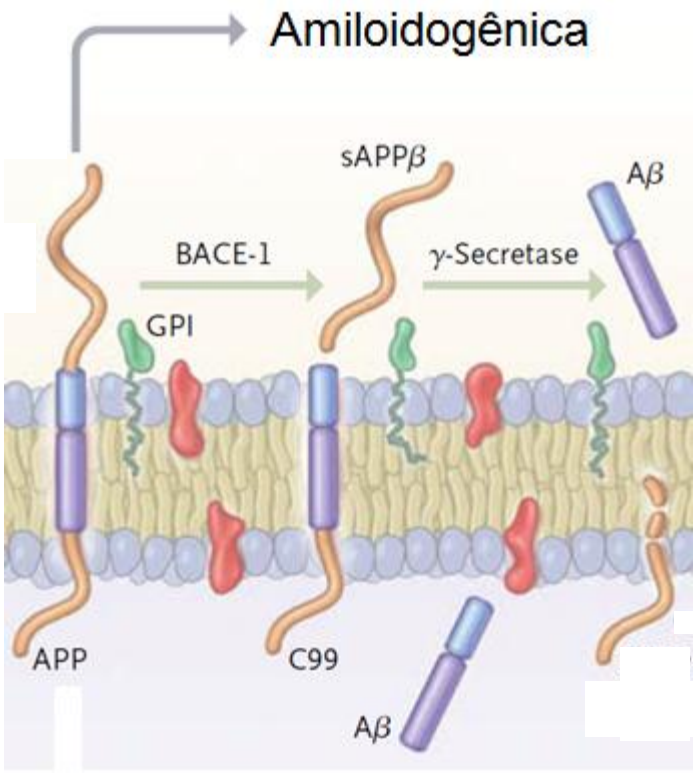
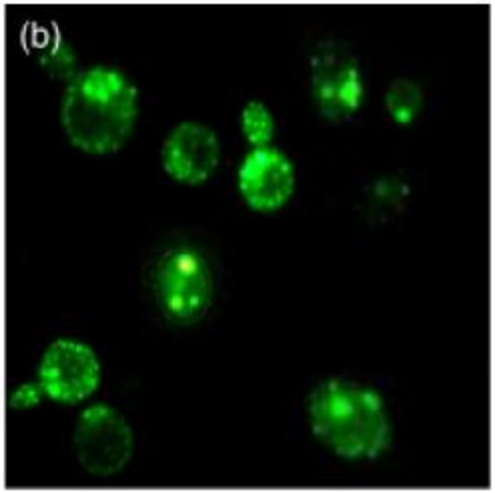
BMM5828/BTC5819 - Utilização de *Saccharomyces cerevisiae* como organismo modelo em biologia molecular

Saccharomyces cerevisiae como modelo de doenças neurodegenerativas





GFP-Aβ

ICB, 18/10/2019

Objetivos

1. Introduzir mecanismos moleculares da Doença de Alzheimer (agregação das proteínas βA42 e Tau);

2. Modelos para o estudo da doença de Alzheimer em levedura;

3. Introduzir conceitos sobre a Doença de Parkinson;

4. Modelos para o estudo da Doença de Parkinson em levedura;

5. Introduzir conceitos sobre a Doença de Huntington;

6. Modelos para o estudo da Doença de Huntington em levedura

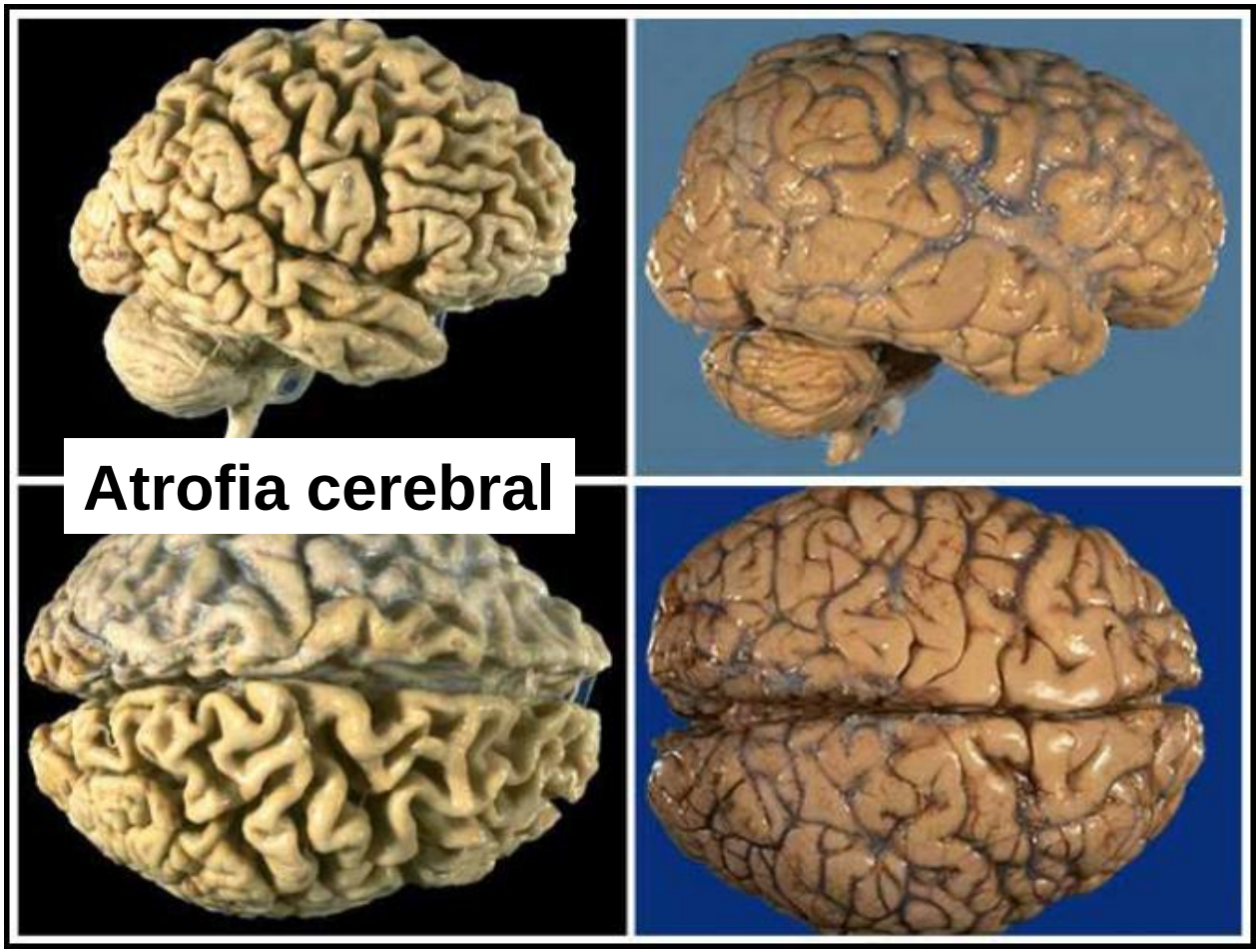
Mecanismos moleculares da Doença de Alzheimer (agregação das proteínas βA42 e Tau)

Critério clínico para diagnóstico da Doença de Alzheimer

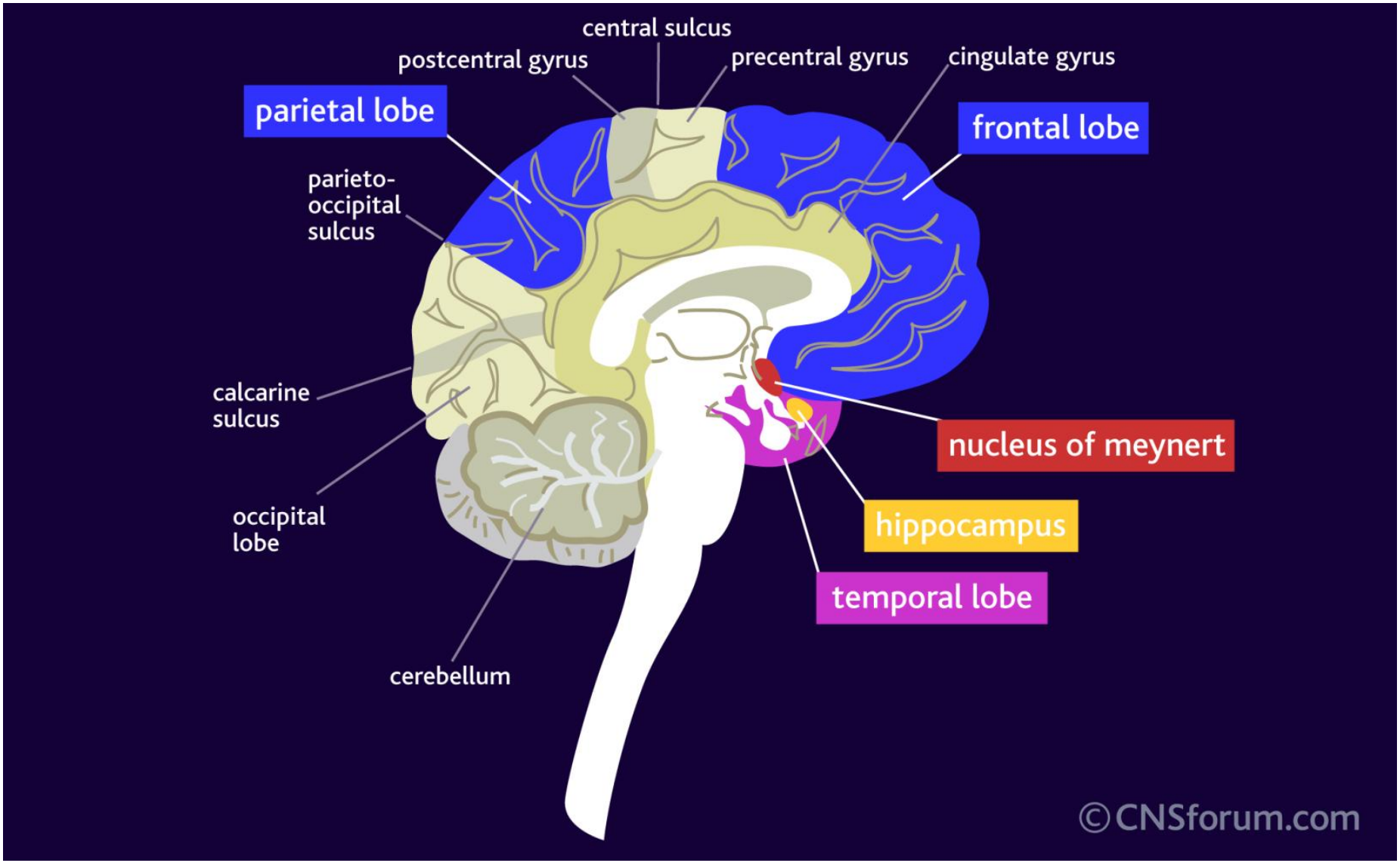
Declínio progressivo de funções cognitivas:

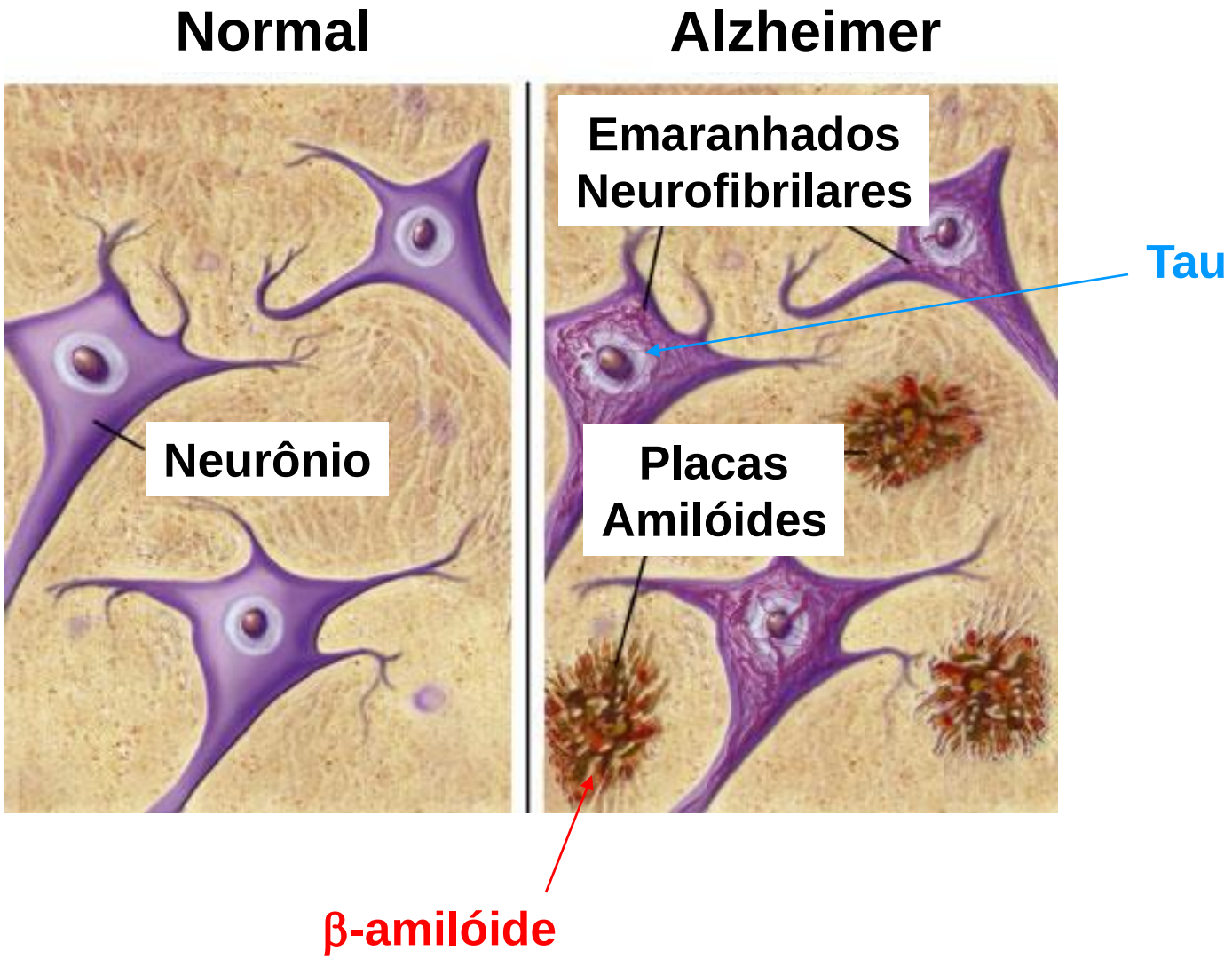
- Aprendizado de novas informações
- Resgate de informações antigas
- Afasia, apraxia, agnosia, perda da capacidade de organizar, planejar e executar atividades cotidianas
- Alterações graduais tornam-se mais severas
- Excluir outras condições que acusam demência: doença de Parkinson, Huntington, hipotiroidismo etc

Alzheimer **Normal**



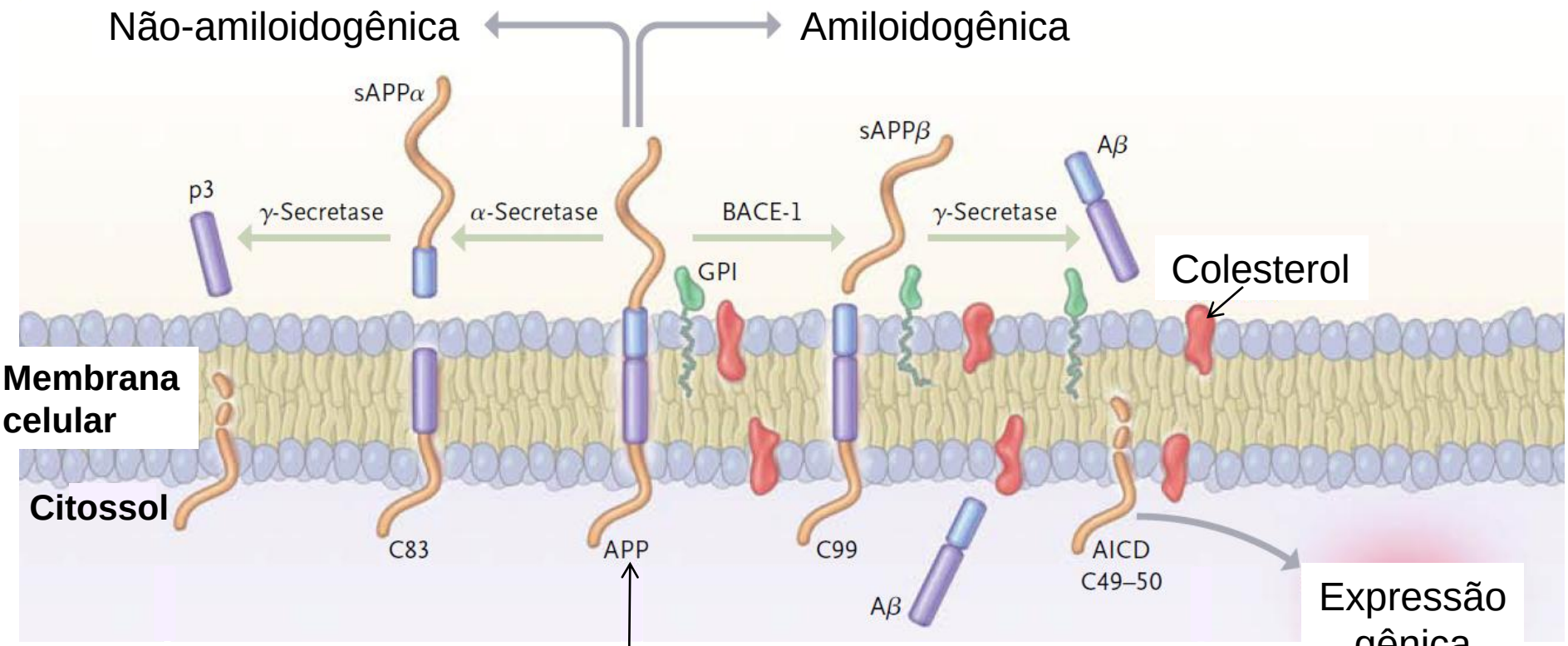
Áreas do cérebro afetadas na doença de Alzheimer



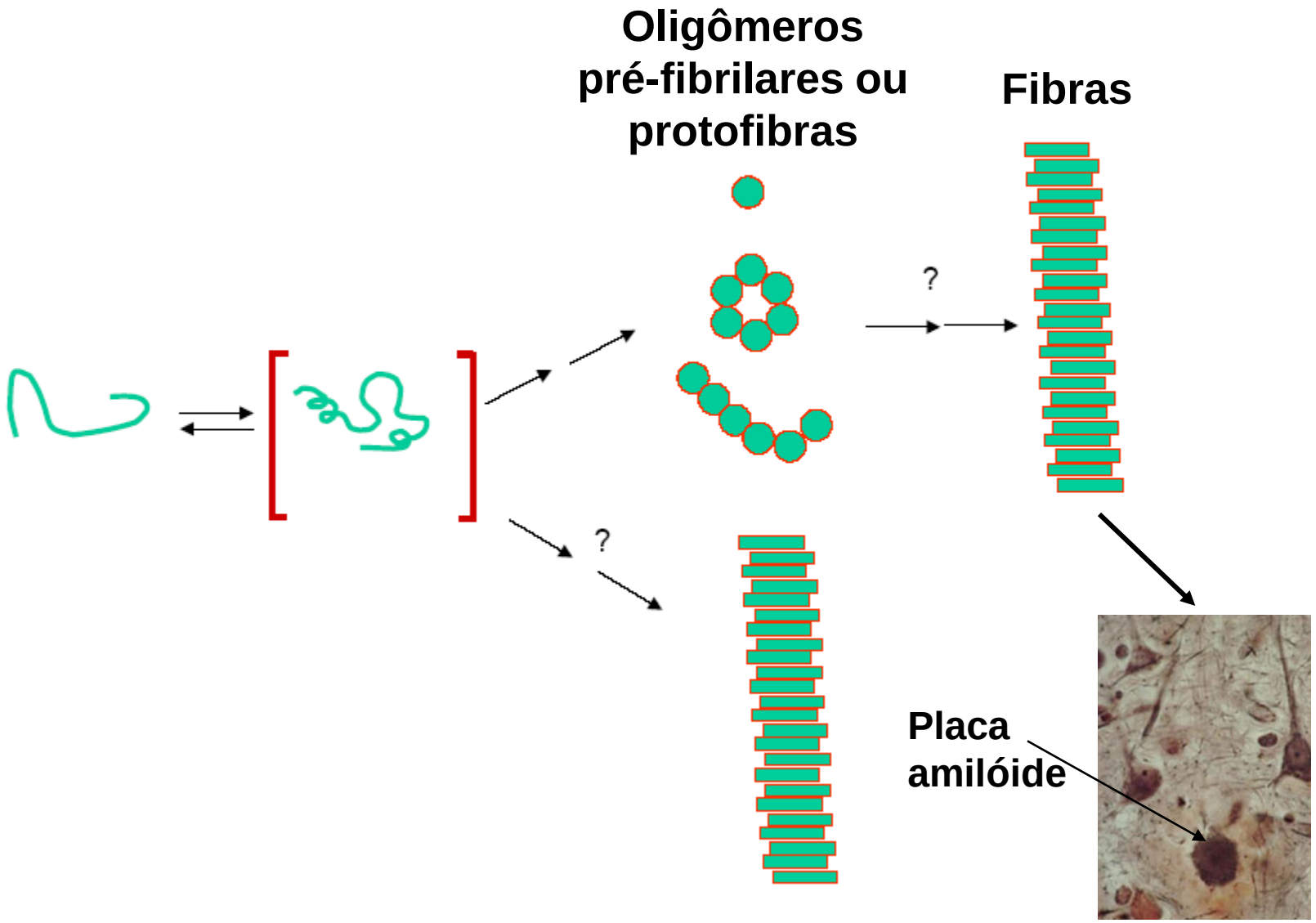

Normal
Alzheimer
Neurônio
Emaranhados
Neurofibrilares
Tau
Placas
Amilóides
β-amilóide

Como se formam as placas constituídas de agregados da proteína β-amilóide?

Mecanismo de clivagem da proteína β-amilóide

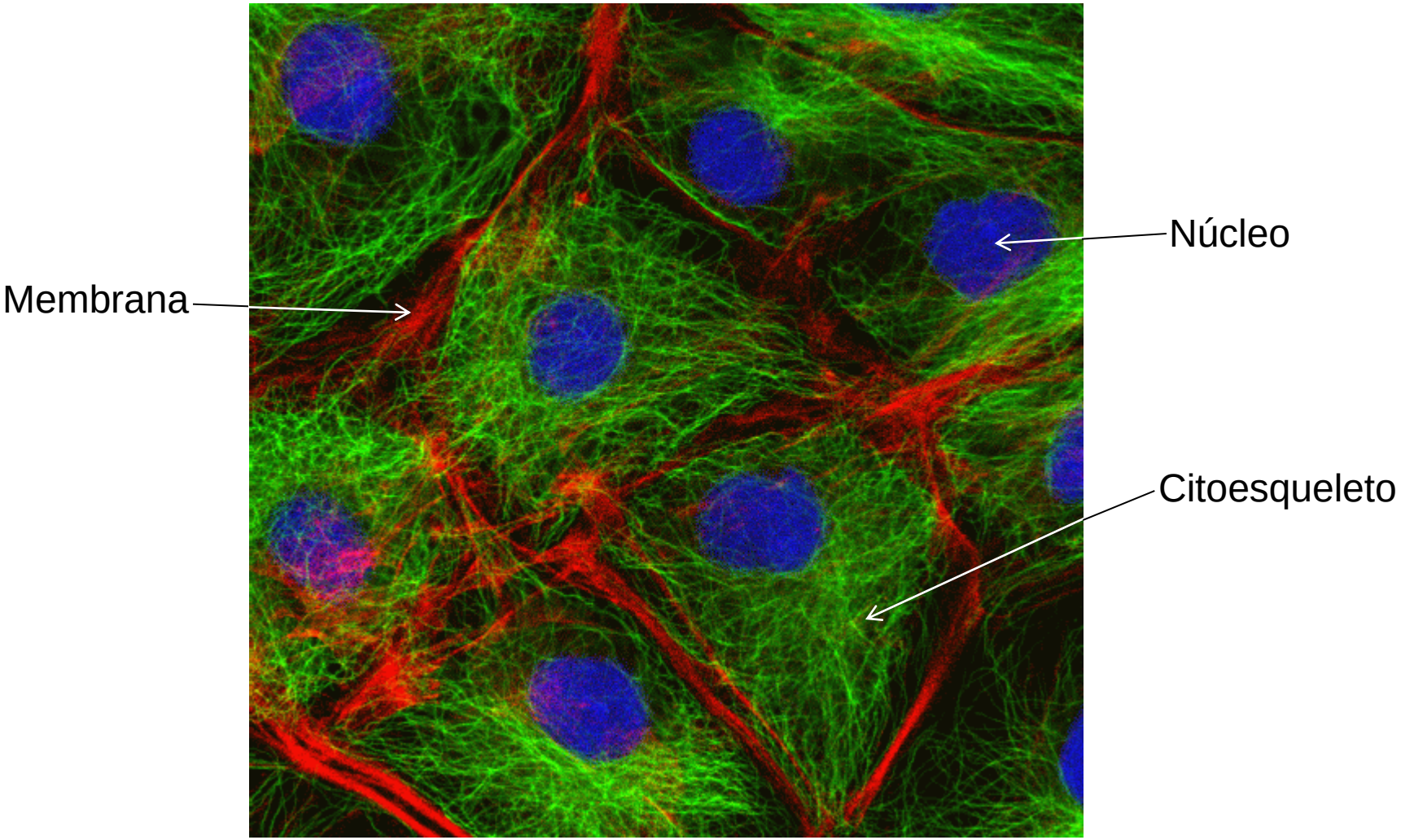


Mecanismo de agregação da proteína β-amilóide

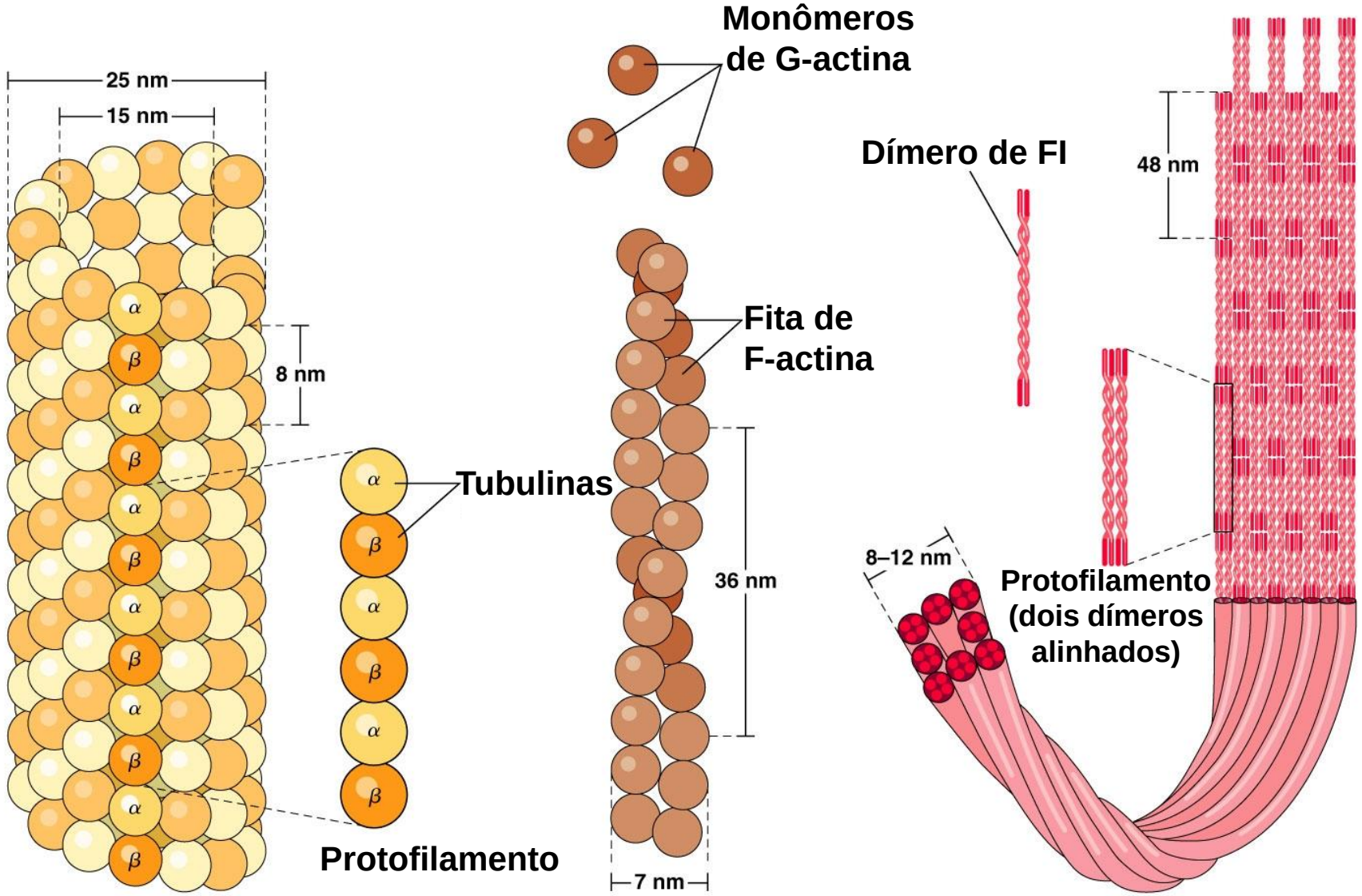


Como se formam os emaranhados neurofibrilares constituídos de agregados da proteína Tau?

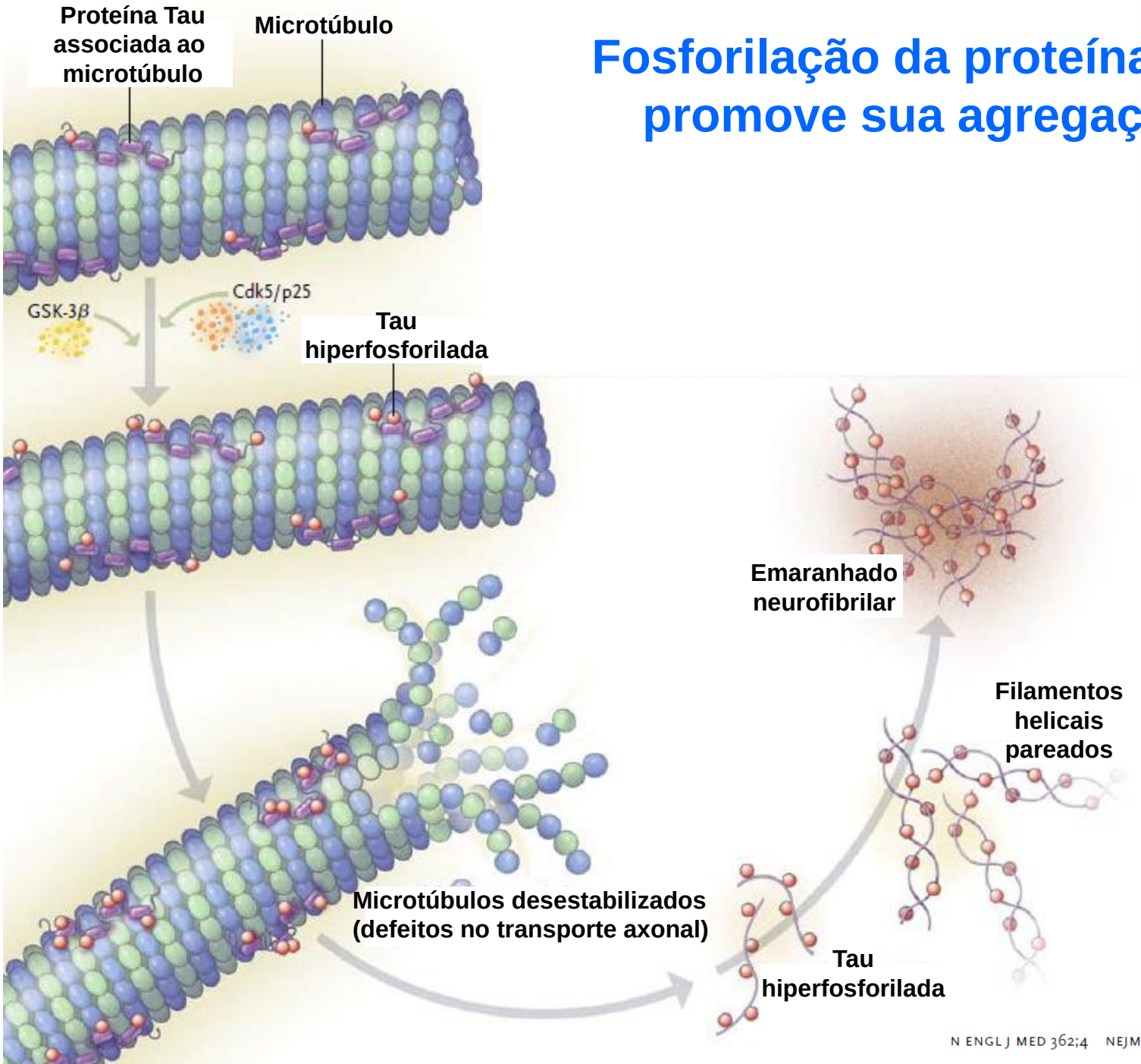
O citoesqueleto mantém a estrutura da célula



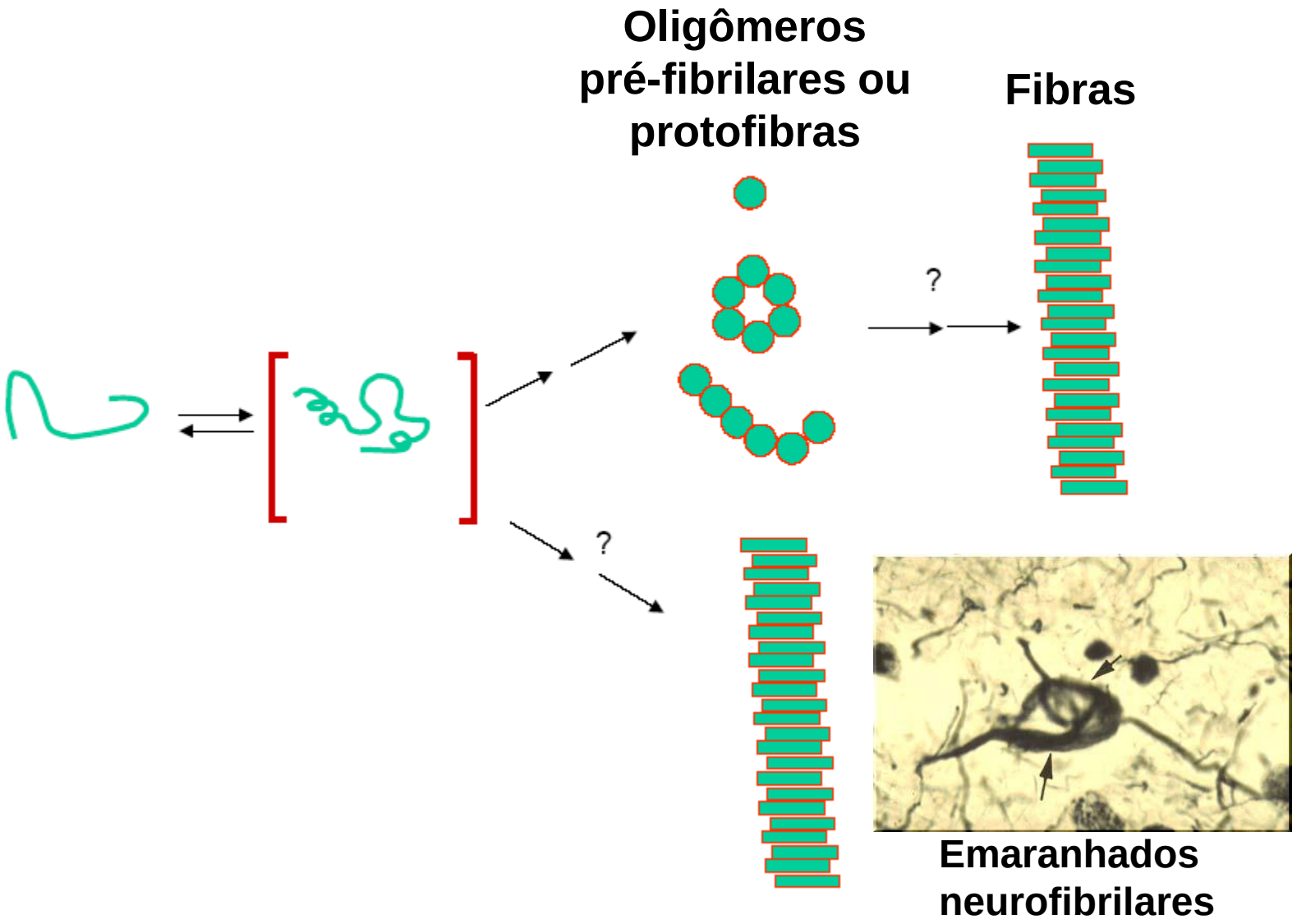
Os componentes do citoesqueleto



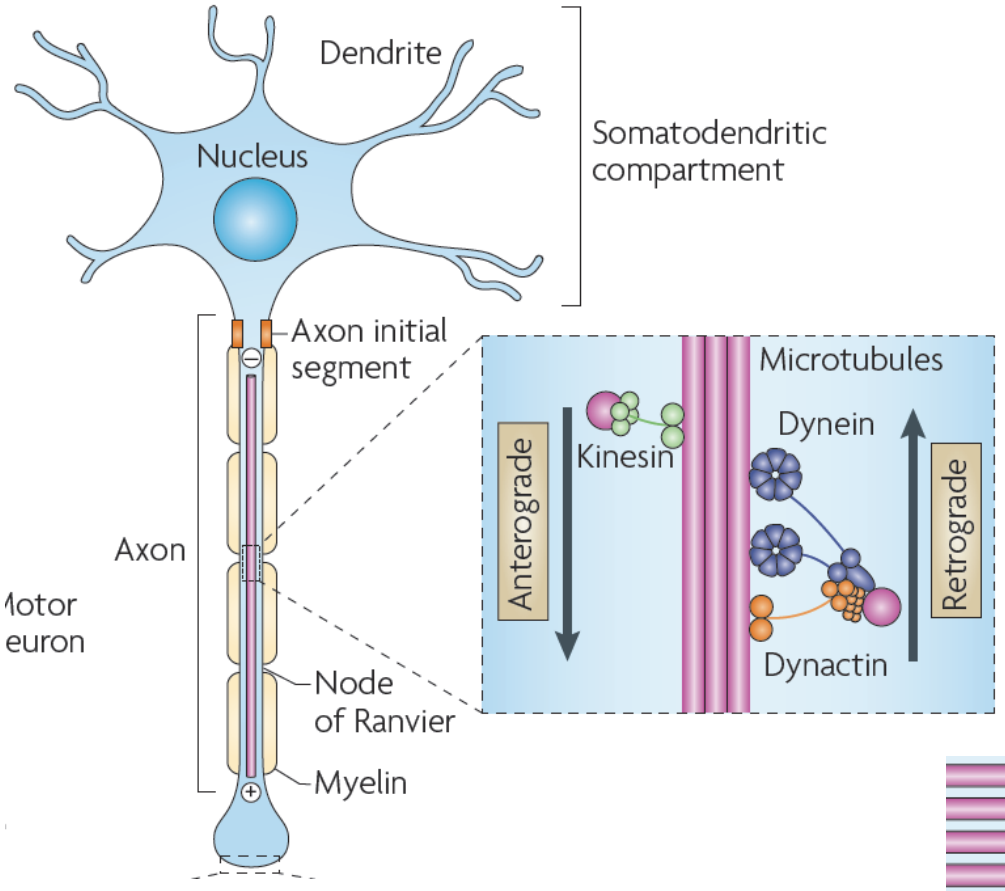
Fosforilação da proteína Tau promove sua agregação

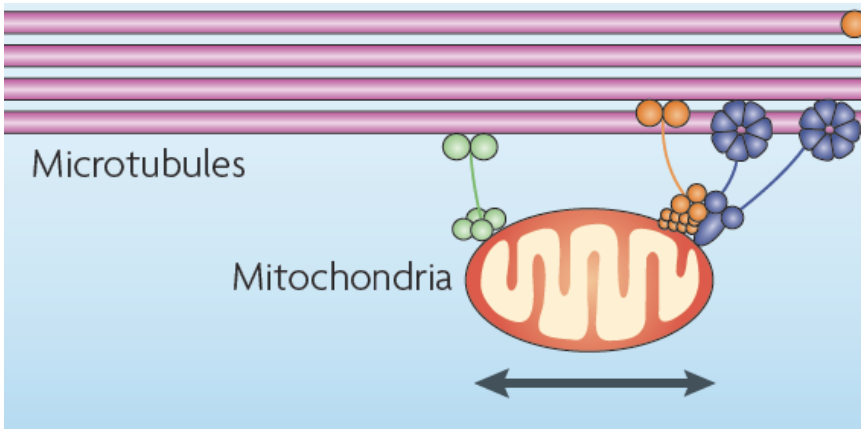


Mecanismo de agregação da Tau



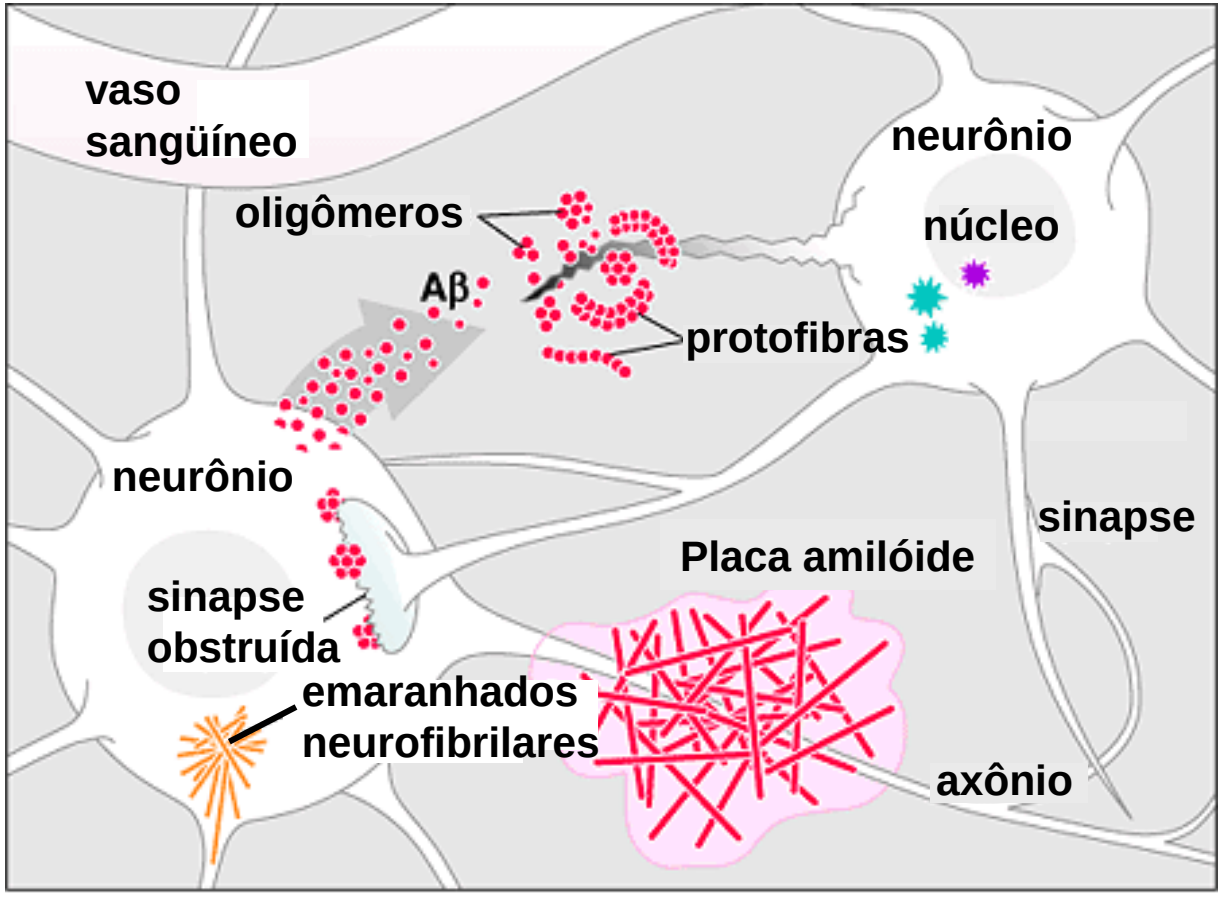
A mitocôndria é transportada nos microtúbulos





www.pnas.org/cgi/doi/10.1073/pnas.1534703100

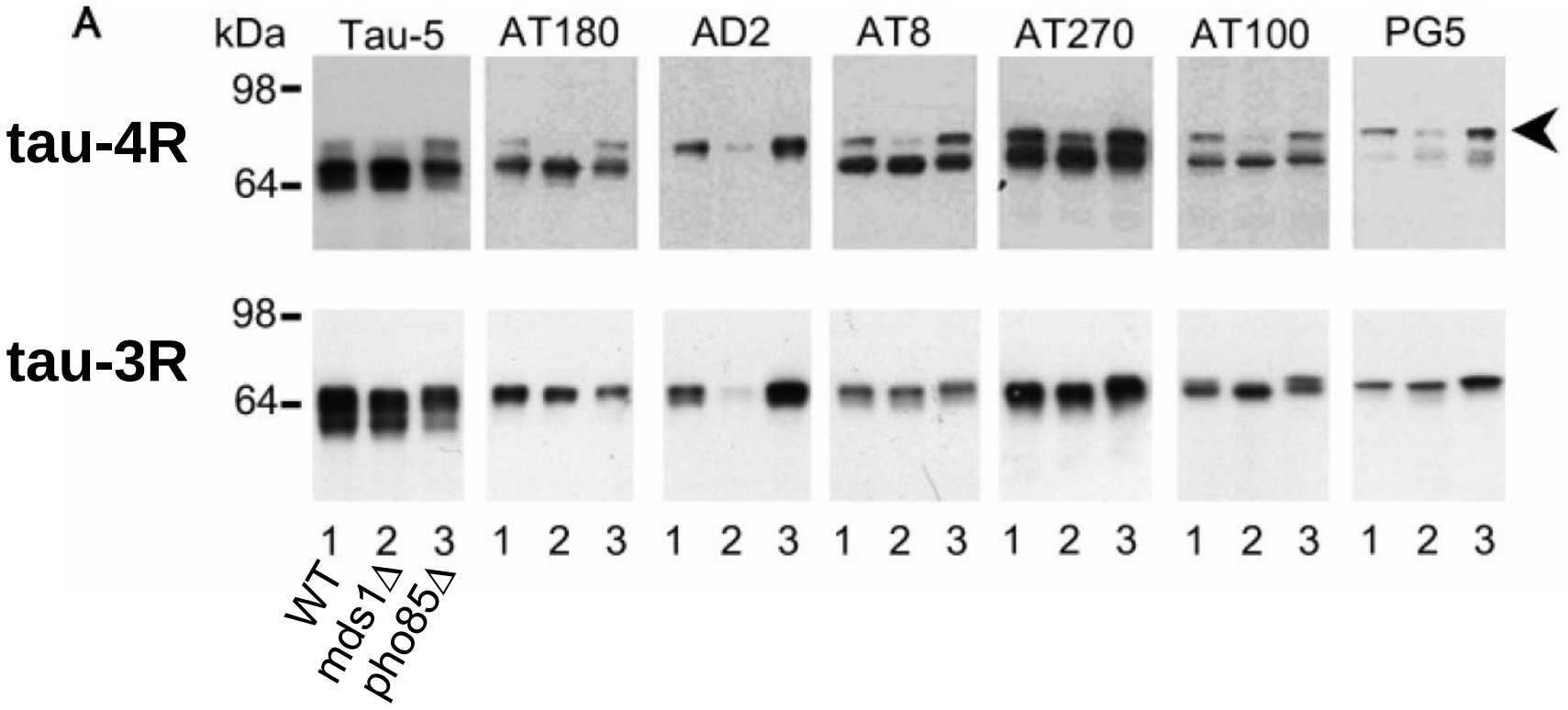
Como os agregados de Aβ42 causam lesão neuronal?



Budding yeast models to study effects of Tau

Human Protein Tau-4R Becomes Hyperphosphorylated inYeast.

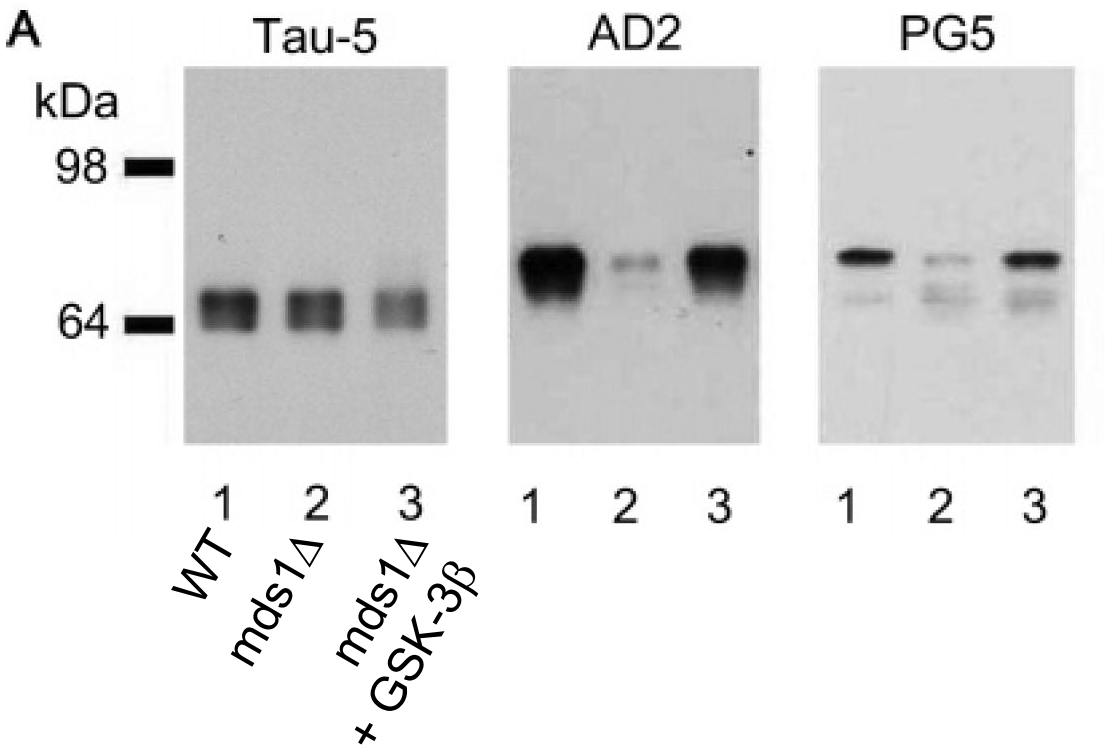
1. Human tau-4R and tau-3R did not affect growth rate
2. Alkaline phosphatase treatment reduced the tau-3R and tau-4R subspecies to single proteins



Vandebroek et al. (2005)Biochemistry **44:** 11466-11475

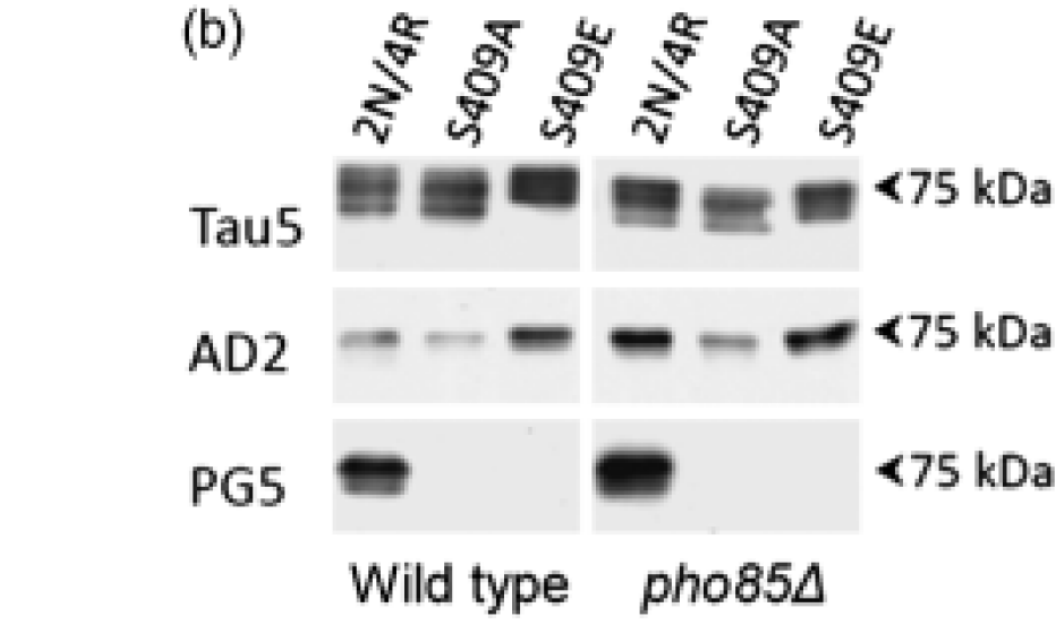
Decrease of epitopes AD2 and PG5 in *mds1Δ* yeast is restored by human GSK-3β[S9A]

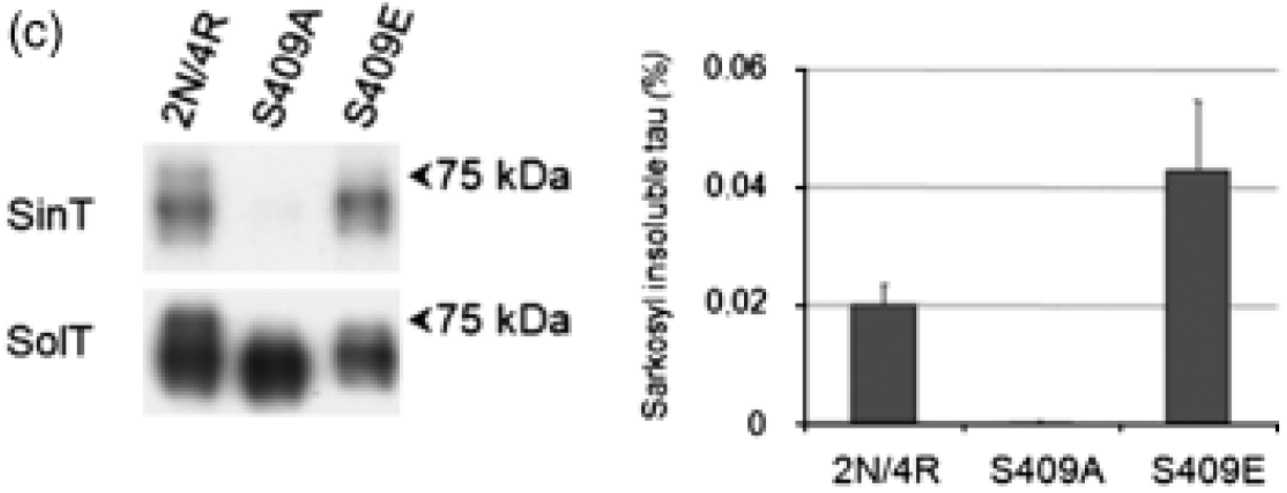
1. Mds1 is an orthologue of mammalian GSK-3β



Vandebroek et al. (2005)Biochemistry **44:** 11466-11475

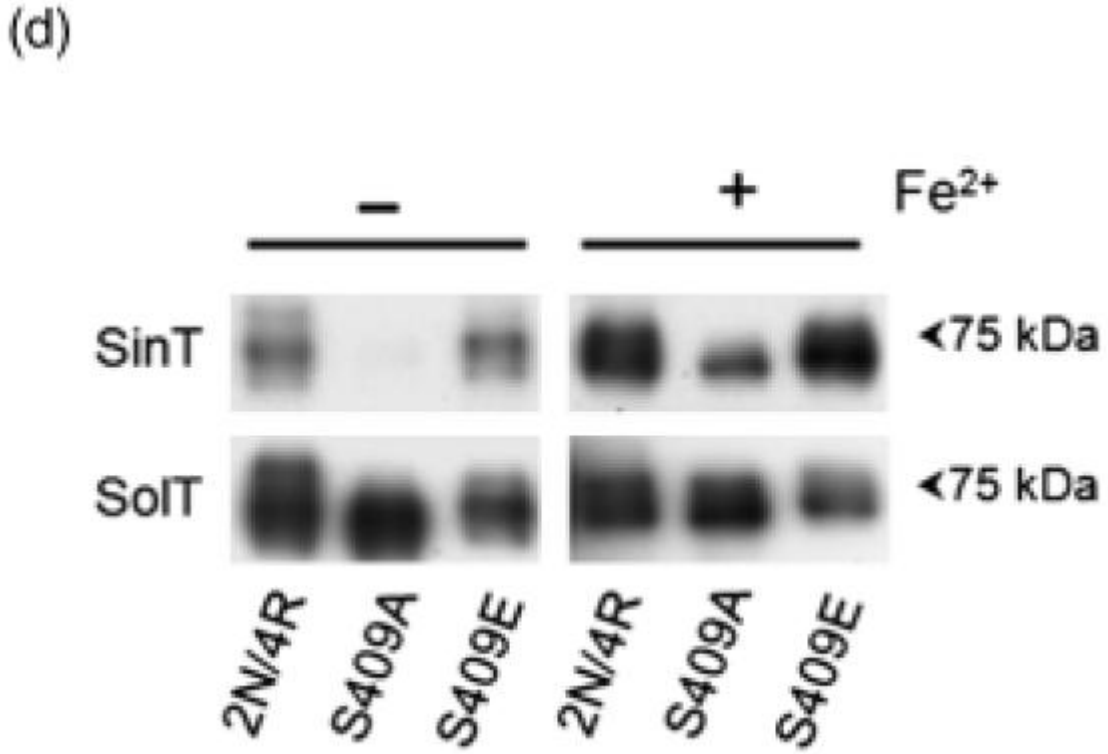
The importance of Ser409 phosphorylation for Tau aggregation.





Vanhelmont et al. (2010) FEMS Yeast Res 10: 992–1005

Oxidative stress triggers an aggregation mechanism parallel to hyperphosphorylation



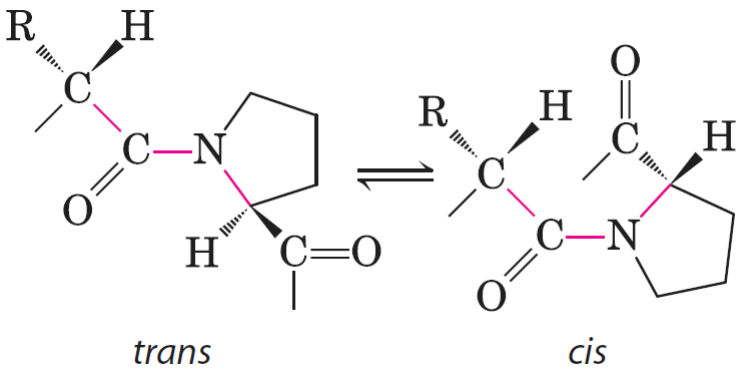
Radical hidroxil

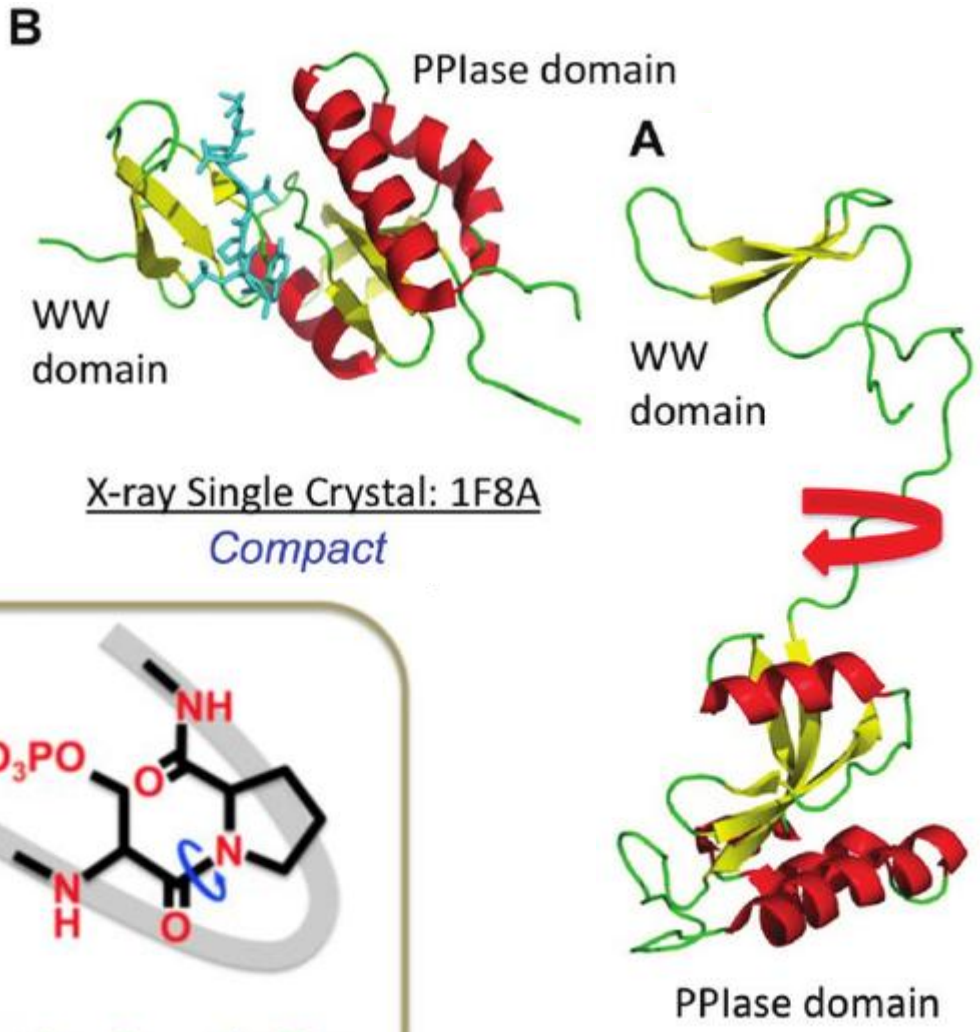
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

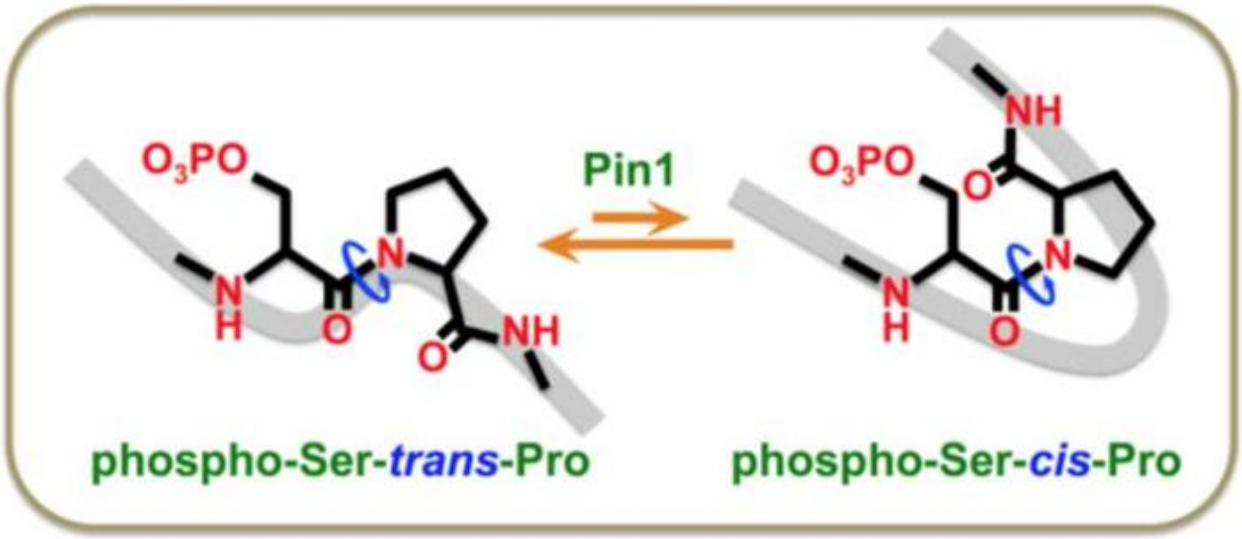
Reação de Fenton

Vanhelmont et al. (2010) FEMS Yeast Res 10: 992–1005

Peptidyl prolyl cis/trans isomerase (PPI) Pin1







Ser/Thr-Pro motifs

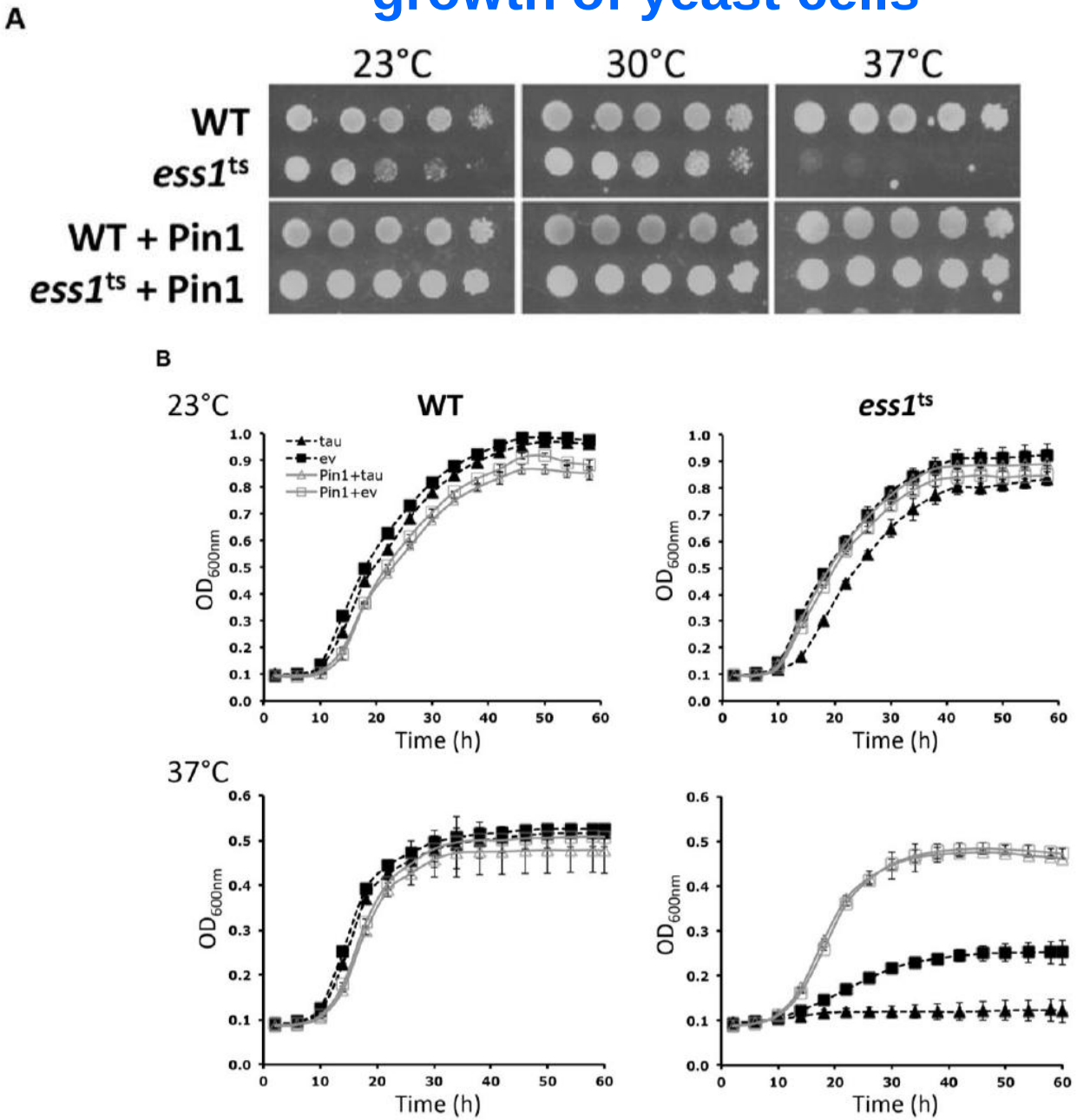
1. Phosphorylation of protein tau on Thr231-Pro232 slows down the conversion from the *cis* to the *trans* state, ultimately making this tau residue a less favorable target for the trans-dependent phosphatase PP2A;

2. Thr231-phosphorylated tau in the cis-state, but not trans-state, is unable to promote MT assembly and is more prone to aggregation, besides being more resistant to dephosphorylation;

3. Pin1$^{-/-}$ mice display an age-dependent full-blown tau pathology whereas a decreased Pin1 expression/activity is reported in case of AD;

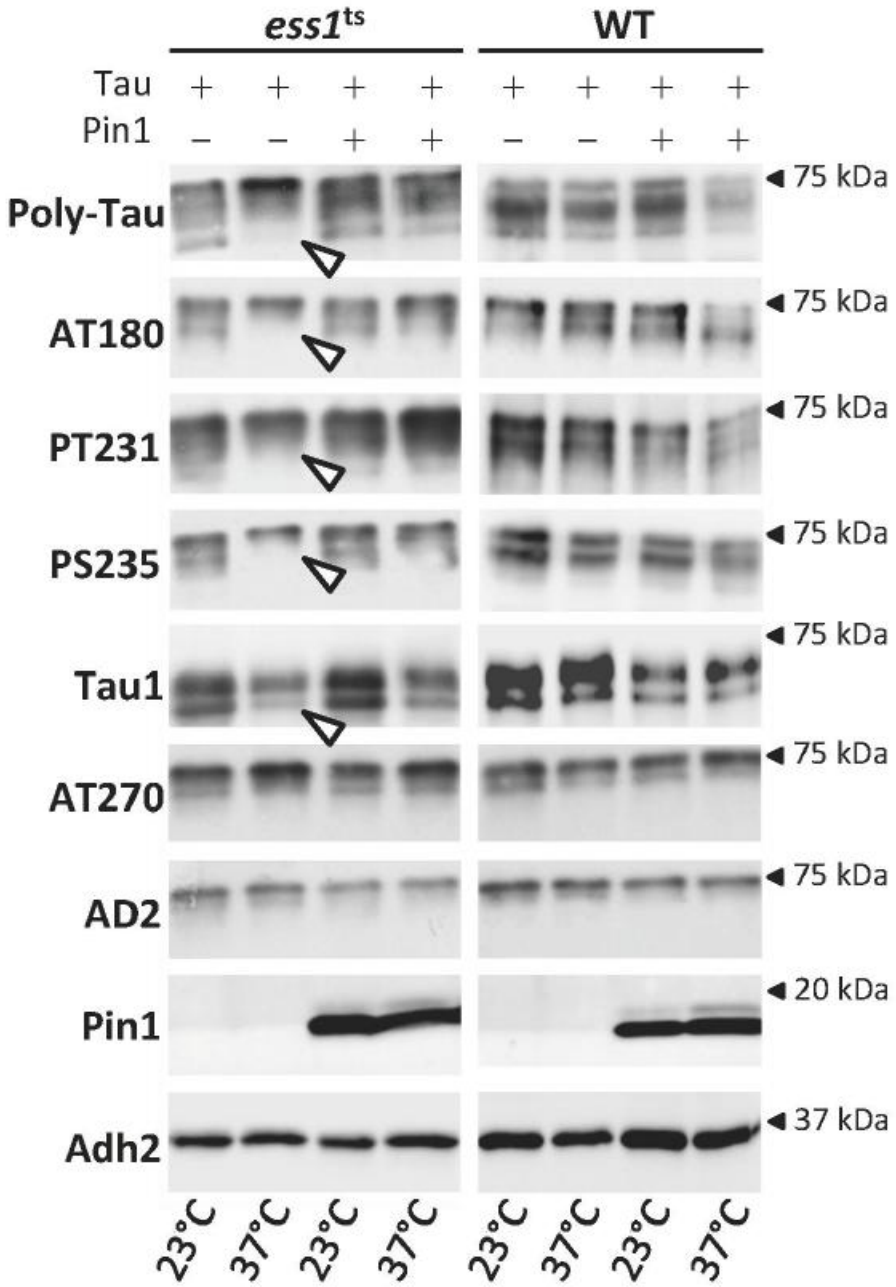
4. Ess1 de *S. cereviaise* é o homólogo de Pin1

The effect of Ess1 depletion and tau expression on the growth of yeast cells



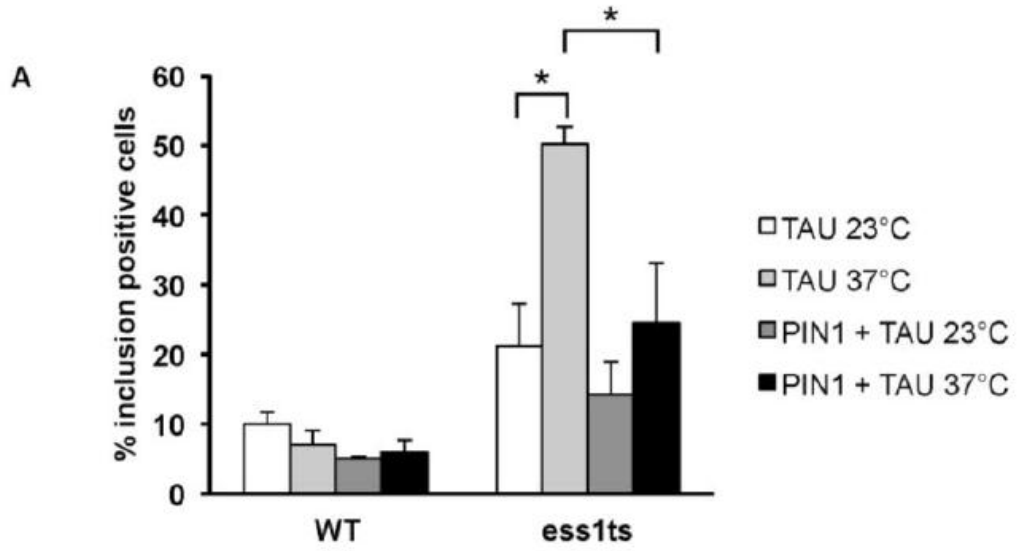
De Vos (2015)AIMS Molecular Science 2:144-160

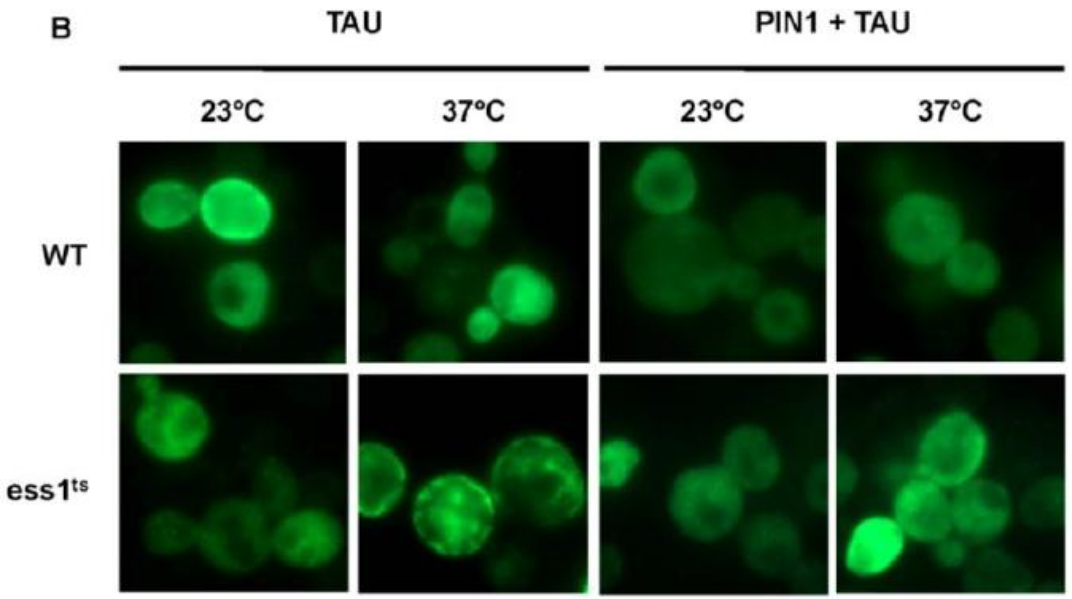
Phosphorylation pattern of tau in wild type and *ess1*^{ts} cells



De Vos (2015)AIMS Molecular Science 2:144-160

Visualisation of tau inclusions in WT and *ess1^{ts}* cells.

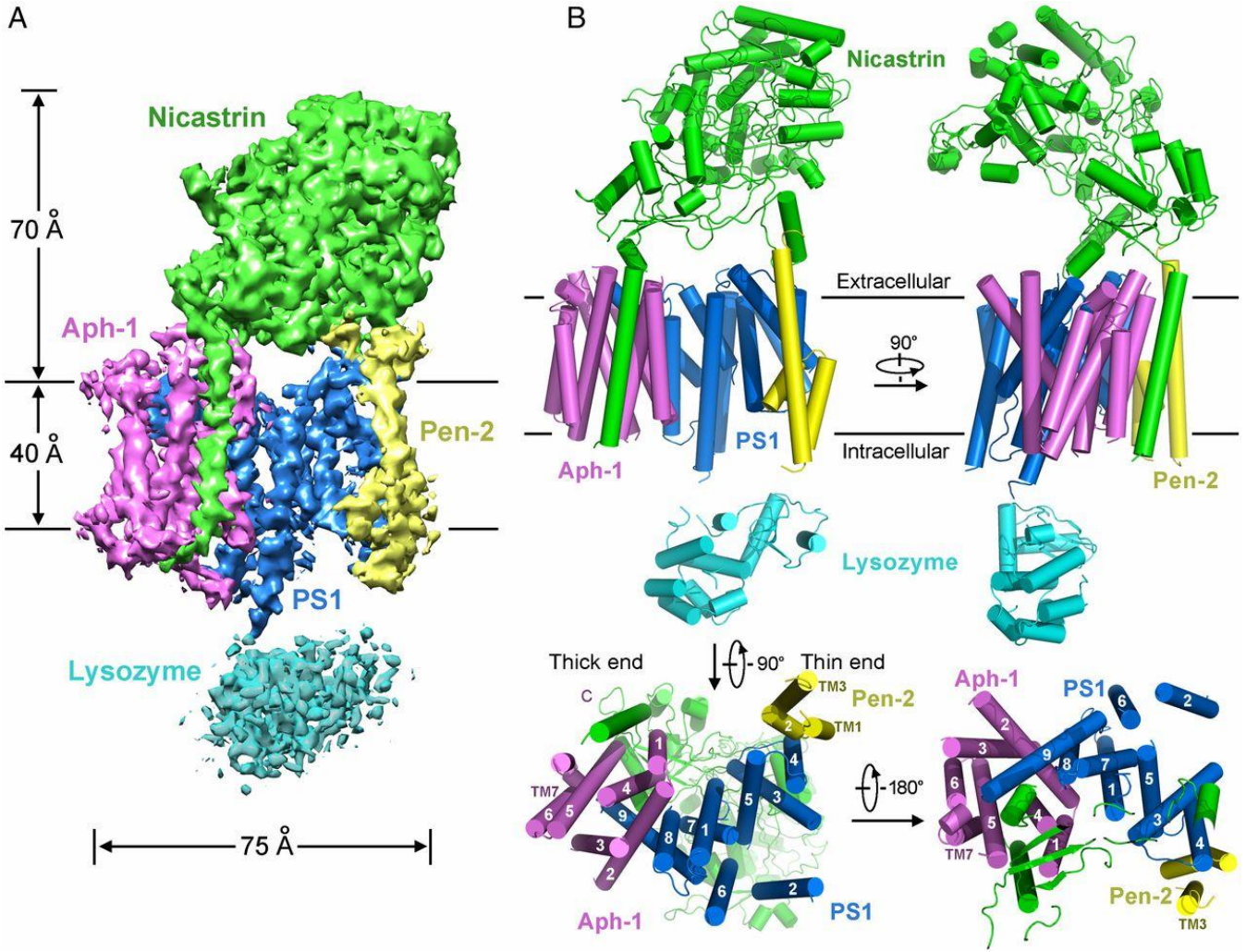




De Vos (2015)AIMS Molecular Science 2:144-160

Budding yeast models to study effects of Aβ

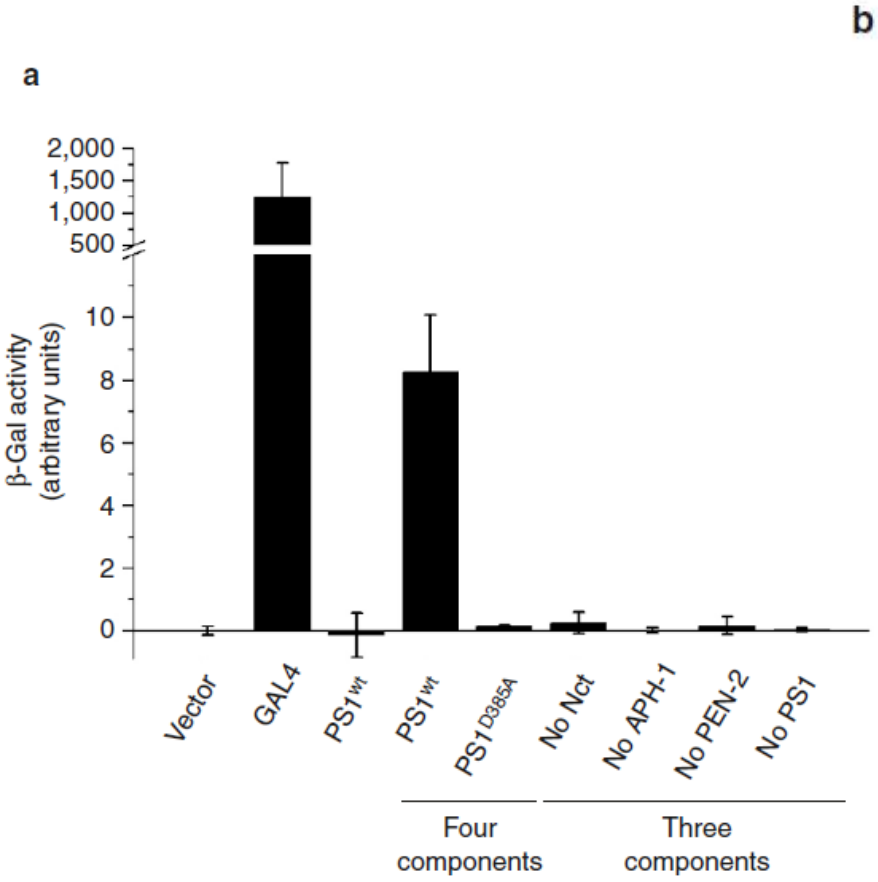
Overall structure of human γ-secretase.

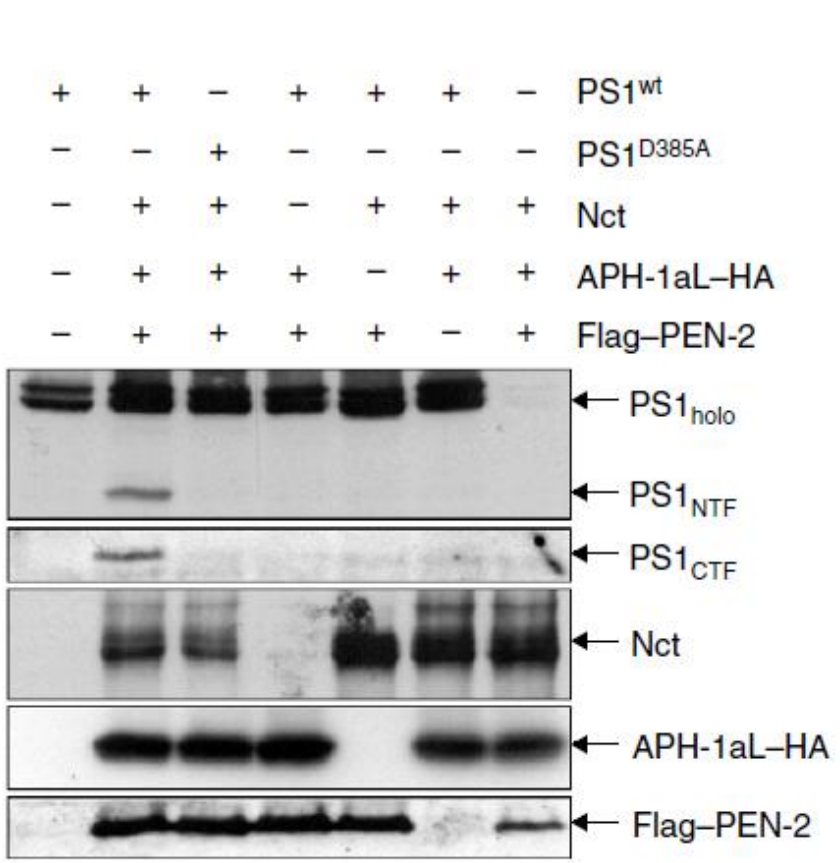


Linfeng Sun et al. PNAS 2015;112:19:6003-6008

PNAS

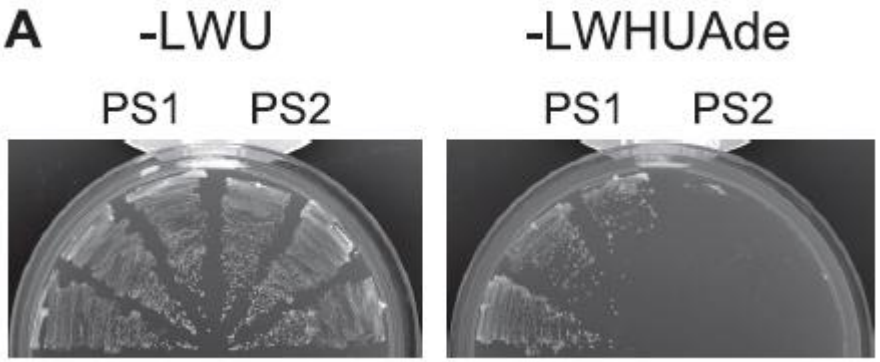
In vivo reconstitution of γ-secretase activity and PS endoproteolysis in yeast

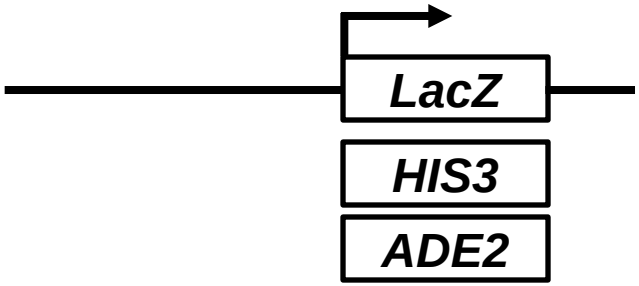


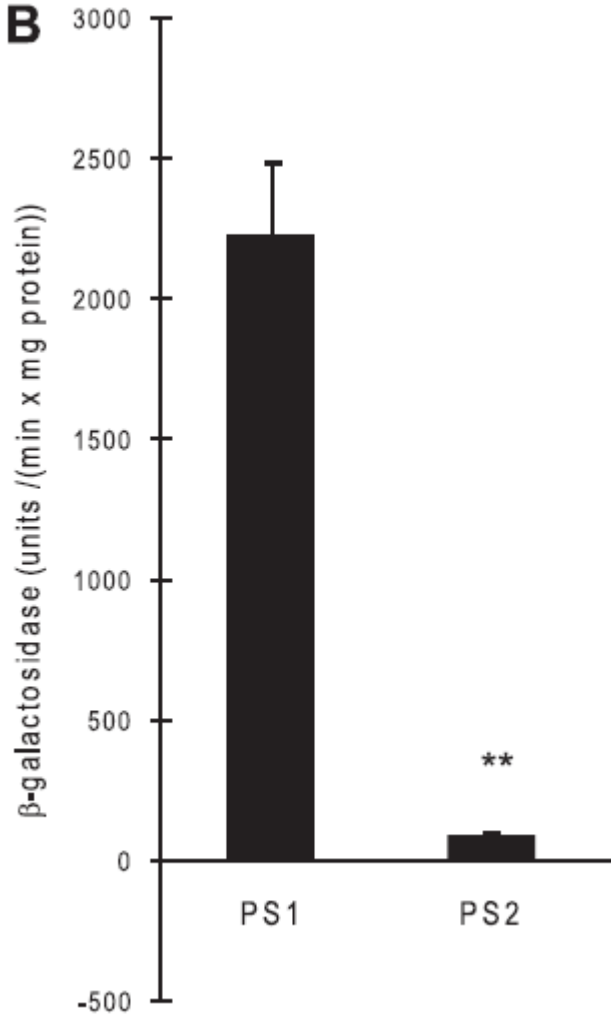


Edbauer et al. (2003) Nat. Cell Biol. **5**: 486–488

Estimate of reconstituted PS1 or PS2 -secretase activity in yeast

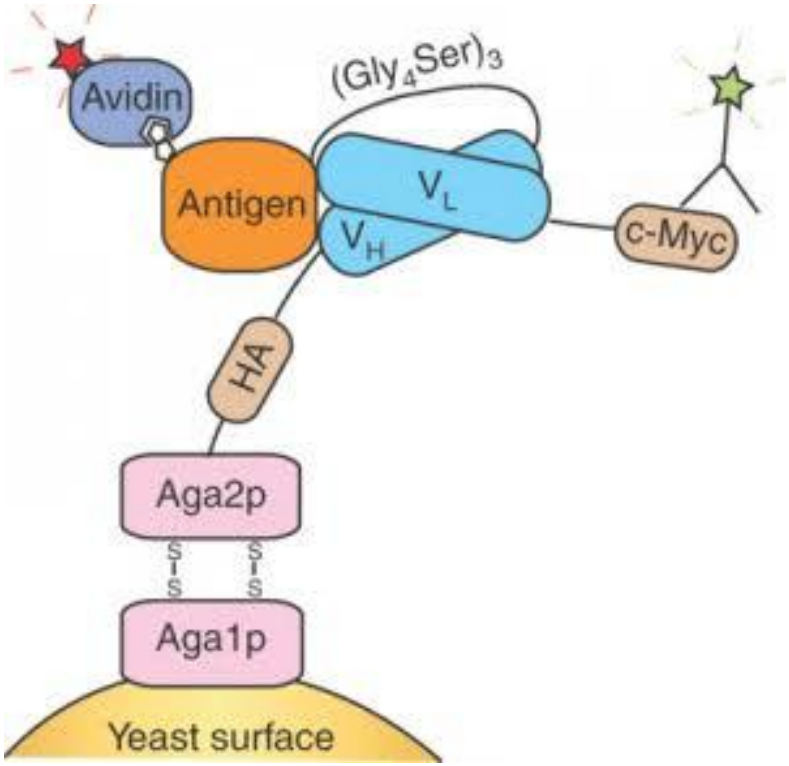




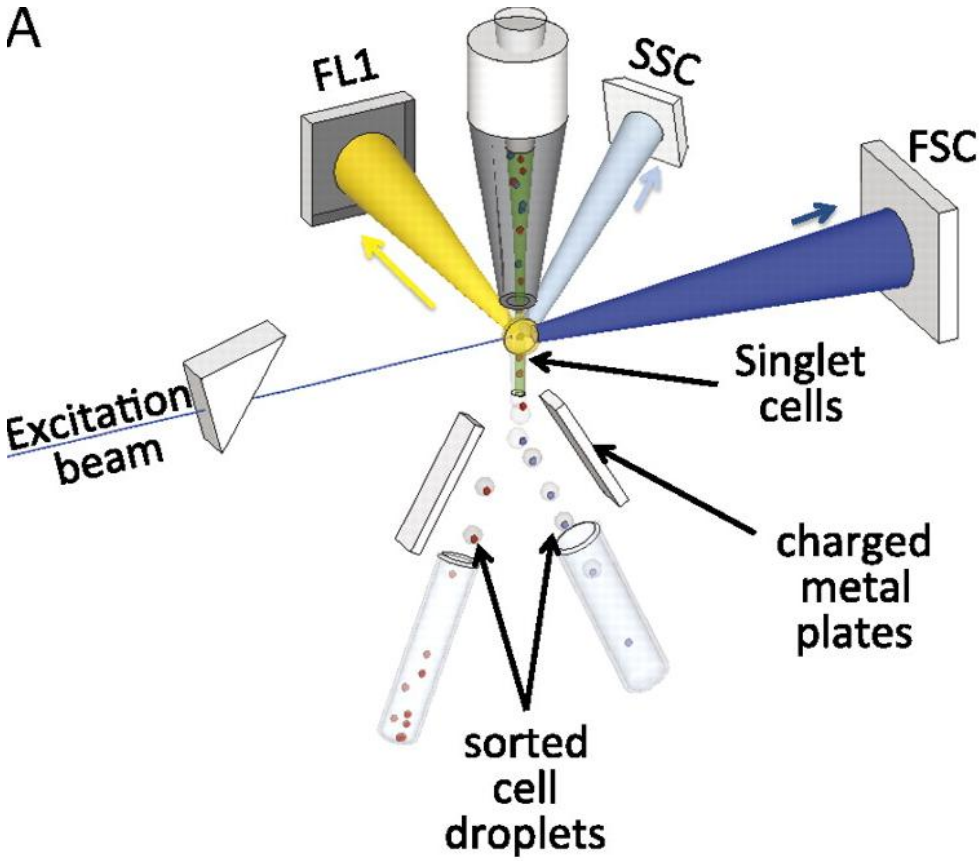


Yonemura et al. (2011) J Biol Cem **286**: 44569–44575

Yeast Display and flow Cytometry



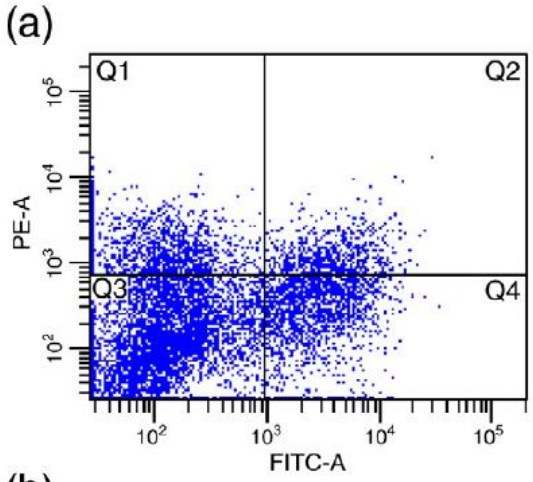
Yeast Display

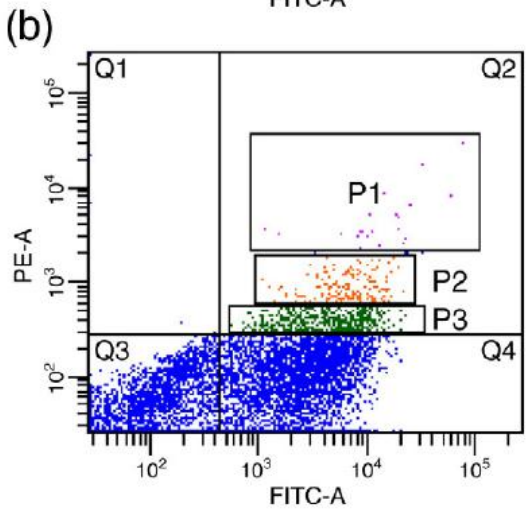


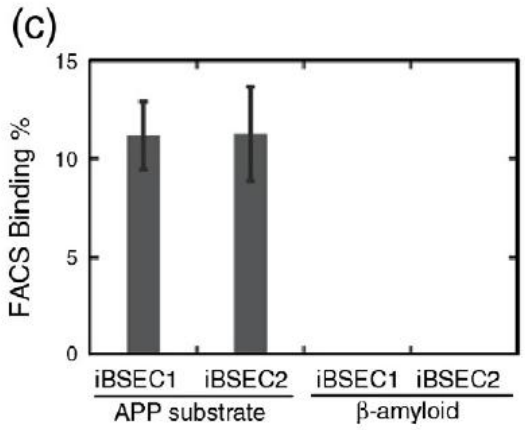
Flow Cytometry

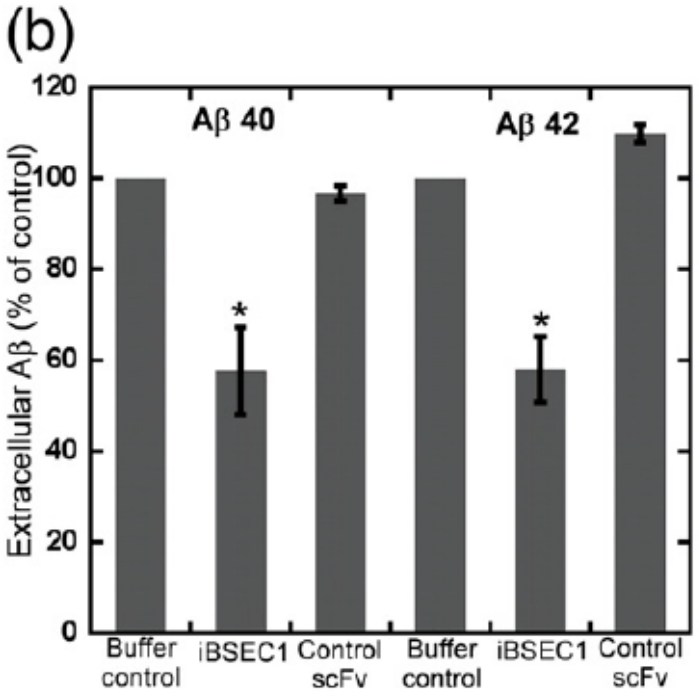
Aβ42

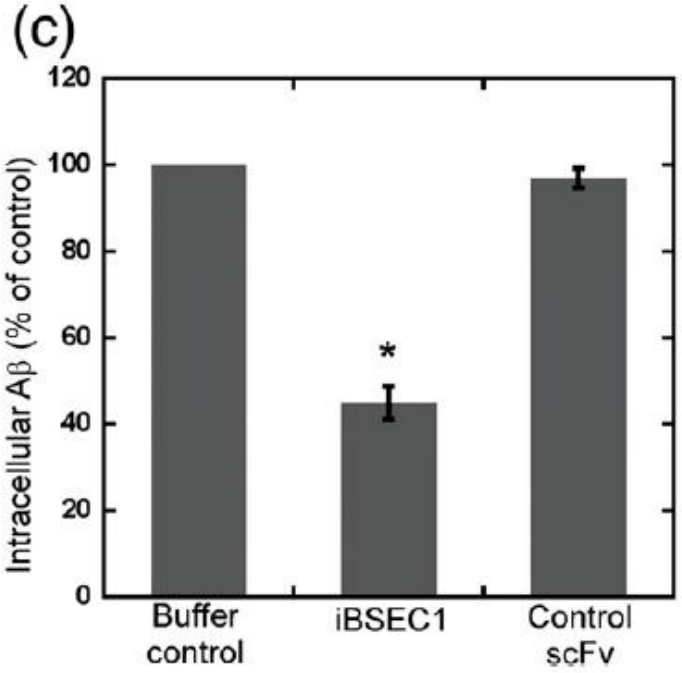
βAPP





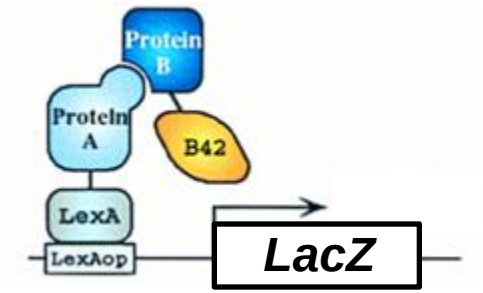


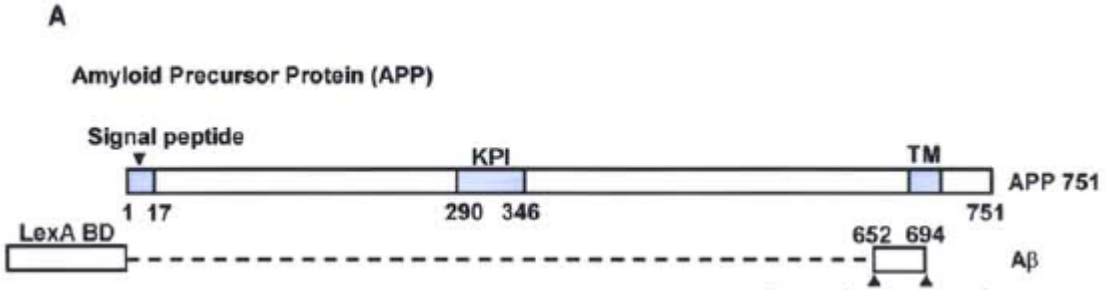


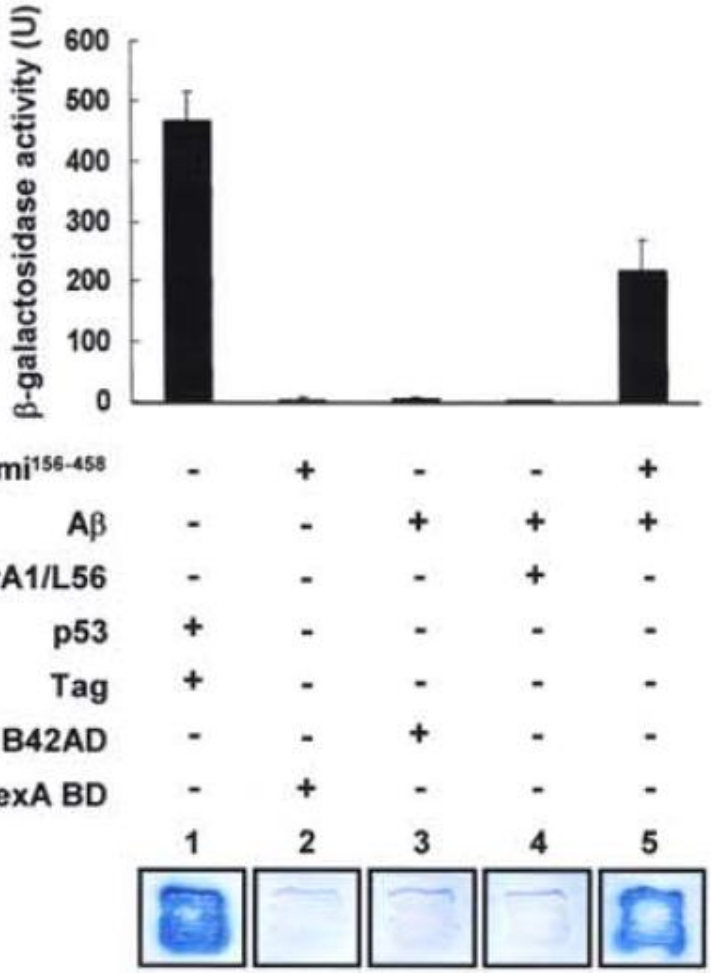


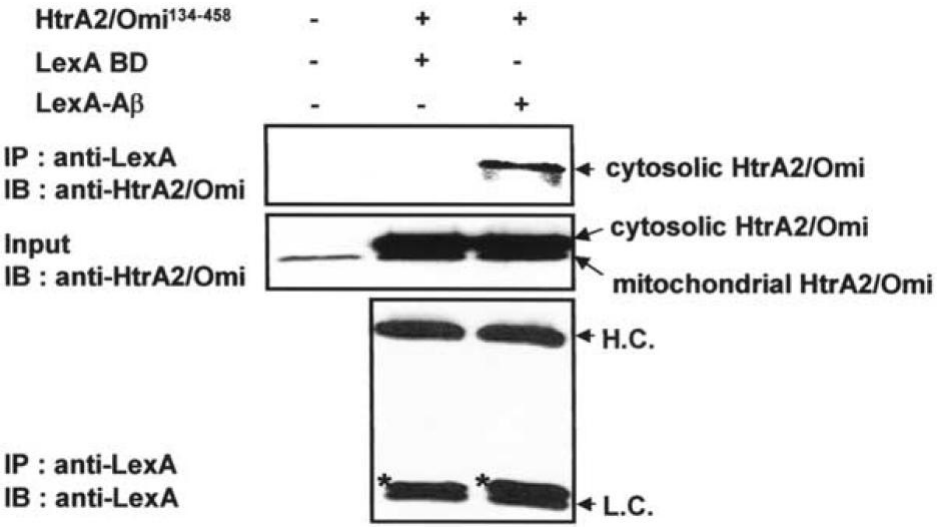
Boddapati et al. (2001) J. Mol. Biol. 405: 436–44

Yeast Two-Hybrid



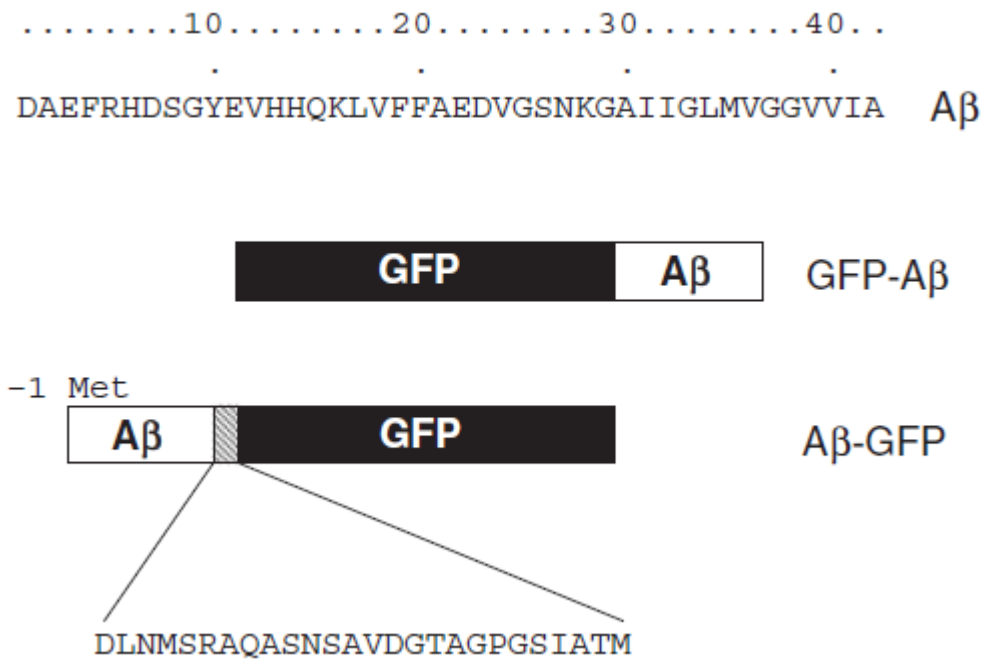


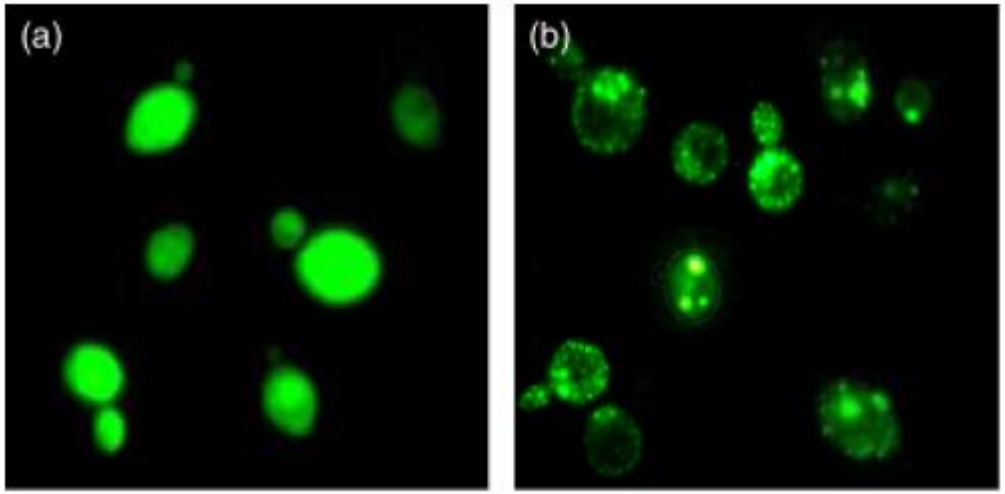




Park et al. (2004)Neurosci. Lett. **357**, 63–67,

Microscopy of yeast with GFP-Aβ

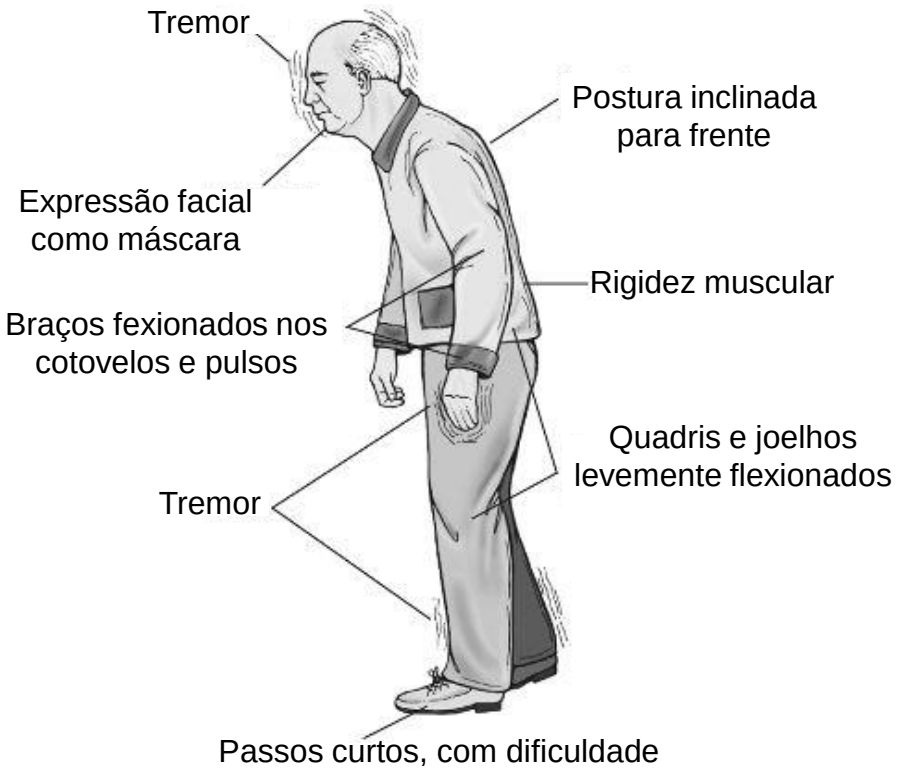




GFP **GFP-Aβ**

Caine et a.. (2007) FEMS Yeast Res **7**: 1230–1236

Introdução à Doença de Parkinson



Características clínicas importantes da Doença de Parkinson

Tremor de repouso

Bradicinesia

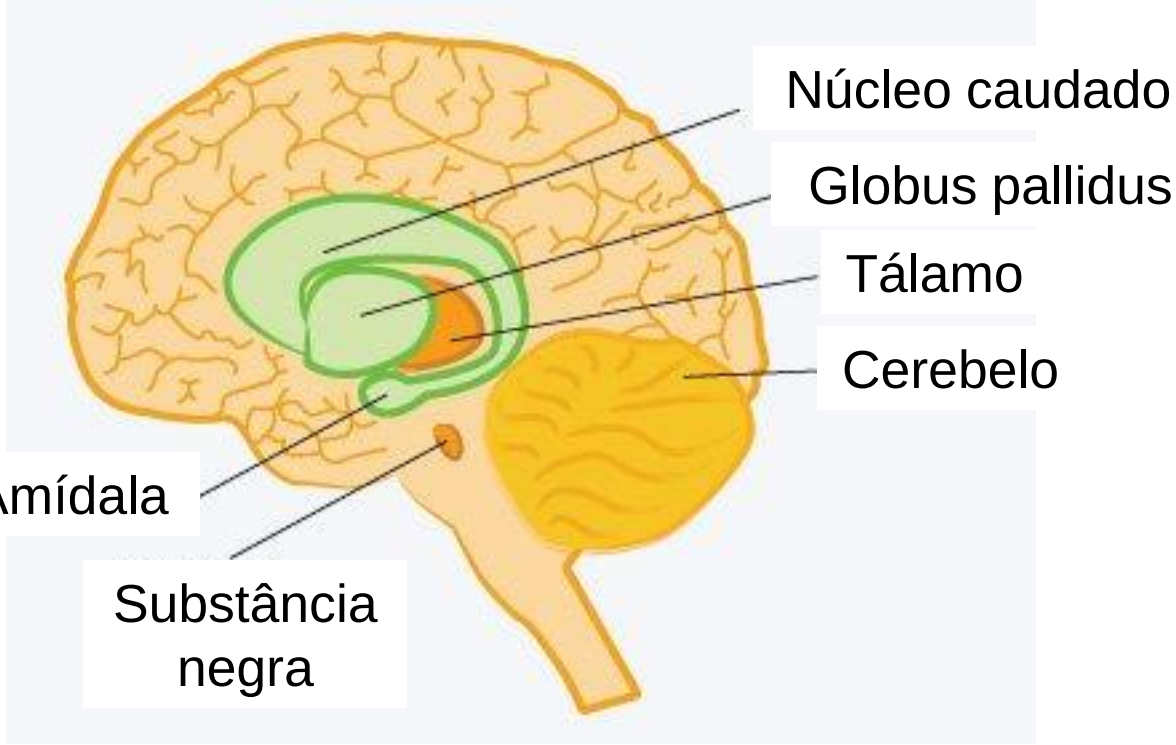
Rigidez muscular

Alteração dos reflexos posturais

Postura flexionada para frente

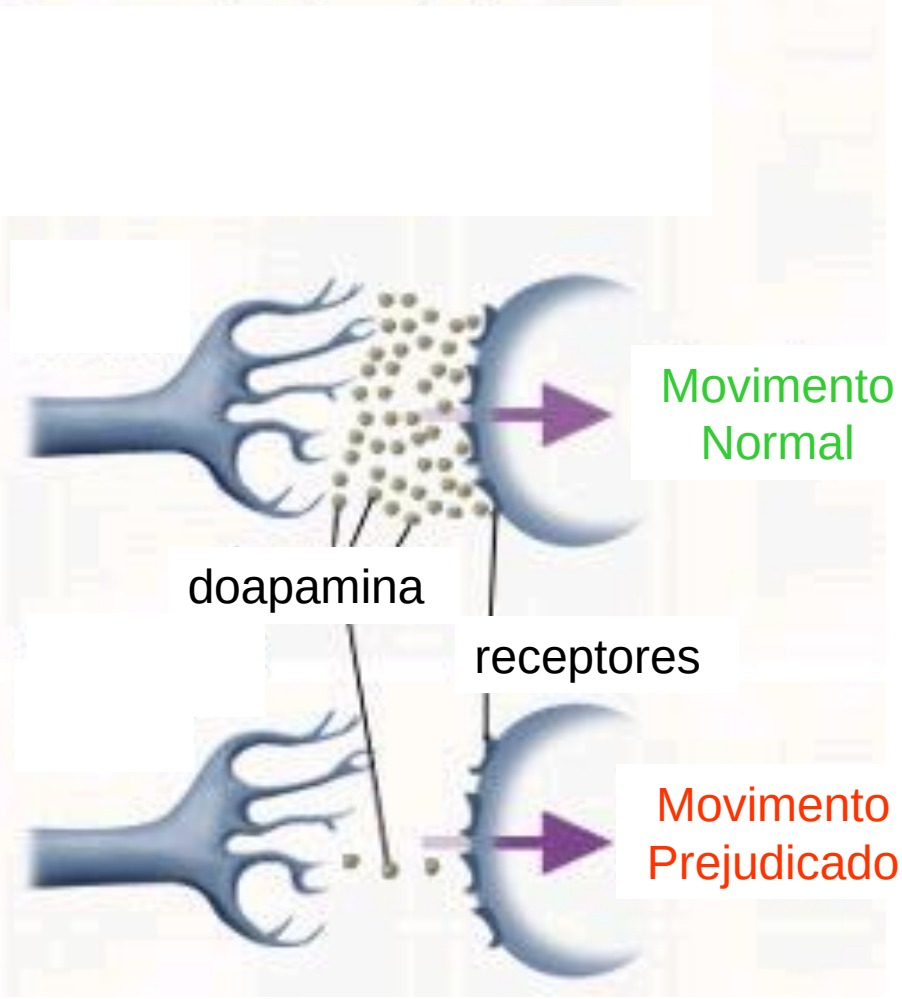
Bloqueio motor

Áreas do cérebro afetadas na doença de Parkinson



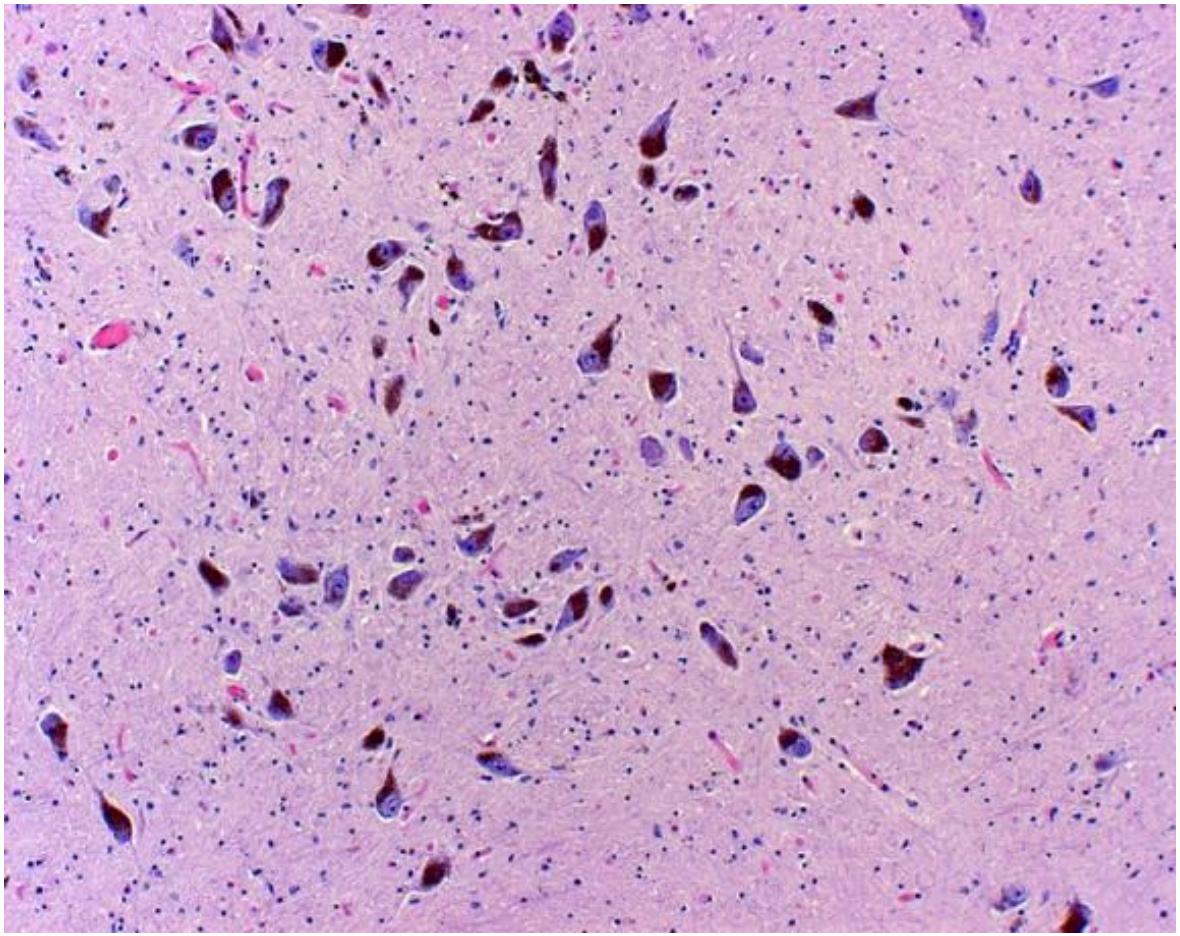
Os níveis de dopamina em neurônios normais e afetados com doença de Parkinson

Neurônio Normal



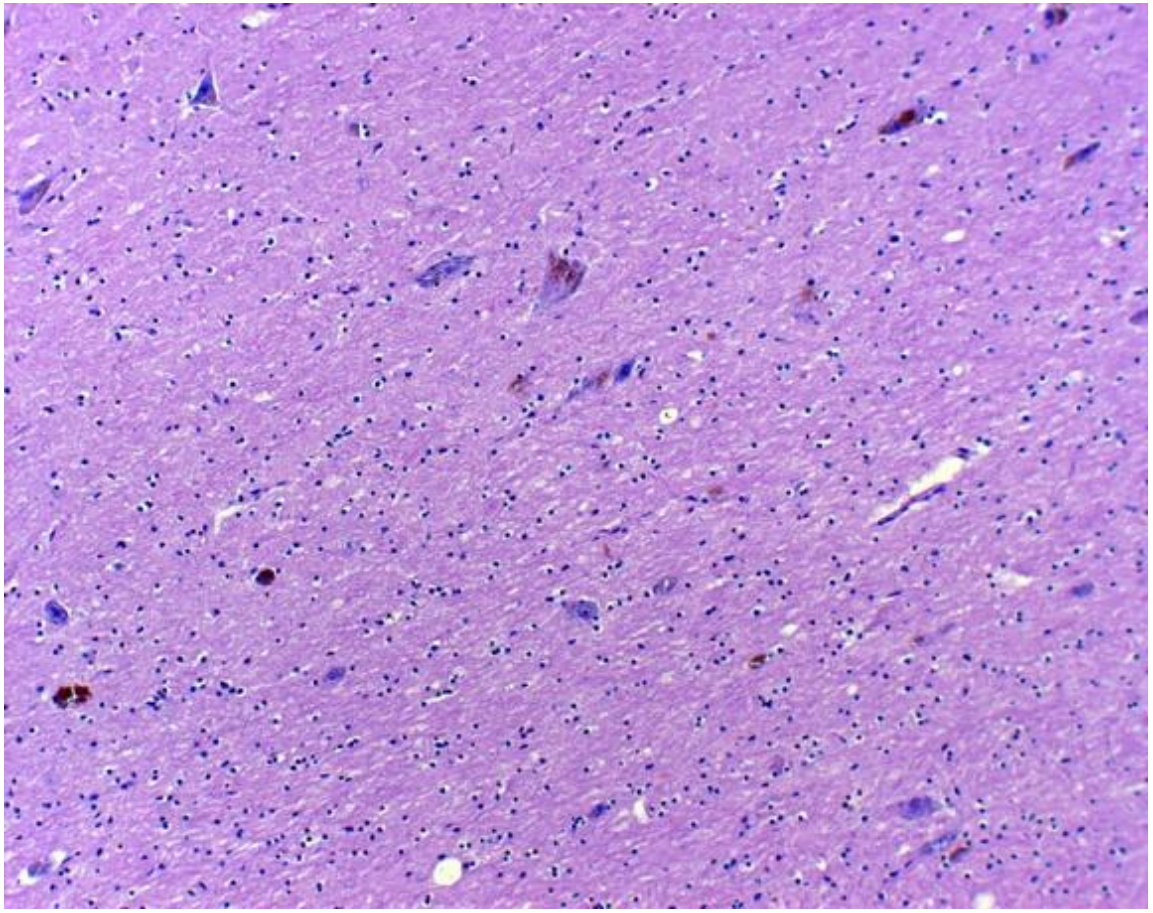
Neurônio afetado com
Doença de Parkinson

Doença de Parkinson



Substância negra normal

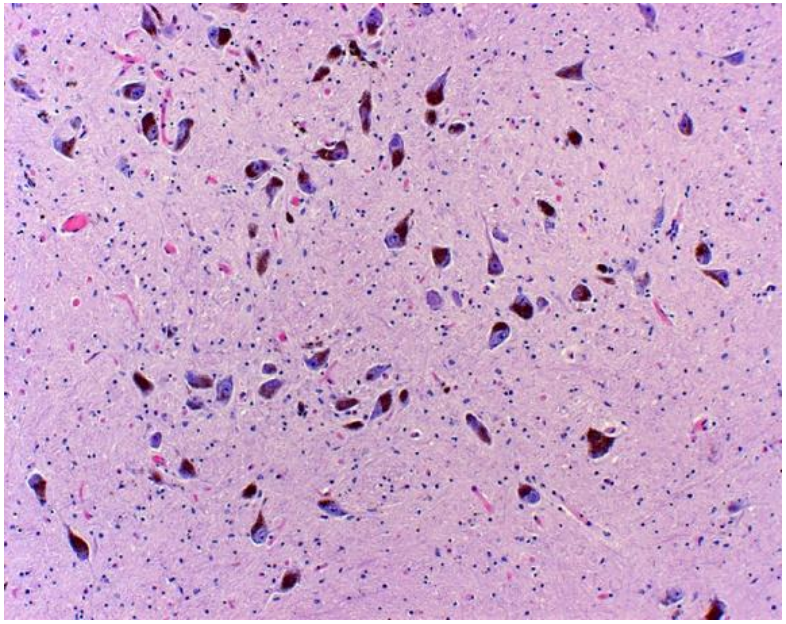
Doença de Parkinson



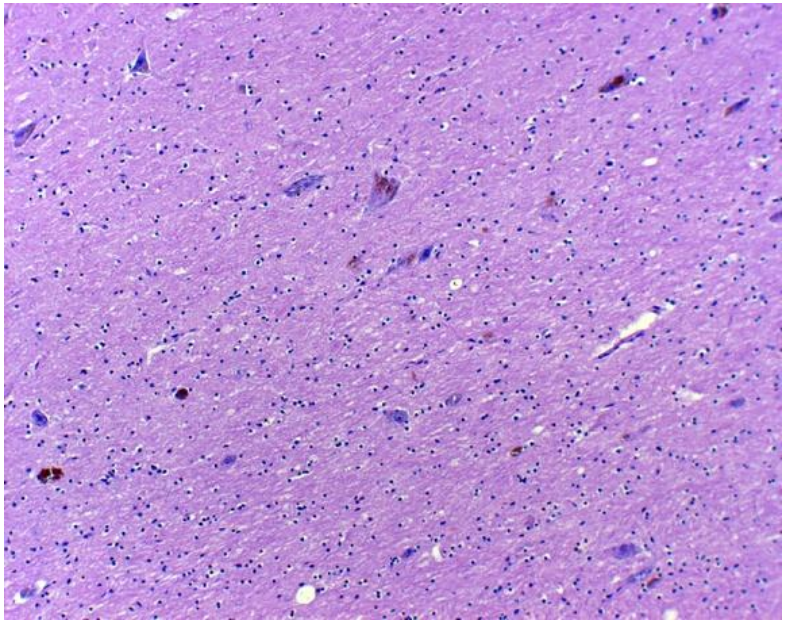
Substância negra com Parkinson

Doença de Parkinson

Substância Negra

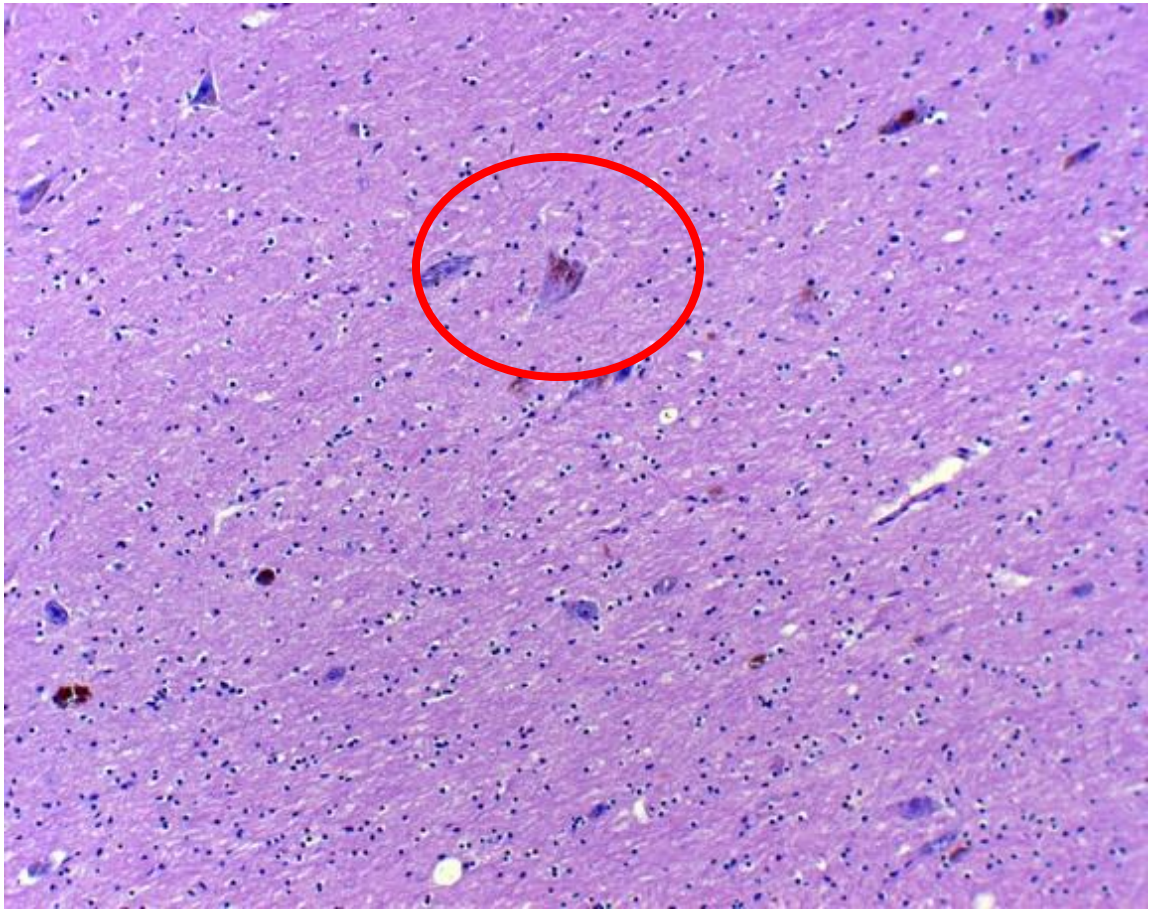


Normal



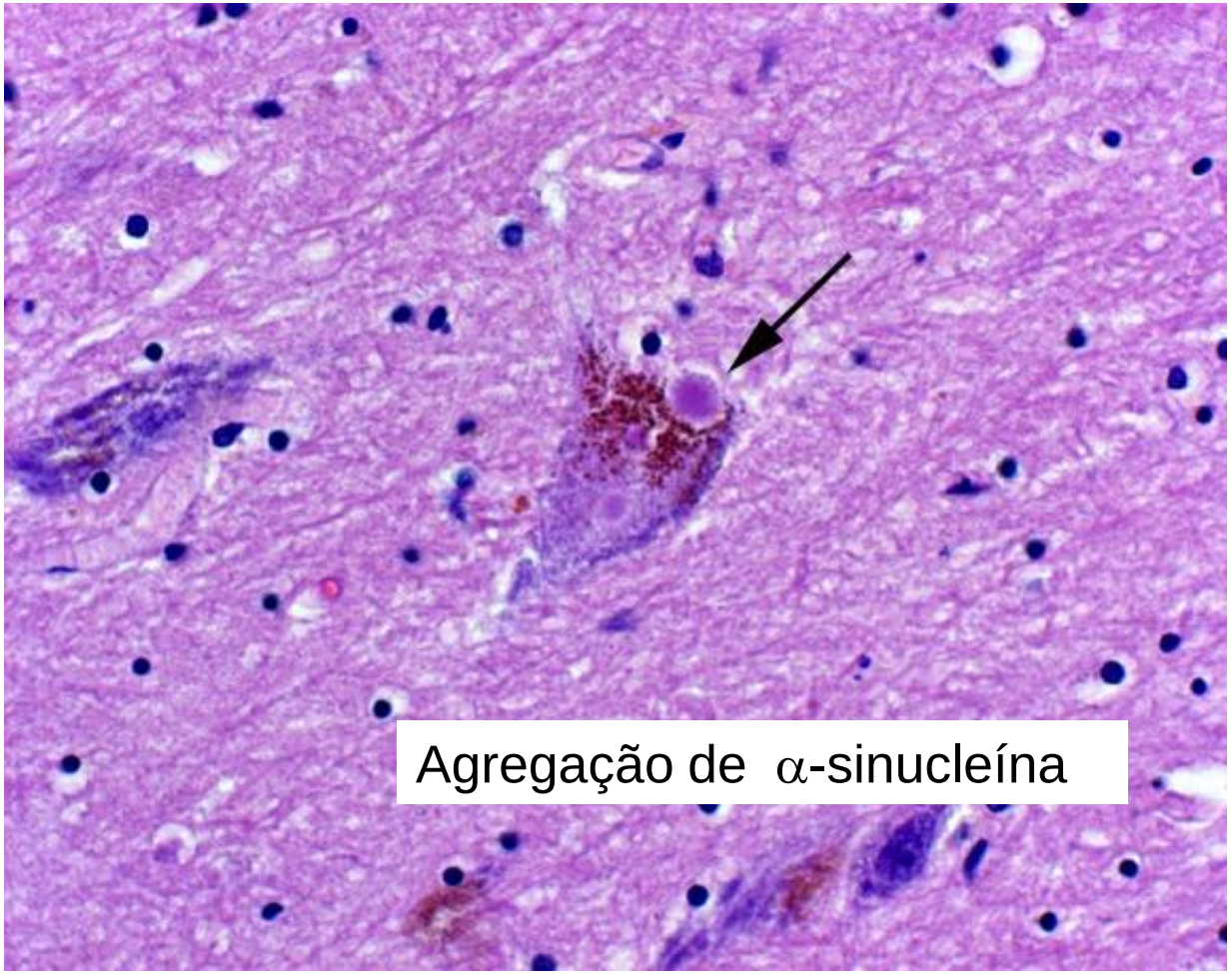
Parkinson

Doença de Parkinson

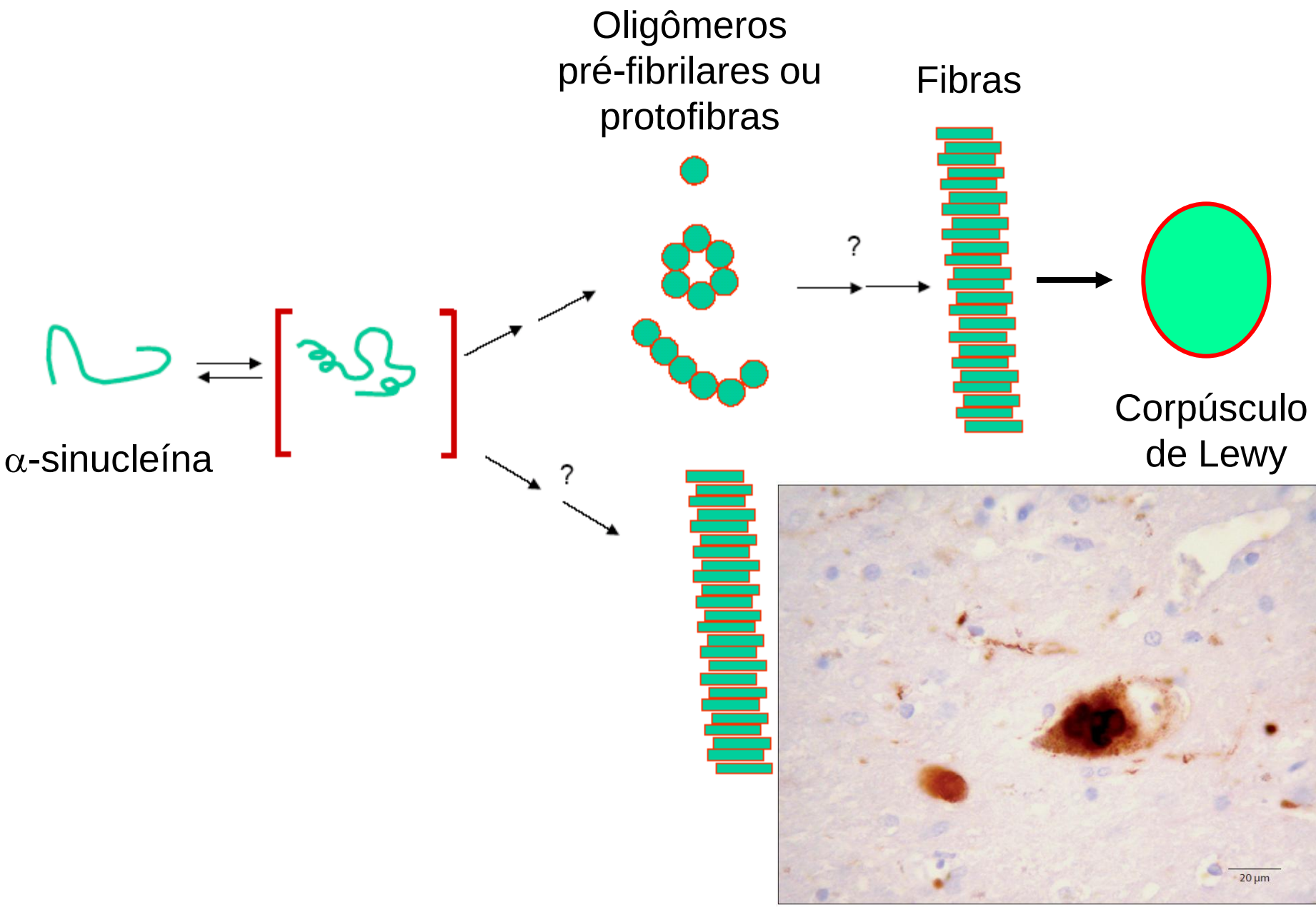


Substância negra com Parkinson

Corpúsculos de Lewy



Mecanismo de agregação de α-sinucleína

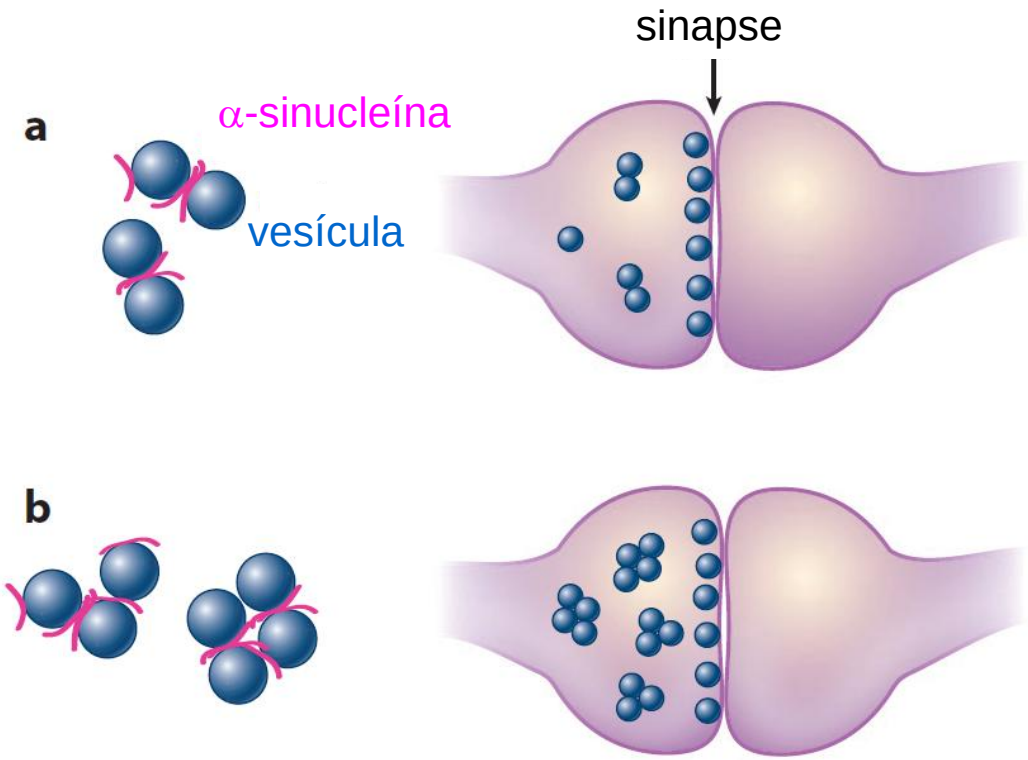


Como os agregados de α-sinucleína causam a Doença de Parkinson?

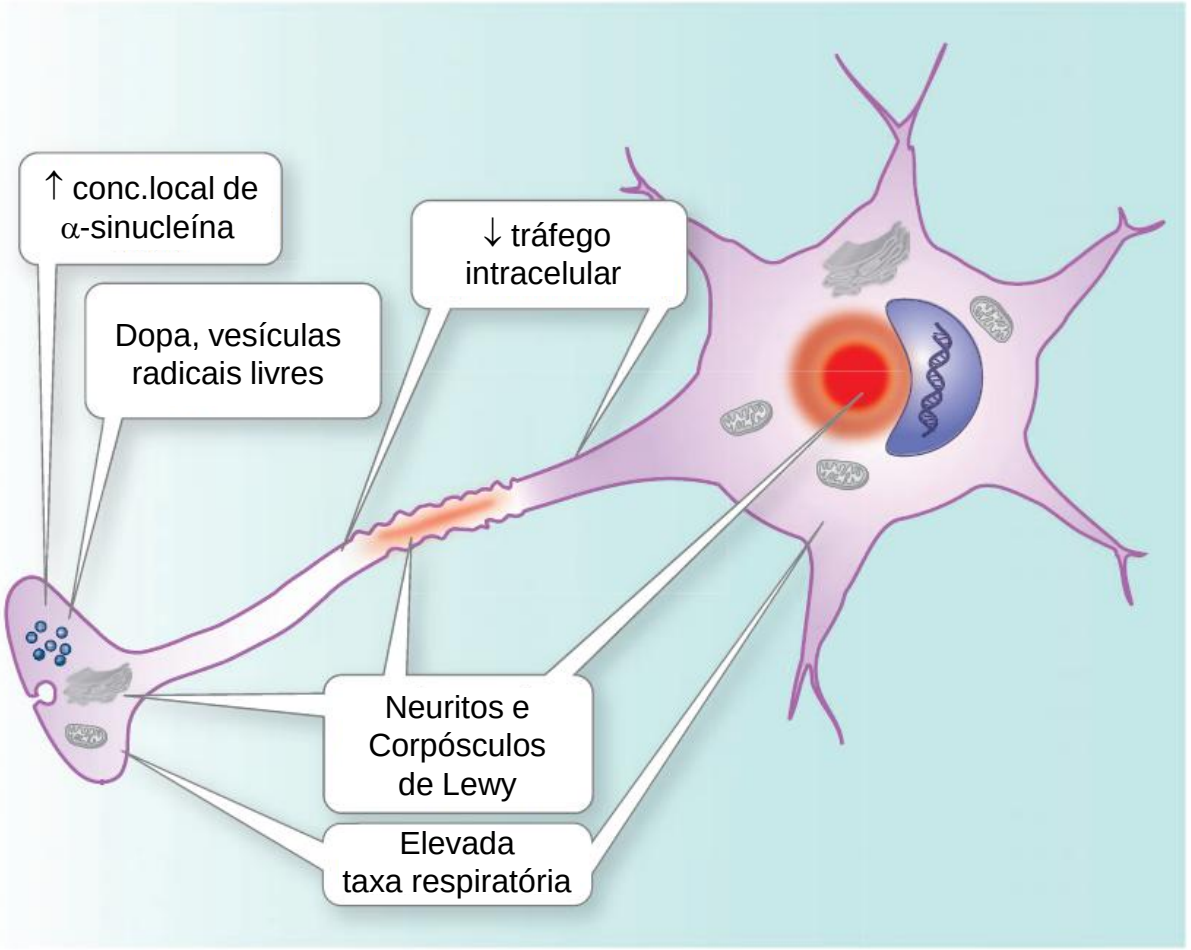
α-sinucleína liga-se às vesículas sinápticas

Normal

Parkinson

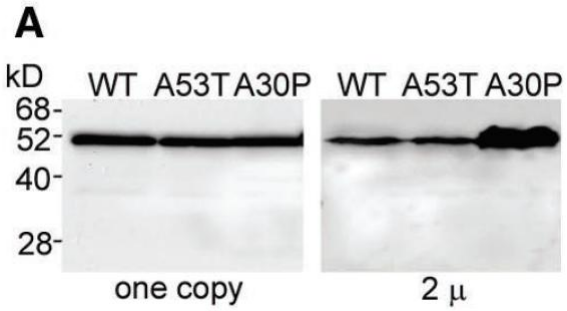


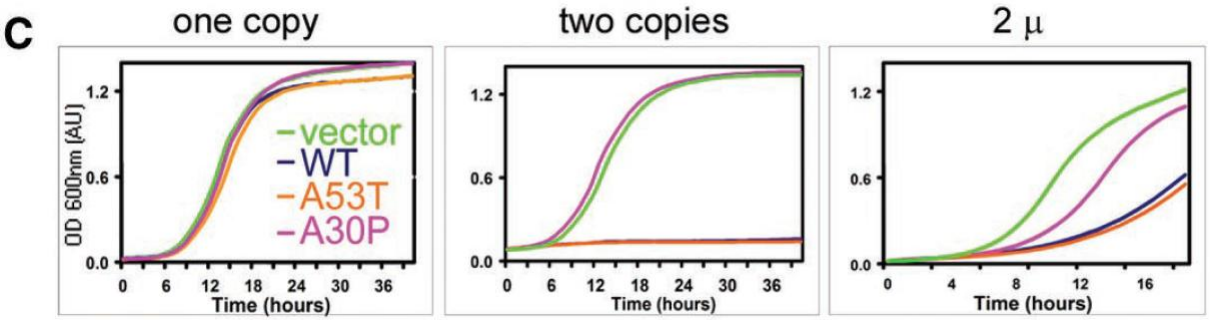
Toxicidade da α-sinucleína em neurônio dopaminérgico

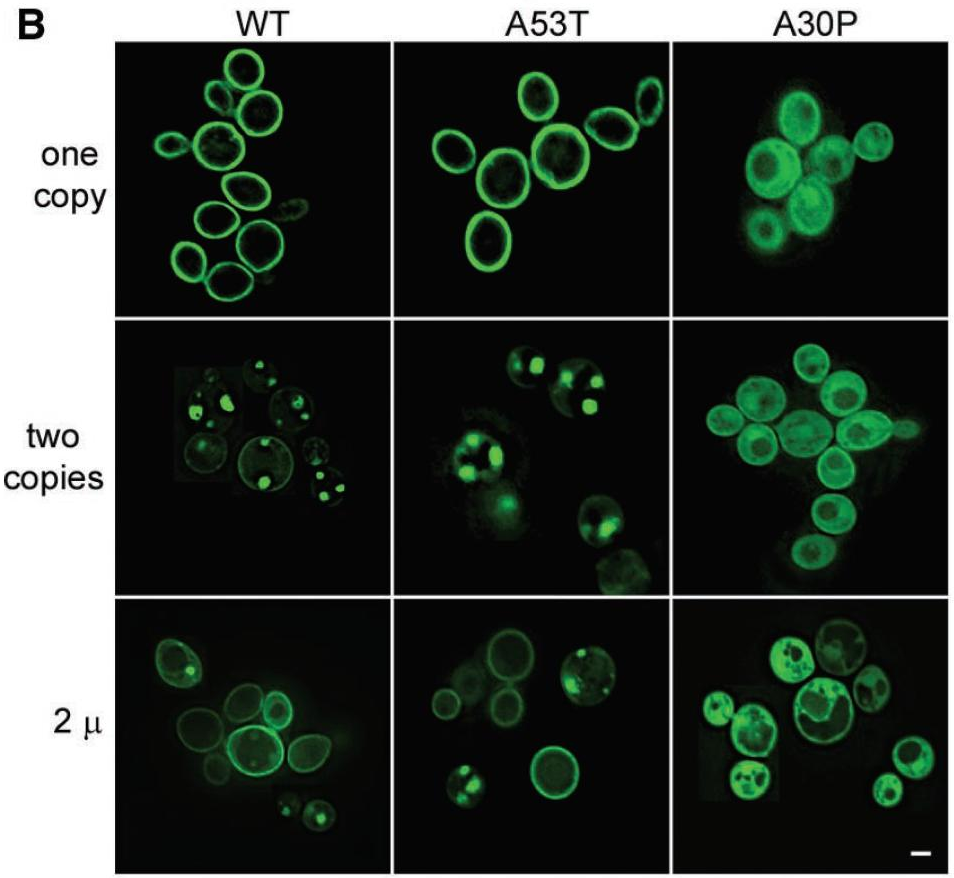


Yeast models to study Parkinson’s disease

Expression of Syn in yeast

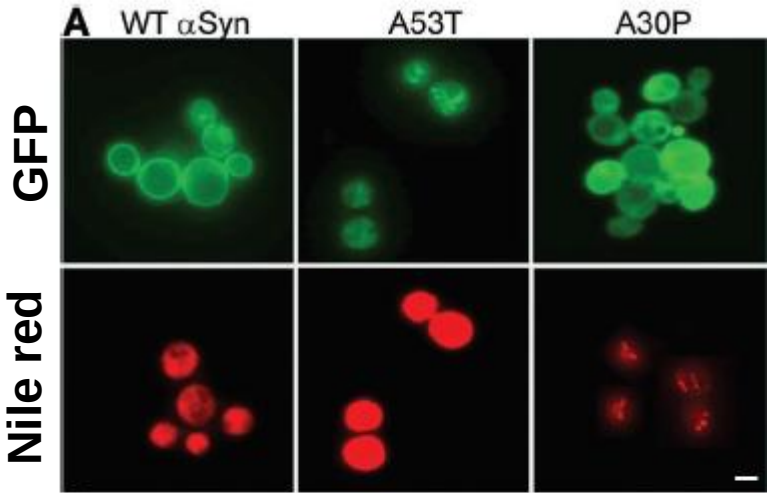


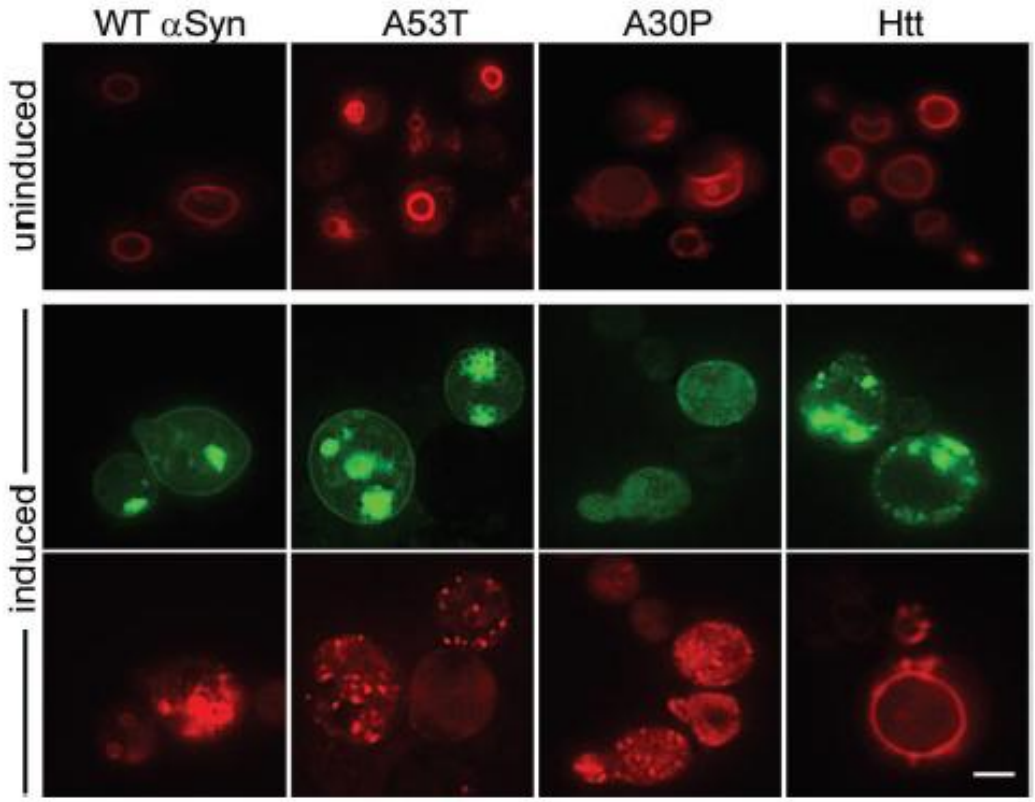




Outeiro and Lindqust Science **302:** 1772-1775

Cells expressing Syn accumulate lipids.
Syn overexpression perturbs the distribution of vesicular pools

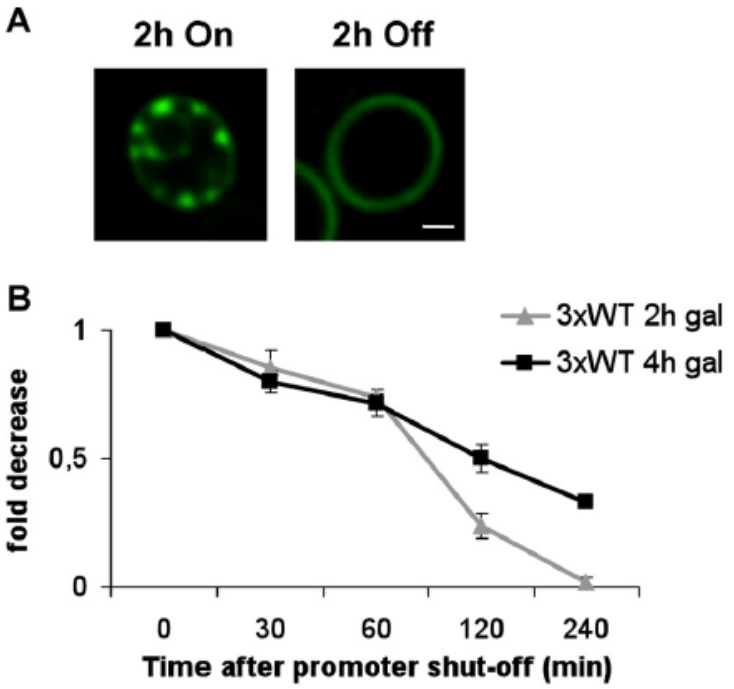


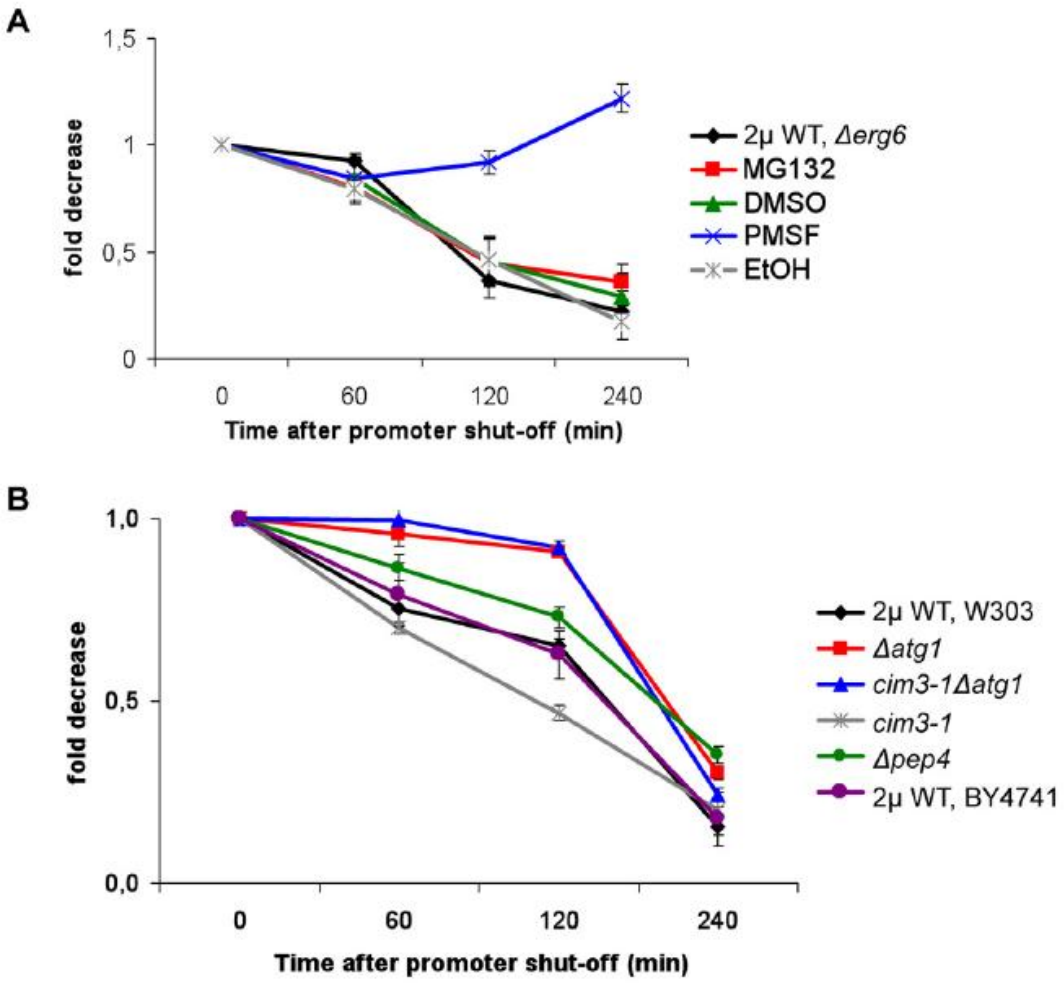


FM4-64

Outeiro and Lindqust Science **302:** 1772-1775

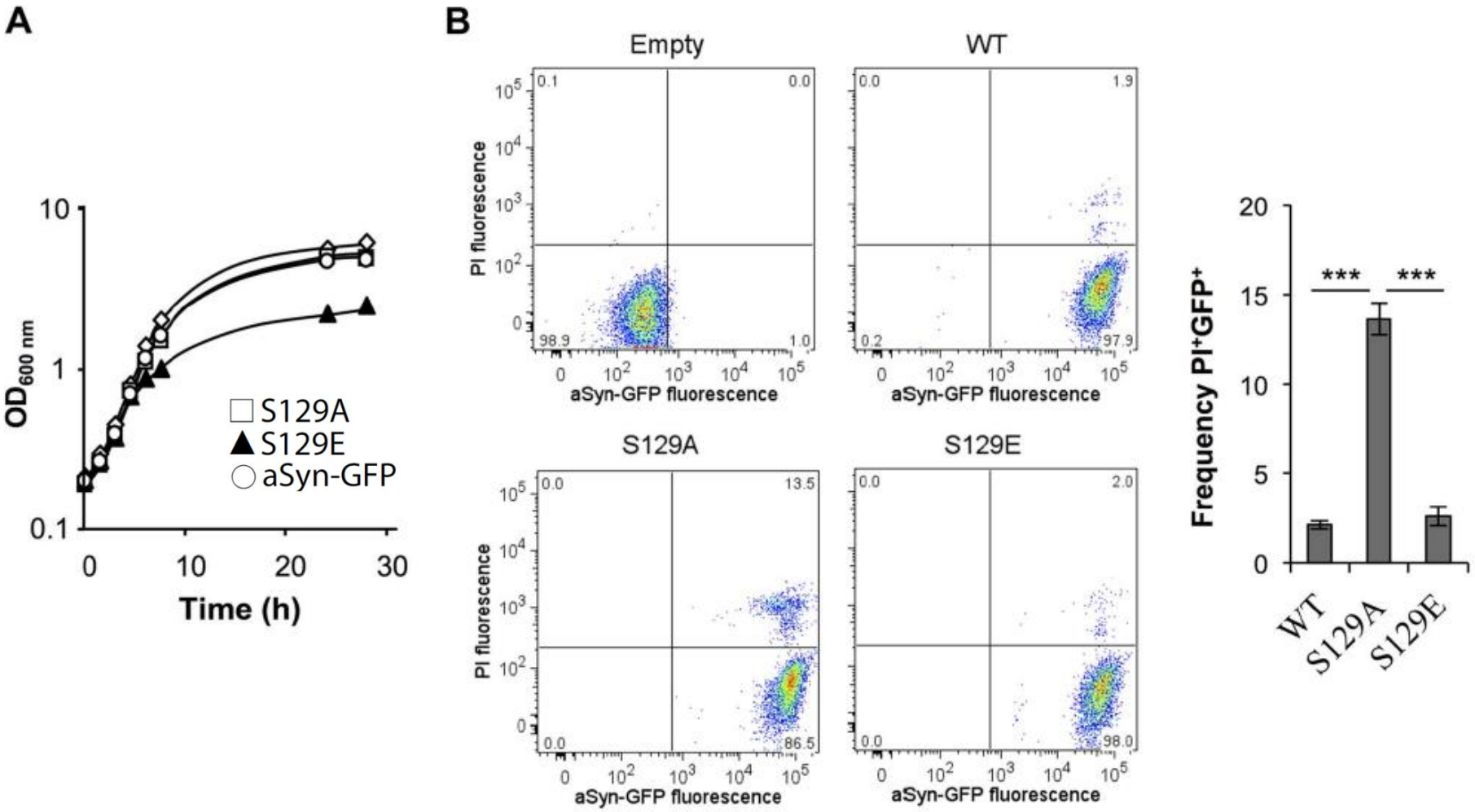
Clearing of -synuclein aggregates by autophagy and vacuole function rather than proteasome



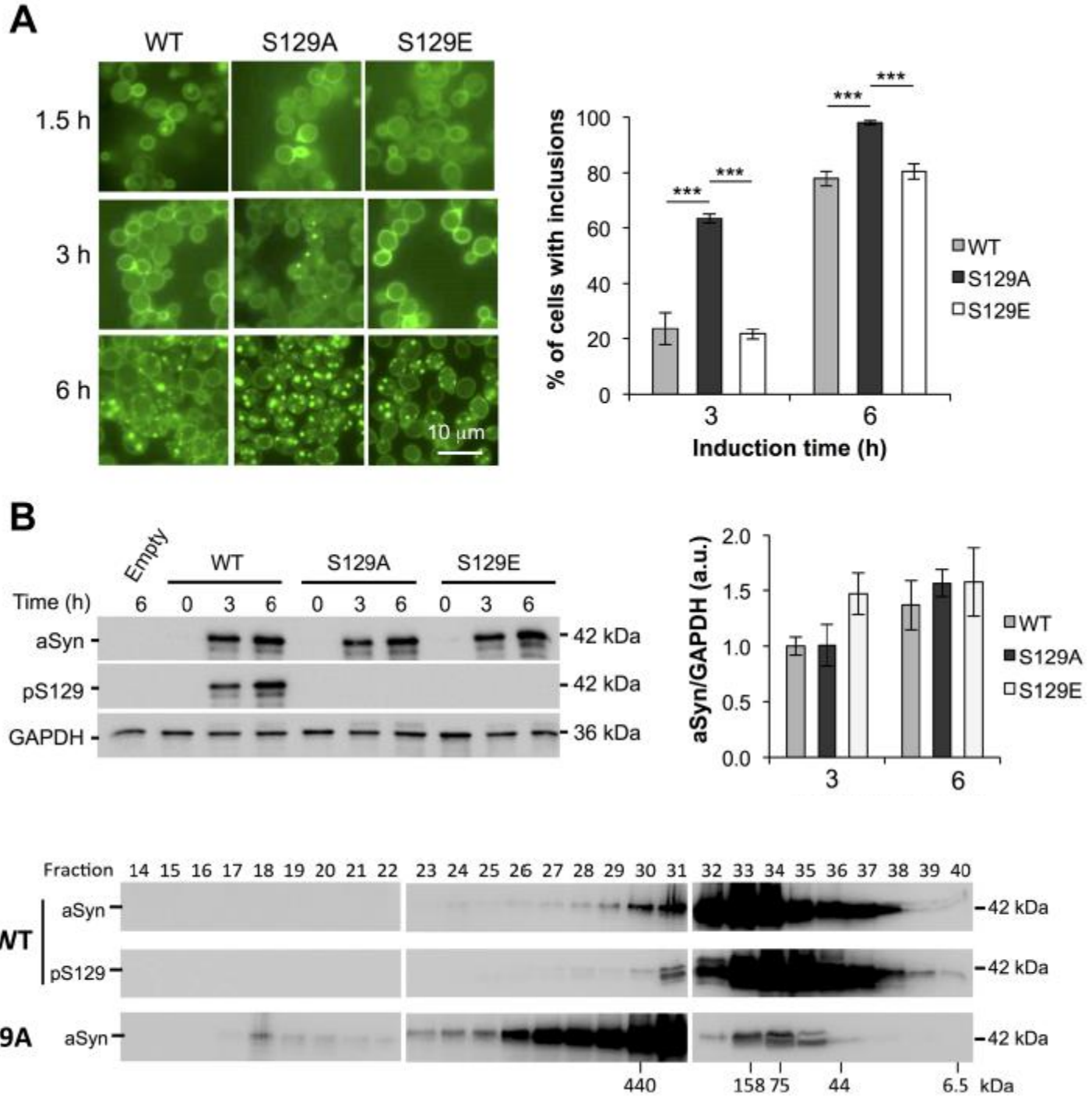


Petroi et al. (2012) J. Biol. Chem. **287**: 27567–27579

S129A αSyn is more toxic for yeast cells than the WT αSyn



Tenreiro et al. (2014) PLoS Genet **10**: e1004302



Tenreiro et al. (2014) PLoS Genet **10**: e1004302

Introdução à Doença de Huntington

Início tardio

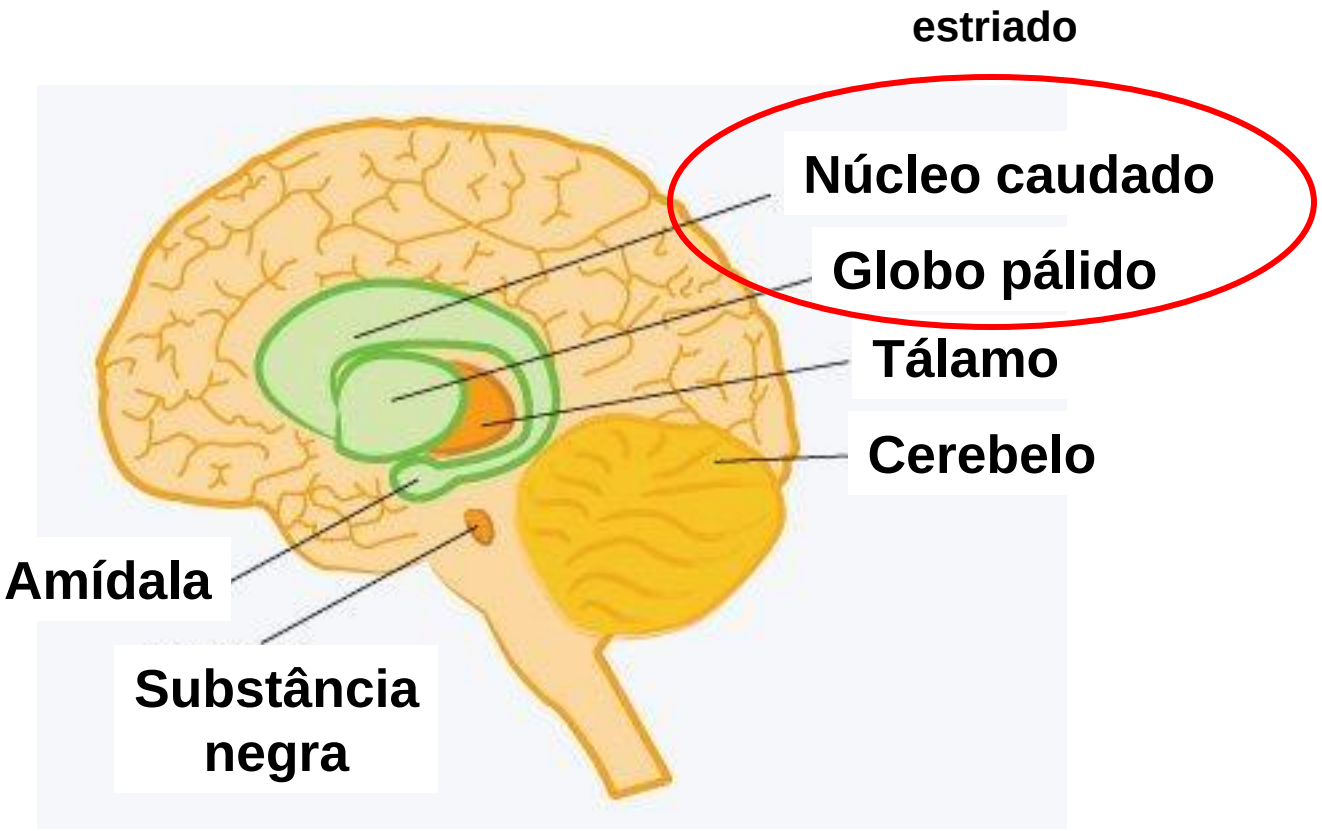
Perda de neurônios do tecido estriado

Perda de peso

Doença com herança autossômica dominante

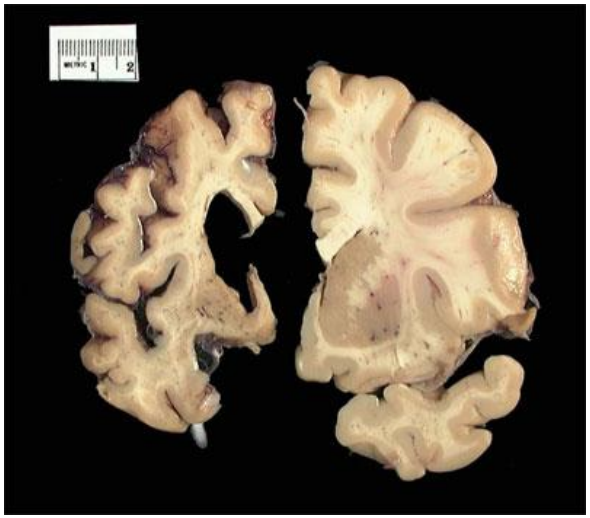
Disfunção motora, psiquiátrica e do sistema cognitivo progressiva $\rightarrow$ morte

Áreas do cérebro afetadas na doença de Huntington



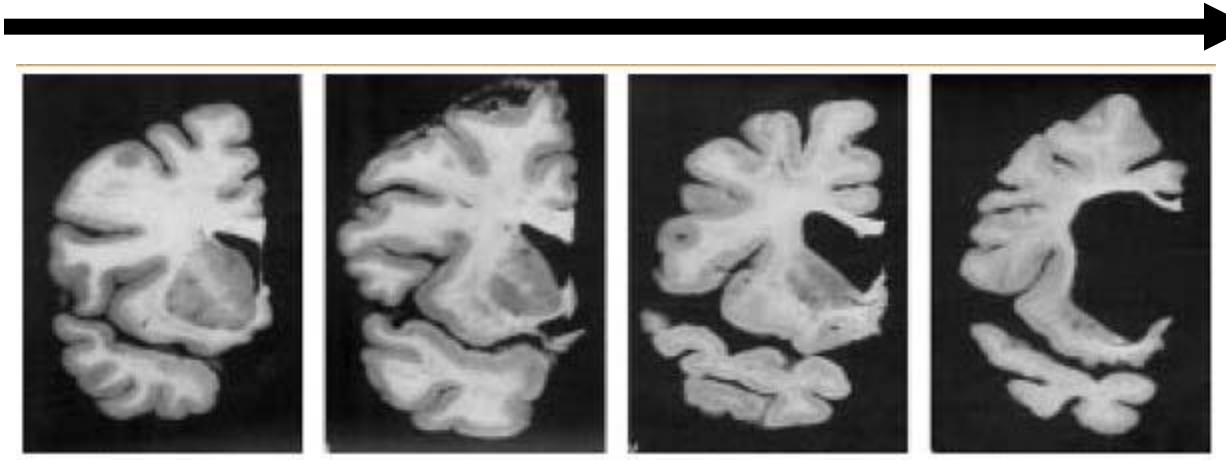
Atrofia do tecido cerebral com o progresso da doença

Huntington

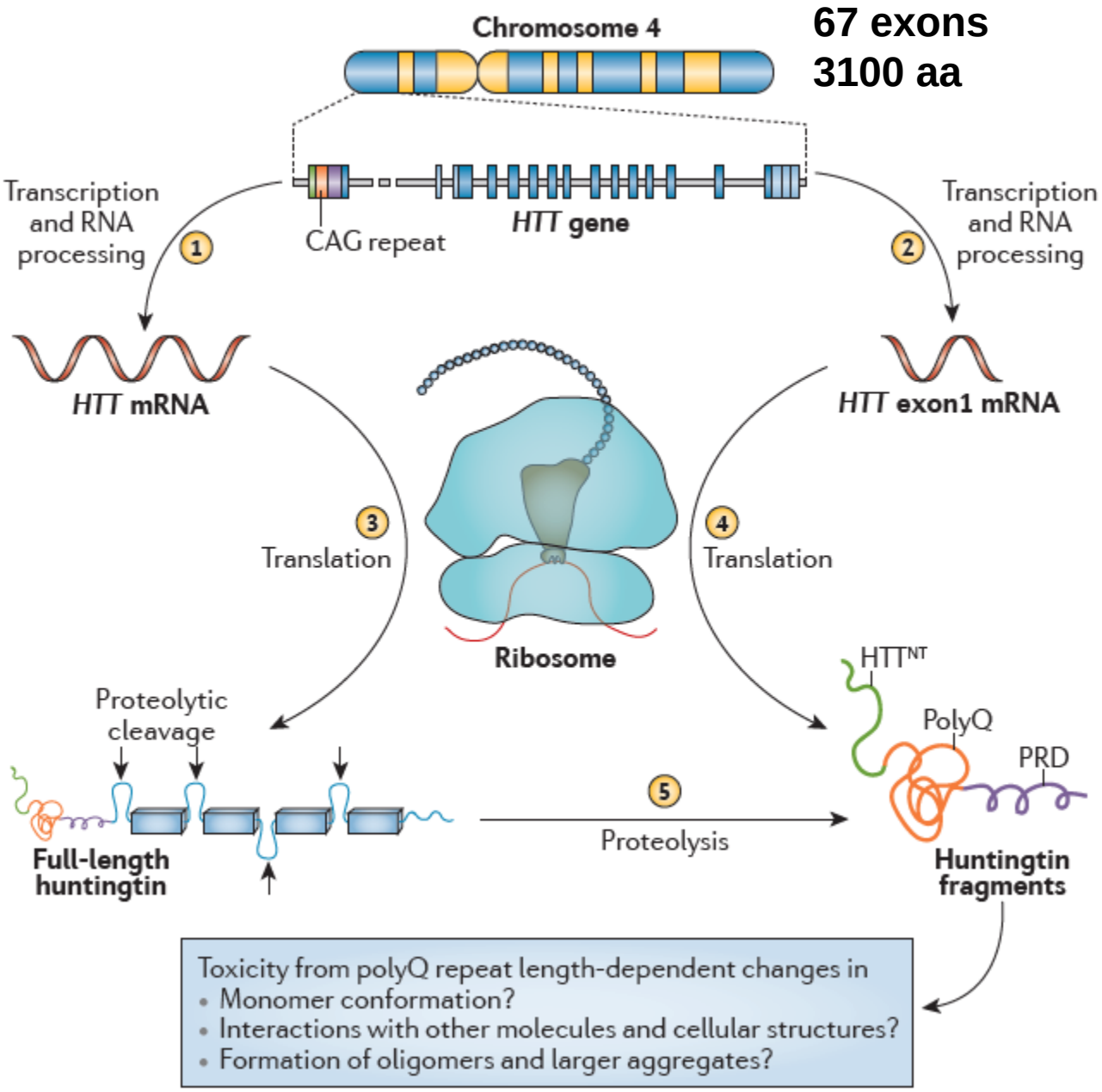


Normal

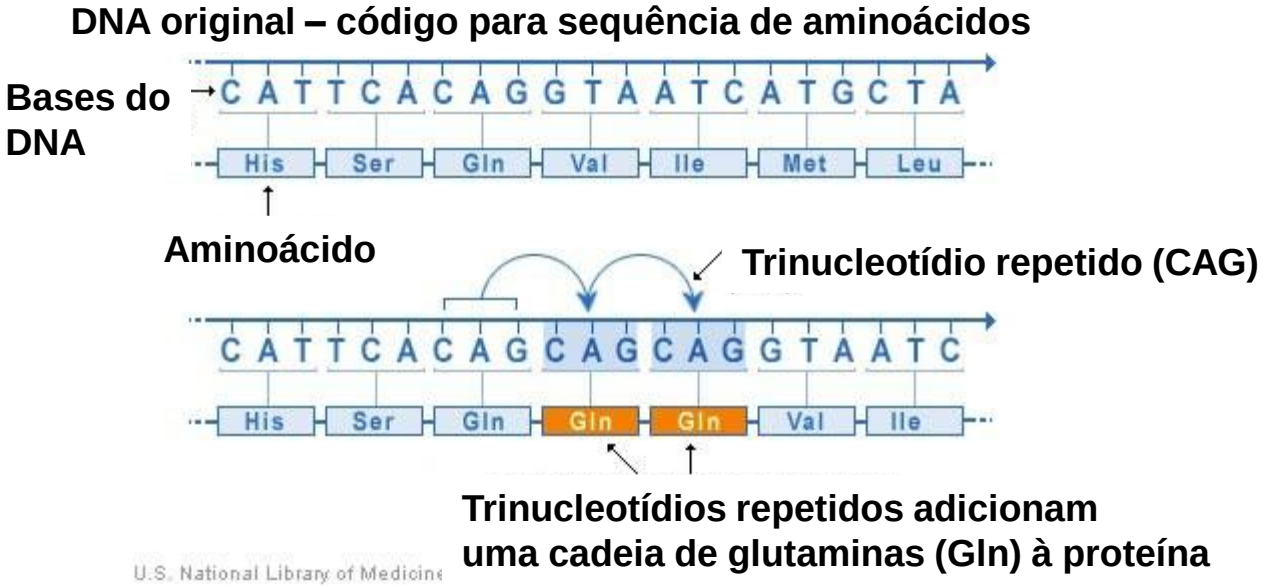
Progresso da doença



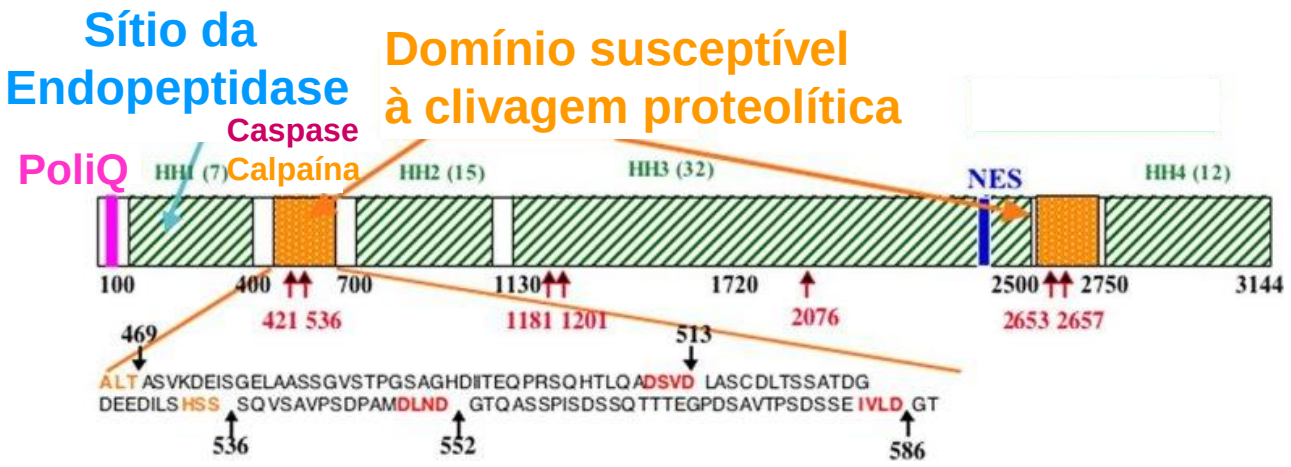
Huntingtin structure and transformations



Mutação por expansão de sequências de trinucleotídios repetidos

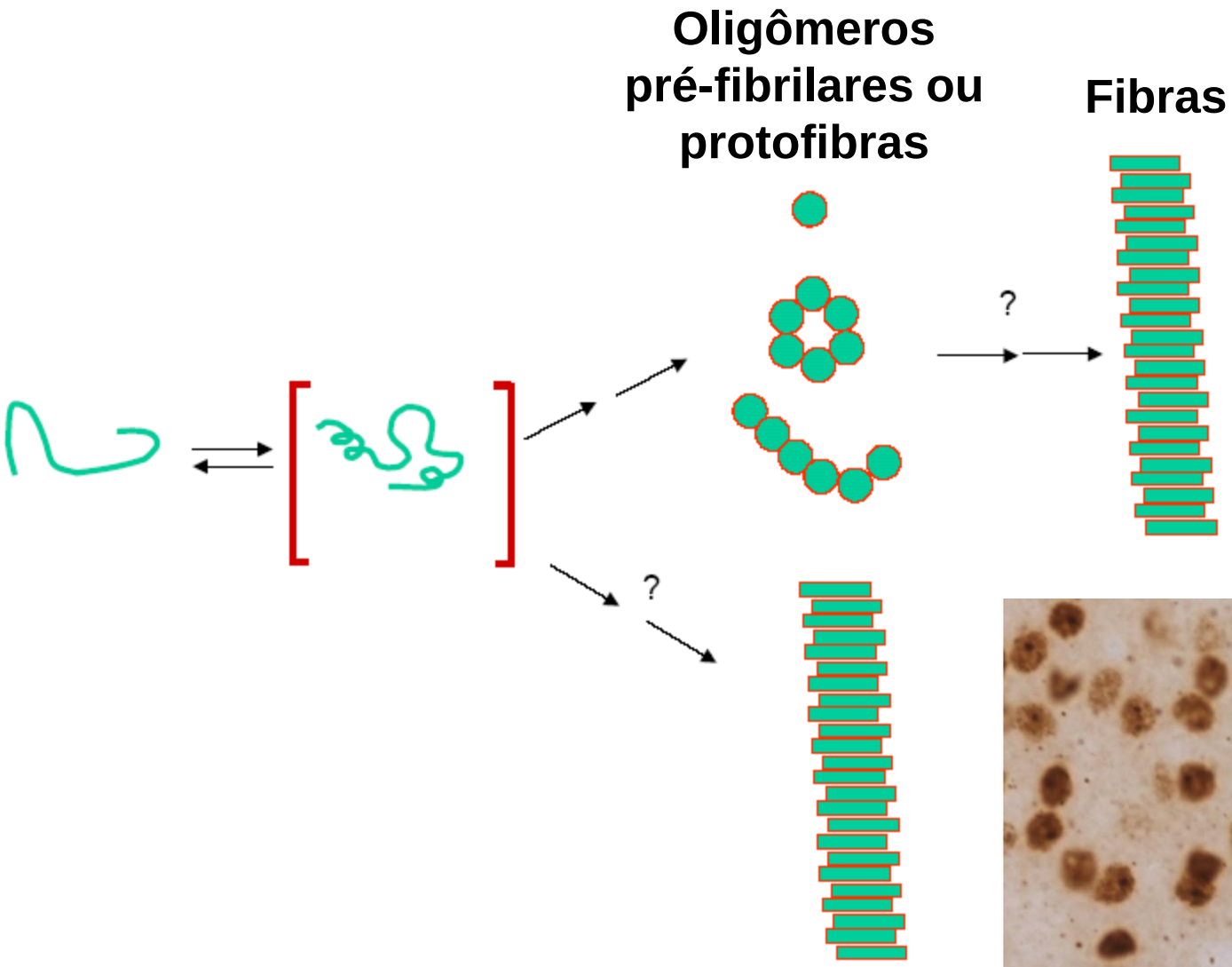


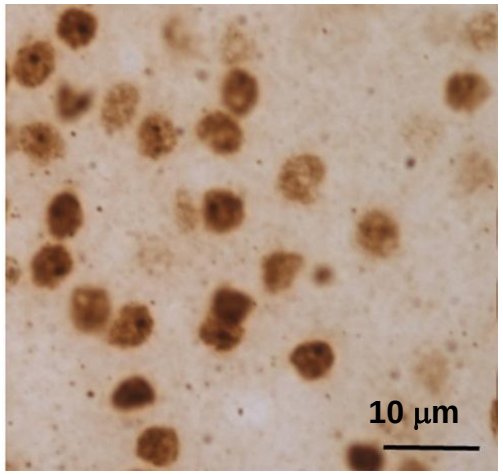
Estrutura da Huntingtina



Quantidade de repetiçoes CAG	Fenótipo
menos de 35	normal
de 36 a 39	aumenta o risco (penetrância incompleta)
mais de 40	desenvolve a doença

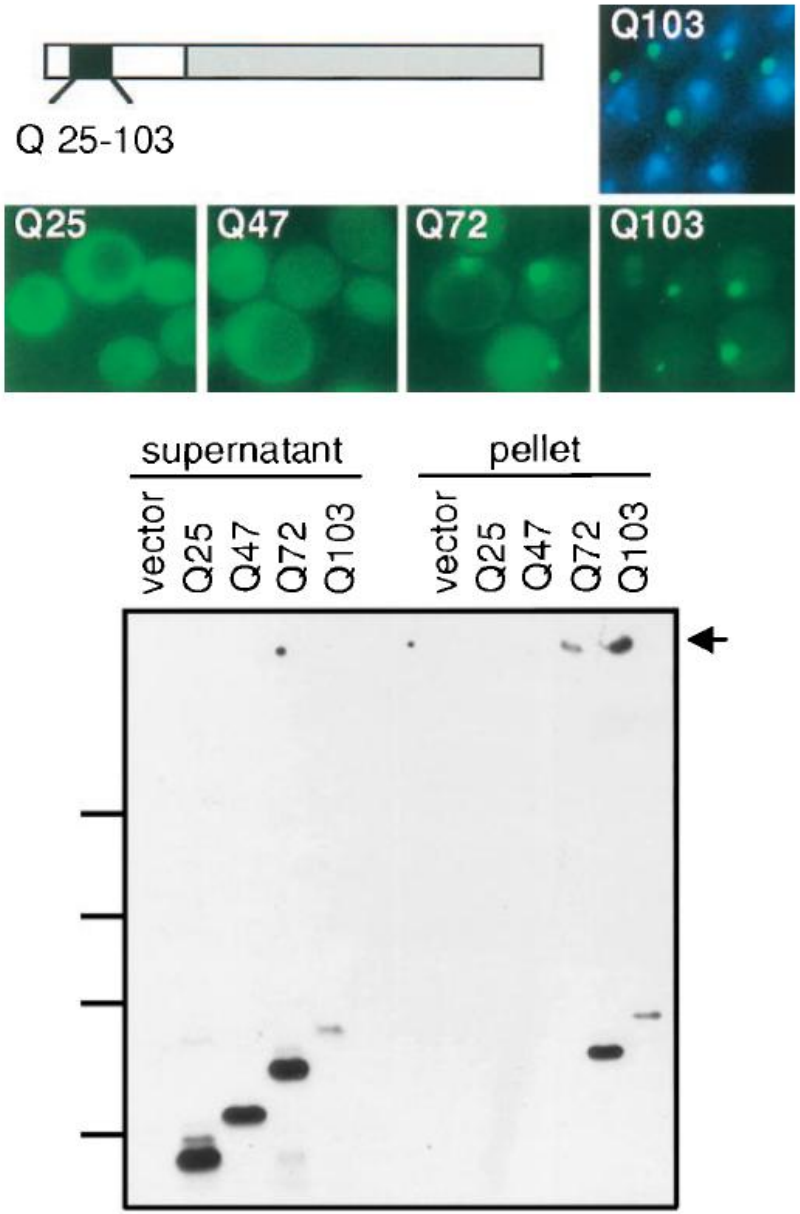
Mecanismo de agregação da Huntingtina





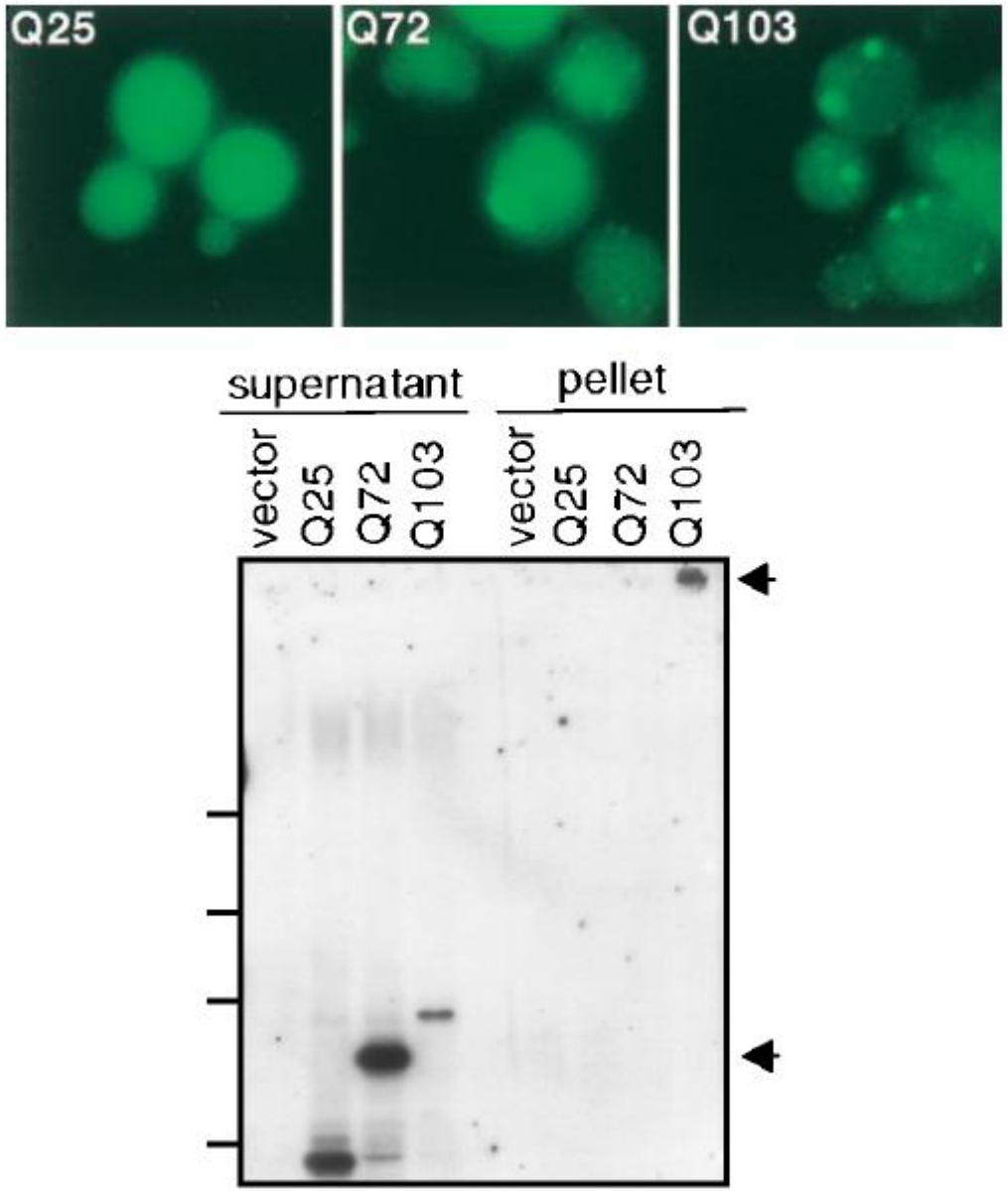
Budding yeast models for the investigation of the mutant Htt-induced toxicity

Expression of Htt fragments in yeast



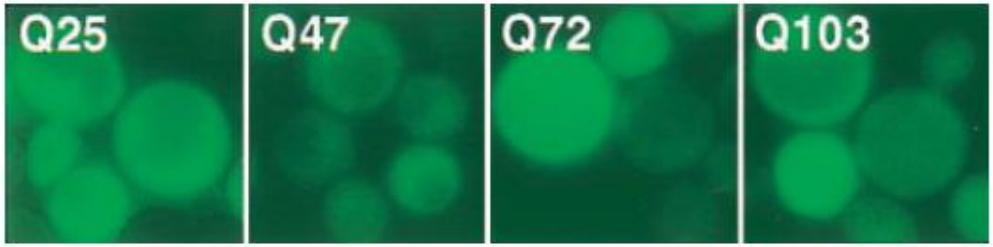
Krobitsch and Lindquist PNAS (2000) **97**:1589-1594

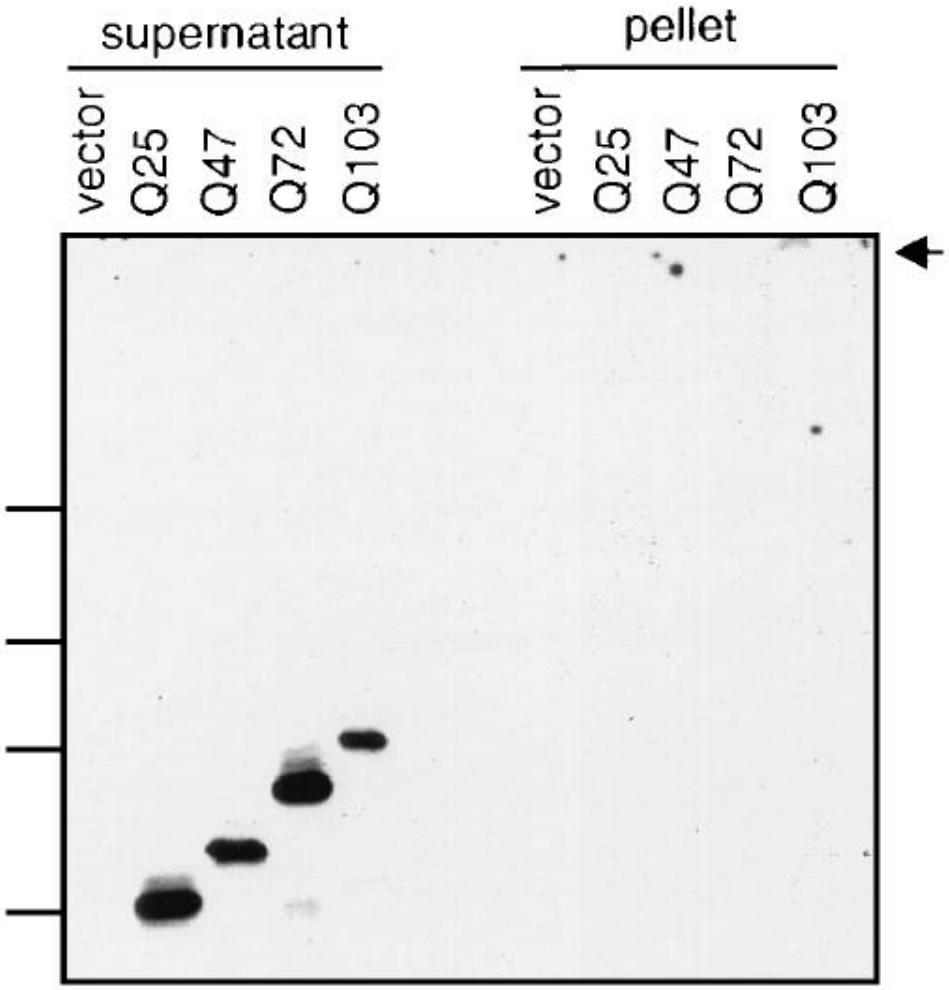
Overexpression of Hsp104 reduces Htt aggregation



Krobitsch and Lindquist PNAS (2000) **97**:1589-1594

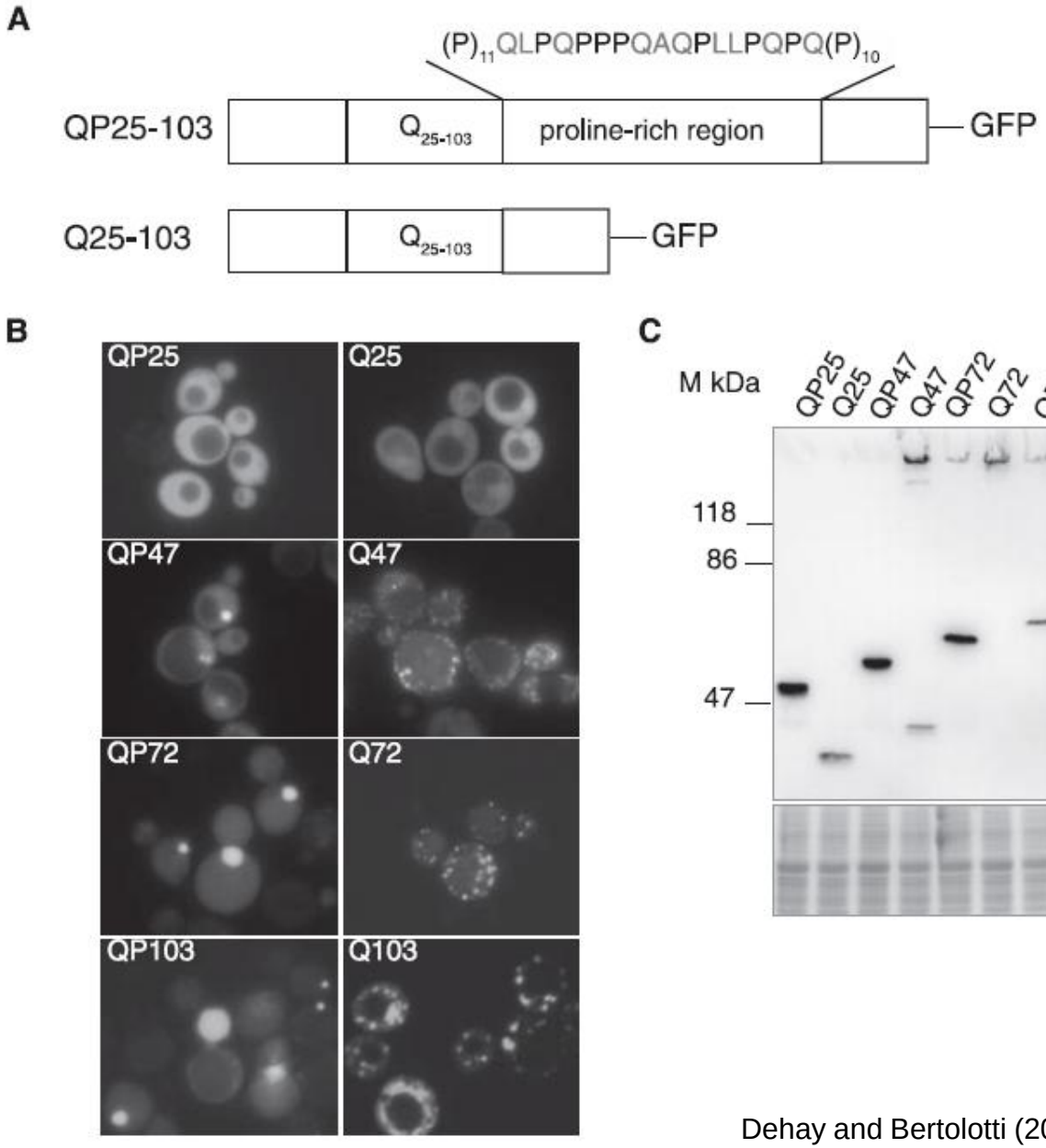
The loss of Hsp104 affects Htt aggregation





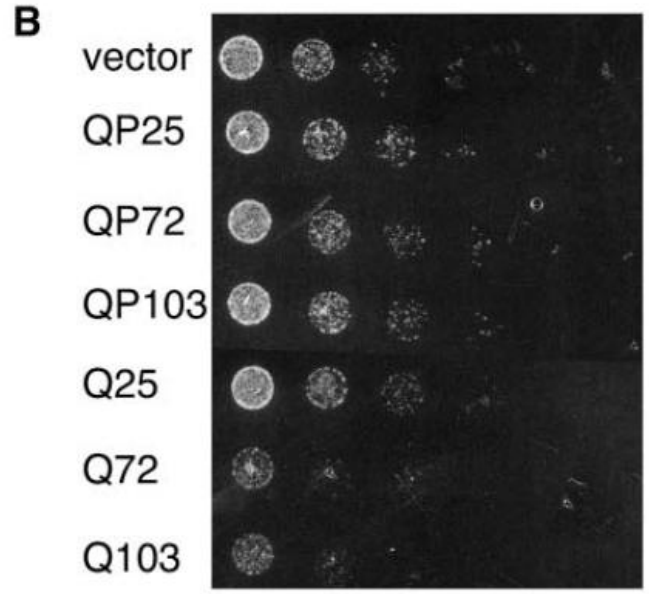
Krobitsch and Lindquist PNAS (2000) **97**:1589-1594

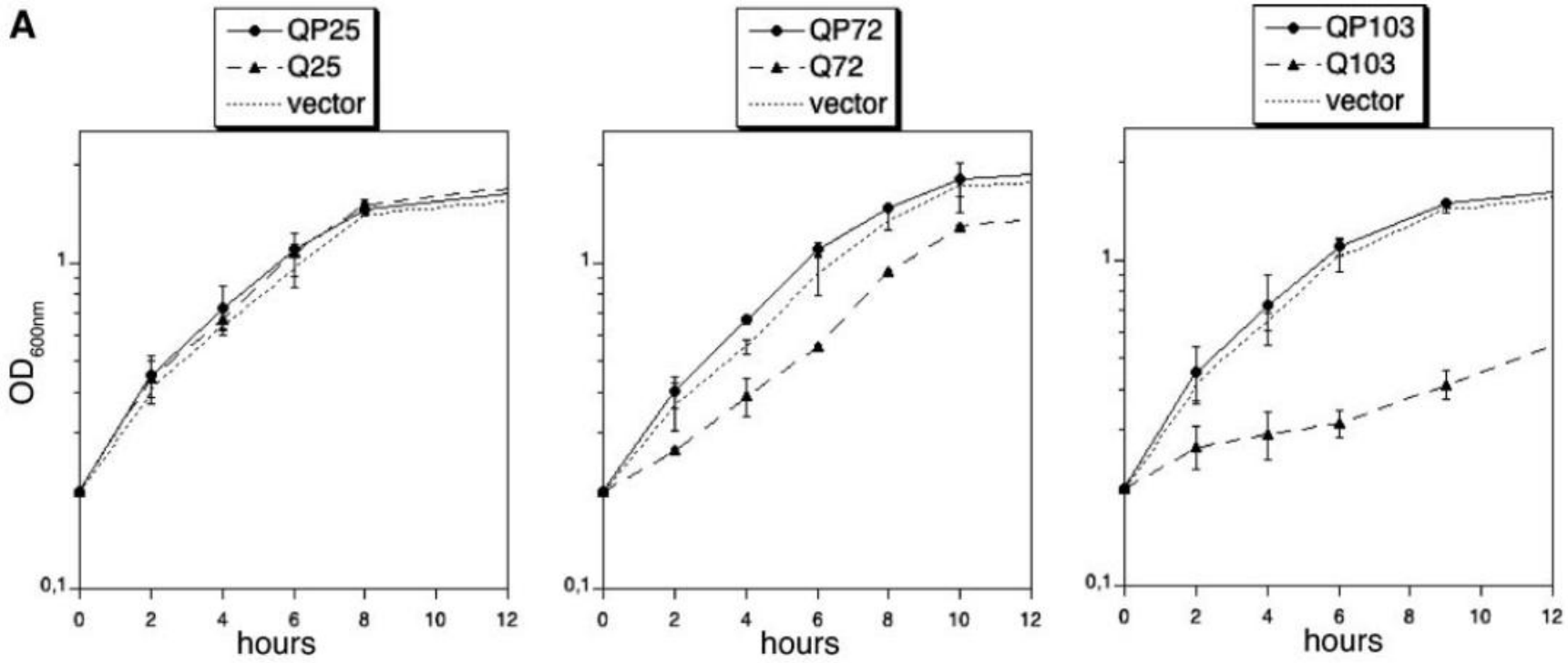
Deletion of the proline-rich region in the amino-terminal region of Htt alters aggregation of expanded poly(Q) in yeast.



Dehay and Bertolotti (2006) J Biol Chem **281**:35608-35615

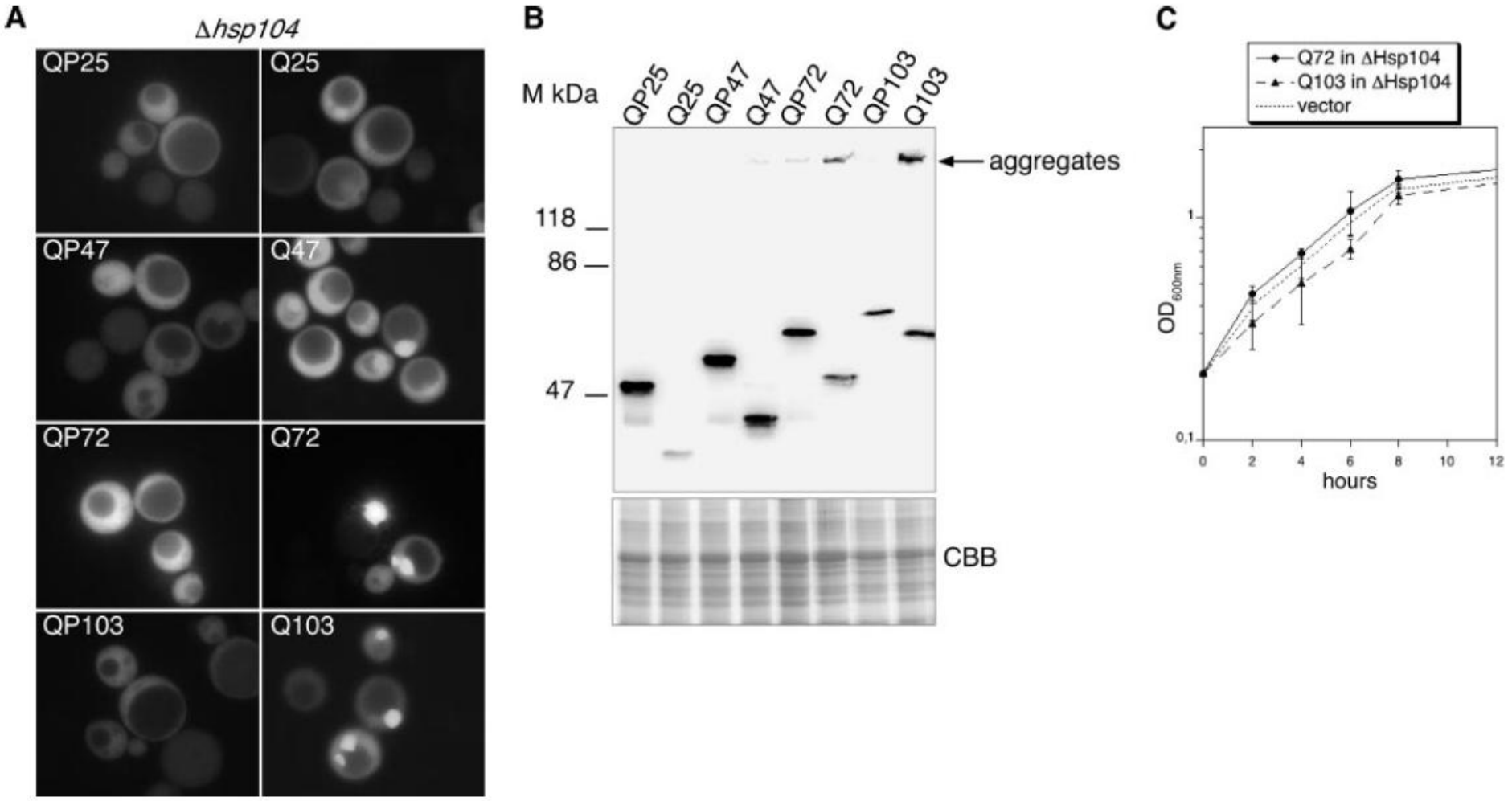
Deletion of the proline-rich region in the amino-terminal region of Htt provokes cytotoxicity of expanded poly(Q) in yeast





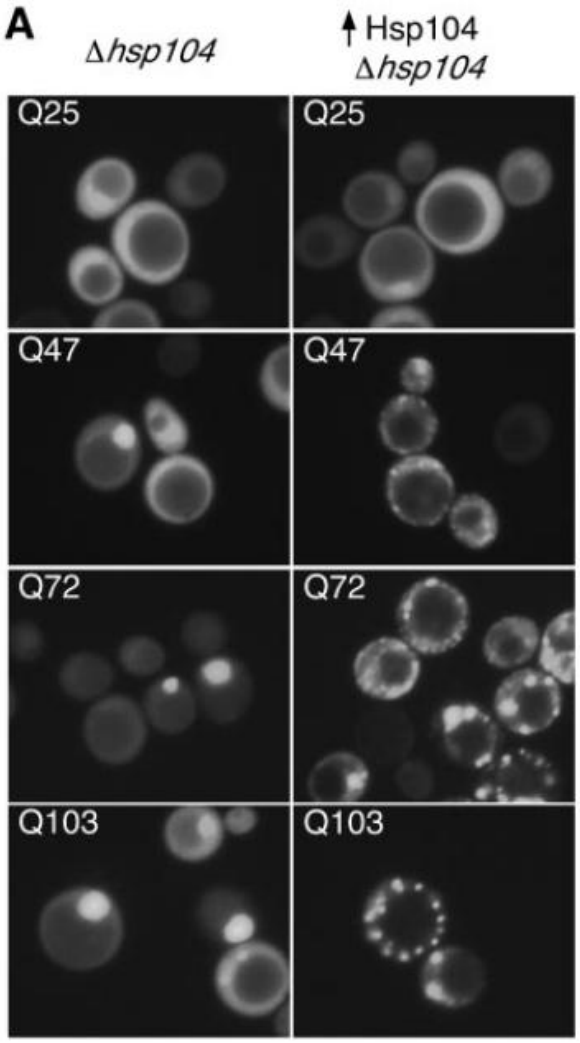
Dehay and Bertolotti (2006) J Biol Chem **281**:35608-35615

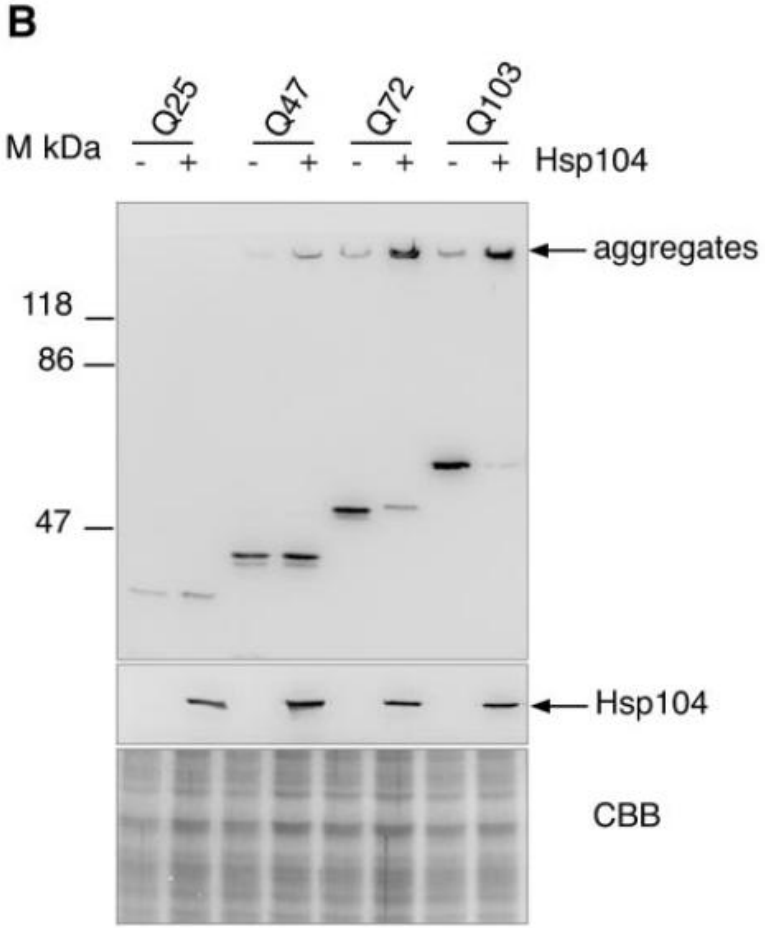
Deletion of the proline-rich region in the amino-terminal region of mutant Htt bypasses the requirement of Hsp104 for aggregate formation

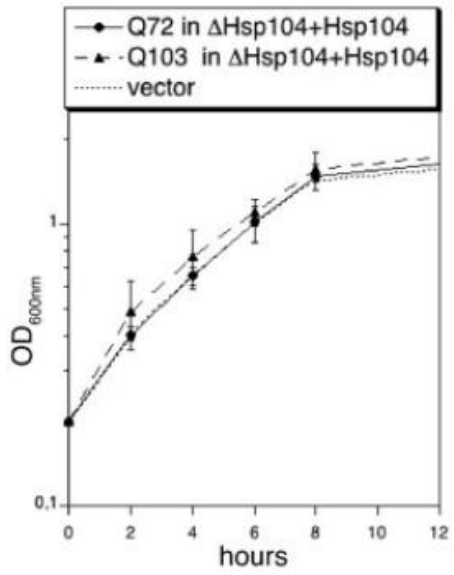


Dehay and Bertolotti (2006) J Biol Chem **281**:35608-35615

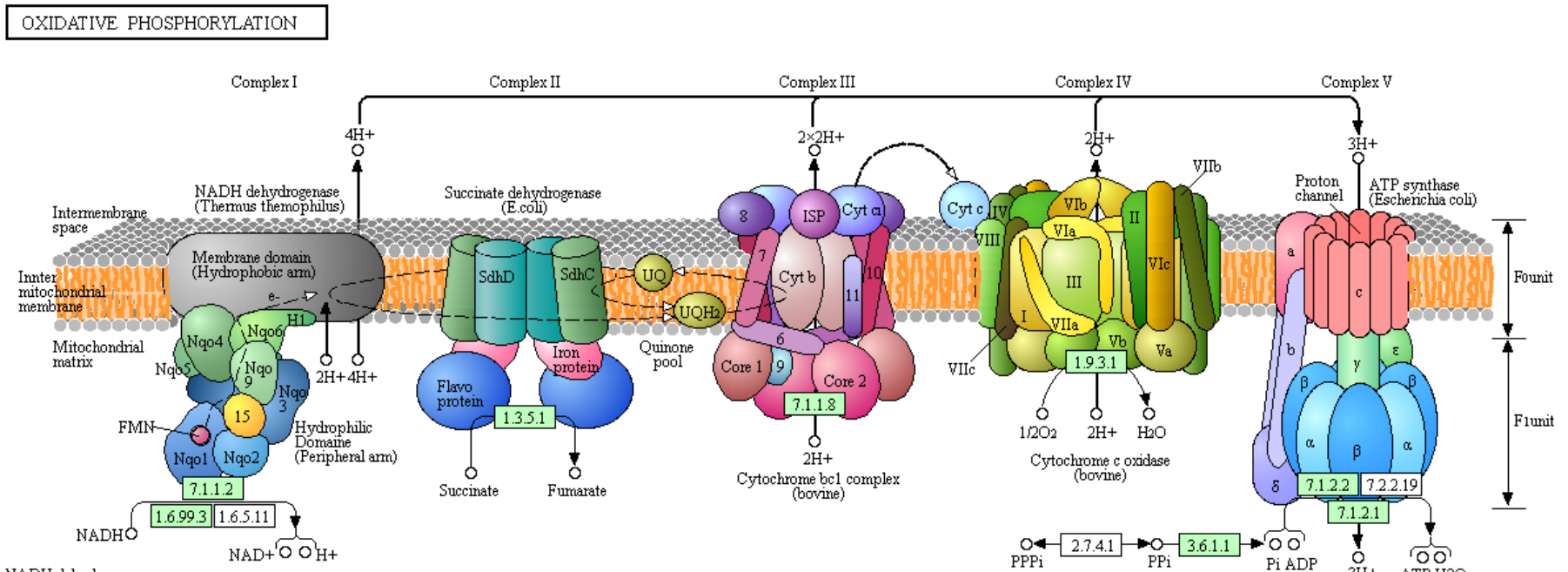
Reintroduction of Hsp104 in hsp104-deleted cells restores the shape of Q47, Q72, and Q103 aggregates observed in wild-type cells







Saccharomyces cerevisiae oxidative phosphorylation complexes



NADH dehydrogenase

E	ND1	ND2	ND3	ND4	ND4L	ND5	ND6										
E	Ndufs1	Ndufs2	Ndufs3	Ndufs4	Ndufs5	Ndufs6	Ndufs7	Ndufs8	Ndufv1	Ndufv2	Ndufv3						
B/A	NuoA	NuoB	NuoC	NuoD	NuoE	NuoF	NuoG	NuoH	NuoI	NuoJ	NuoK	NuoL	NuoM	NuoN			
B/A	NdhC	NdhK	NdhJ	NdhH	NdhA	NdhI	NdhG	NdhE	NdhF	NdhD	NdhB	NdhL	NdhM	NdhN	HoxE	HoxF	HoxU
E	Ndufa1	Ndufa2	Ndufa3	Ndufa4	Ndufa5	Ndufa6	Ndufa7	Ndufa8	Ndufa9	Ndufa10	Ndufab1	Ndufa11	Ndufa12	Ndufa13			
E	Ndufb1	Ndufb2	Ndufb3	Ndufb4	Ndufb5	Ndufb6	Ndufb7	Ndufb8	Ndufb9	Ndufb10	Ndufb11	Ndufc1	Ndufc2				

Succinate dehydrogenase / Fumarate reductase

E	SDHC	SDHD	SDHA	SDHB
B/A	SdhC	SdhD	SdhA	SdhB
			FrdA	FrdB	FrdC	FrdD

Cytochrome c reductase

E/B/A	ISP	Cyt b	Cyt 1						
E				COR1	QCR2	QCR6	QCR7	QCR8	QCR9	QCR10

Cytochrome c oxidase

E	COX10		COX3	COX1	COX2	COX4	COX5A	COX5B	COX6A	COX6B	COX6C	COX7A	COX7B	COX7C	COX8	E/B/A: COX11	COX15	COX17
B/A	CyoE	CyoD	CyoC	CyoB	CyoA													
		CoxD	CoxC	CoxA	CoxB													
		QoxD	QoxC	QoxB	QoxA													

Cytochrome c oxidase , cbb3-type

B	I	II	IV	III

Cytochrome bd complex

B/A	CydA	CydB	CydX

F-type ATPase (Bacteria)

alpha	beta	gamma	delta	epsilon
a	b	c		

F-type ATPase (Eukaryotes)

alpha	beta	gamma	delta	epsilon	
OSCP	a	b	c	d	e
f	g	f6/h	j	k	8

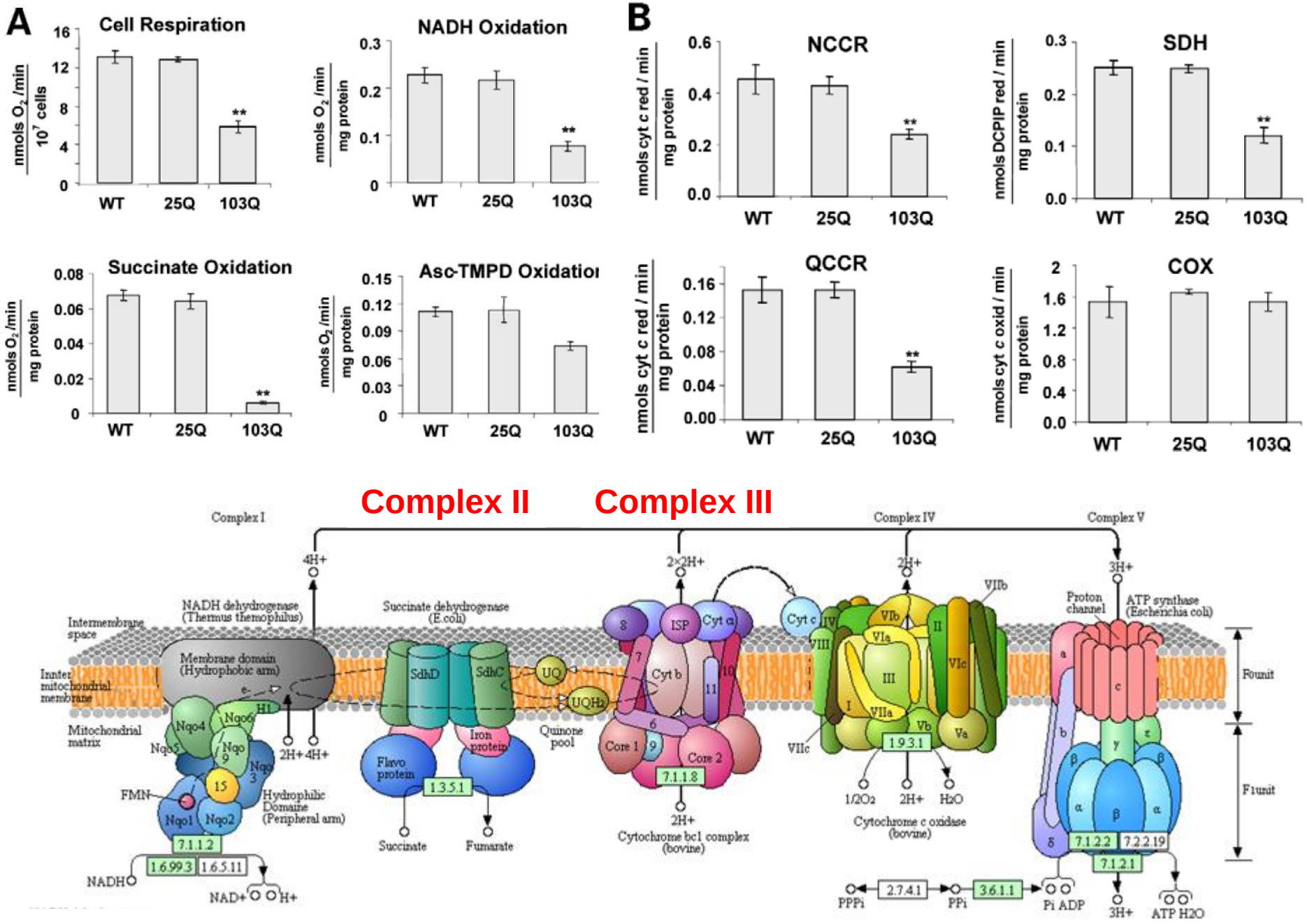
V/A-type ATPase (Bacteria, Archaeas)

A	B	C	D	E	F	G/H
I	K					

V-type ATPase (Eukaryotes)

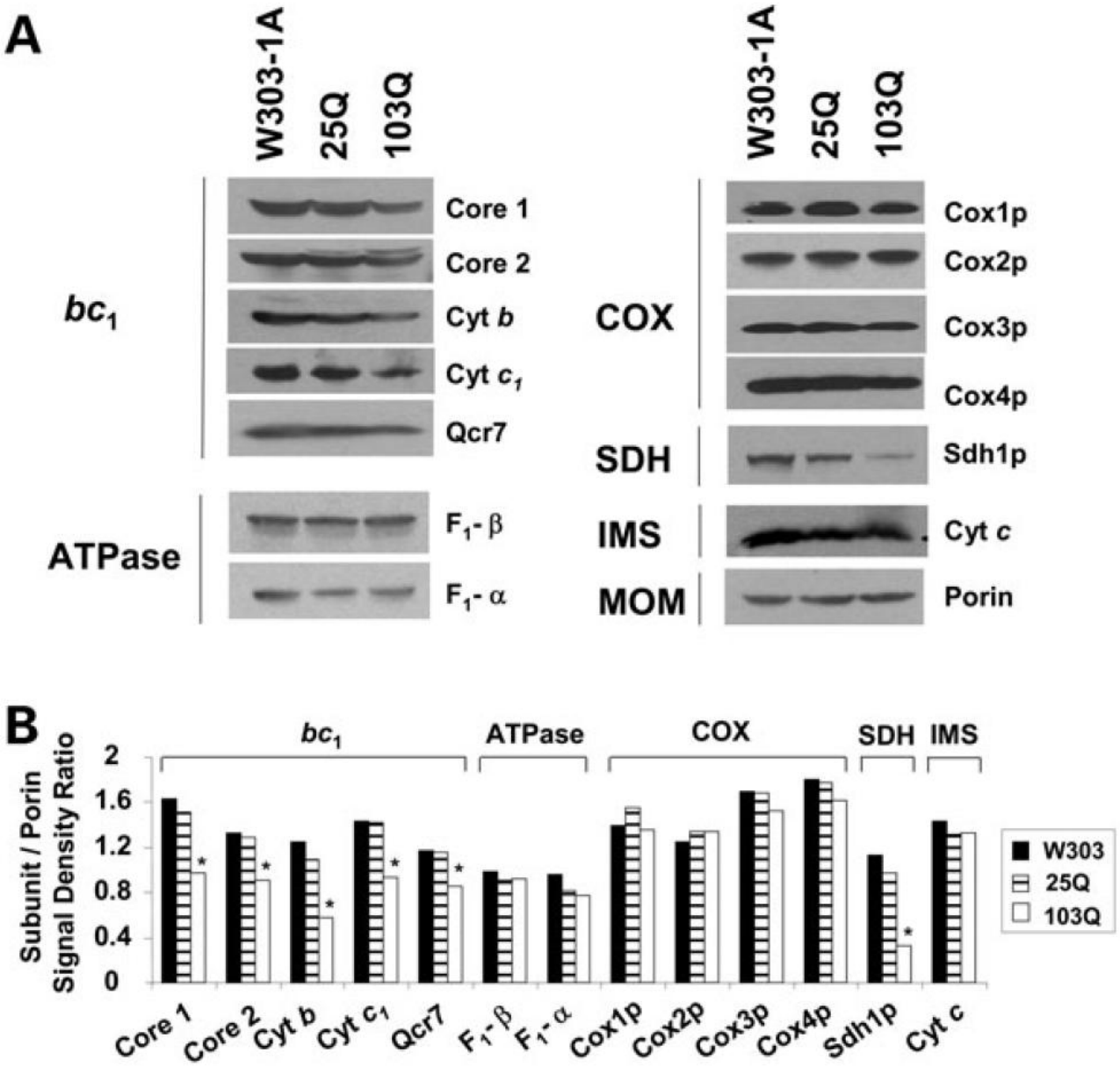
A	B	C	D	E	F	G	H
a	c	d	e	S1			

Mitochondrial functional characterization of the wild-type yeast strain W303 expressing wild-type and mutant PolyQ domains from integrative plasmids.

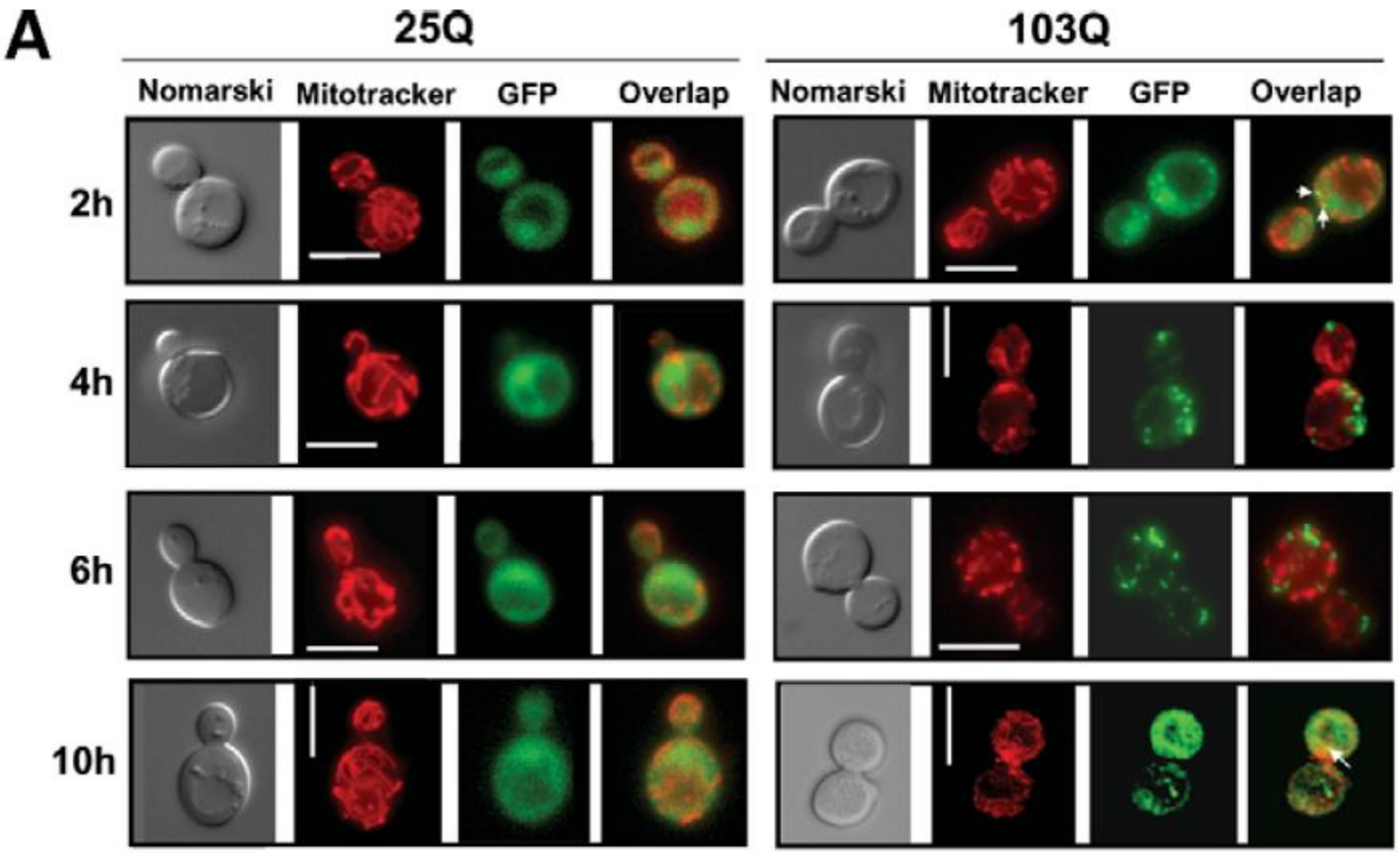


Solans, et al. (2006) Hum Mol Genet **15**:3063-3081

Steady-state concentrations of mitochondrial respiratory chain components



Mitochondrial distribution is altered in cells expressing 103Q



Resumo

1. Em doenças neurodegenerativas há formação de agregados proteicos;

2. Fusão de Tau, Aβ42, α-synucleína ou huntingttina com GFP mostraram formação de agregados por microscopia de fluorescência;

3. Em levedura, mostrou-se que Tau é hiperfosforilada e que a Ser409 é um determinante de sua agregação;

4. Yeast Display permitiu identificar fragmentos de anticorpos que inibem a clivagem de βAPP;

5. A formação de agregados de α-synucleína depende do número de cópias expressas; os agregados são depurados majoritariamente por autofagia e a mutação S129A é mais tóxica que α–syn WT;

6. Expressão de Htt em levedura mostrou que a agregação depende de Hsp104; o PRD inibe a formação de agregados e Htt-GFP diminui as atividades dos complexos II e III da cadeia respiratória;