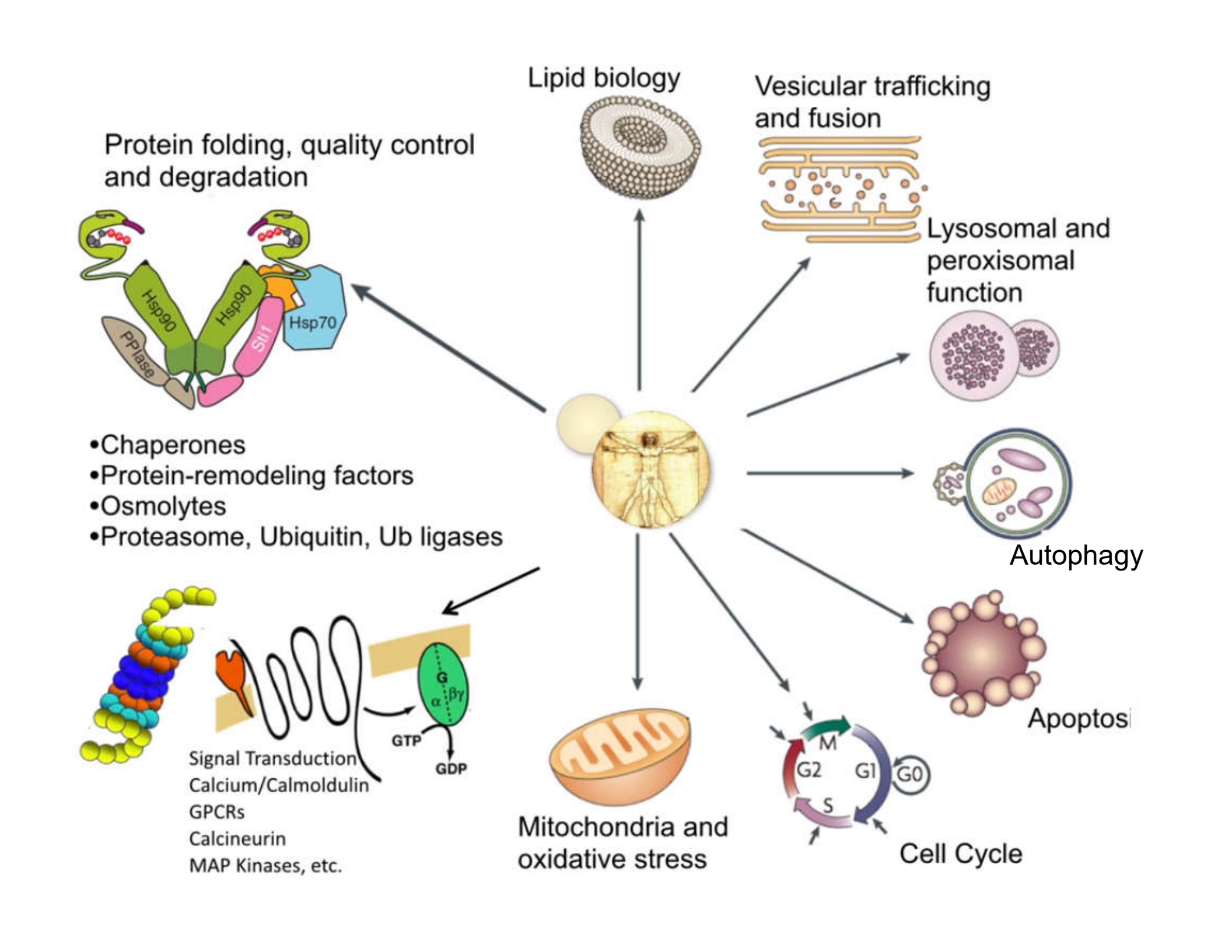
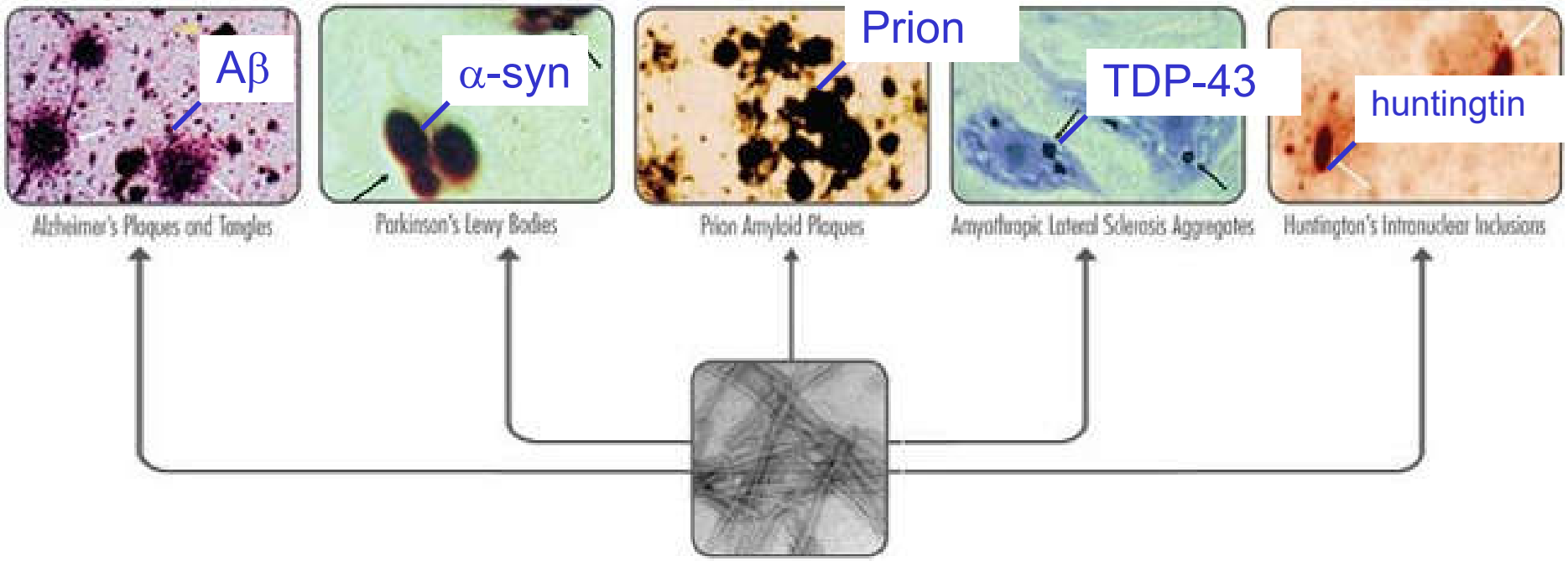
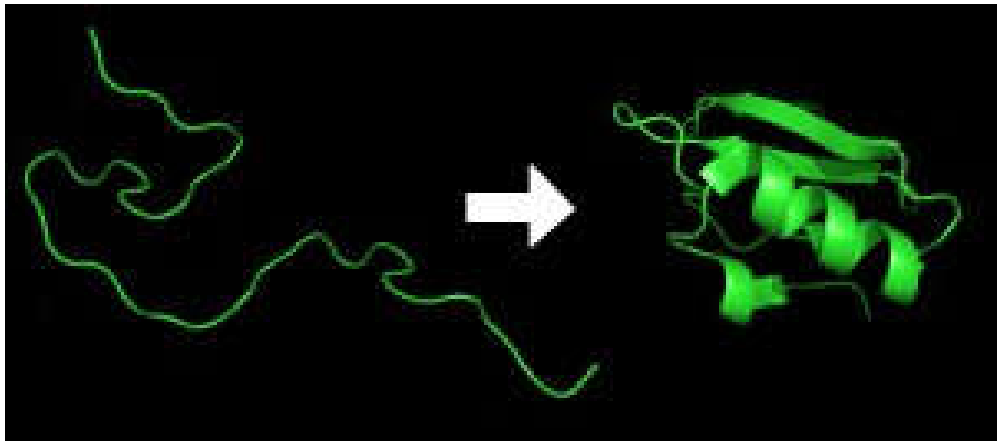
Saccharomyces cerevisiae como um modelo para estudos de *proteostasis* e neurodegeneação

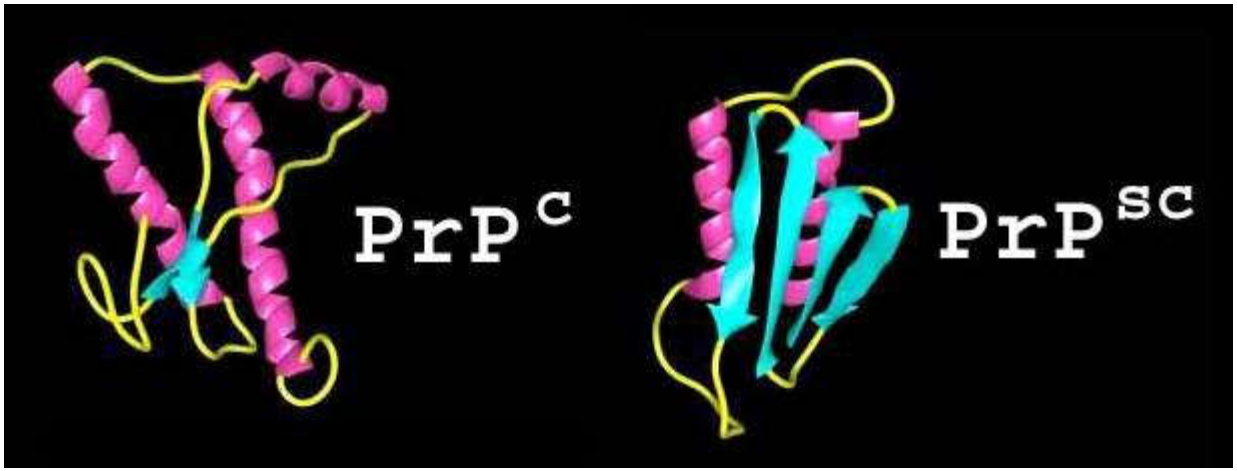

Lipid biology
Vesicular trafficking and fusion
Protein folding, quality control and degradation
Hsp90
Hsp90
Hsp70
Sti1
Pplase
Lysosomal and peroxisomal function
•Chaperones
•Protein-remodeling factors
•Osmolytes
•Proteasome, Ubiquitin, Ub ligases
Autophagy
G
α
βγ
GTP
GDP
Signal Transduction
Calcium/Calmoldulin
GPCRs
Calcineurin
MAP Kinases, etc.
Apoptosis
Mitochondria and oxidative stress
M
G2
G1
G0
S
Cell Cycle

Protein Folding and Neurodegeneration



A simple exemple of a folding event





Geral mechanism of protein aggregation

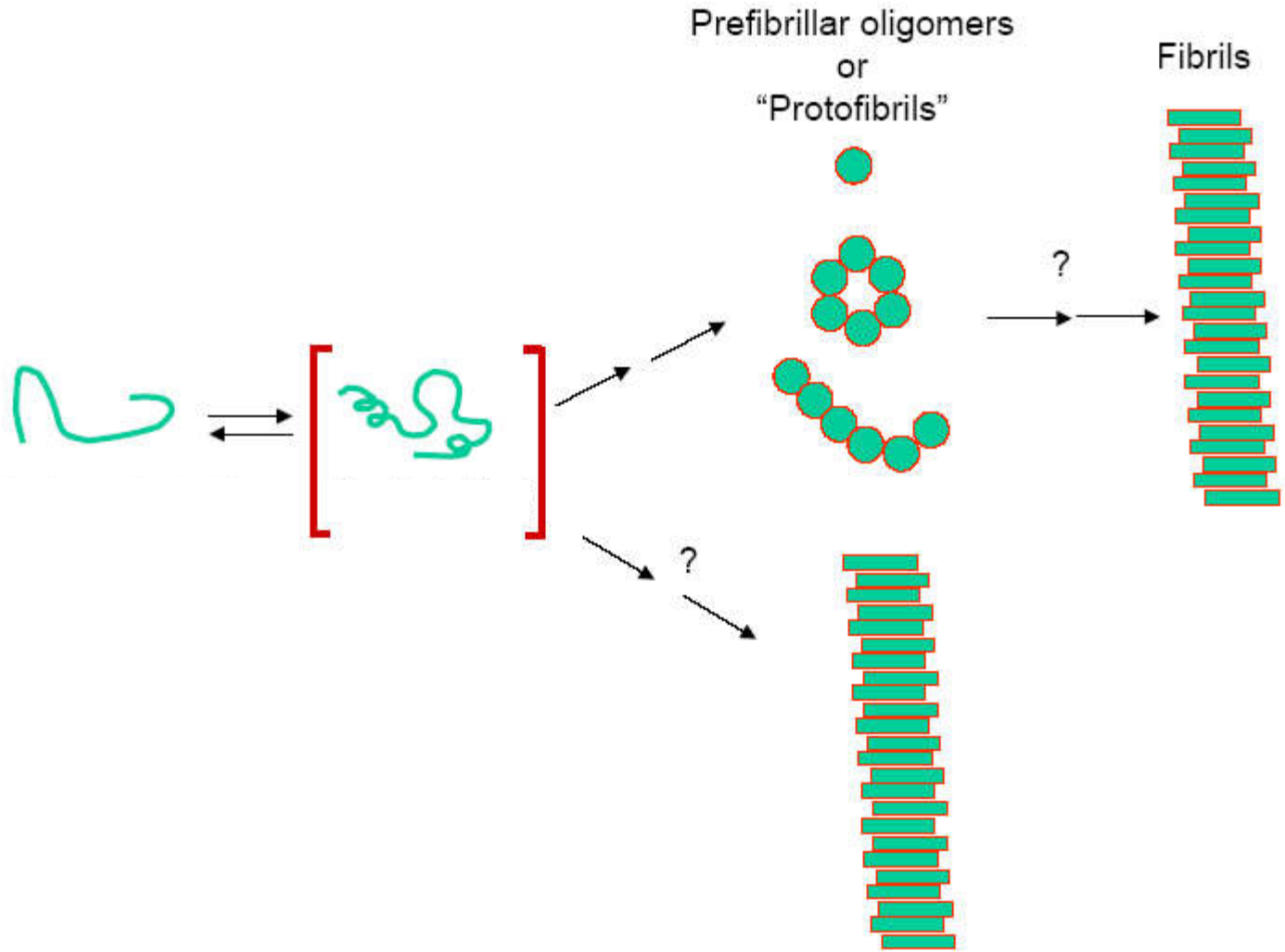
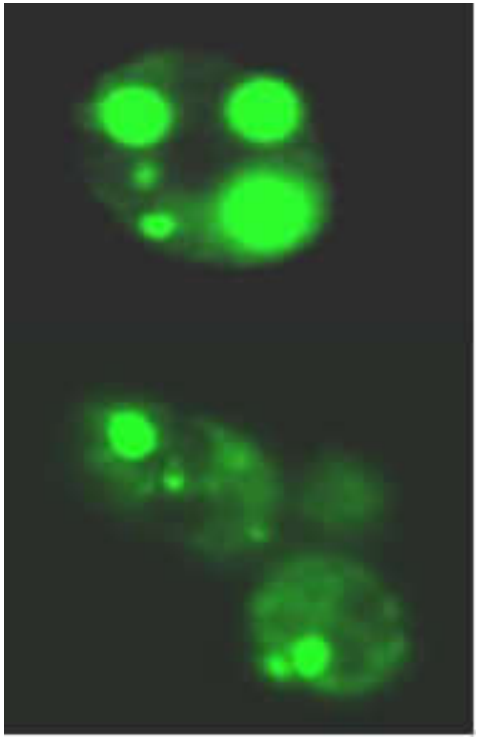
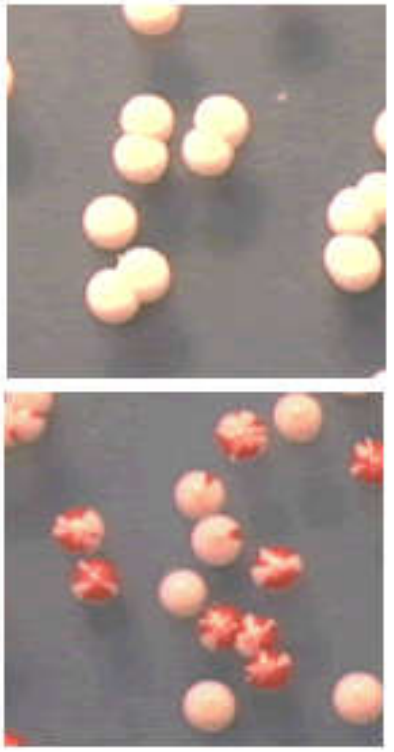


Table 1 Glossary

Prion protein	Any polypeptide that, in addition to its normal conformation (which is typically soluble), can access at least one conformation (which is typically β-sheet rich and insoluble) that is self-perpetuating and infectious.
Amyloid	A highly stable structure composed of many protein monomers arranged into β-sheet–rich fibrils such that the β-strands from different monomers stack perpendicularly to the fibril axis.
Prion strains (variants)	Distinct prion diseases or phenotypes that are caused by unique β-sheet–rich conformations of infectious prion proteins with identical amino acid sequence.
Prion species barriers	A phrase describing the inefficient transmission of infectious prions between different species.
Templating	The process by which infectious prions catalyze the conformational change of proteins (that are typically identical in amino acid sequence) from their soluble, non-prion conformation to their insoluble, prion conformation.

Prions - *S. cerevisiae*





Fator [psi]
Brian Cox

-[URE3]

HSPs
Susan Lindquist

Reed B. Wickner,

3 Critérios genéticos para definição de prions
(Distinguindo de ac. Nucleícos , plasmídios ou vírus)

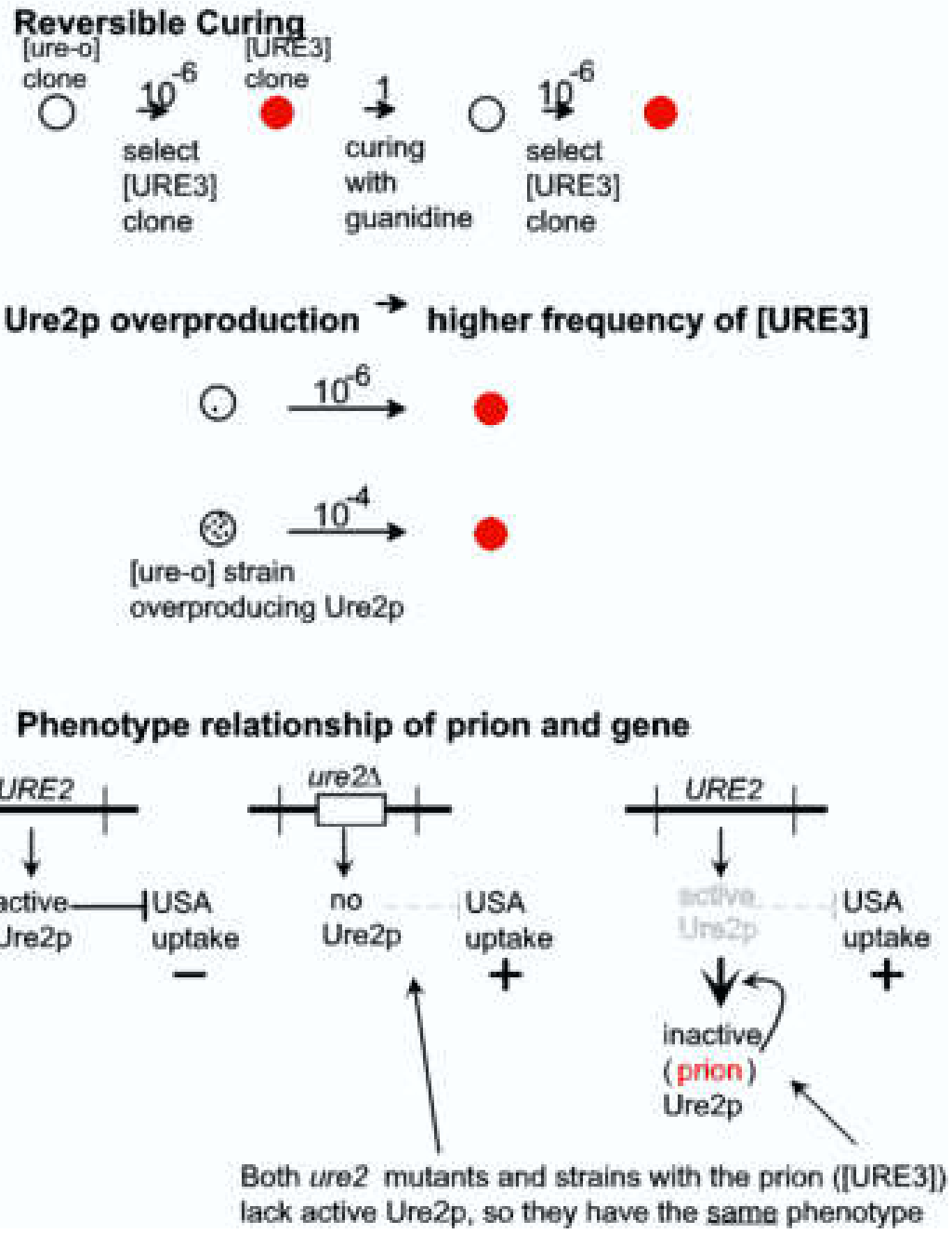
1 – Cura reversível: Um prion pode ser “curado” e pode surgir espontaneamente novamente. Uma vez “curado” vírus ou plasmídios não surgem “*de novo*”.

2 –Super produção da proteína deve aumentar o frequência na qual o prion surge.

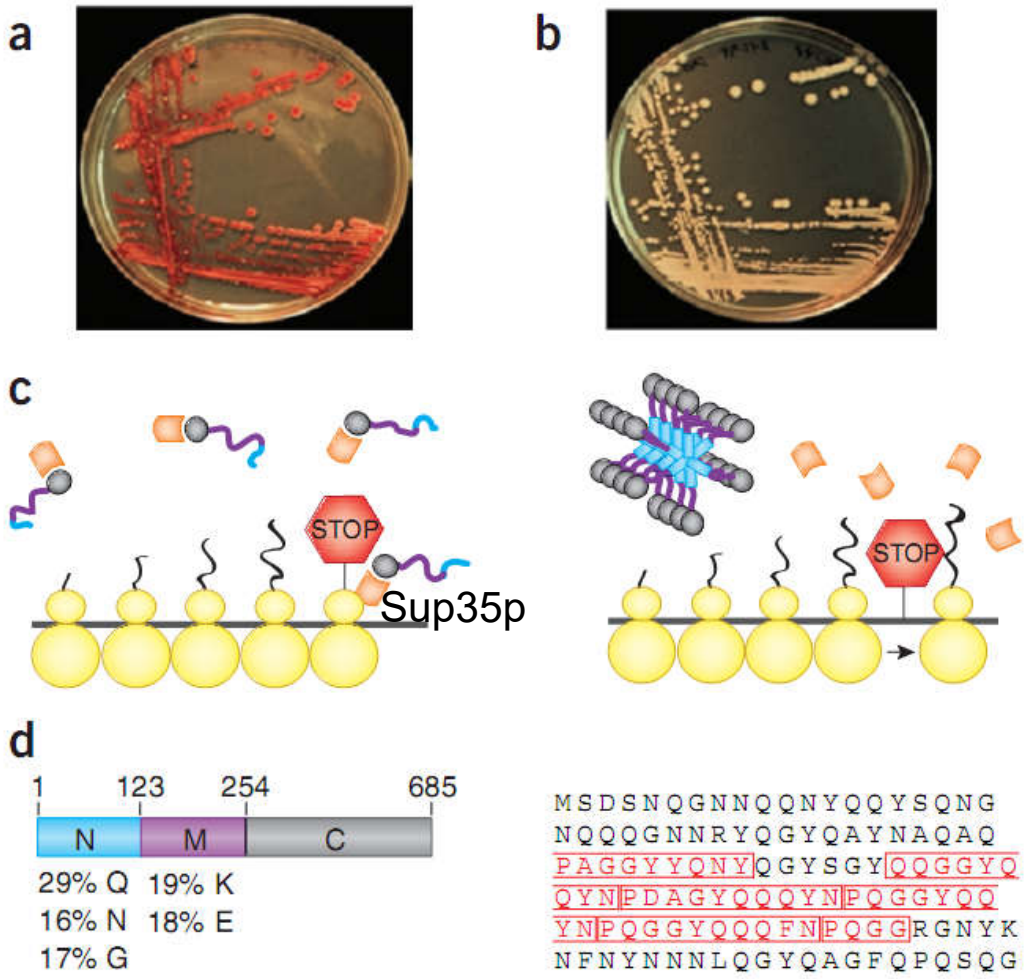
3- O fenótipo produzido pelo prion deve sr o mesmo fenótipo produzido por uma mutação no gene codificante para a respectiva proteína, pois em ambos os casos a forma natural da proteína não está presente.

Genetic properties of a prion

Wickner, RB (1994) *Science* **264**:566-9

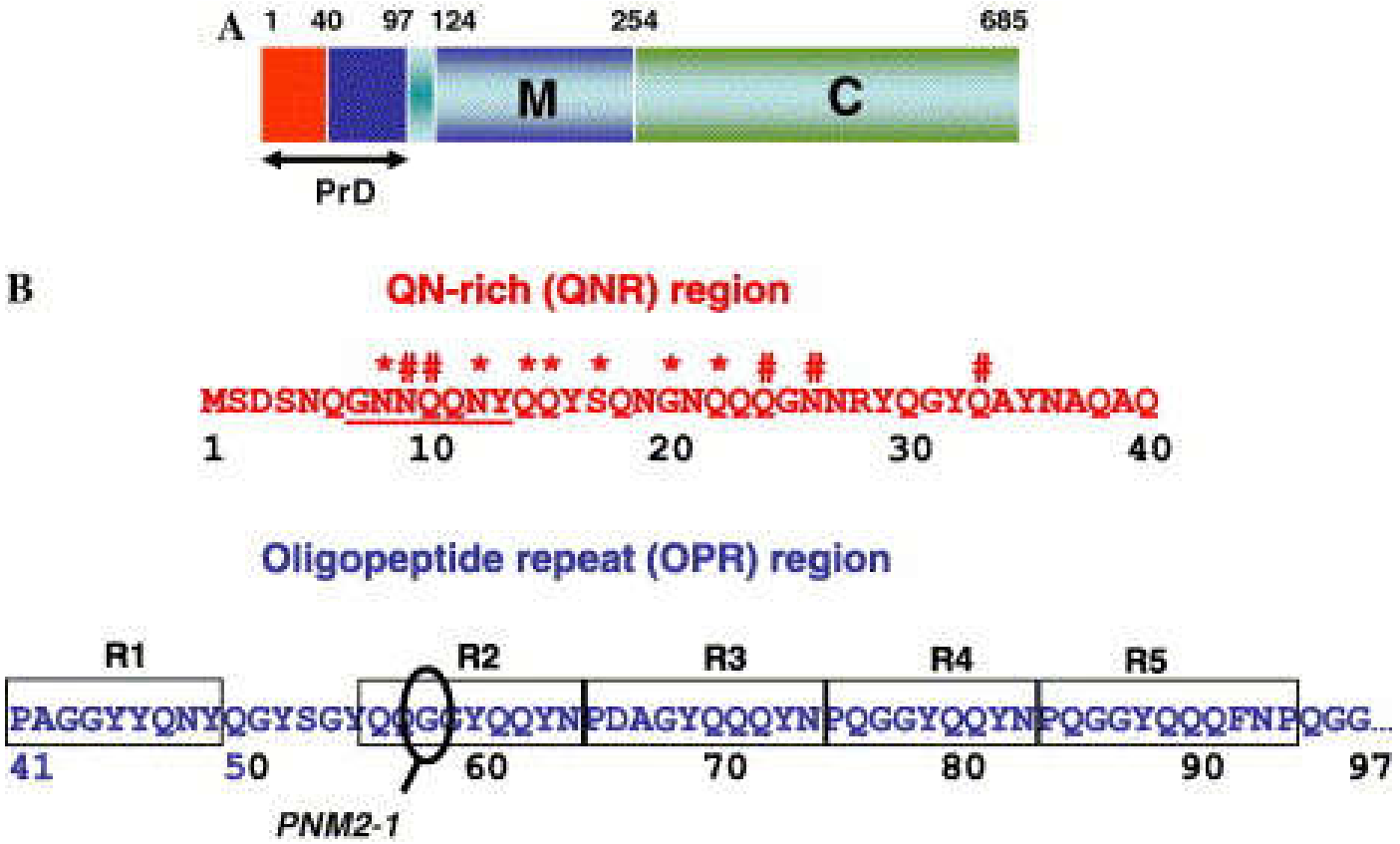


Fator psi→ 1º prion de levedura a ser descrito



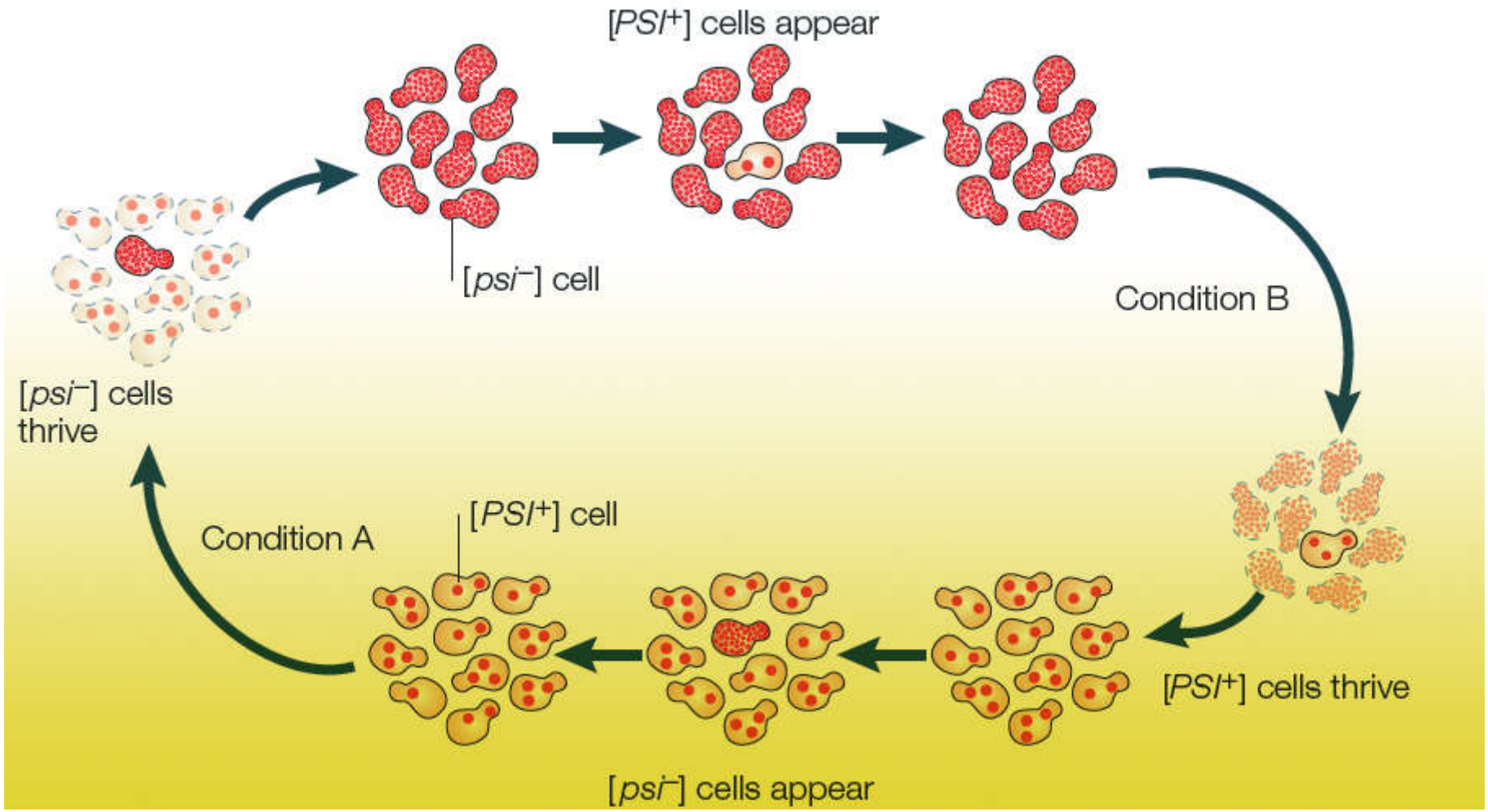
volume 16 number 6 june 2009 nature structural & molecular biology

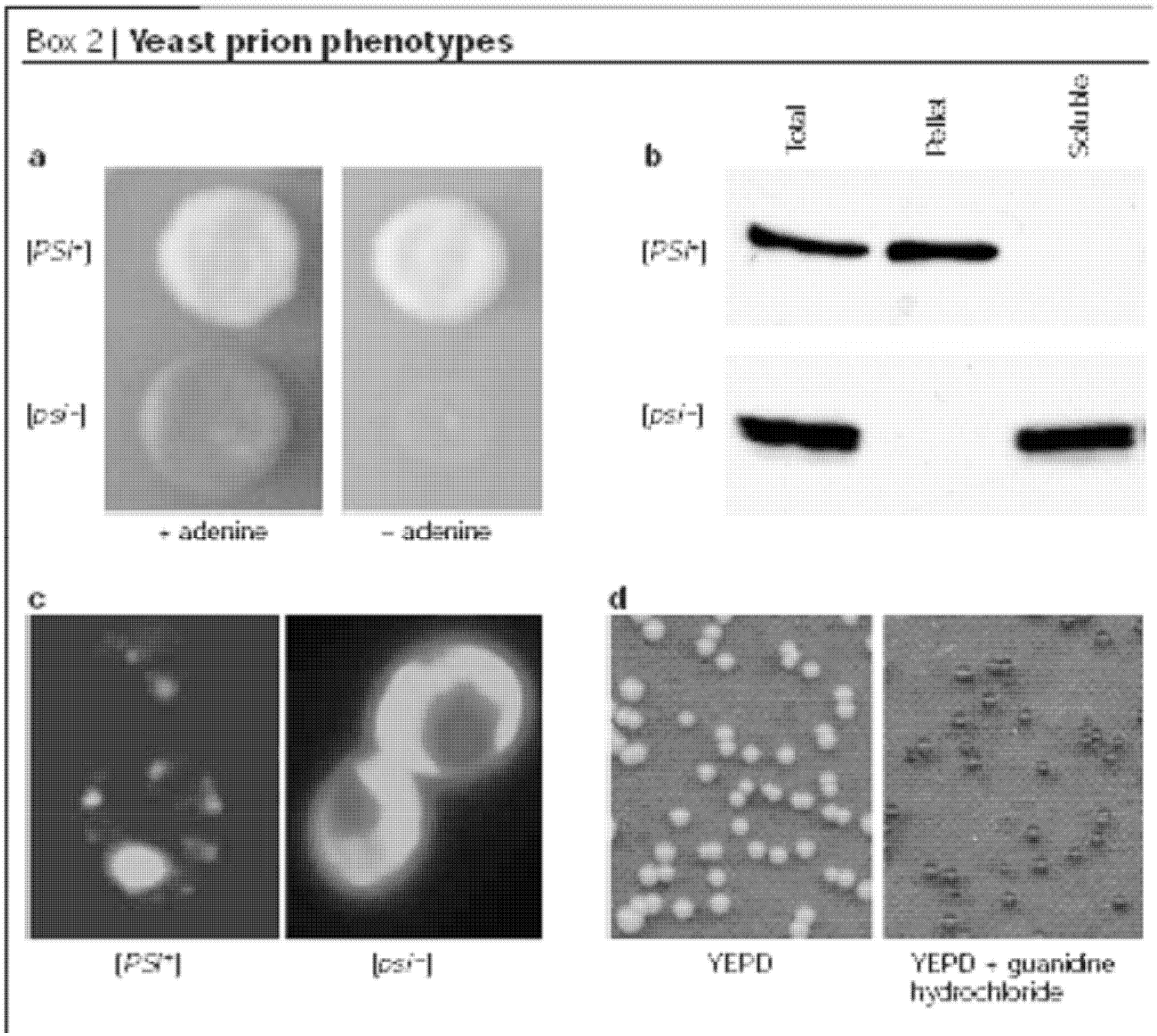
prion conversion domain:



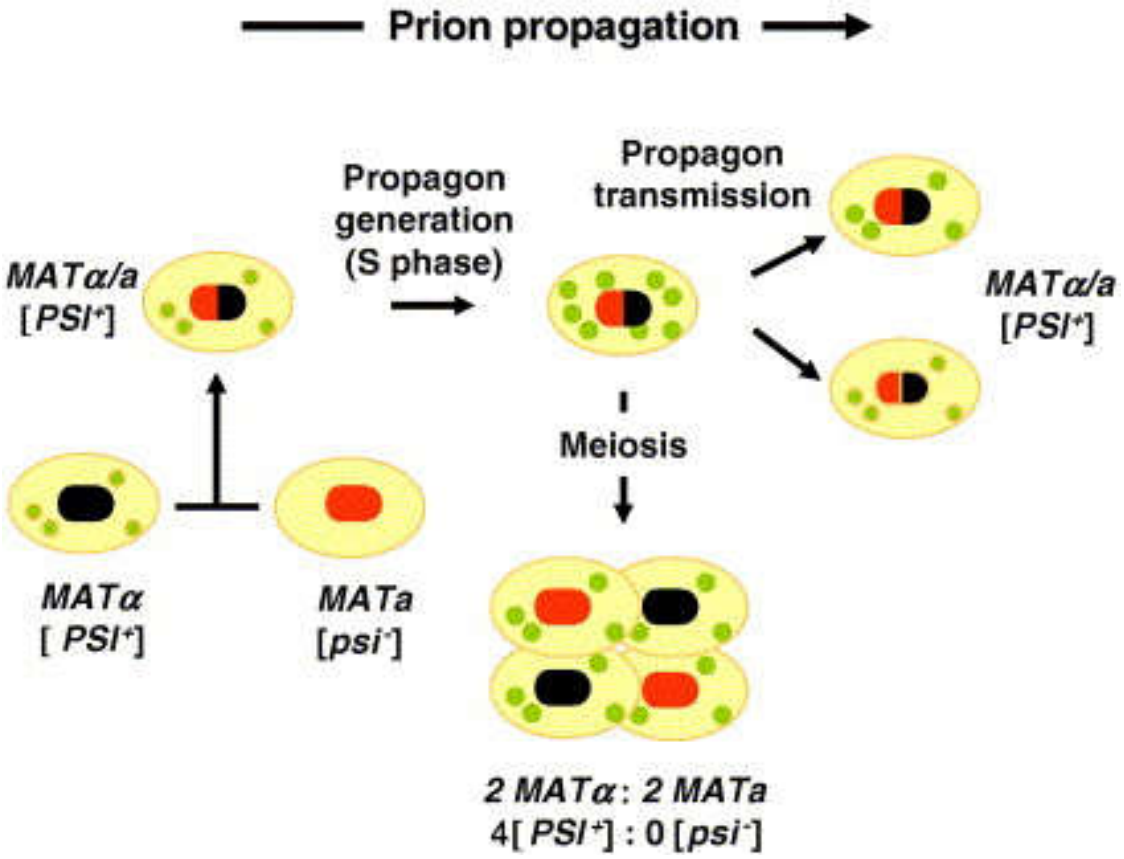
Methods
Volume 39, Issue 1, May 2006, Pages 9-22

Fator Psi - super supressor - mascara mais de 150 fenótipos - Herança Epigenética.





Nature Reviews Molecular Cell Biology 4, 878-890



Methods
Volume 39, Issue 1, May 2006, Pages 9-22

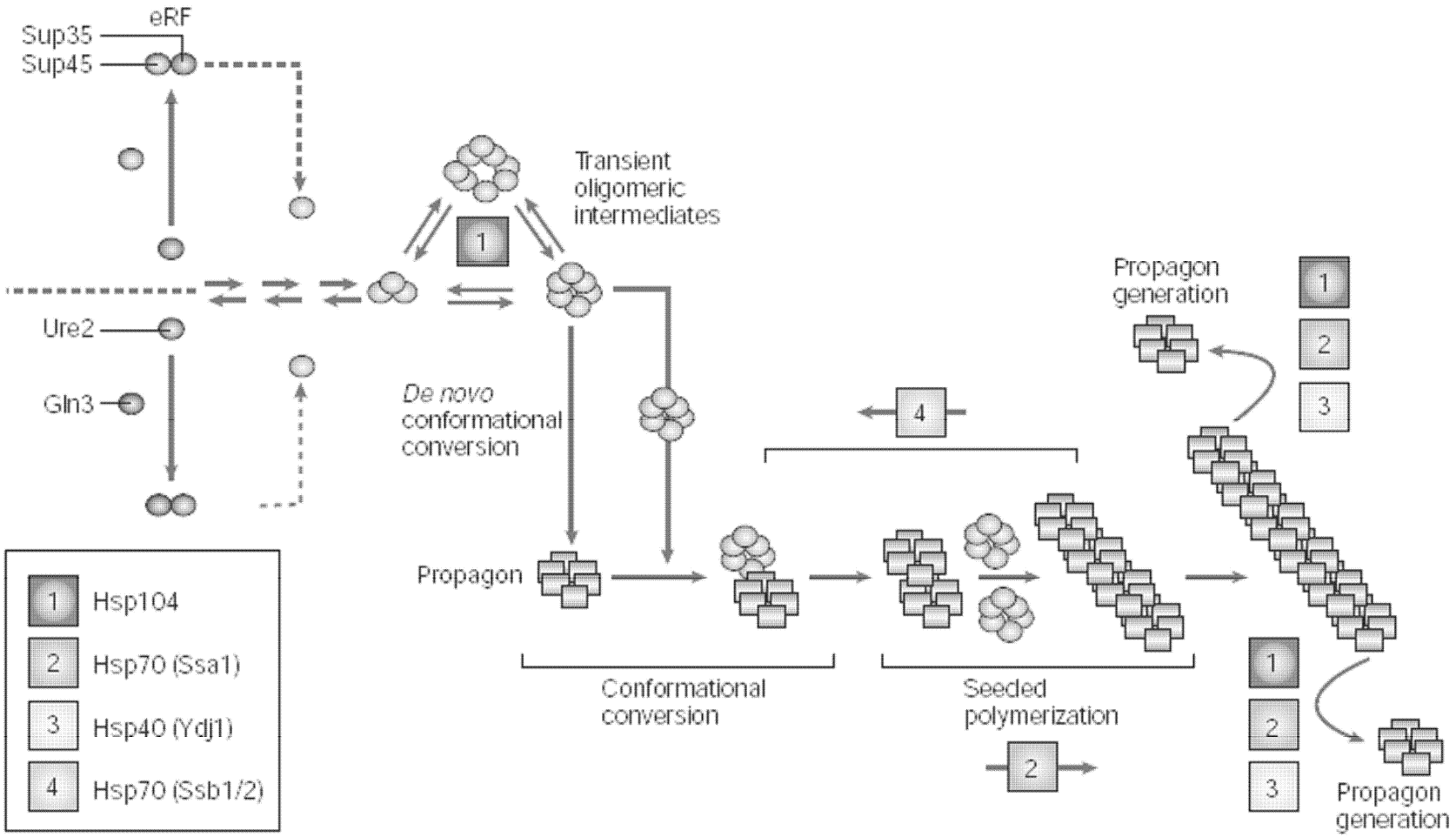


Figure 4 | **Model for yeast prion conversion and propagation?** A prion protein can switch to its prion state (orange circles)

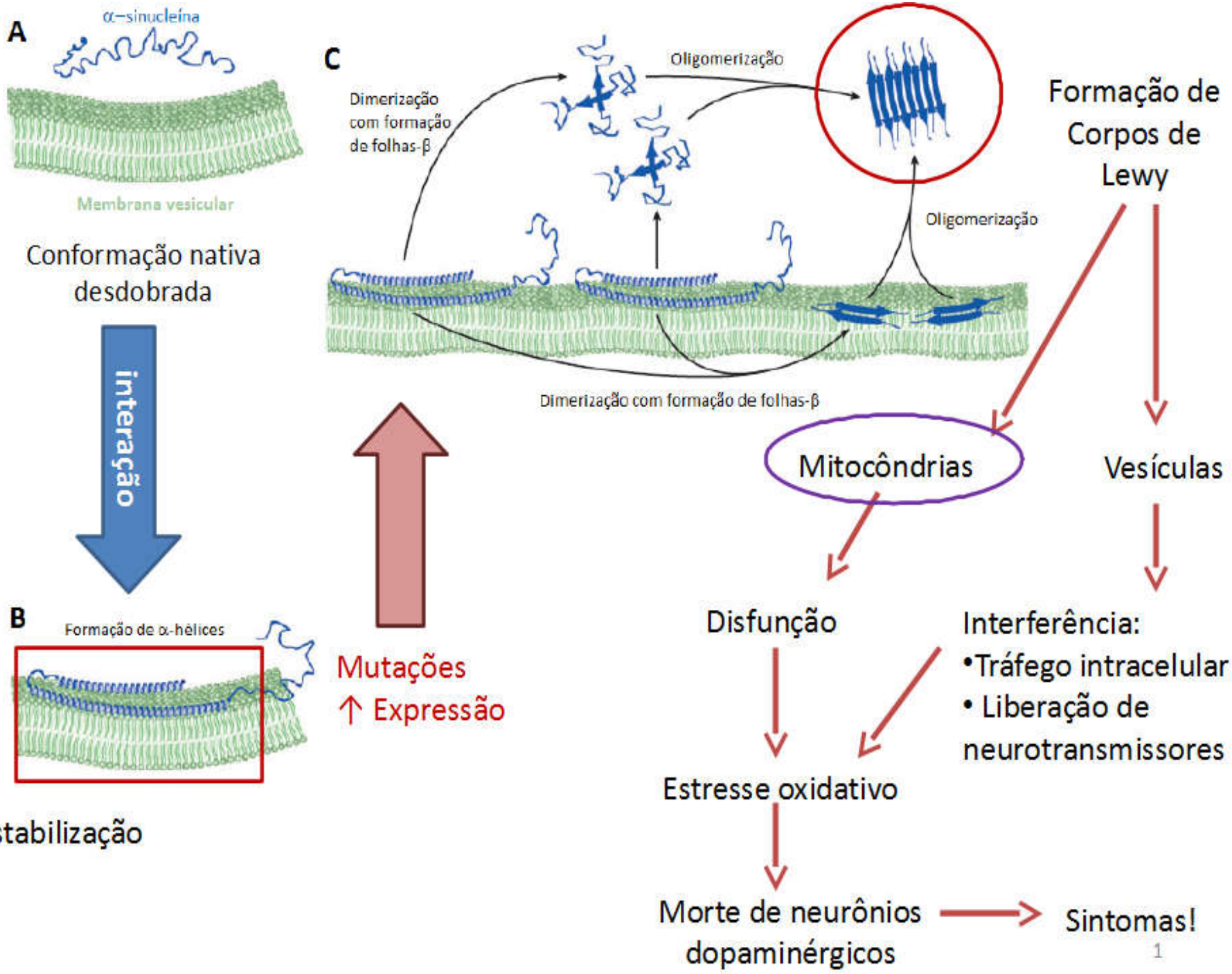
Nature Reviews Molecular Cell Biology 4, 878-890

Susan Lindquist

Leveduras: tubos de ensiaos para estudos de *proteostasis*.

→ Kit genético sem rivais

→Melhor amigo do homem.

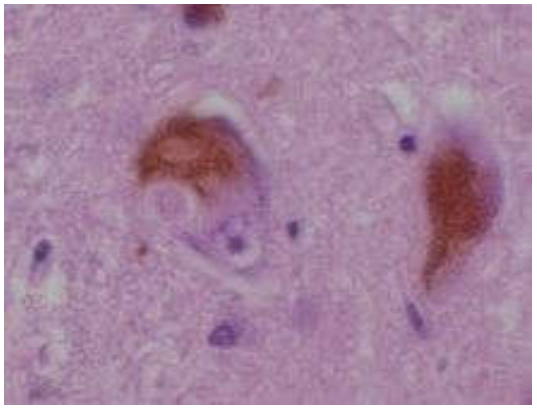

A
α–sinucleína
Membrana vesicular
Conformação nativa desdobrada
interação
B
Formação de α-hélices
↑Estabilização
Mutações
↑ Expressão
C
Oligomerização
Dimerização com formação de folhas-β
Oligomerização
Dimerização com formação de folhas-β
Formação de Corpos de Lewy
Mitocôndrias
Vesículas
Disfunção
Interferência:
•Tráfego intracelular
• Liberação de neurotransmissores
Estresse oxidativo
Morte de neurônios dopaminérgicos
Sintomas!

Synocleionopathies

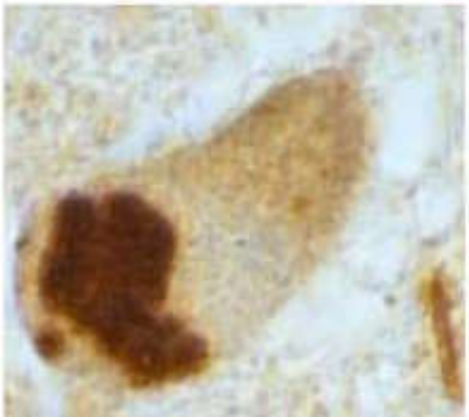
Parkinson's disease

Dementia with Lewy bodies

Multiple system atrophy (MSA)

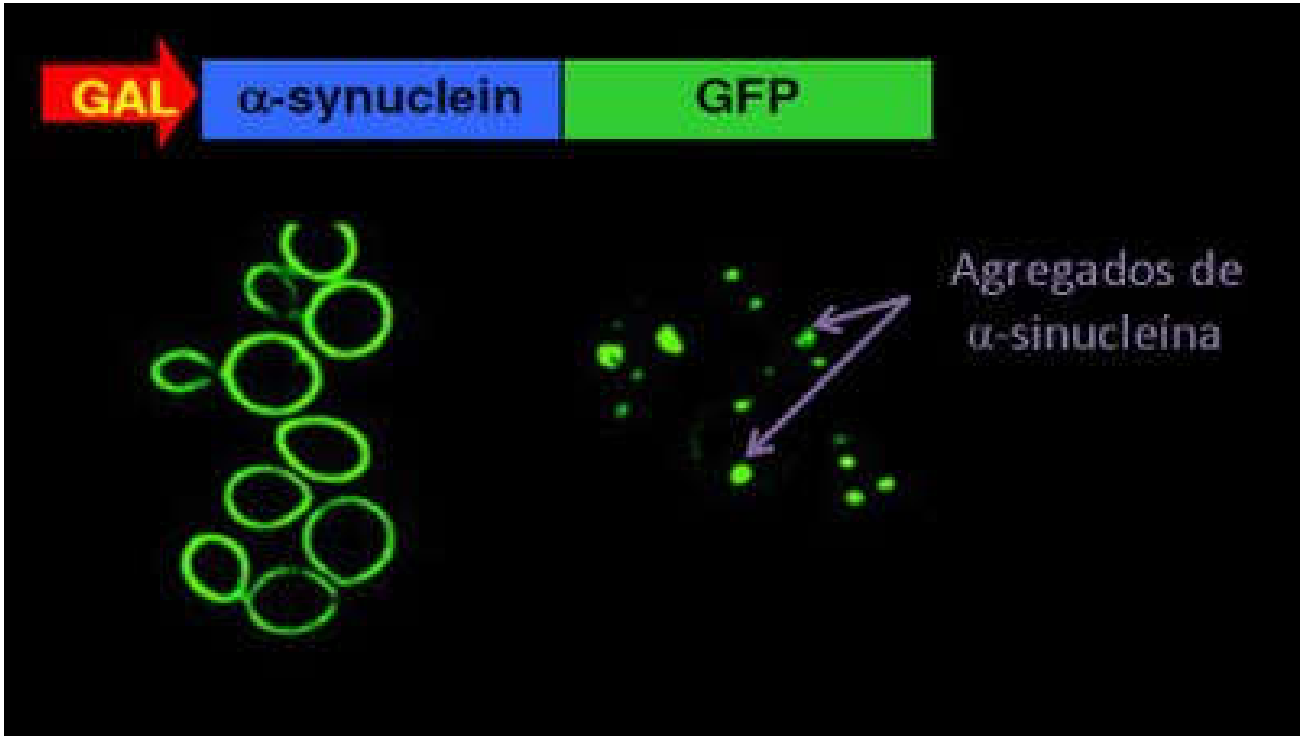


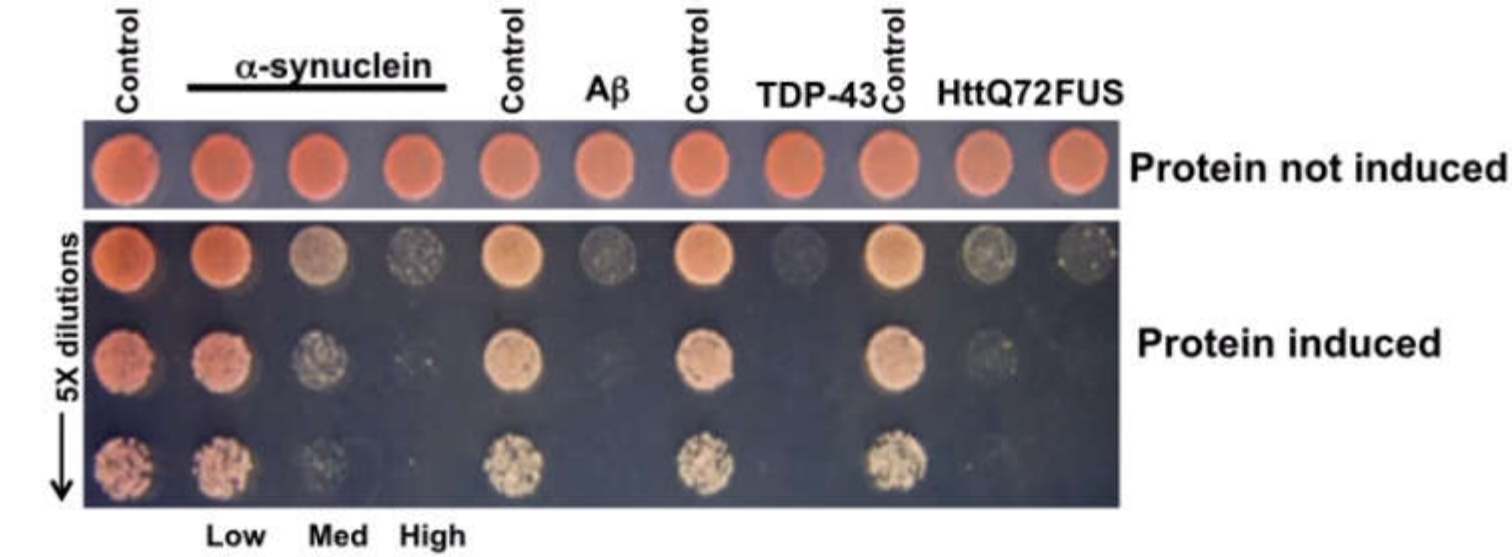
Lewy bodies

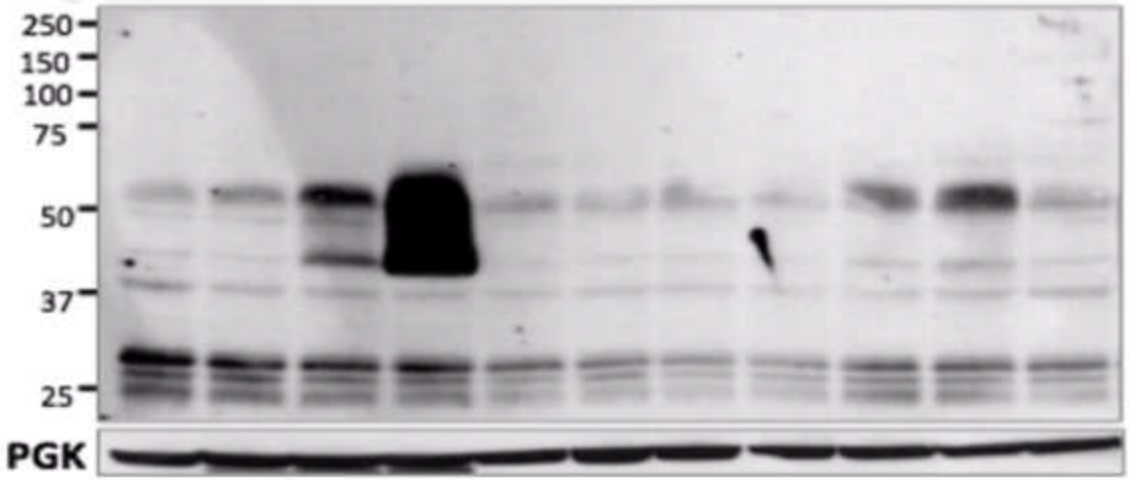


Glial cytoplasmic inclusions (GCI) bodies

Expression of α-synuclein in yeast cells

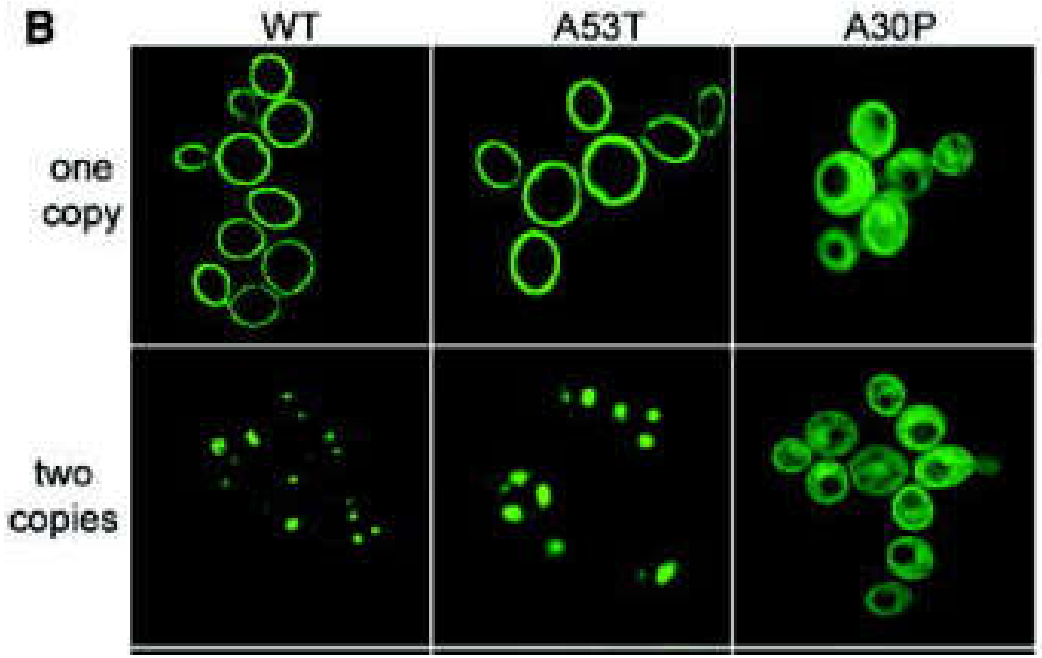






Unique and specific cellular pathologies *directly* related to human disease.

Replicates dosage-toxicity in man



Science. Dec 5, 2003; 302(5651): 1772–1775.

Search for genetic interactors

→ Plasmid over-expression:

Toxicity enhancer
Toxicity suppressor

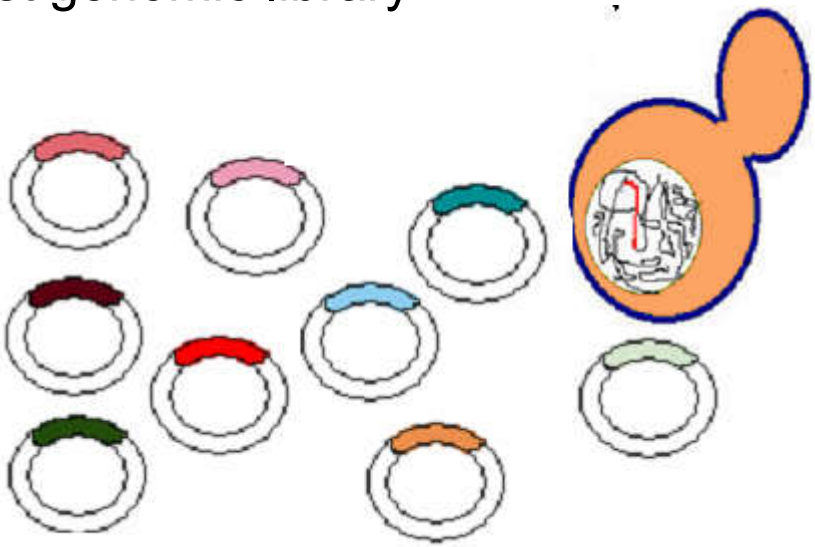
→Gene deletion:

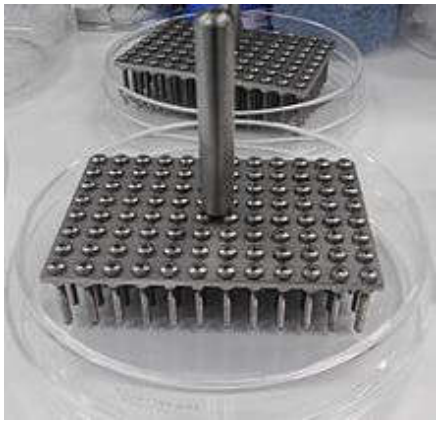
Toxicity enhancer
Toxicity suppressor

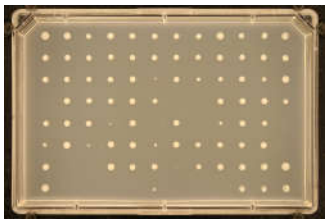
Search for genetic interactors

Yeast genomic library

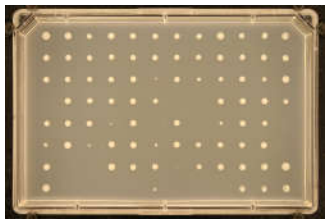
Yeast expressing α-syn







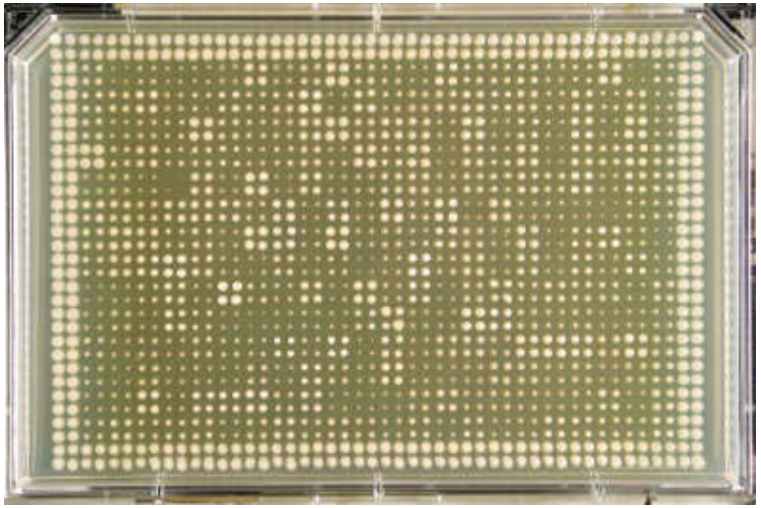
glucose



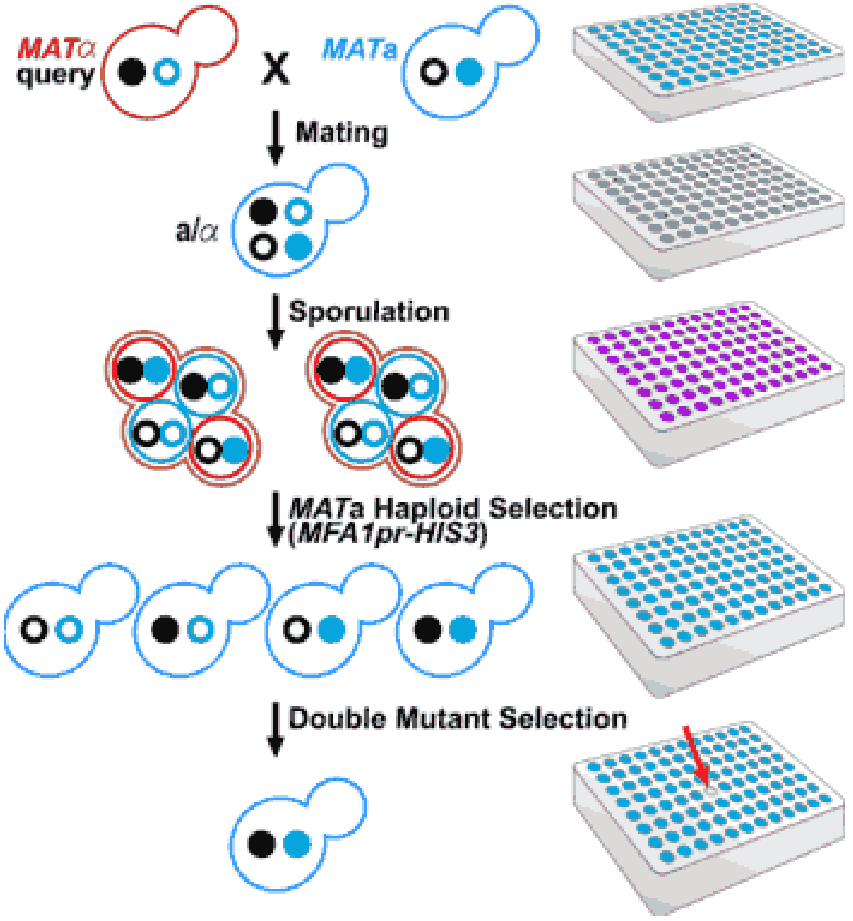
galactose

Search for genetic interactors

Effect of specific gene deletion in α-syn toxicity



Mapping Synthetic Lethality using Synthetic Genetic Array (SGA) Analysis



Interactors involved in

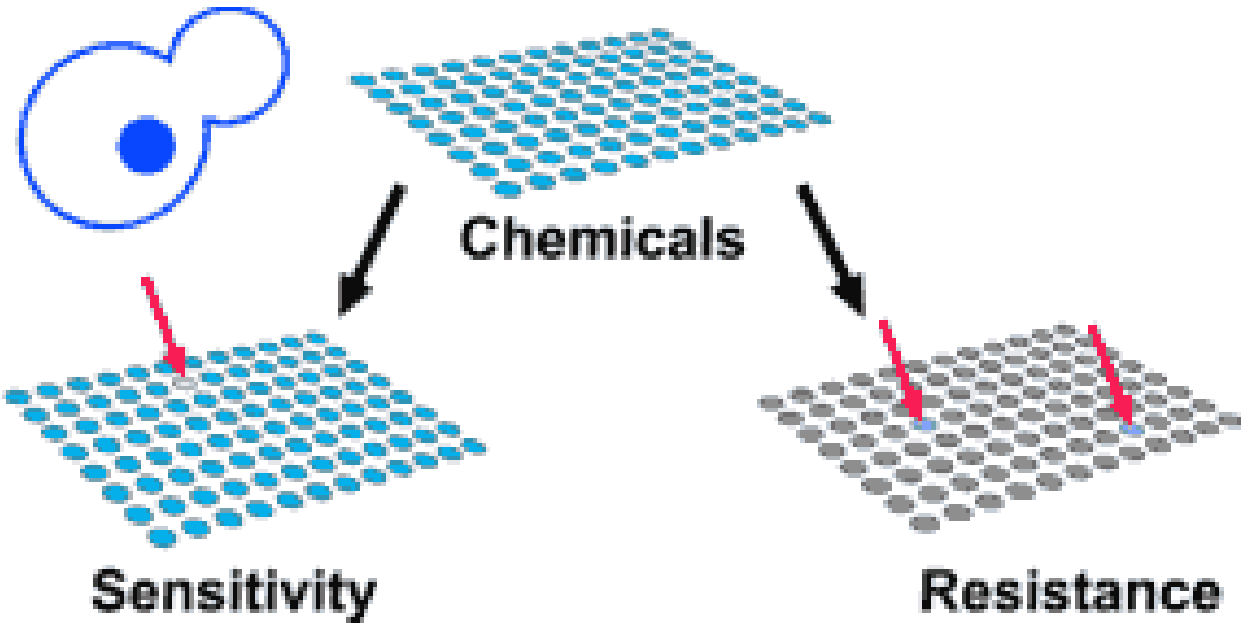
ER→ Golgi vesicle trafficing

Lysossomes

Mitochondria oxidative damages

Search for new drugs

Yeast expressing α-syn

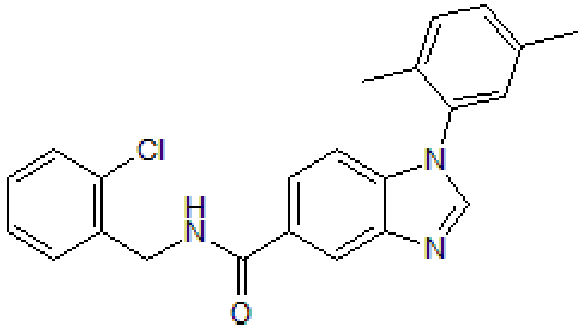


In 550.000 library compound

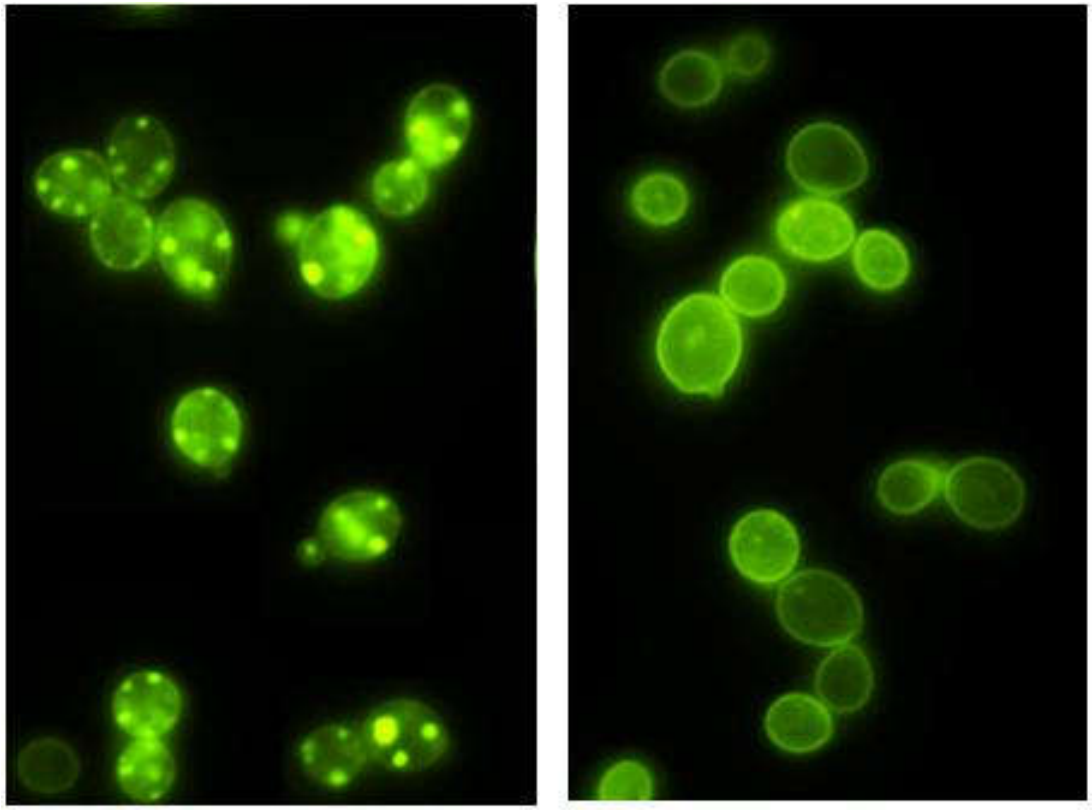
→Fix trafficking deffect

→ Fix mitochondrial damages

→ Work in nematodes – rats - neurons



N-[(2-Chlorophenyl)methyl]-1-(2,5-dimethylphenyl)-1*H*-benzimidazole-5-carboxamide



Untreated

Treated

Genetic and chemical screening

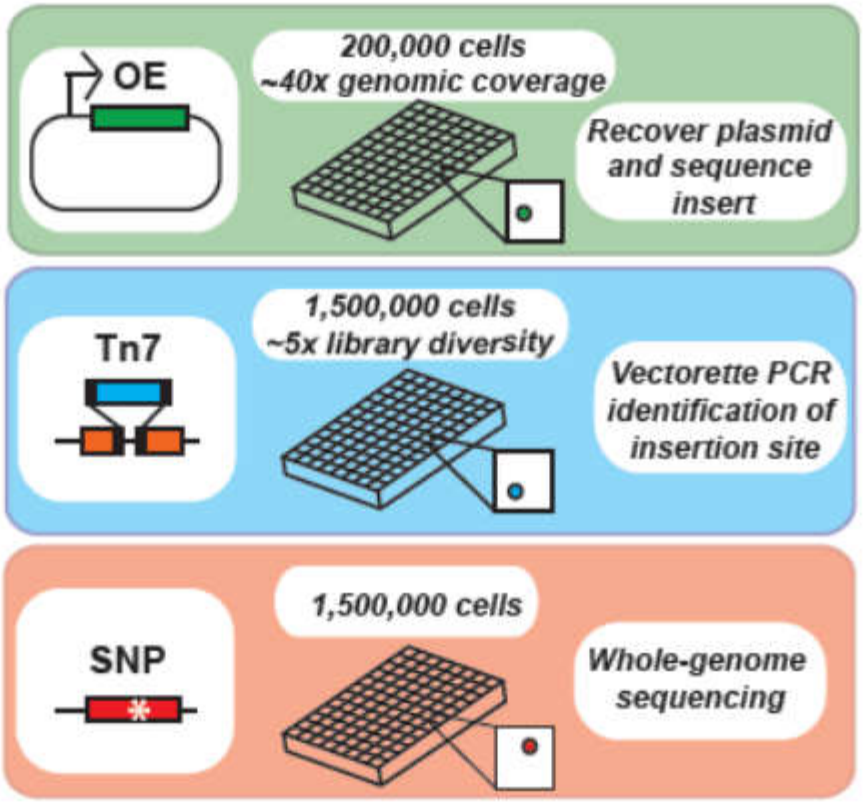
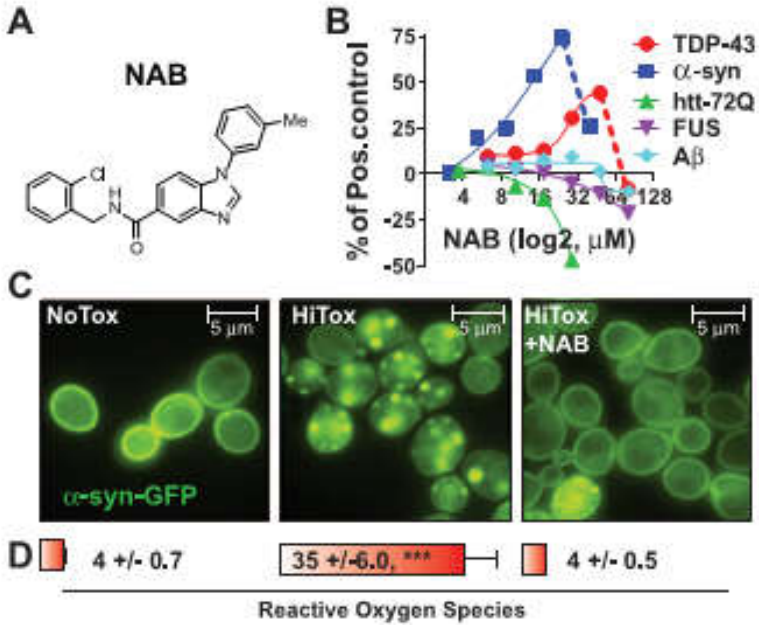


Fig. S4

Summary of three chemical genetic screens. Overexpression (green), transposon insertion (blue) and spontaneous NAB-resistant mutant selections were performed using fully inhibitory concentrations of NAB2 (40 μM). Multiple fold coverage of each library was used to ensure full sampling of their diversity. The nature of each screen hit was identified using the indicated approaches. Due to the stringent conditions of the screen, nearly all arising colonies were successfully, independently verified, both as screen isolates, and subsequently in de novo genetic alterations.

Yeast Reveal a "Druggable" Rsp5/Nedd4 Network that Ameliorates α-Synuclein Toxicity in Neurons

Daniel F. Tardiff,[1] Nathan T. Jui,[2] Vikram Khurana,[1,3] Mitali A. Tambe,[4] Michelle L. Thompson,[5]* Chee Yeun Chung,[1] Hari B. Kamadurai,[6] Hyoung Tae Kim,[7] Alex K. Lancaster,[1]† Kim A. Caldwell,[5] Guy A. Caldwell,[5] Jean-Christophe Rochet,[4] Stephen L. Buchwald,[2] Susan Lindquist[1,8]‡



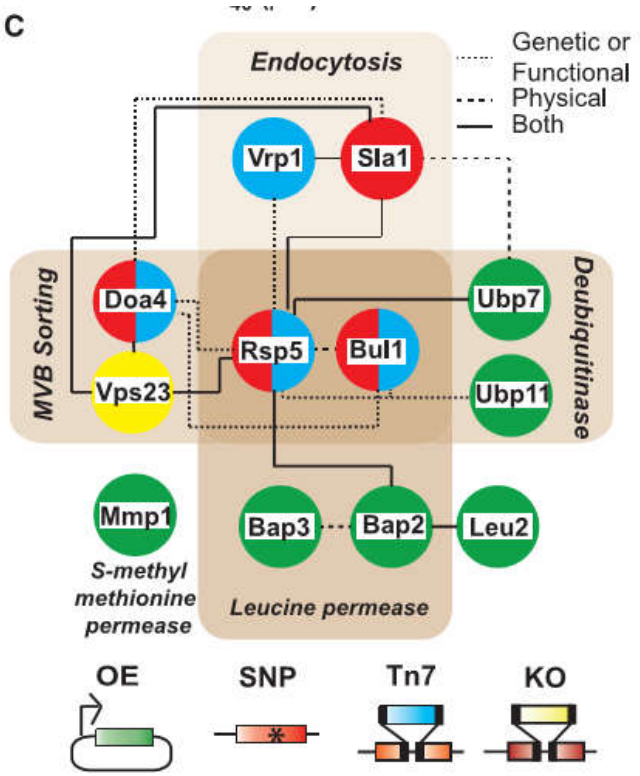


Fig. 2. Chemical genetic screens of NAB2 reveal a network centere the E3 ligase, Rsp5. (**A**) Efficacy (EC_{40}) in α-syn cells versus growth bition (IC_{40}) in WT cells for active analogs. NAB1 is the screen hit, and NA the most potent analog. (**B**) Viable cells recovered after prolonged N treatment. (**C**) NAB2 interaction network. Node color reflects screen of o indicated below. Edges are interactions (legend, top right) according to S database and literature. *VPS23* was deleted after identification of other

NAB2 helps Rsp5 E3 ubiquitin-ligase in the formation of endossomic vesicles

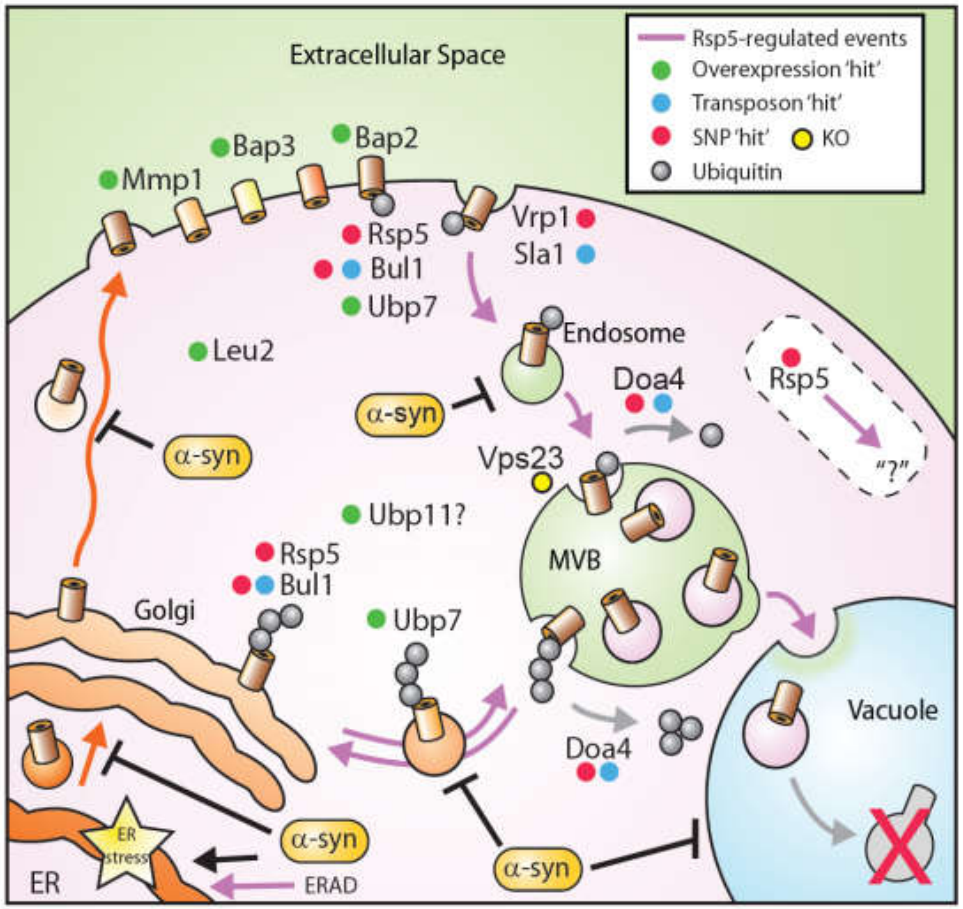


Fig. S8

Cellular schematic of Rsp5-regulated and α-syn-inhibited processes. Rsp5 ubiquitinates diverse membrane proteins to promote endocytosis and endosomal transport to multivesicular bodies, eventually leading to their destruction in the vacuole/lysosome (see purple arrows) (*15*). Rsp5 also ubiquitinates proteins at the Golgi network, including

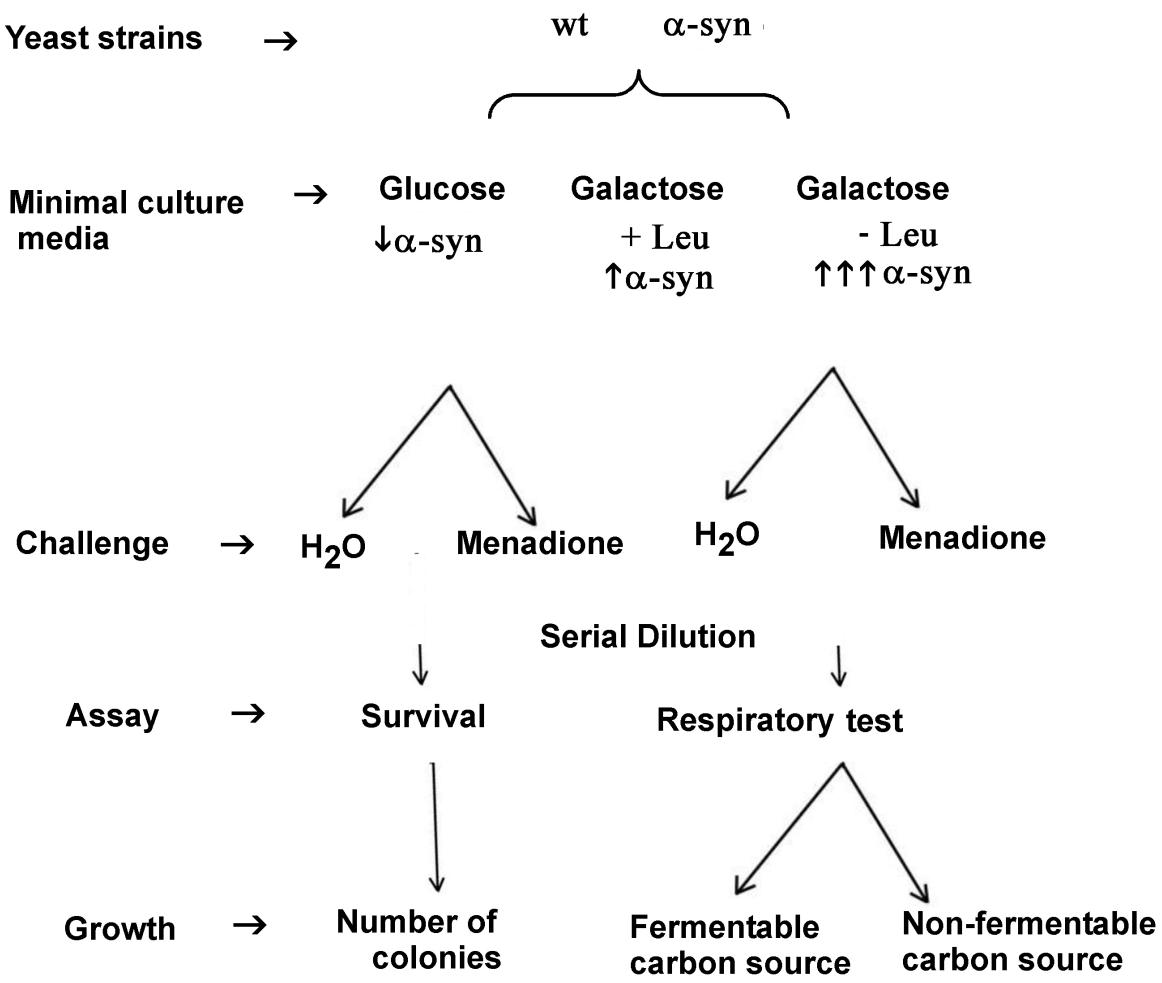
α-syn expression in yeast lead to

→Vesicle trafficking problems

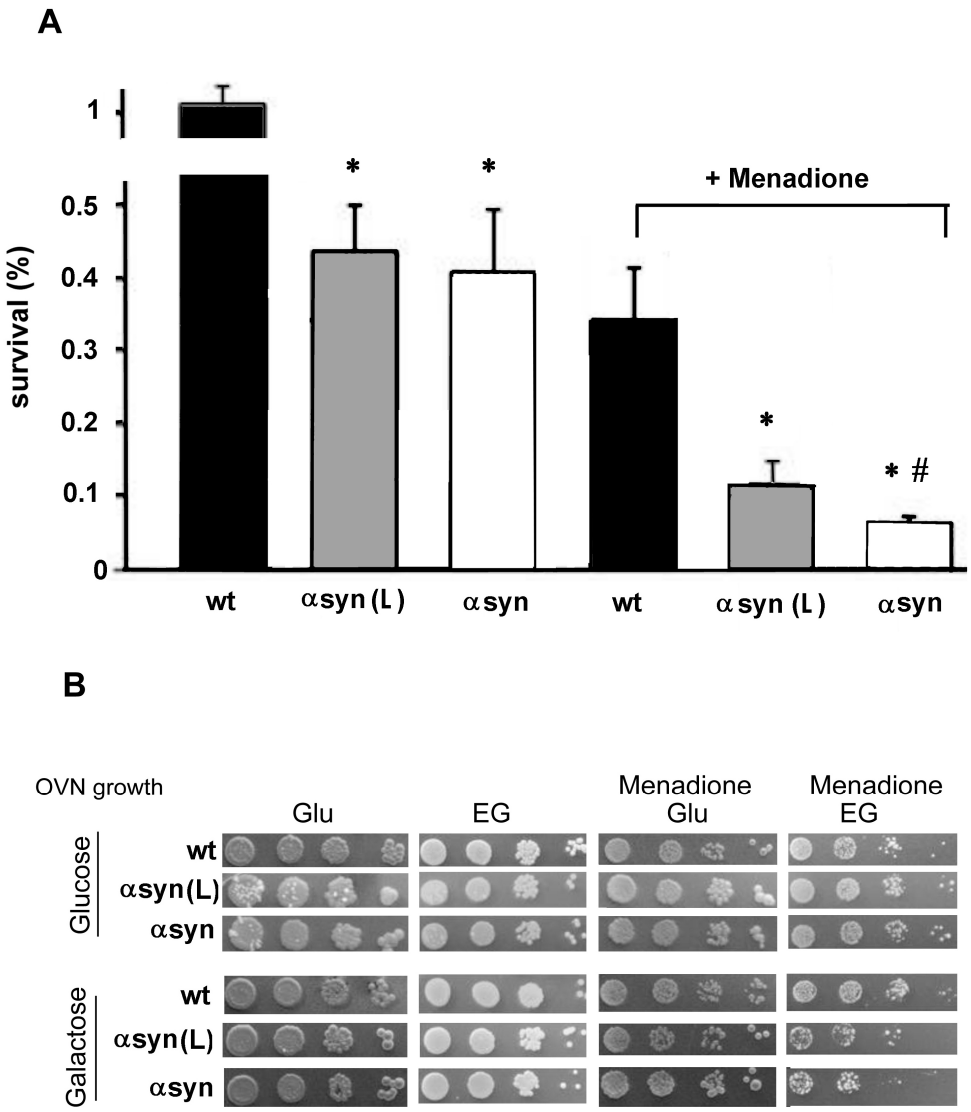
→Mitochondrial dysfuncion

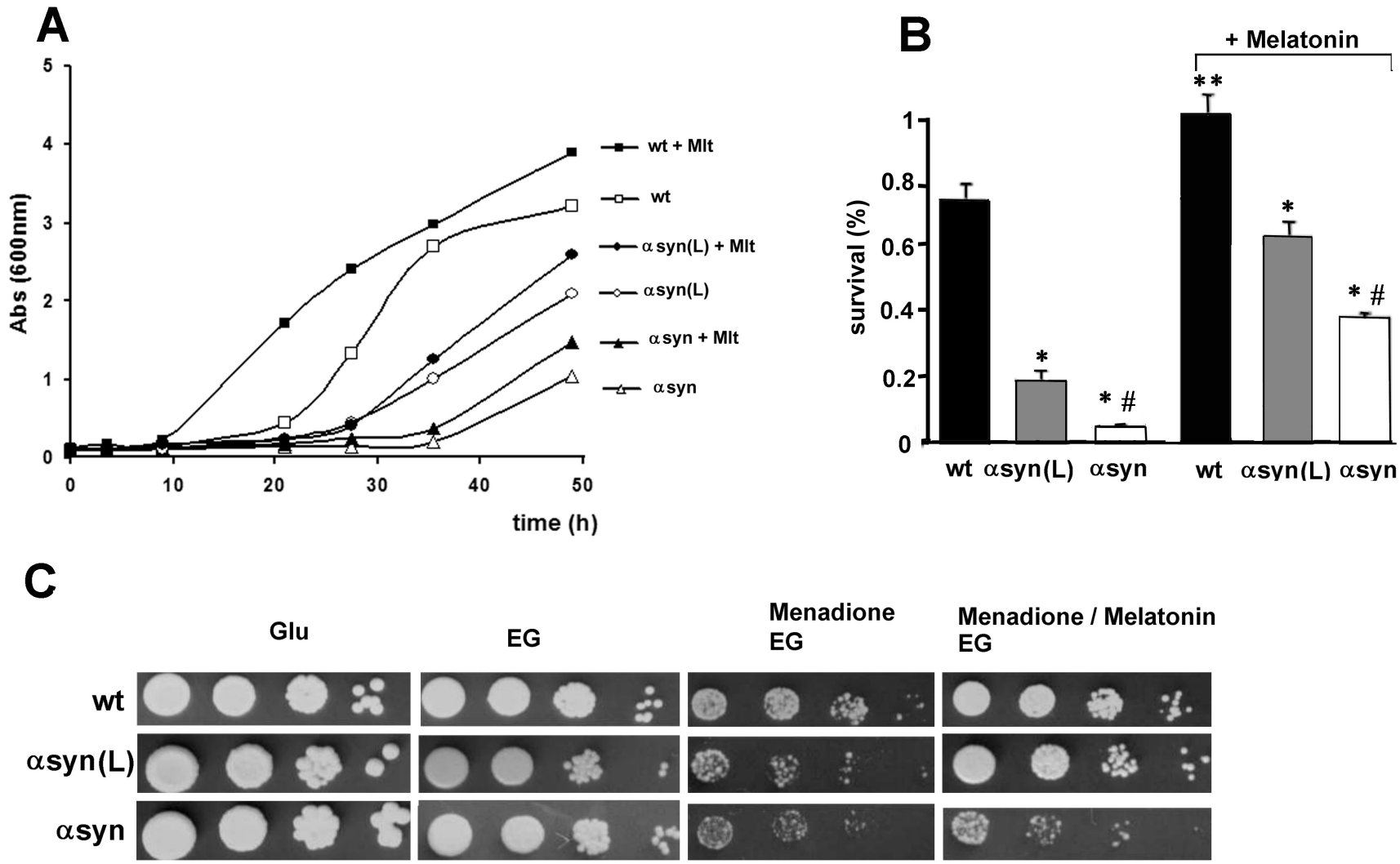
→Manganese homeostasis defects

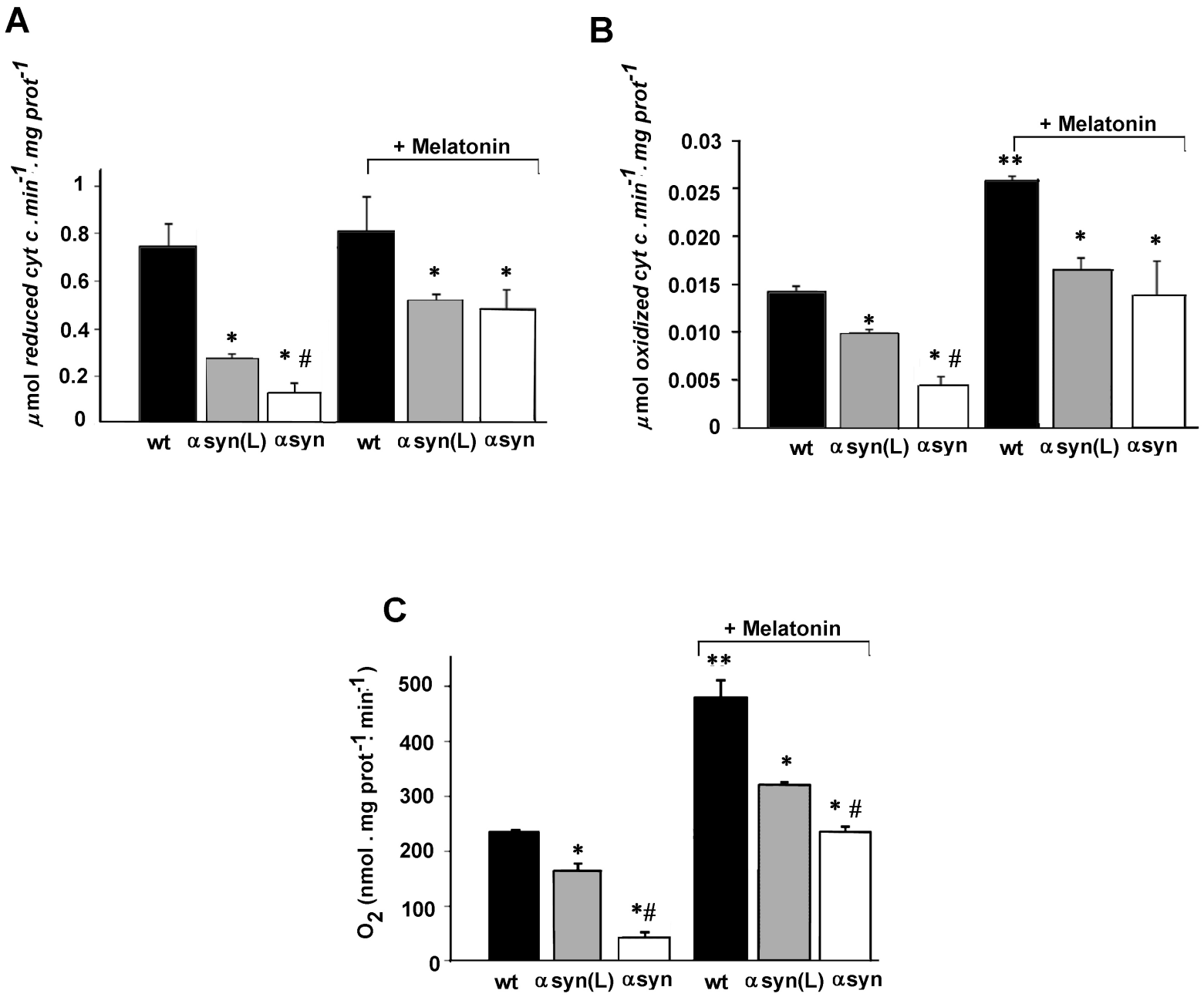
→Iron and Ca++ homeostasis

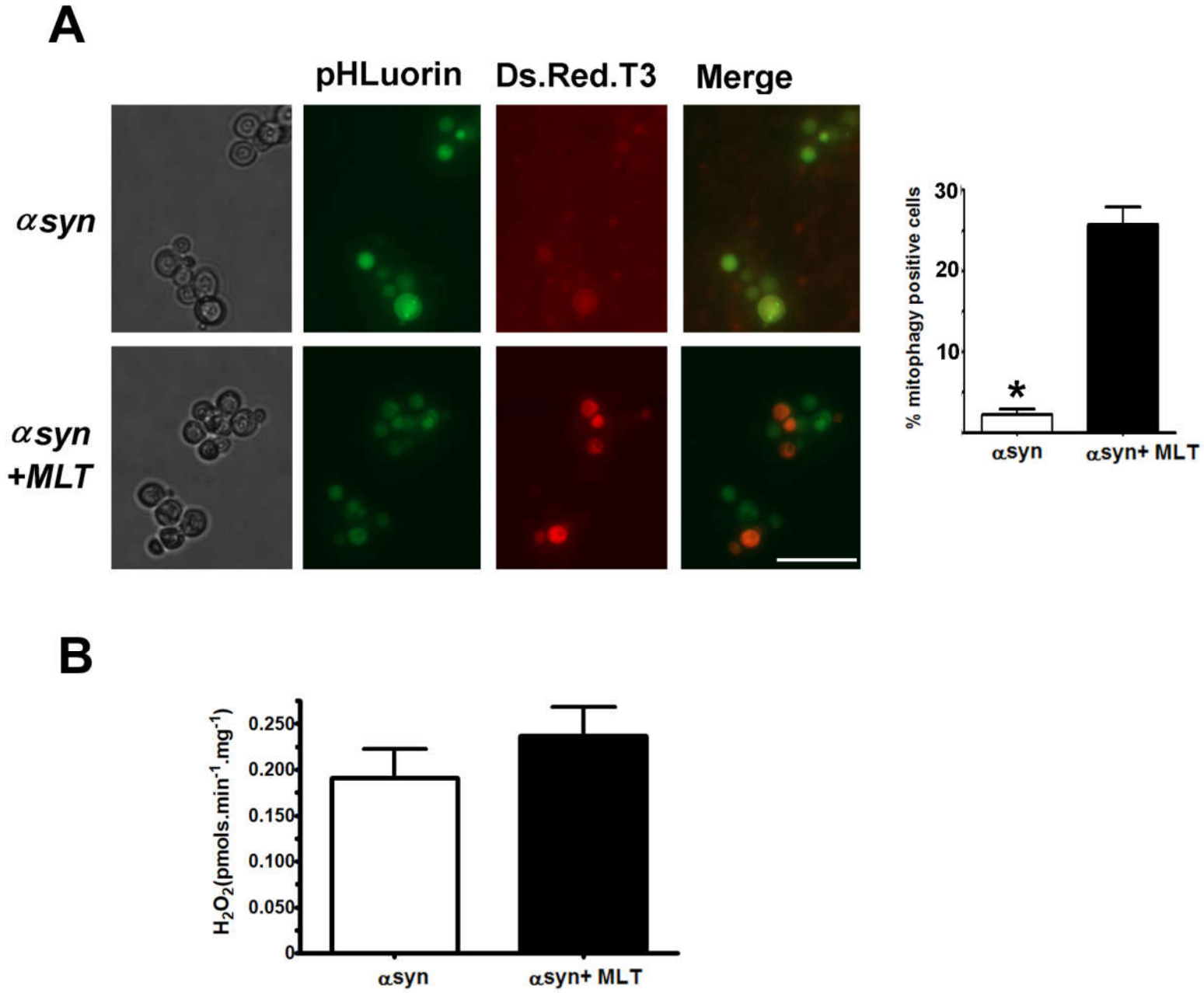


Mariana Zampol


A
survival (%)
1
0.5
0.4
0.3
0.2
0.1
0
*
*
+ Menadione
*
* #
wt
αsyn (L)
αsyn
wt
αsyn (L)
αsyn
B
OVN growth
Glu
EG
Menadione Glu
Menadione EG
Glucose
wt
αsyn(L)
αsyn
Galactose
wt
αsyn(L)
αsyn


A
Abs (600nm)
time (h)
wt + Mlt
wt
αsyn(L) + Mlt
αsyn(L)
αsyn + Mlt
αsyn
B
+ Melatonin
survival (%)
wt αsyn(L) αsyn wt αsyn(L) αsyn
C
Glu
EG
Menadione EG
Menadione / Melatonin EG
wt
αsyn(L)
αsyn


A
μmol reduced cyt c . min-1 . mg prot-1
1
0.8
0.6
0.4
0.2
0
+ Melatonin
*
* #
*
*
wt
αsyn(L)
αsyn
wt
αsyn(L)
αsyn
B
μmol oxidized cyt c . min-1 . mg prot-1
0.03
0.025
0.020
0.015
0.010
0.005
0
+ Melatonin
**
*
* #
*
*
wt
αsyn(L)
αsyn
wt
αsyn(L)
αsyn
C
O2 (nmol . mg prot-1 min-1)
500
400
300
200
100
0
+ Melatonin
**
*
*#
*
* #
wt
αsyn(L)
αsyn
wt
αsyn(L)
αsyn


A
pHLuorin
Ds.Red.T3
Merge
αsyn
αsyn +MLT
% mitophagy positive cells
30
20
10
*
αsyn
αsyn+ MLT
B
H2O2(pmols.min-1.mg-1)
0.250
0.200
0.150
0.100
0.050
0
αsyn
αsyn+ MLT