Overview

1. In aging, the issue with neurons is not dysregulated mitosis; it is inclusions and apoptosis

2. Very small amounts of over-expressed protein can drive inclusions

3. The paradox of marking a protein for degradation & not degrading it

4. A single molecule decision for a protein in jeopardy-degrade v. refold

5. An ensemble decision for a cell—compromised survival v. apoptosis

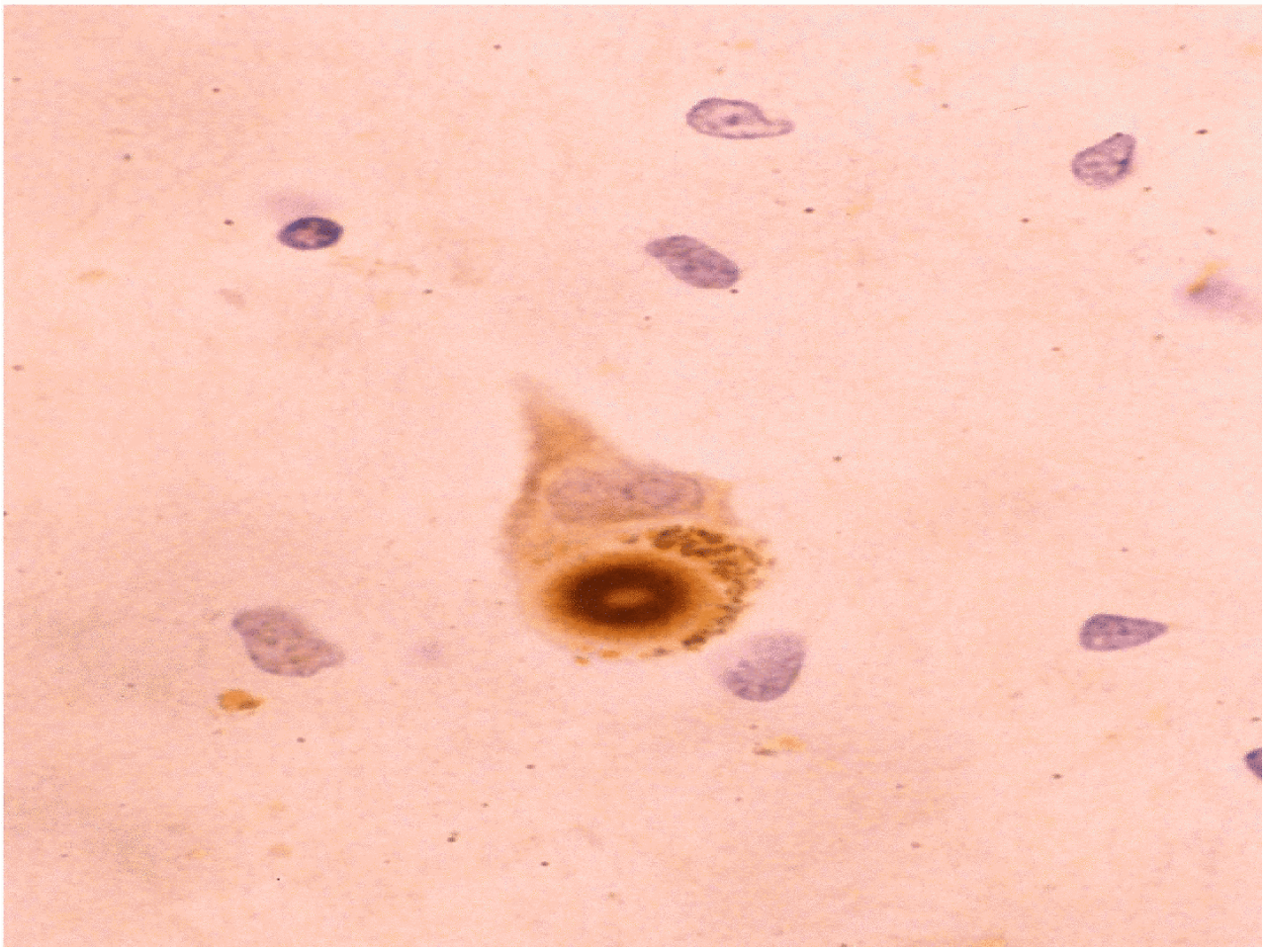
6. Reducing chaperone levels & phenotypic emergence

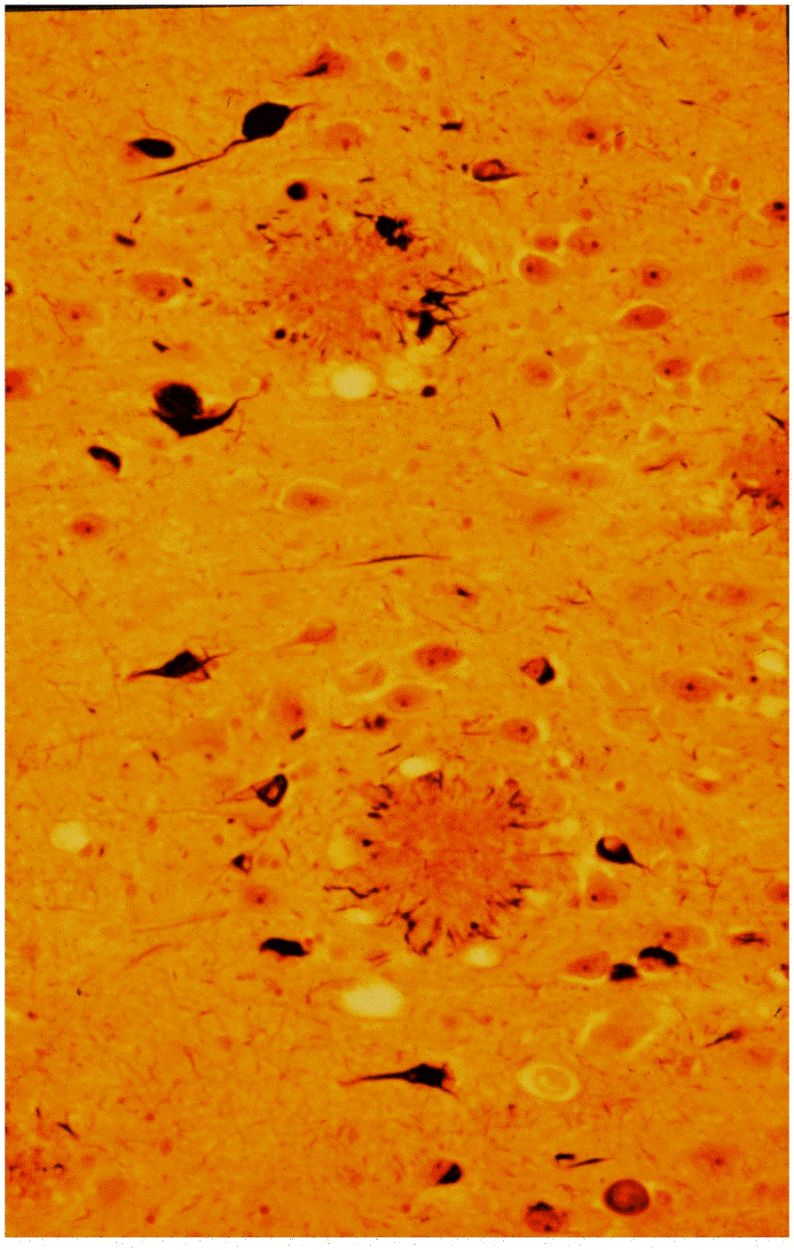
Disease	**Inclusions-intracellular**
Parkinson Disease/ Lewy Body Dementia	α-synuclein
Frontotemporal Dementia/ tangle diseases	Tau
ALS	Supra-oxide dismutase
Polyglutamine repeat diseases (Huntington's disease; spinocerebellar ataxia)	Huntingtin ataxin

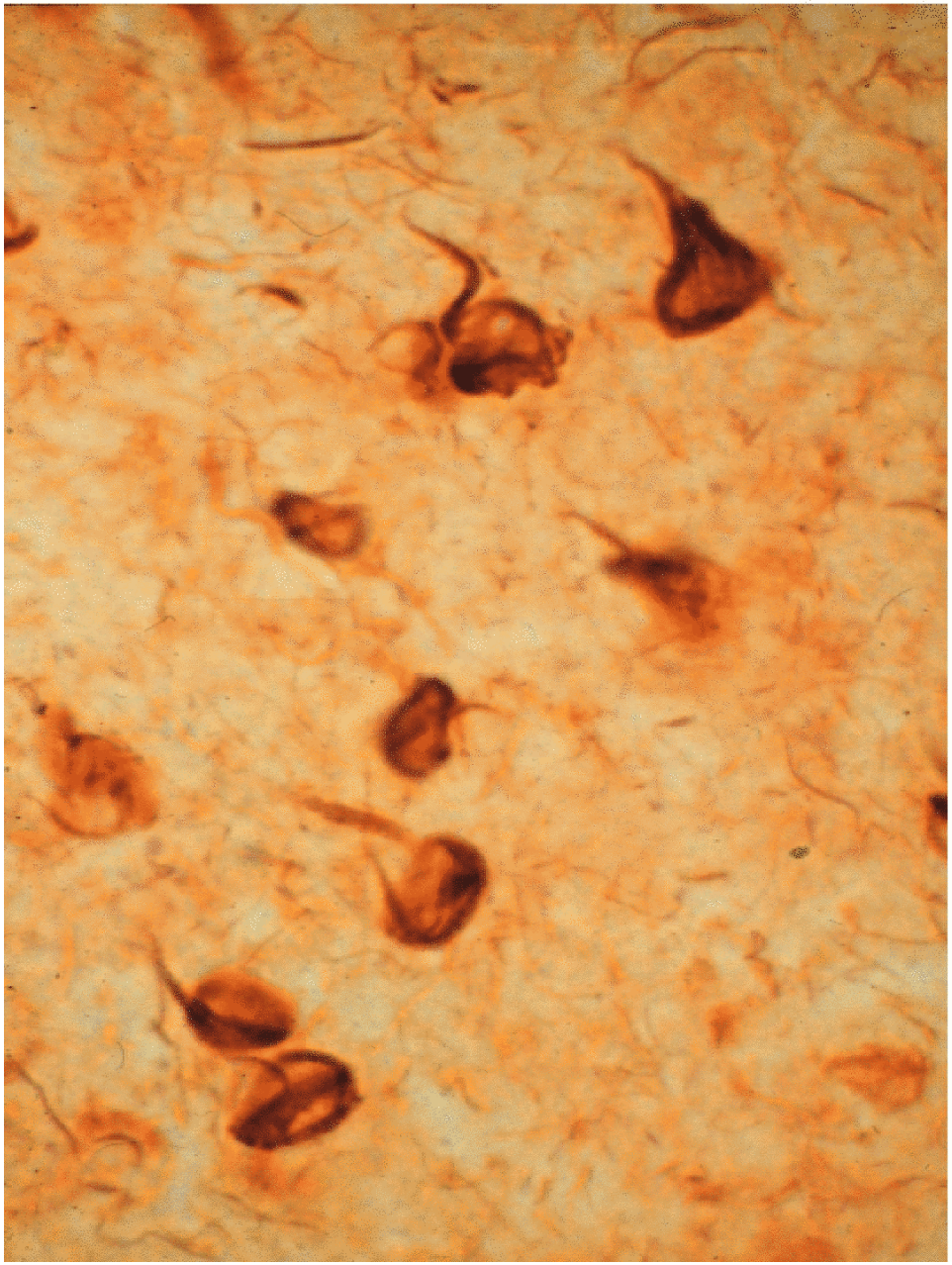
Exist as rare autosomal dominant forms and common sporadic forms except CAGn diseases exist only as genetic forms

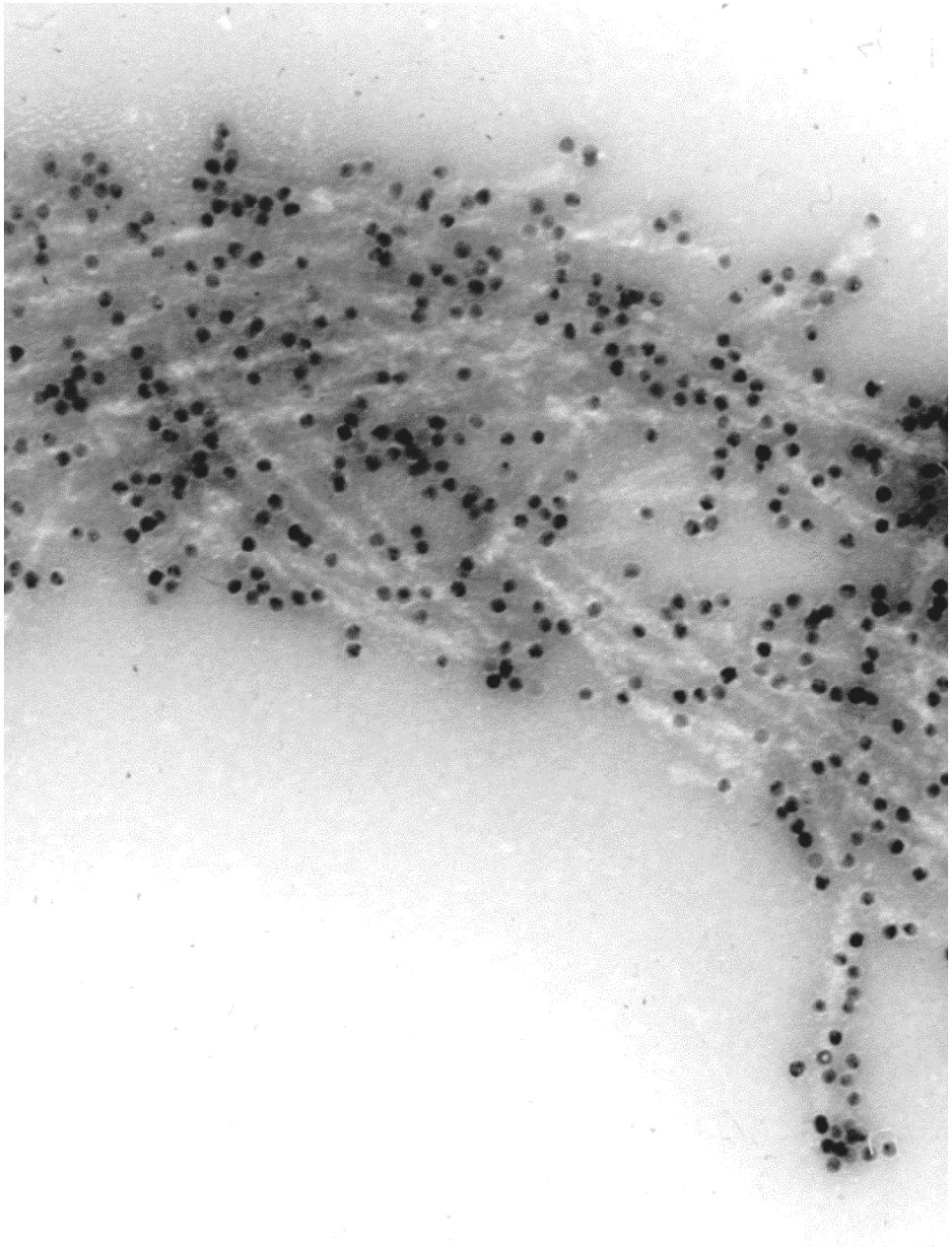
Disease	Inclusions-extra-cellular
Alzheimer Disease	A-beta
Hereditary Creuzfeldt-Jacob/ Gerstmann Straussler	Prion protein
CADASIL	Notch 3

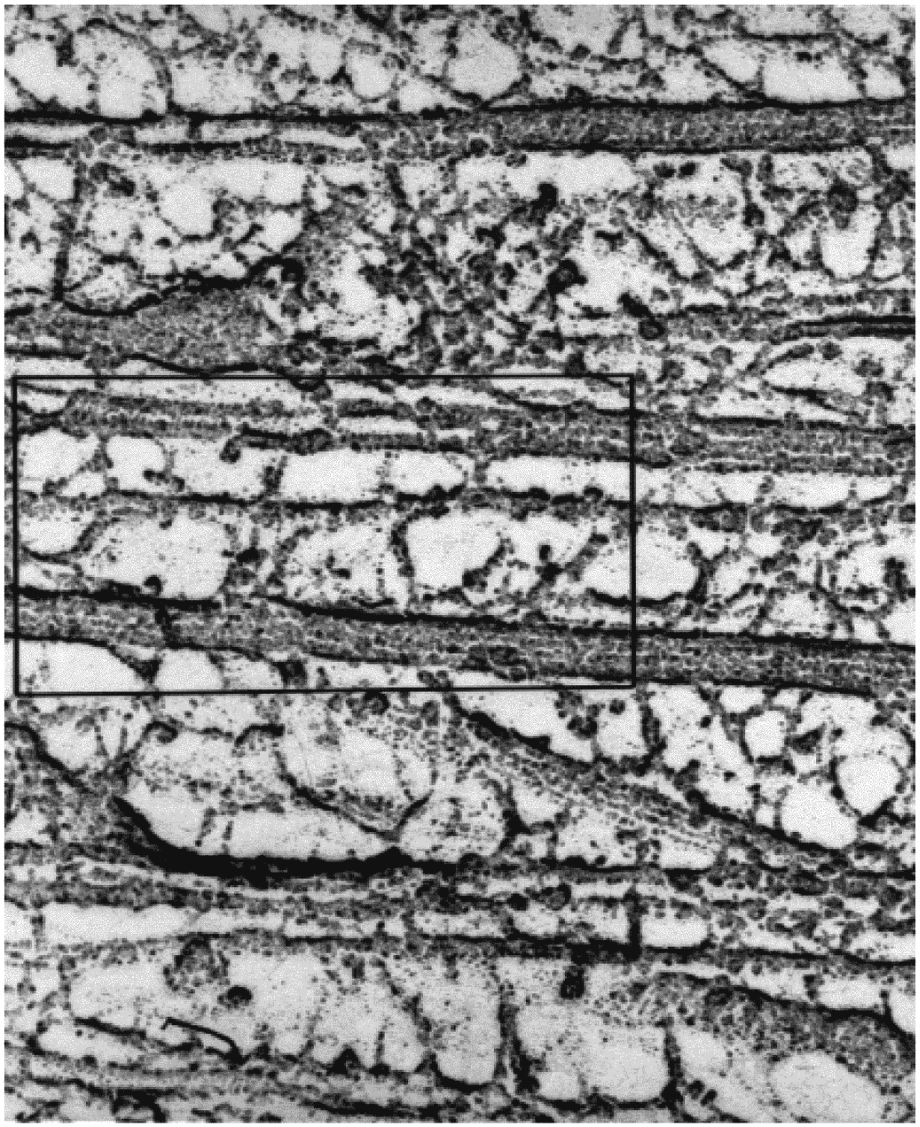
Exist as rare autosomal dominant forms and sporadic forms which are common (AD), rare (CJ), or unknown (CADASIL)

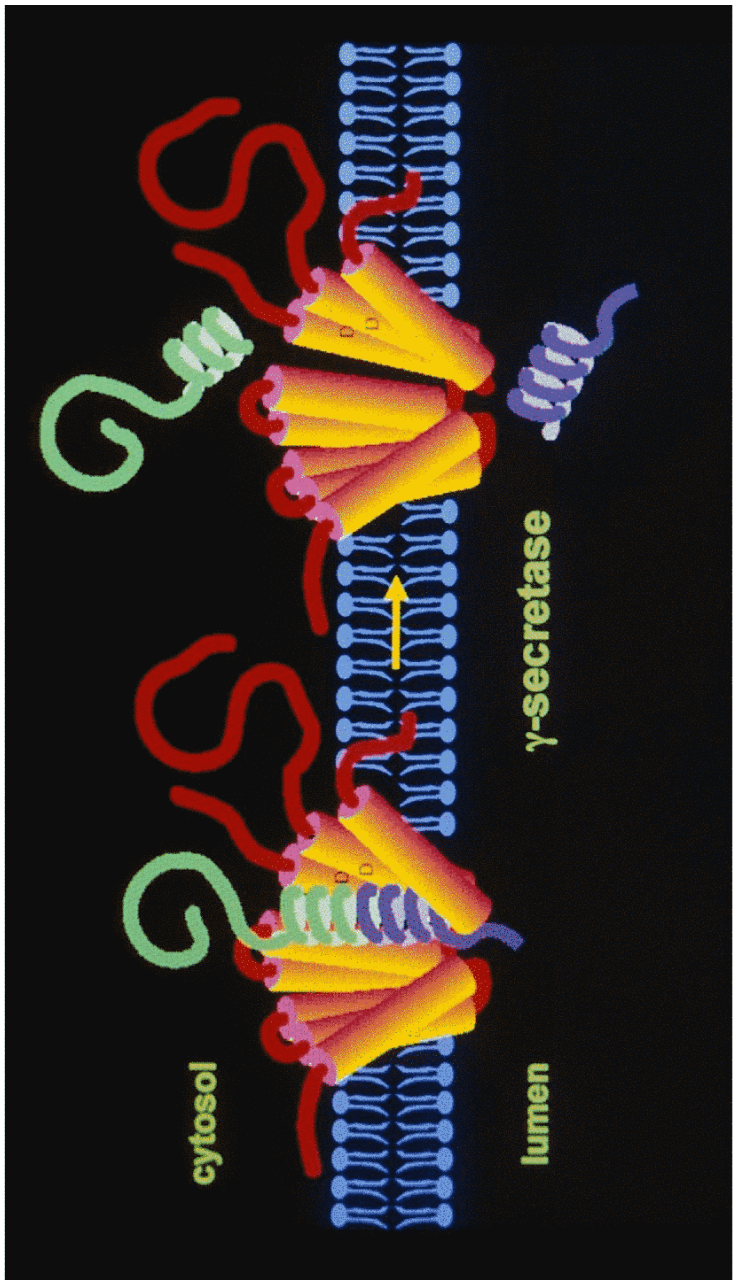


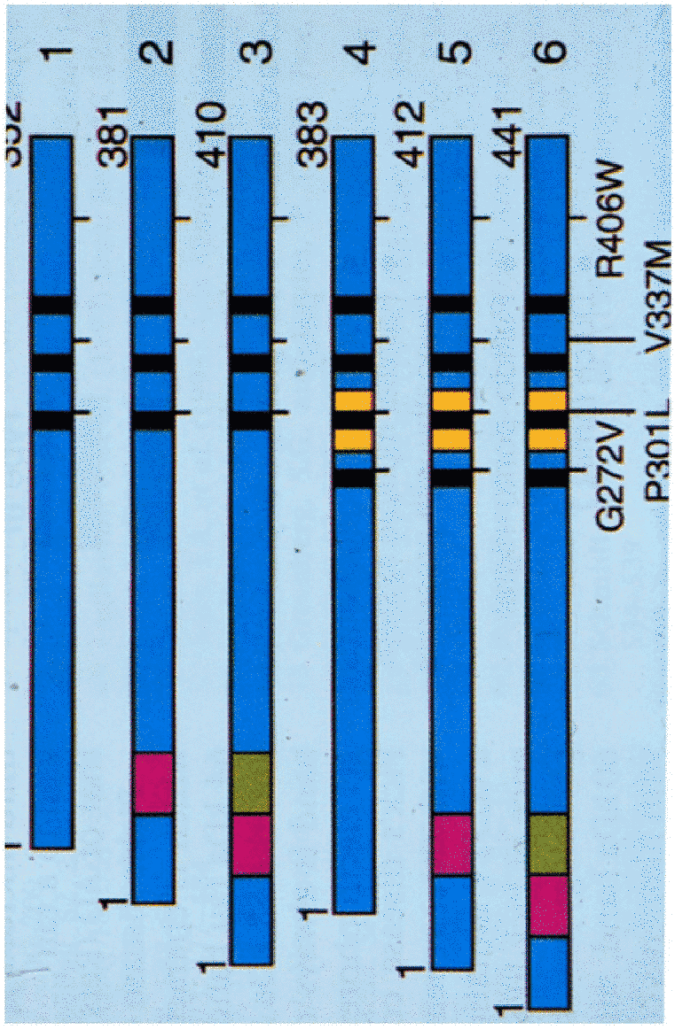


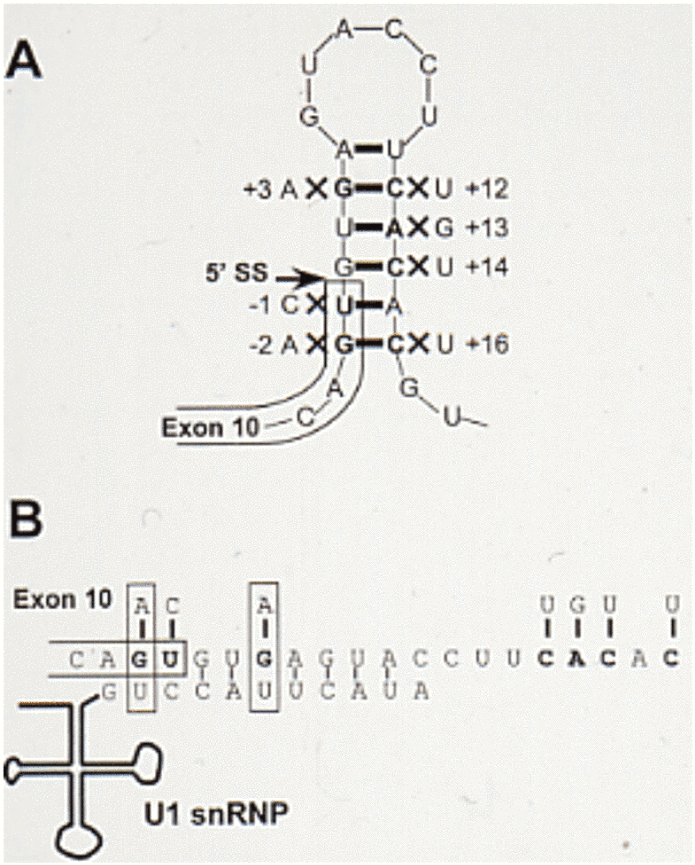







cytosol
lumen
γ-secretase


1
381
2
1
410
3
1
383
4
1
412
5
1
441
6
G272V
P301L
R406W
V337M

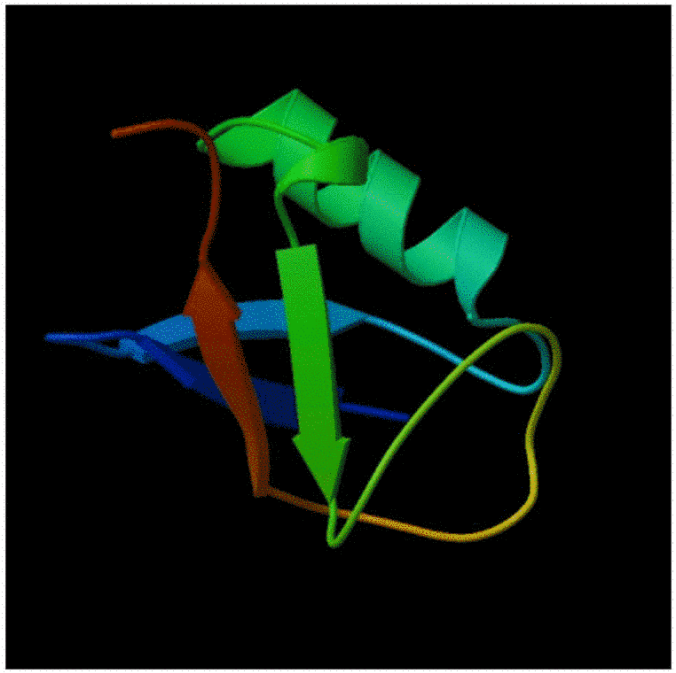


The haplotype of the Mendelian pathogenic locus influences the disease risk for sporadic disease. In the absence of coding changes, a haplotypic association implies there is genetic variability either in the amount of expression of the protein, or in the alternate splicing of the protein, and that this contributes to disease risk.

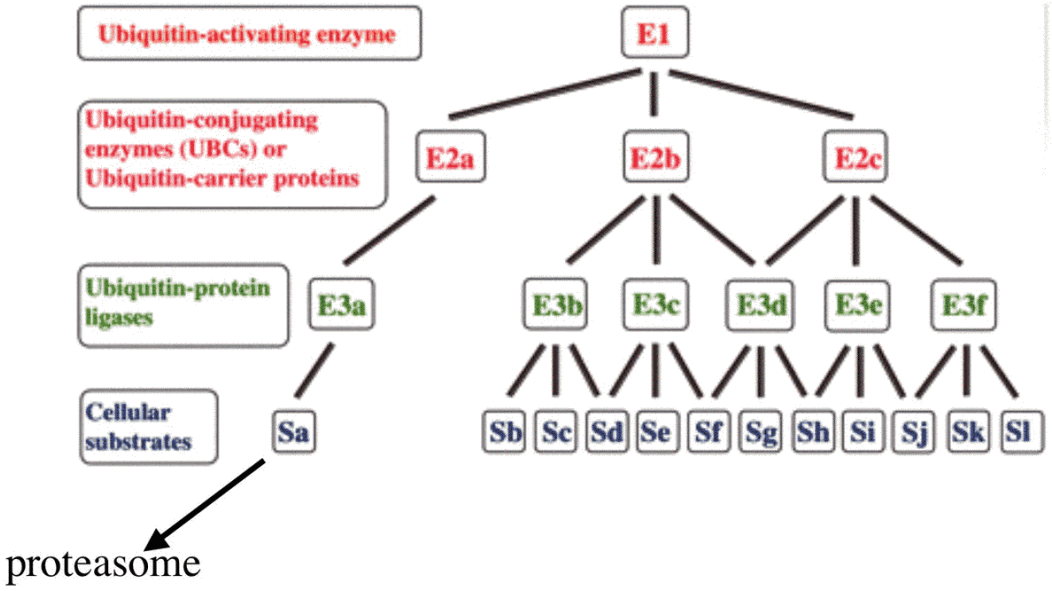
The case for:

1. Parkinson's disease—α-synuclein (gene triplication)
2. Alzheimer disease—amyloid precursor protein (trisomy 21)
3. Fronto-temporal dementia—tau

Andrew Singleton, Amanda Myers and John Hardy
Human Molecular Genetics, 2004, Vol. 13, Review Issue

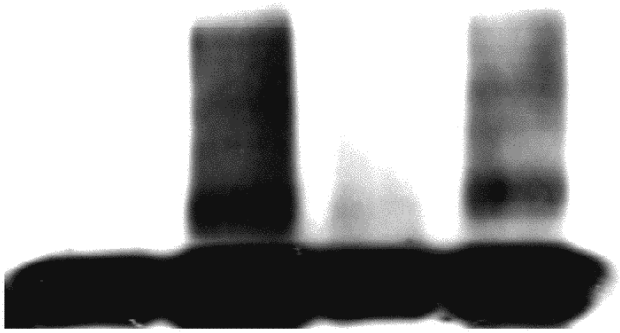


Ubiquitin—76 amino acids



The EMBO Journal Vol. 17,pp. 7151-7160, 1998
The ubiquitin-proteasome pathway: on protein death and cell life
Aaron Ciechanover

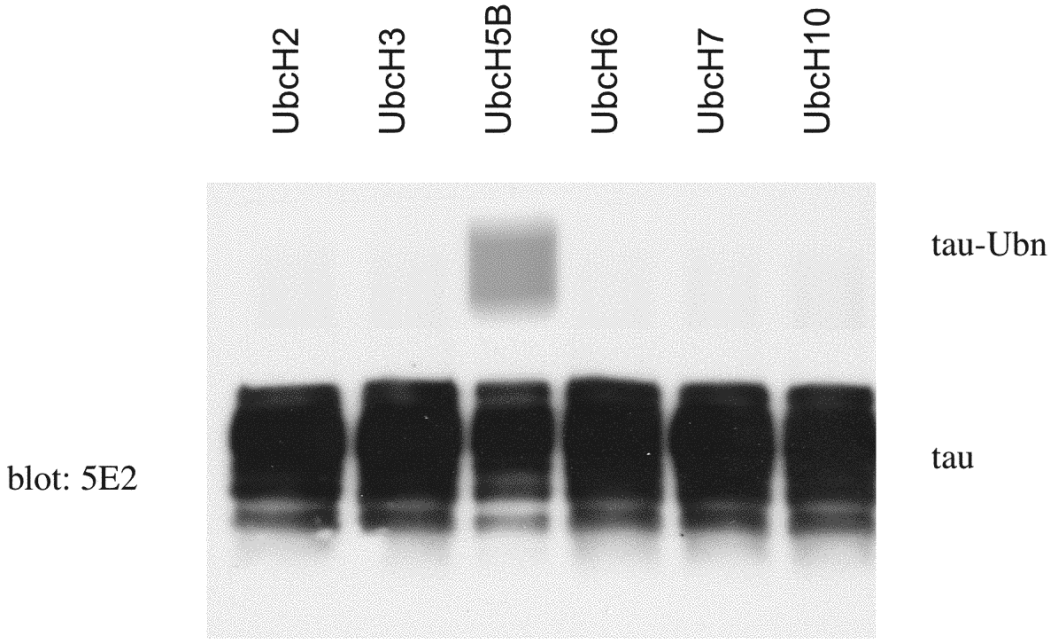
C AD C AD



tau-Ubn

tau

Blot 5E2



Many proteins are targeted by phosphorylation:

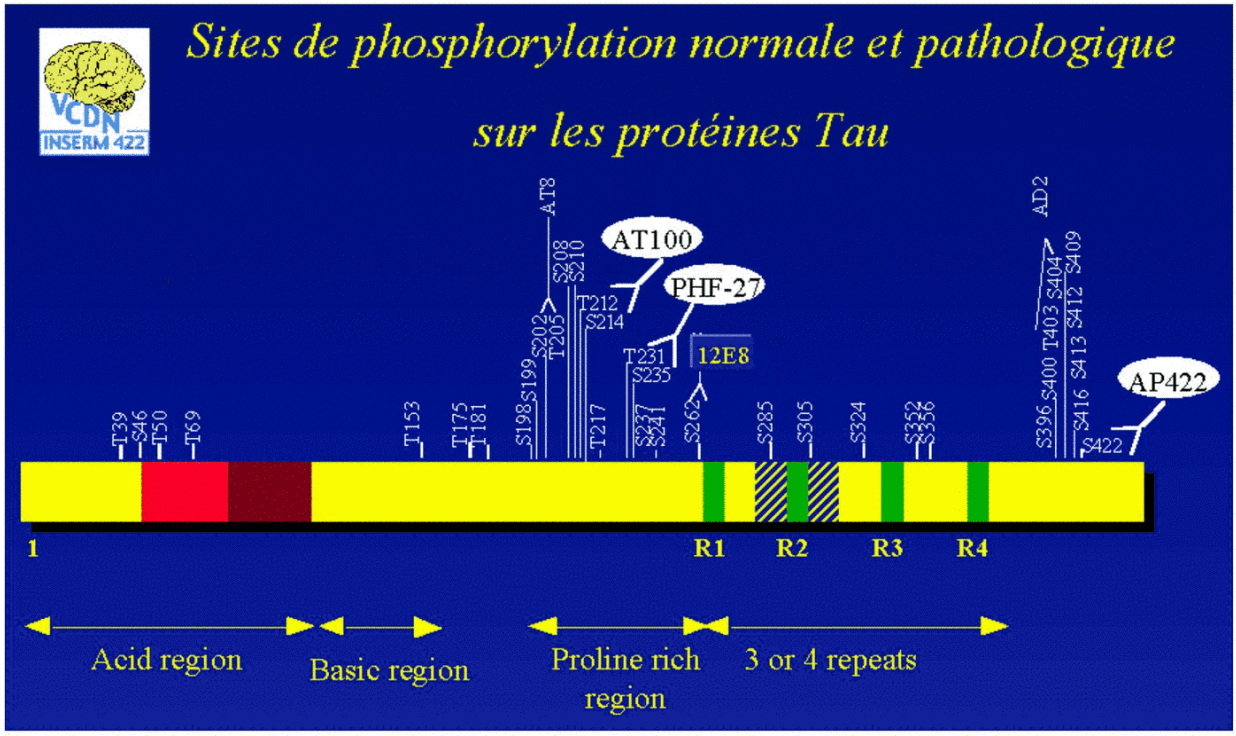
The yeast G1 cyclin CLN3 (Yaglom *et al.*, 1995) and the GCN4 transcriptional activator (Kornitzer *et al.*, 1994) are degraded following phosphorylation at a PEST sequence.
The mammalian G1 cyclin D1 is targeted for degradation by phosphorylation on a Thr residue that does not reside within a PEST [Pro(P), Glu(E), Ser(S), Thr(T)] sequence (Diehl *et al.*, 1997).

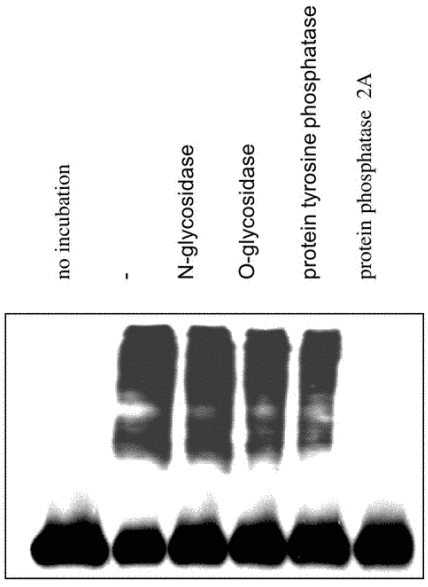
Phosphorylation of Ser32 and Ser36 targets IκBα (Chen *et al.*, 1995).

Degradation of b-catenin is mediated by phosphorylation at Ser37

Phosphorylation at Ser3 of c-Mos (Nishizawa *et al.*, 1992) or multiple phosphorylations of c-Jun (Musti *et al.*, 1997) suppress their ubiquitination and degradation.

How about tau?


Sites de phosphorylation normale et pathologique
sur les protéines Tau
VCDN
INSERM 422
AT8
AT100
PHF-27
12E8
AD2
AP422
T39
S46
T50
T69
T153
T175
T181
S198
S199
S202
T205
S208
S210
T212
S214
T217
T231
S235
S237
S241
S262
S285
S305
S324
S352
S356
S396
S400
T403
S404
S409
S413
S412
S416
S422
1
R1
R2
R3
R4
Acid region
Basic region
Proline rich region
3 or 4 repeats


no incubation
-
N-glycosidase
O-glycosidase
protein tyrosine phosphatase
protein phosphatase 2A
tau-ubn
tau

GSK3b & Cdk5 phosphorylate sites dephosphorylated by PP2A

Brain homogenate

↓

recombinant tau—PO4

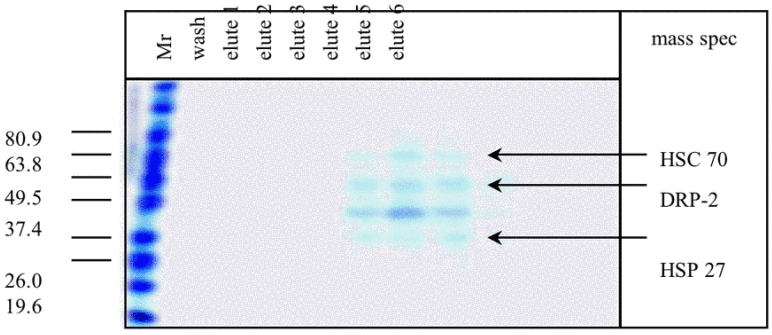
↓

UbcH5B

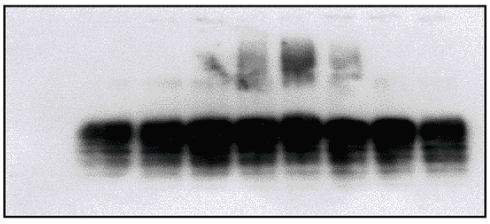
↓

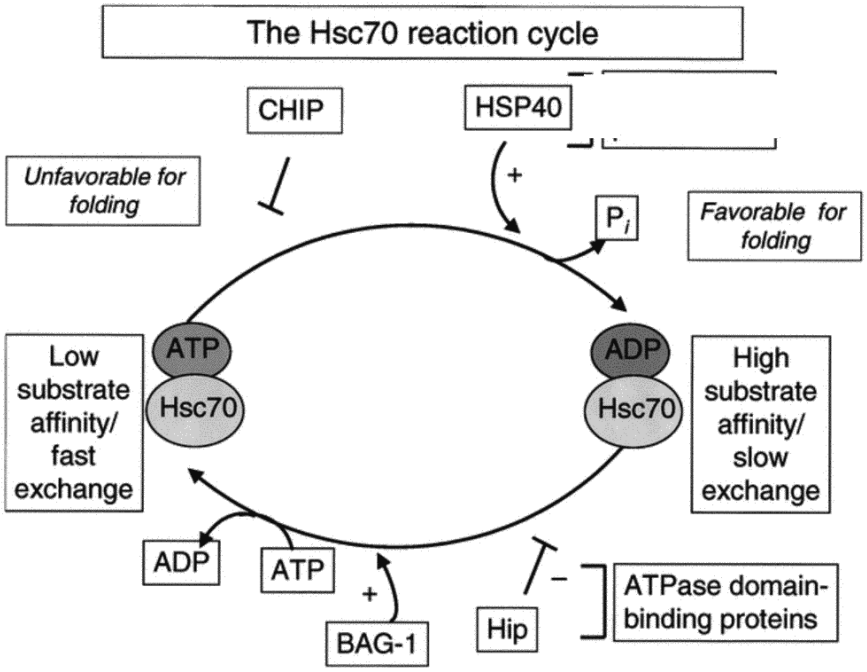
Mass spect

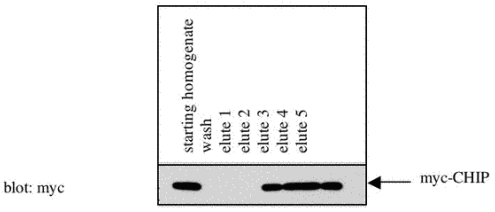
A)



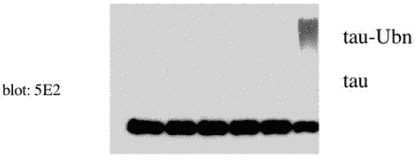
B)

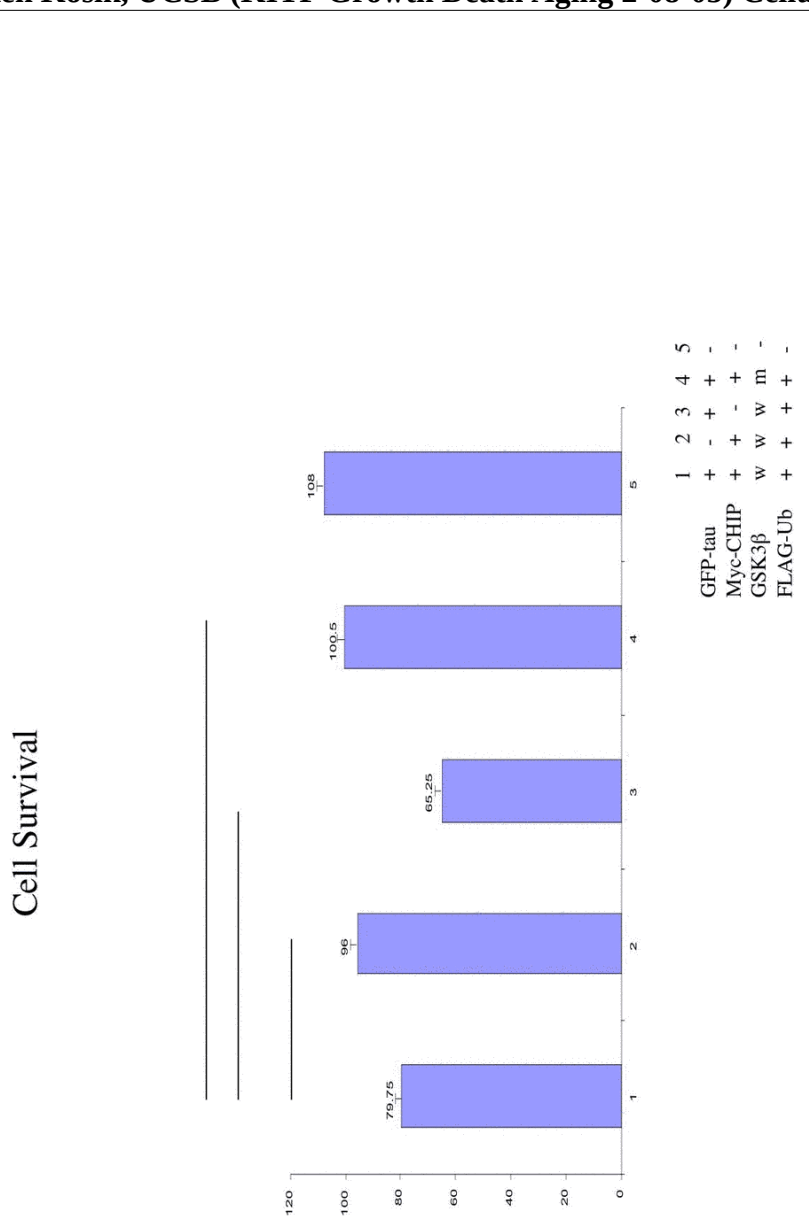


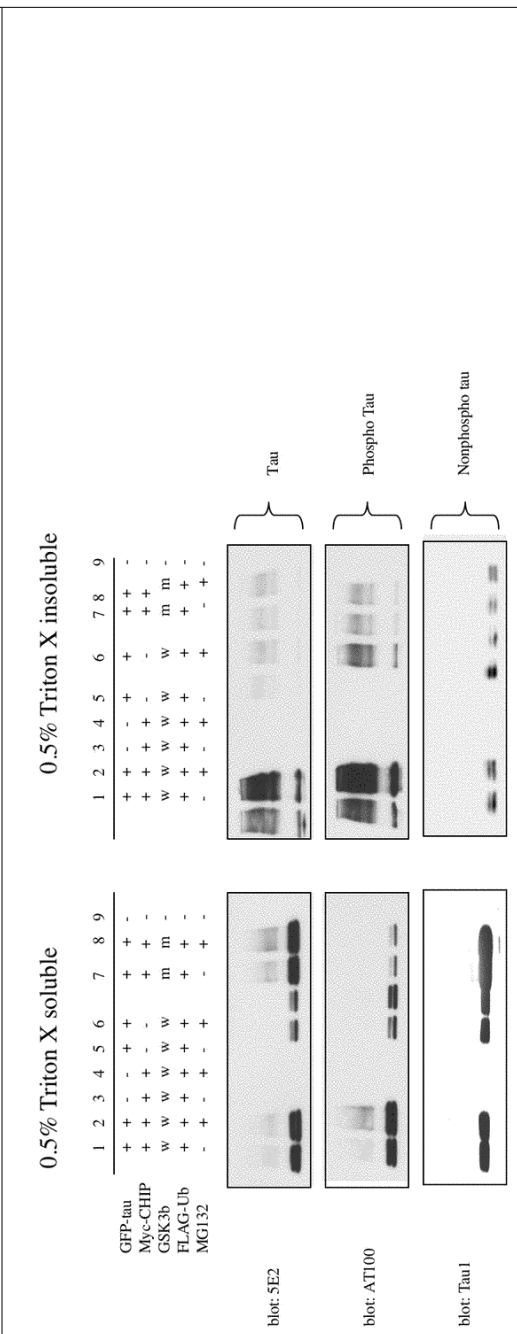


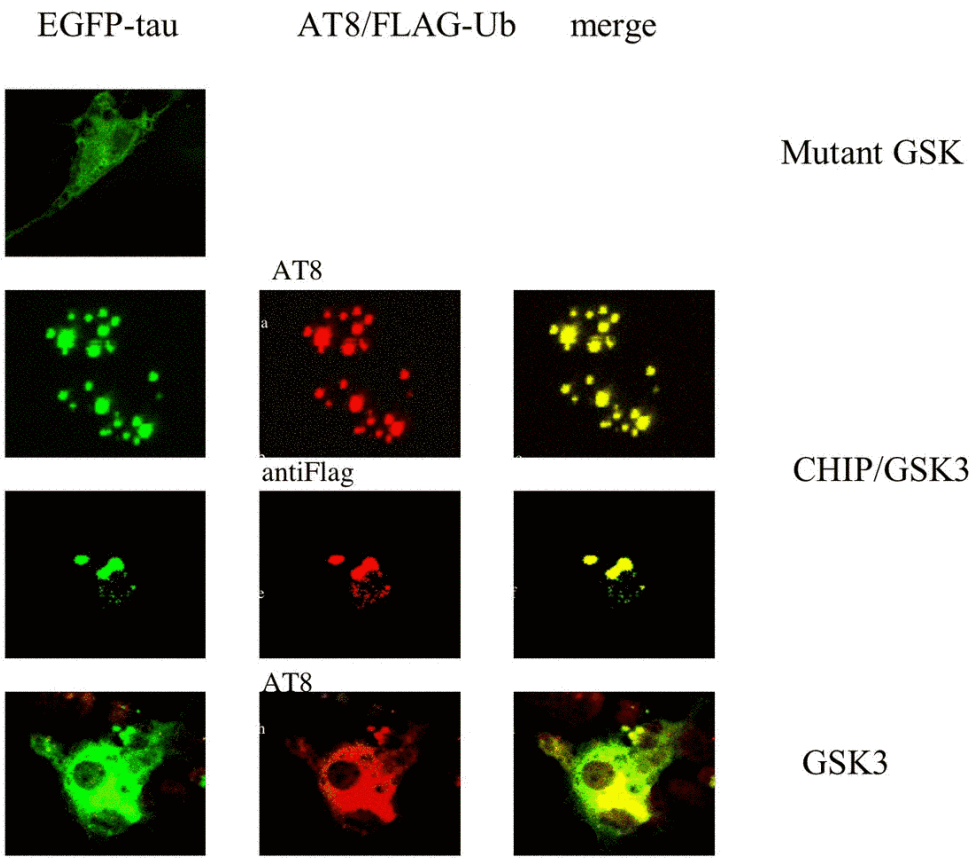


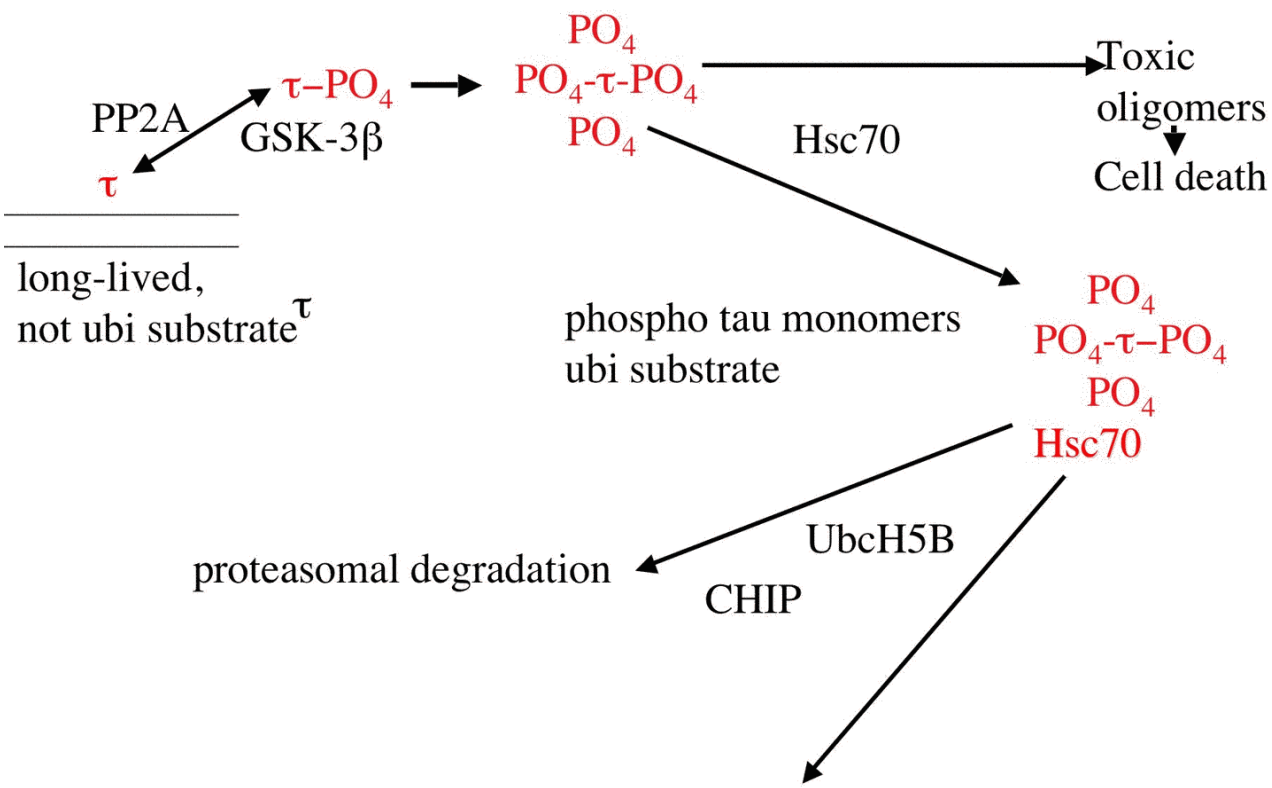
E1	-	+	+		+	+	+
UbcH5B	+	-	+		+	+	+
GST-CHIP	+	+	-		+	+	+
Hsc70	+	+	+		-	+	+
FLAG-Ub	+	+	+		+	+	+
phospho tau	+	+	+		+	+	+
time (min)	30	30	30		30	0	30




Cell Survival
120
100
80
60
40
20
0
79.75
96
65.25
100.5
108
1
2
3
4
5
1 2 3 4 5
GFP-tau + - + + -
Myc-CHIP + + - + -
GSK3β w w m - -
FLAG-Ub + + + - -


0.5% Triton X soluble
0.5% Triton X insoluble
1 2 3 4 5 6 7 8 9
GFP-tau + + - - + + + + -
Myc-CHIP + + + + - + + + -
GSK3b w w w w w m m m -
FLAG-Ub + + + + + + + + -
MG132 - + - + - + - + -
Tau
Phospho Tau
Nonphospho tau
blot: 5E2
blot: AT100
blot: Tau1


EGFP-tau
AT8/FLAG-Ub
merge
Mutant GSK
AT8
CHIP/GSK3
antiFlag
AT8
GSK3


PO4
PO4-τ-PO4
PO4
τ–PO4
PP2A
GSK-3β
τ
Hsc70
Toxic
oligomers
Cell death
long-lived,
not ubi substrate τ
phospho tau monomers
ubi substrate
PO4
PO4-τ–PO4
PO4
Hsc70
UbcH5B
CHIP
proteasomal degradation
Sequestered ubiquitinated tau aggregates

Partitioning into an aggregate to prevent neurotoxity

a. Amyloid plaques present without clinical symptoms of AD, and severity of AD does not correlate well with plaque density.

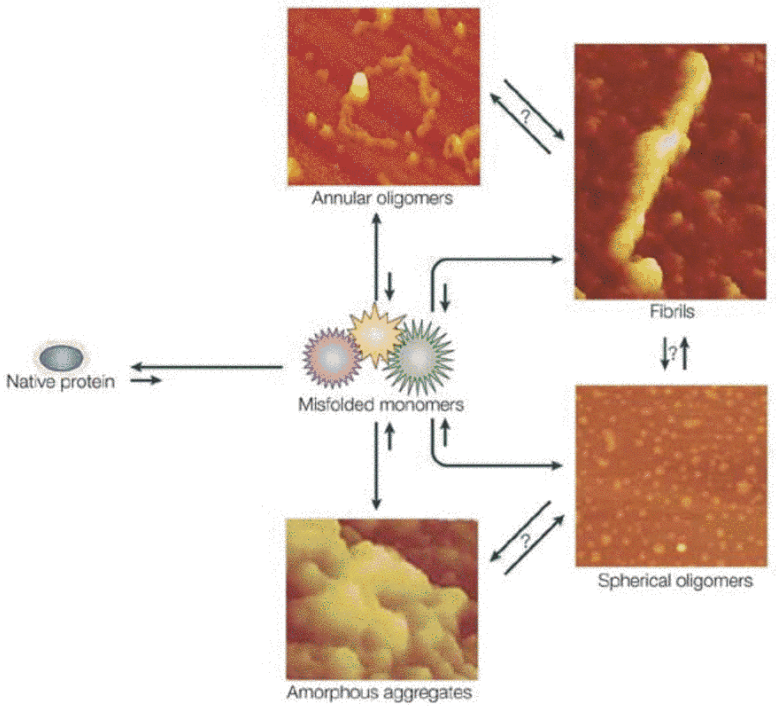
b. In PD, neurons that contain Lewy bodies are healthier than surrounding cells.

c. In a cell culture model of HD, inclusion body formation predicts neuronal survival, whereas the level of diffuse huntingtin correlates with cell death. Arrasate *et al*.,

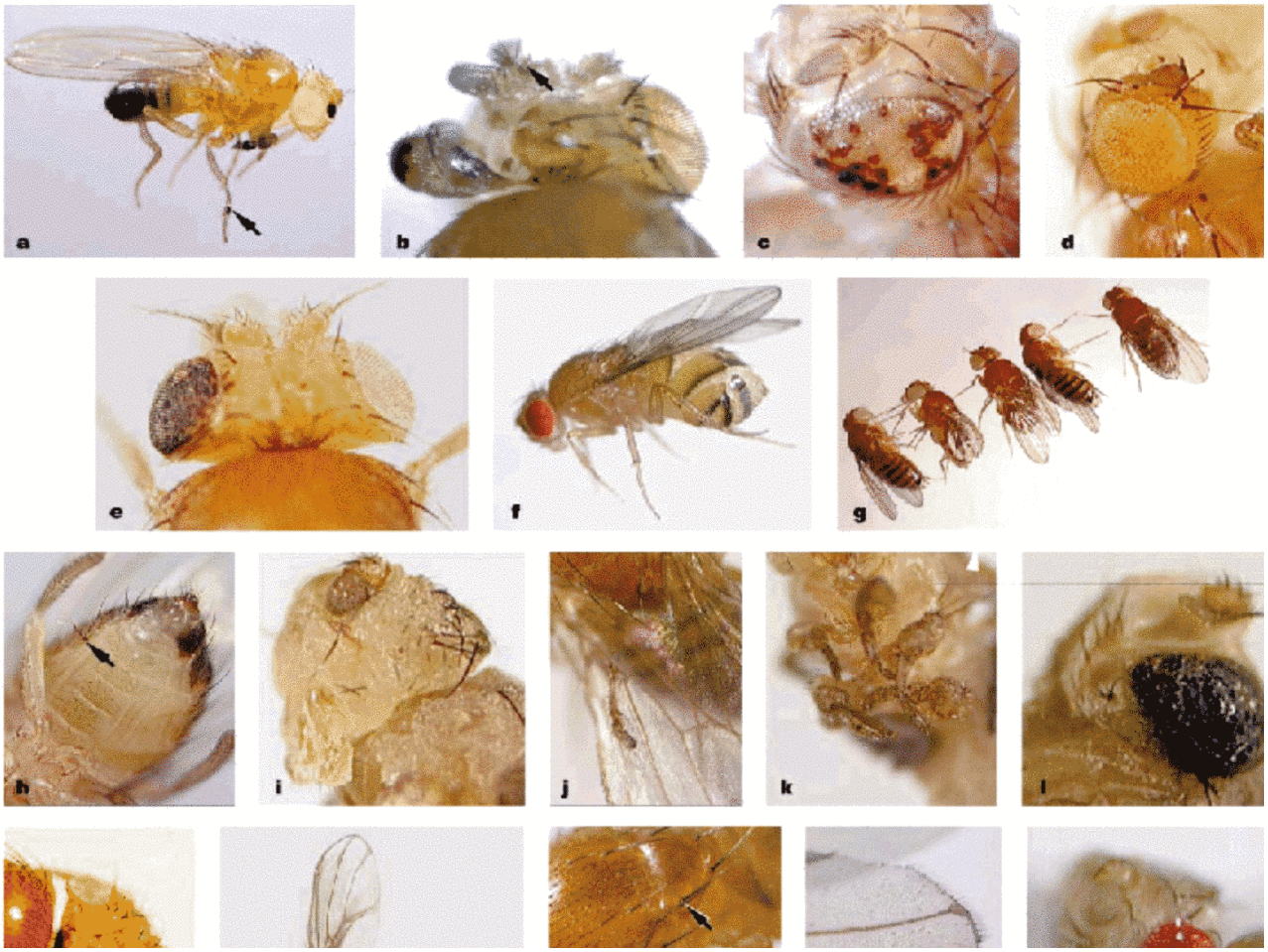
d. In a polyglutamine disease model neuronal dysfunction occurs in the absence of ubiquitin-proteasome system impairment and inversely correlates with the degree of nuclear inclusion formation. Bowman AB, et al Hum Mol Genet. 2005 Jan 20;

d. aggregates protect cells from the toxicity of soluble phosphorylated tau.

e. Tau inclusions associated with Hsp27



Caughey, B. & Lansbury, P. T. *Annu. Rev. Neurosci.* **26**, 267_298 (2003).



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