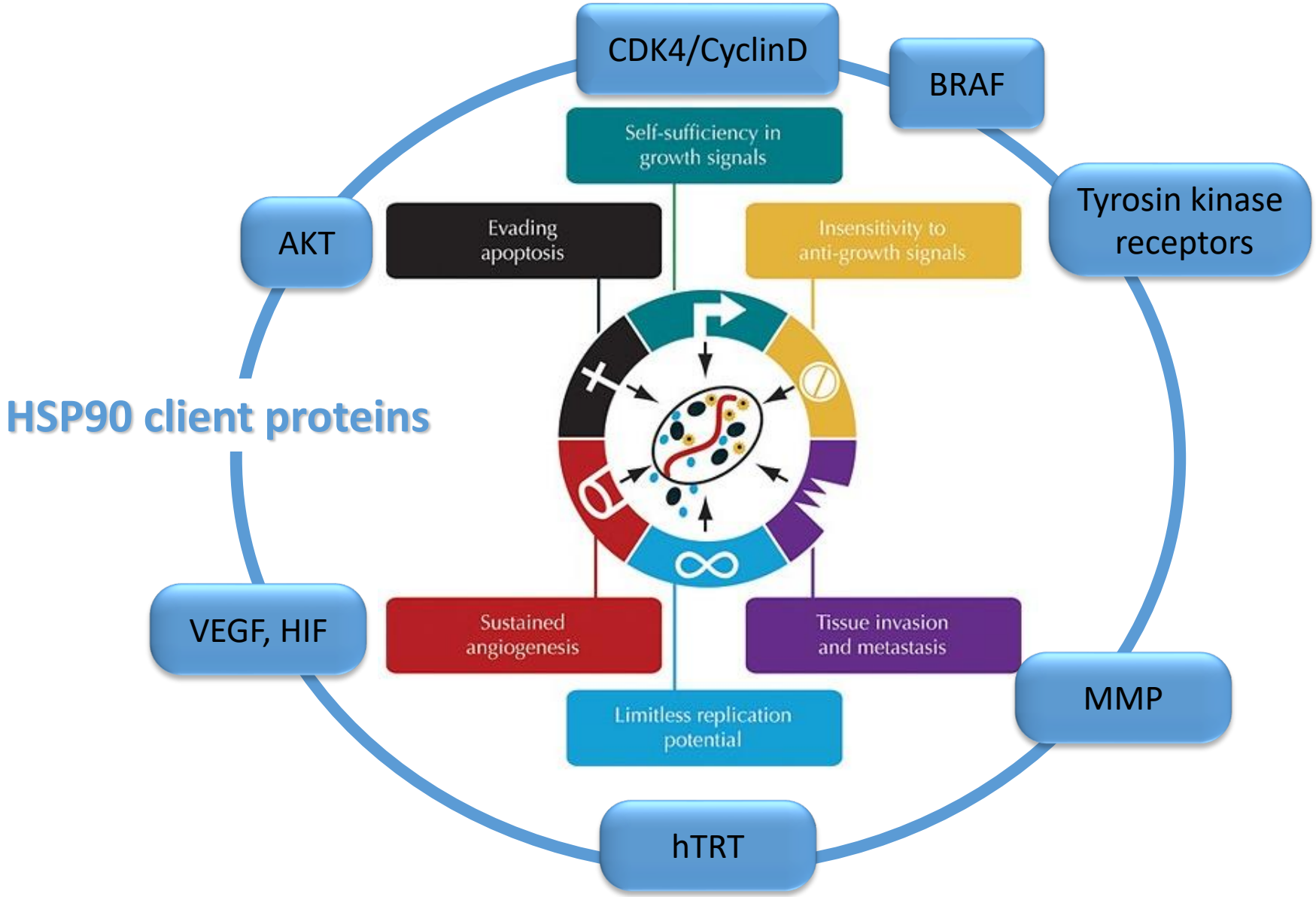


Enhanced proteosynthesis
Production of mutated, conformational instable protins



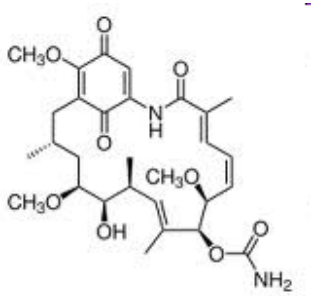
Hanahan D, Weinberg RA.: Cell. 2000 Jan 7;100(1):57-70.

Activity of Hsp90 is essential for expression of cancer phenotype

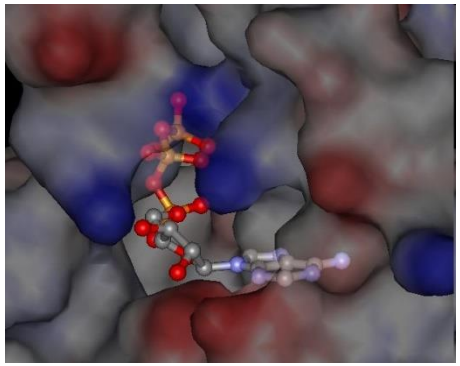


Specific inhibitors Hsp90

Isolation of Geldanamycin (1970)



Geldanamycin binds ATP cavity of Hsp90 (1997)

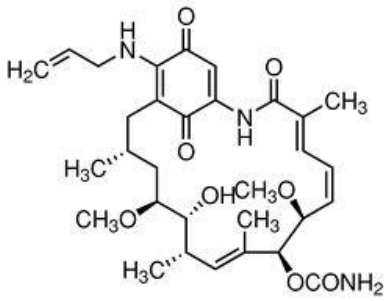


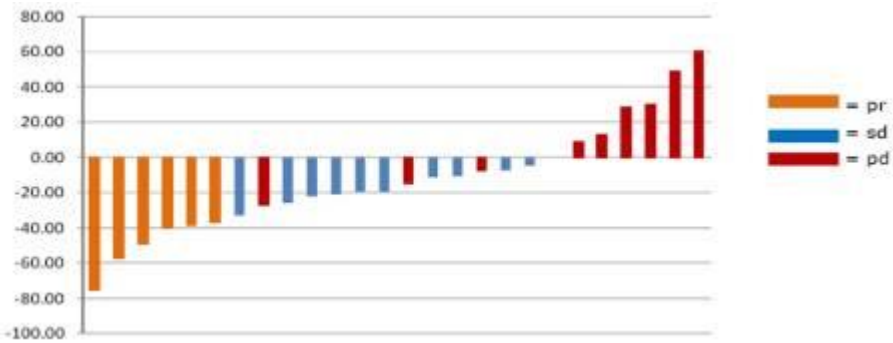
Clinical trials with Geldanamycin(2000)

	inhibitor	No of studies	phase	Company
1	tanespimycin (17AAAG)	36	III	Bristol-Myers Squibb, Kosan
2	retaspimycin (IPI-504)	11	II/III*	Infinity Pharmaceuticals
3	alvespimycin (17DMAG)	7	II	Bristol-Myers Squibb, Kosan
4	STA-9090	14	II	Synta Pharmaceuticals Corp.
5	AUY922	11	II	Novartis Pharmaceuticals
6	CNF2024 (BIIB021)	7	II	Biogen Idec
7	SNX-5422	4	I	Pfizer, Serenex, Inc.
8	AT13387	3	I	Astex Therapeutics
9	KW-2478	2	I/II	Kyowa Hakko Kirin Pharma, Inc.
10	IPI-493	2	I	Infinity Pharmaceuticals
11	HSP990	2	I	Novartis Pharmaceuticals
12	MPC-3100	1	I	Myrexis Inc.
13	Debio 0932	1	I	Debiopharm S.A.
15	BIIB028	1	I	Biogen Idec

Hsp90 is unique therapeutic target for anti-cancer therapy

more than 17 different molecules in clinical trials





Variable response
need for predictive markers

What does kill the cells:

- apoptosis, aggregation, ….

Different assembly of Hsp90 machinery ?

- posttranlational modifications
- expression pattern of co-chaperones

Client spectrum ?

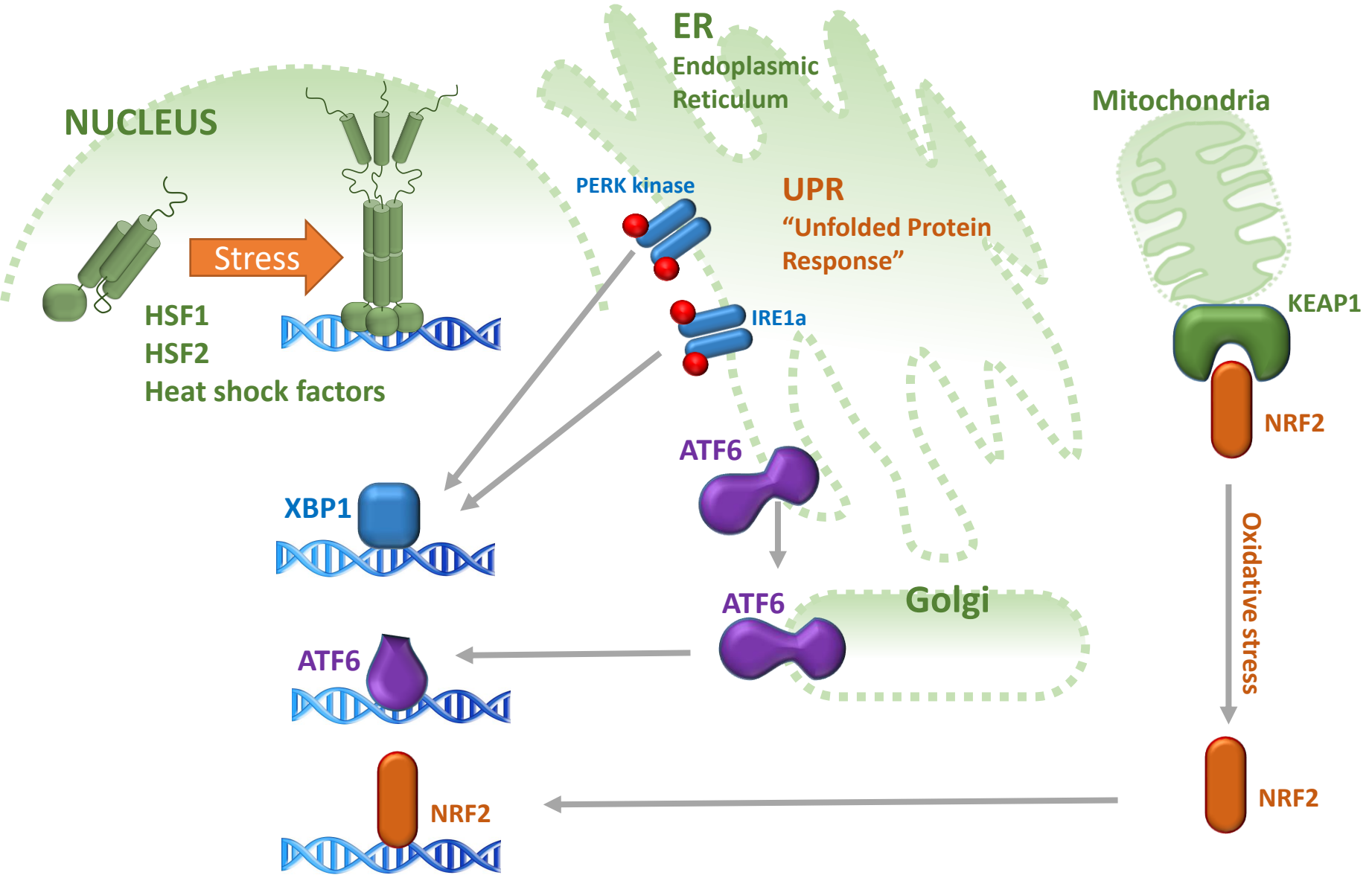
Sensors of proteotoxic stress

Metabolic stress

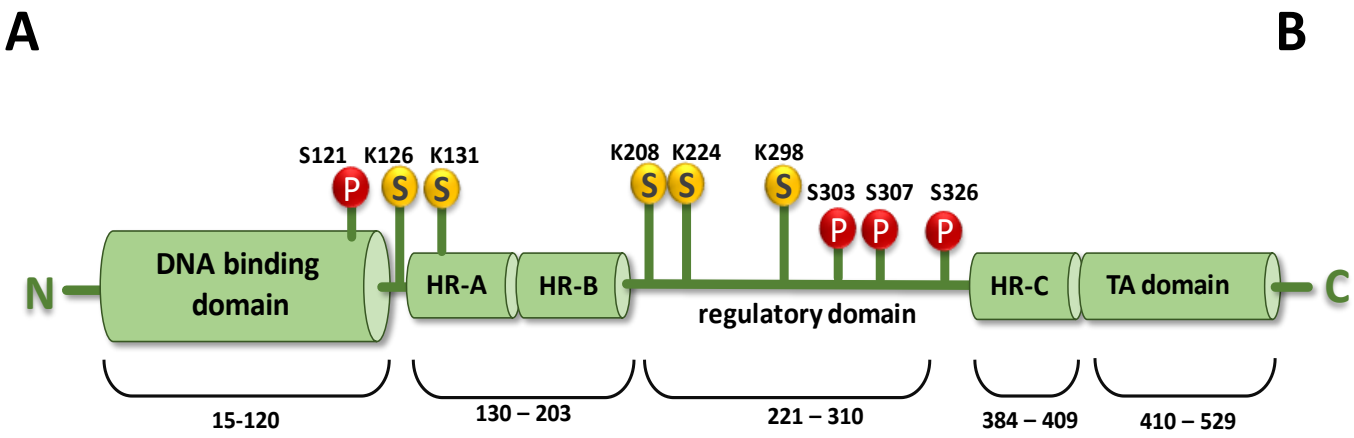
Increased temperature

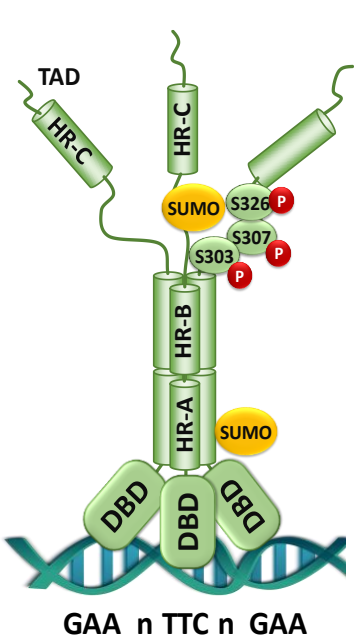
Mutations and genomic instability

Oxidative stress



HSF1 is the main regulator of gene expression responsible for maintaining protein homeostasis

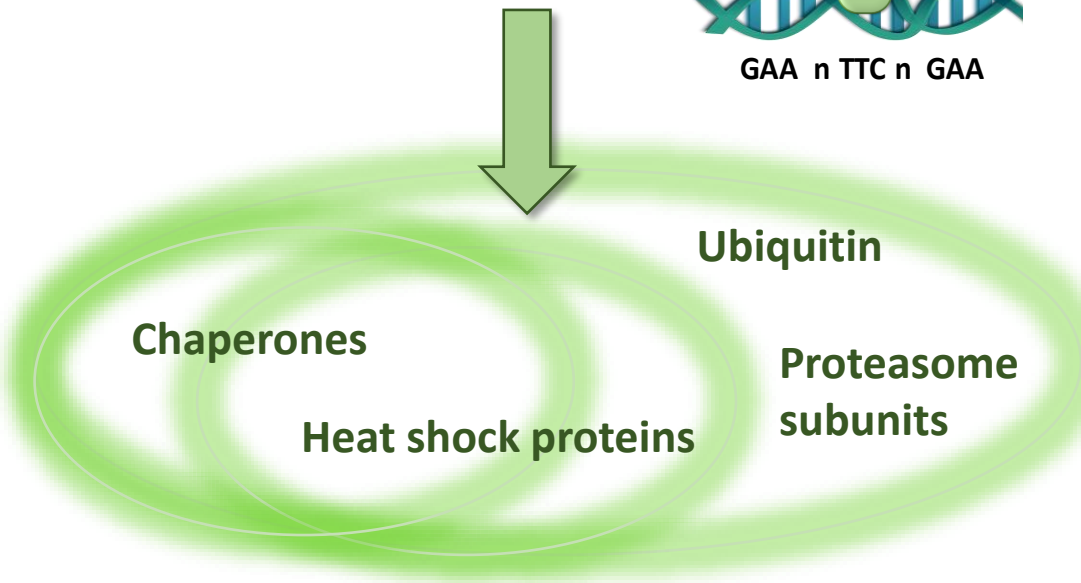




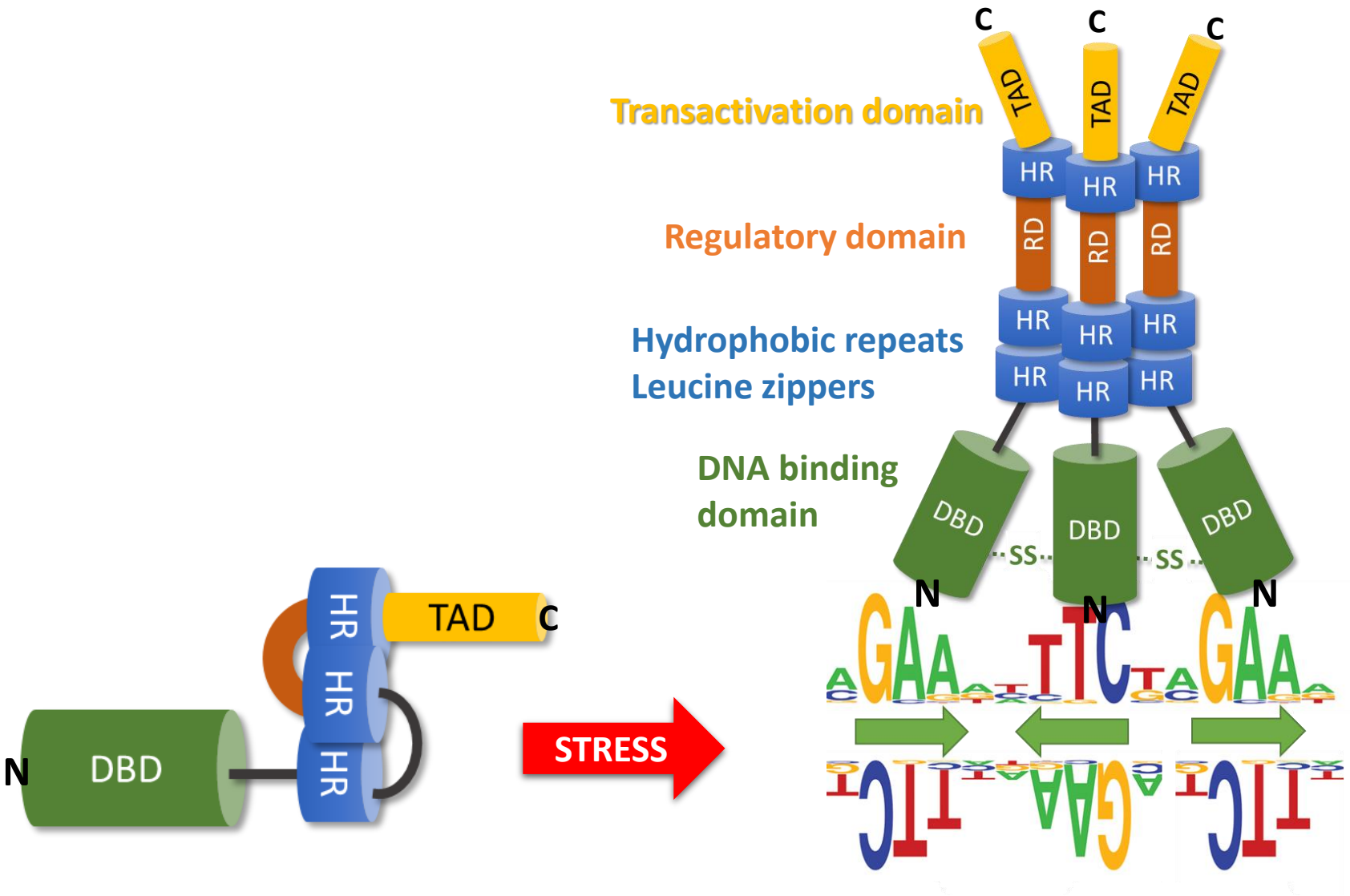
HSF1
HSF2
HSF4
HSF-X-linked
HSF-Y-linked
HSF-5

NRF2
NRF1

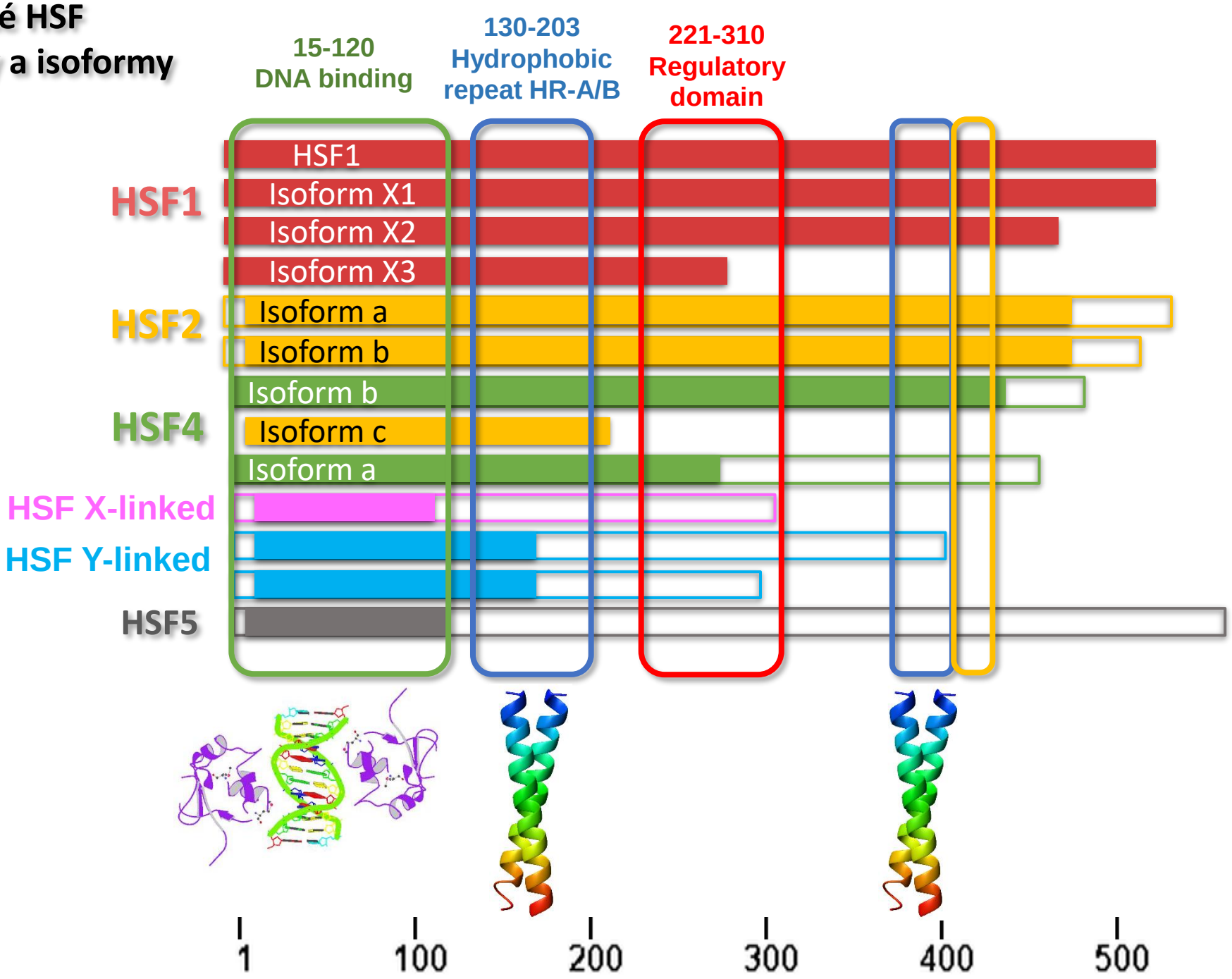
HIF1
ARNT



Regulation of chaperone gene expression



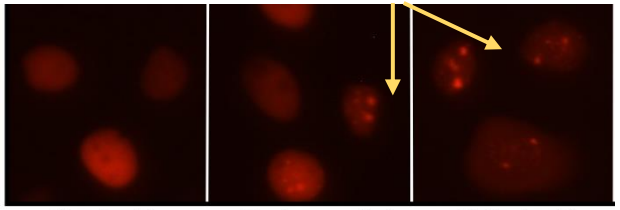
Lidské HSF
Geny a isoformy



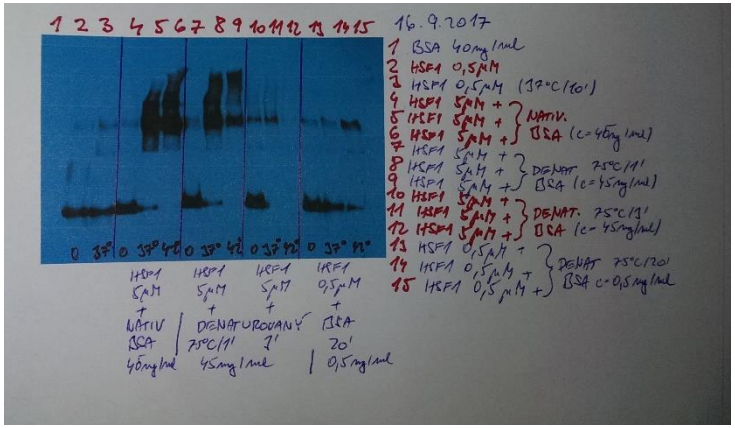
Analysis of HSF1 activation

HSF1-mCherry in A375 and H1299

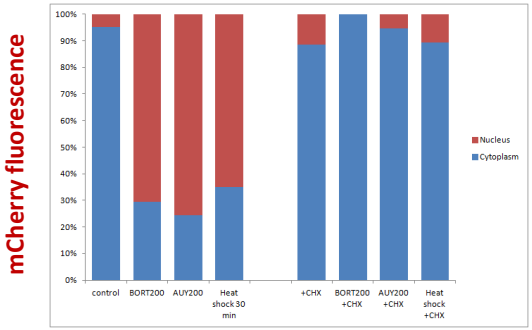
Nuclear stress bodies



Native gel, detection of trimers



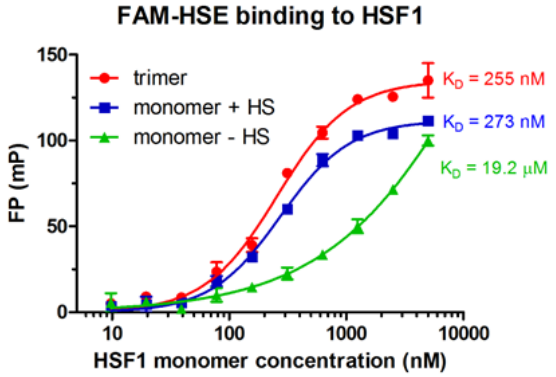
Cell fractionation



WB

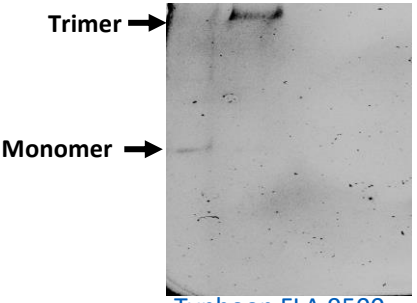
- Crosslinkink
- Fractionation
- phosphorylation

Measurement of DNA binding capacity



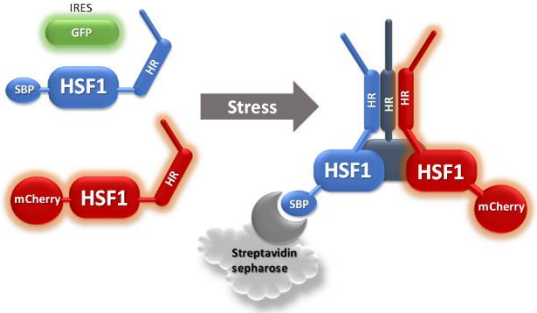
Fluorescence polarization

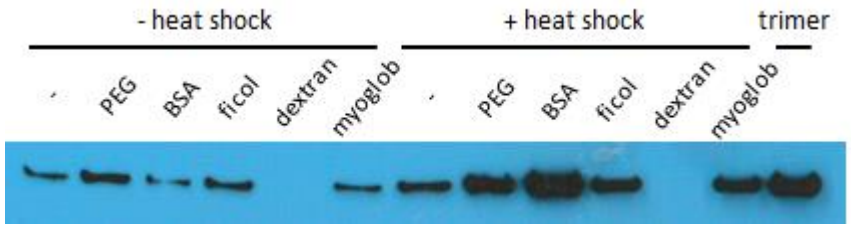
Native gel - mCherry



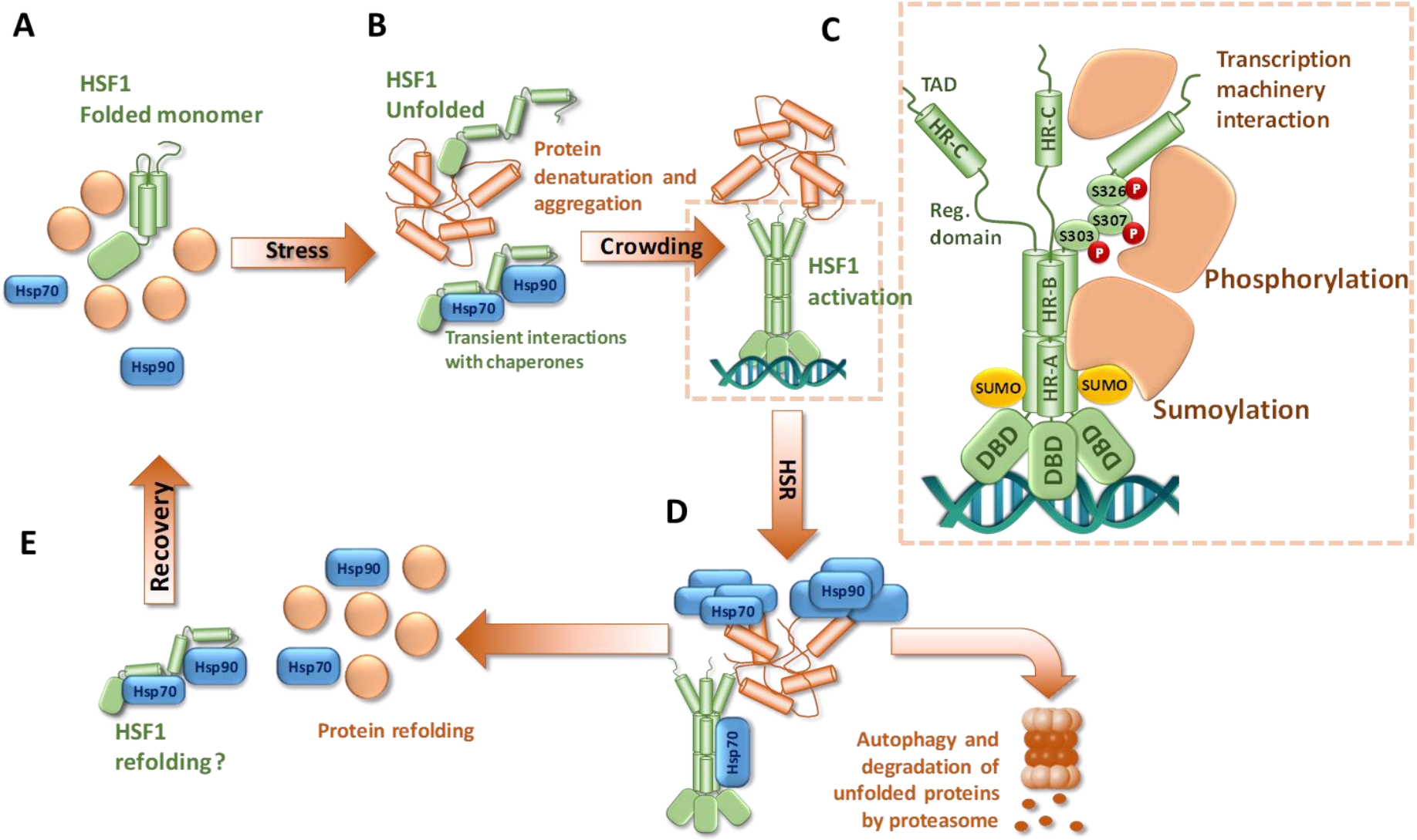
Typhoon FLA 9500

HSF1-mCherry + SBP-HSF1

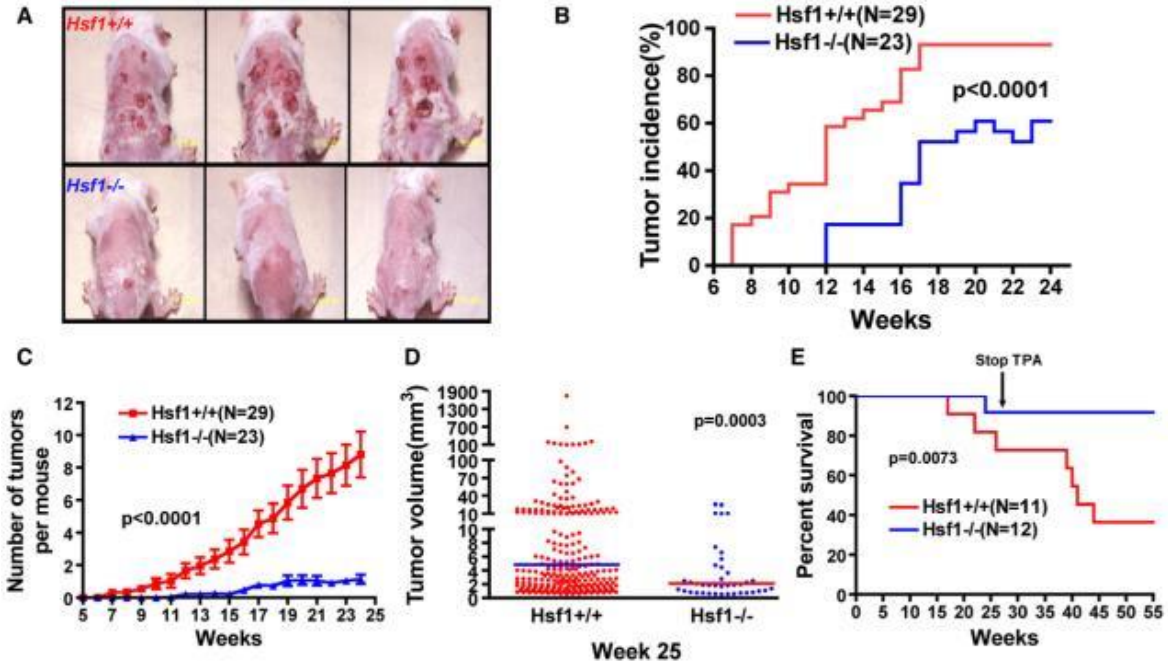




Mechanisms of HSF1 activation



HSF1 is essential for carcinogenesis and tumour progression



Cell

Heat Shock Factor 1 Is a Powerful Multifaceted Modifier of Carcinogenesis

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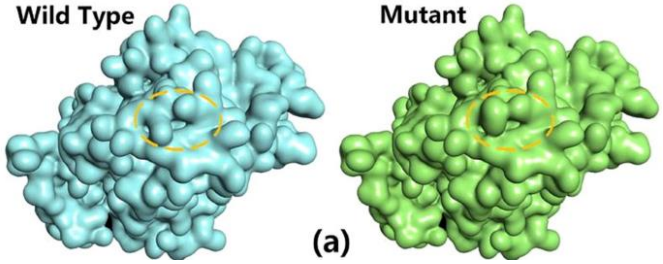
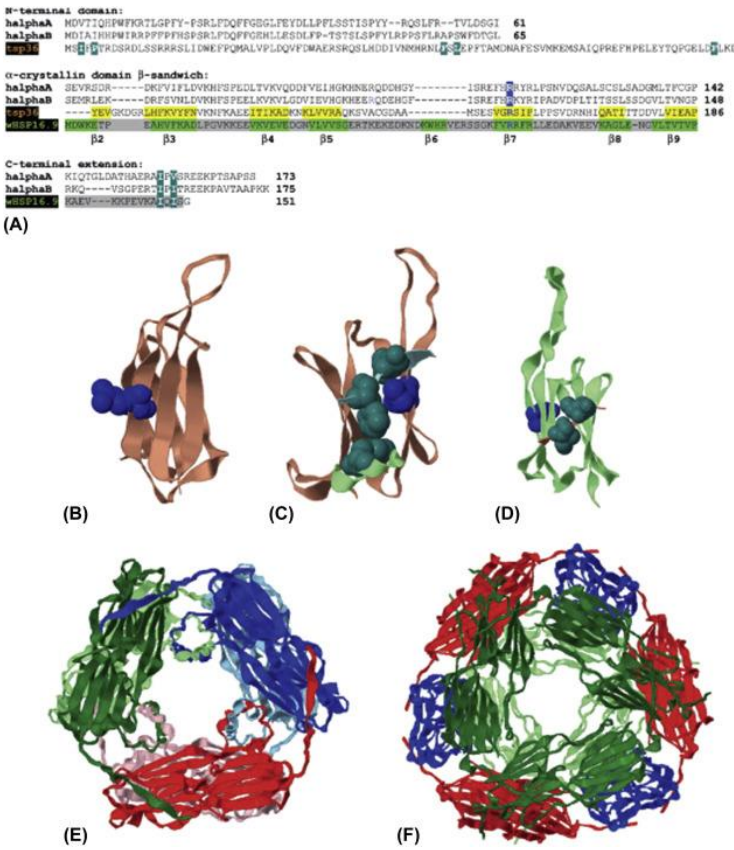
DOI 10.1016/j.cell.2007.07.020

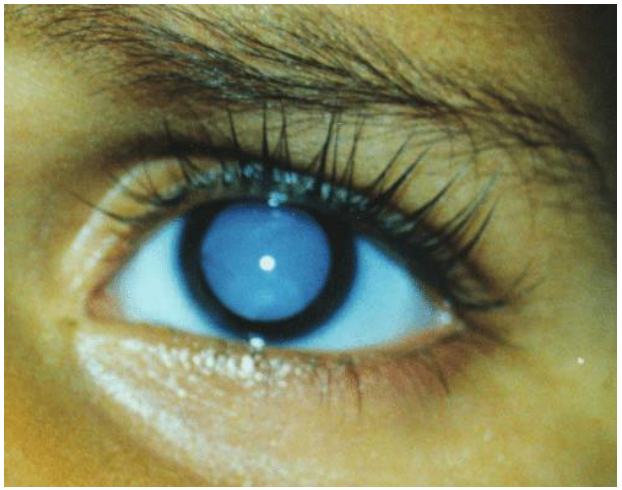
HSF4



Mutation in HSF4 leads to decreased expression of crystalline genes in the lens, resulting in congenital cataracts

Crystalline alpha/beta (CRYAB, CRYAA)

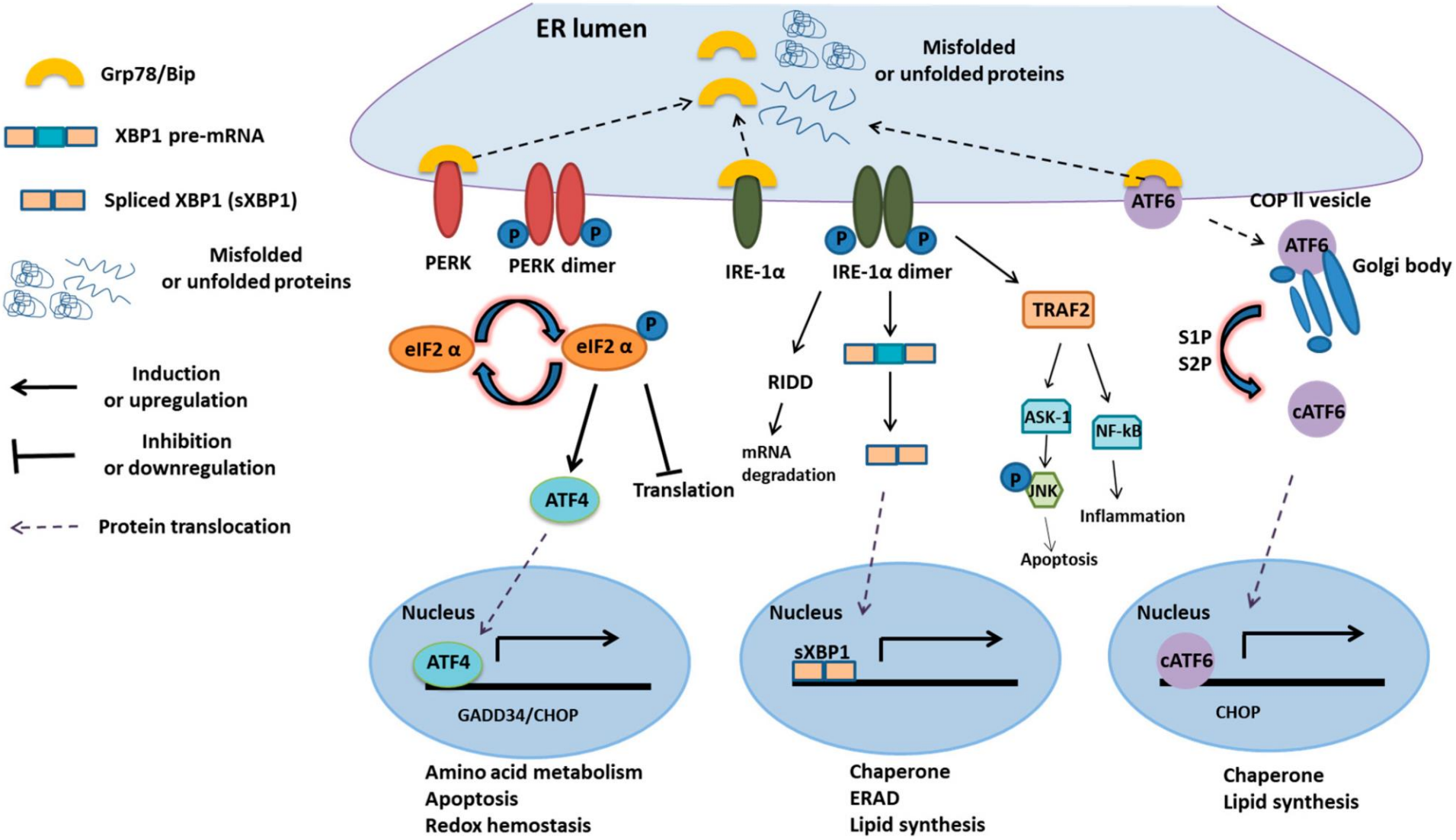




A Homozygous Splice Mutation in the HSF4 Gene Is Associated with an Autosomal Recessive Congenital Cataract

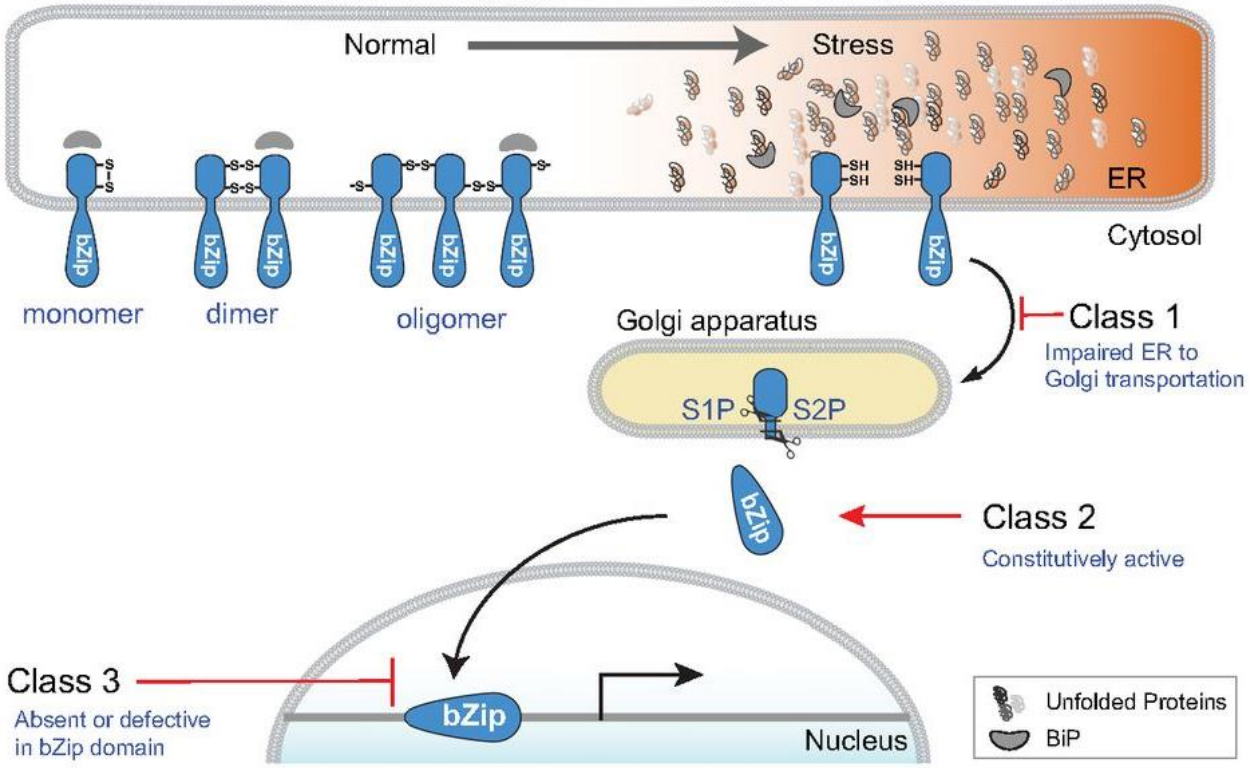
Congenital Cataract in Australian Shepard

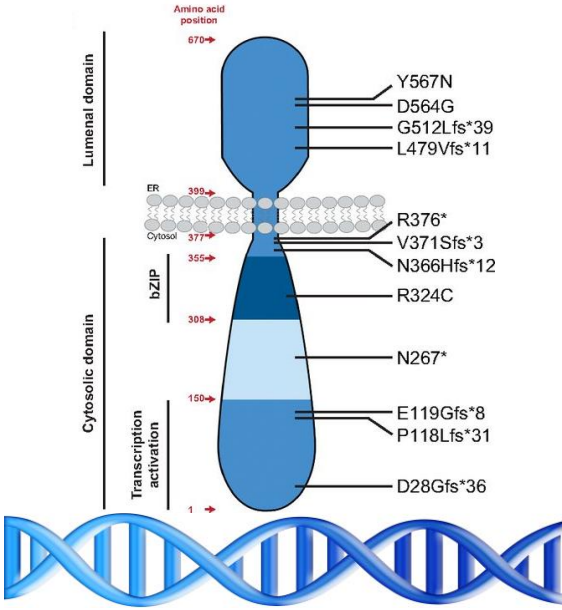
Unfolded protein response and autophagy



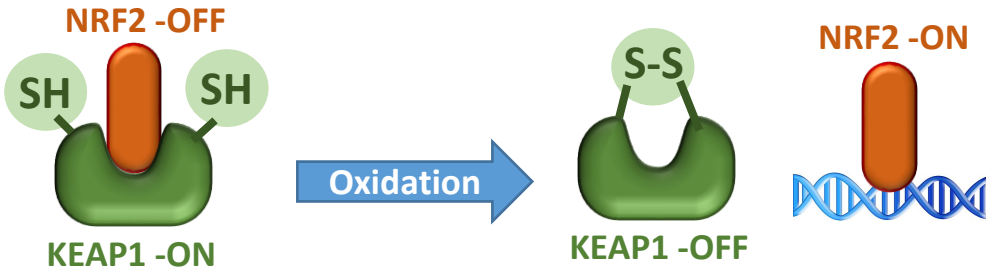
ATF6

- **ATF6 is an endoplasmic reticulum (ER) stress-regulated transmembrane transcription factor that activates the transcription of ER molecules.**
- **Accumulation of misfolded proteins in the Endoplasmic Reticulum results in the proteolytic cleavage of ATF6.**
- **The cytosolic portion of ATF6 will move to the nucleus and act as a transcription factor to cause the transcription of ER chaperones.**

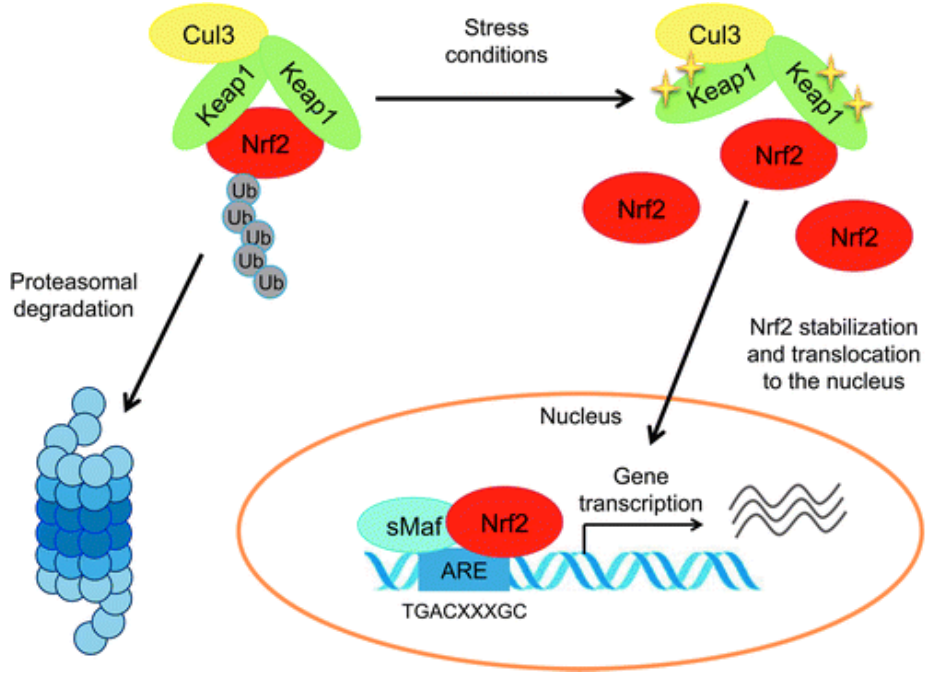


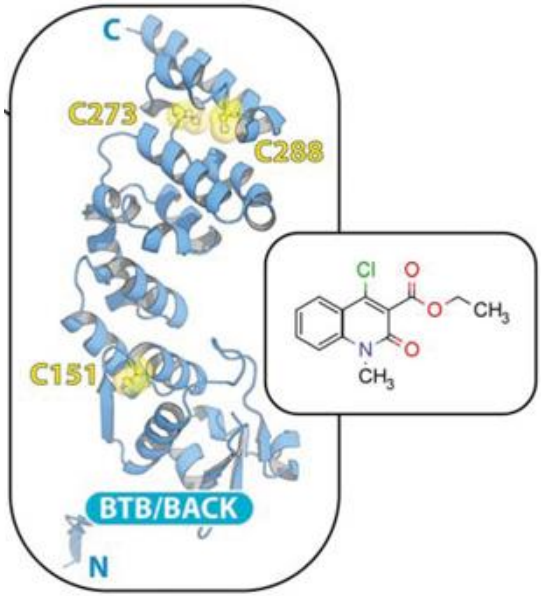


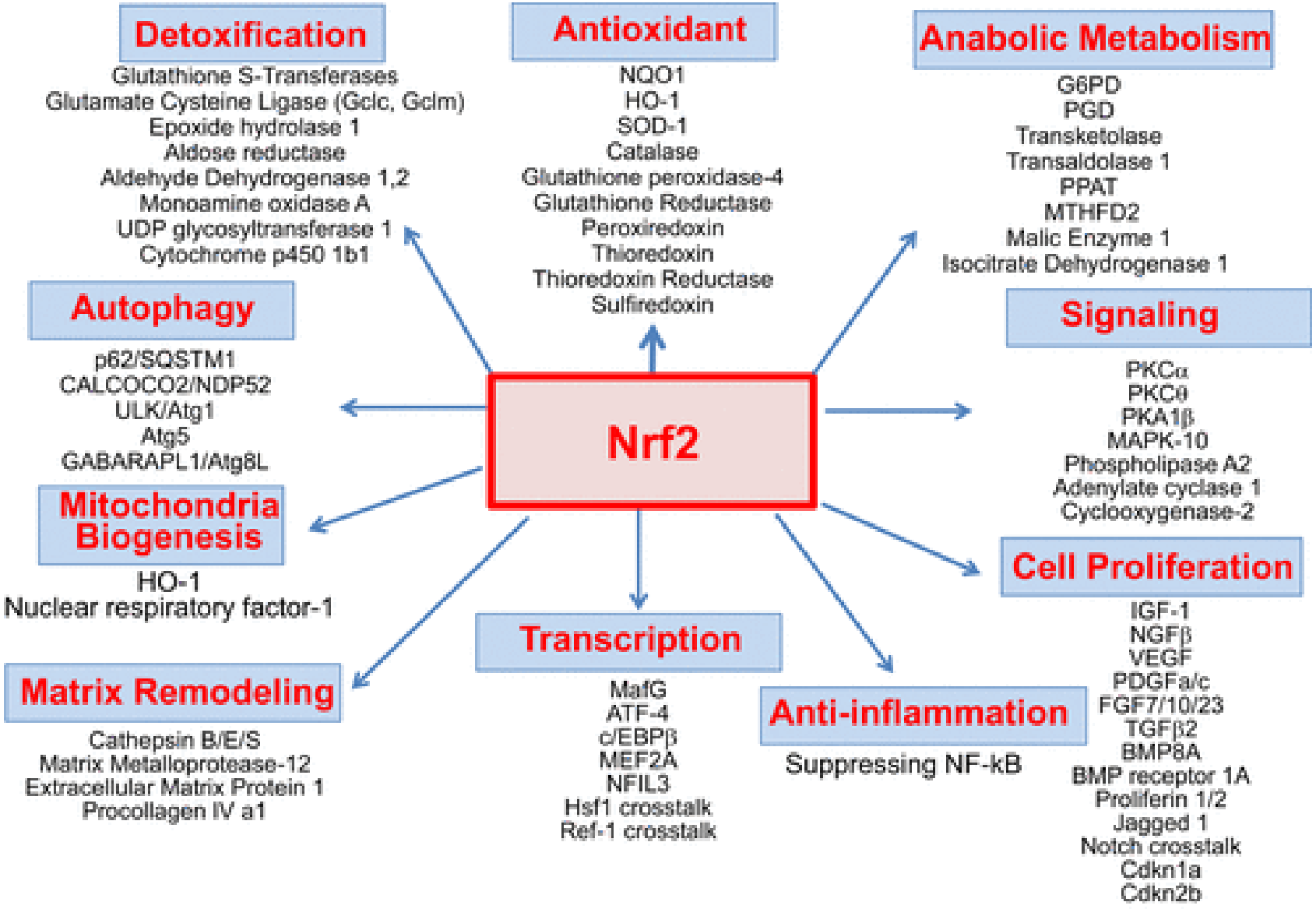
NRF2



NRF2 is a transcription factor that regulates the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation






Detoxification
Glutathione S-Transferases
Glutamate Cysteine Ligase (Gclc, Gclm)
Epoxide hydrolase 1
Aldose reductase
Aldehyde Dehydrogenase 1,2
Monoamine oxidase A
UDP glycosyltransferase 1
Cytochrome p450 1b1
Antioxidant
NQO1
HO-1
SOD-1
Catalase
Glutathione peroxidase-4
Glutathione Reductase
Peroxiredoxin
Thioredoxin
Thioredoxin Reductase
Sulfiredoxin
Anabolic Metabolism
G6PD
PGD
Transketolase
Transaldolase 1
PPAT
MTHFD2
Malic Enzyme 1
Isocitrate Dehydrogenase 1
Autophagy
p62/SQSTM1
CALCOCO2/NDP52
ULK/Atg1
Atg5
GABARAPL1/Atg8L
Nrf2
Signaling
PKCα
PKCθ
PKA1β
MAPK-10
Phospholipase A2
Adenylate cyclase 1
Cyclooxygenase-2
Mitochondria Biogenesis
HO-1
Nuclear respiratory factor-1
Cell Proliferation
IGF-1
NGFβ
VEGF
PDGFa/c
FGF7/10/23
TGFβ2
BMP8A
BMP receptor 1A
Proliferin 1/2
Jagged 1
Notch crosstalk
Cdkn1a
Cdkn2b
Transcription
MafG
ATF-4
c/EBPβ
MEF2A
NFIL3
Hsf1 crosstalk
Ref-1 crosstalk
Anti-inflammation
Suppressing NF-kB
Matrix Remodeling
Cathepsin B/E/S
Matrix Metalloprotease-12
Extracellular Matrix Protein 1
Procollagen IV a1