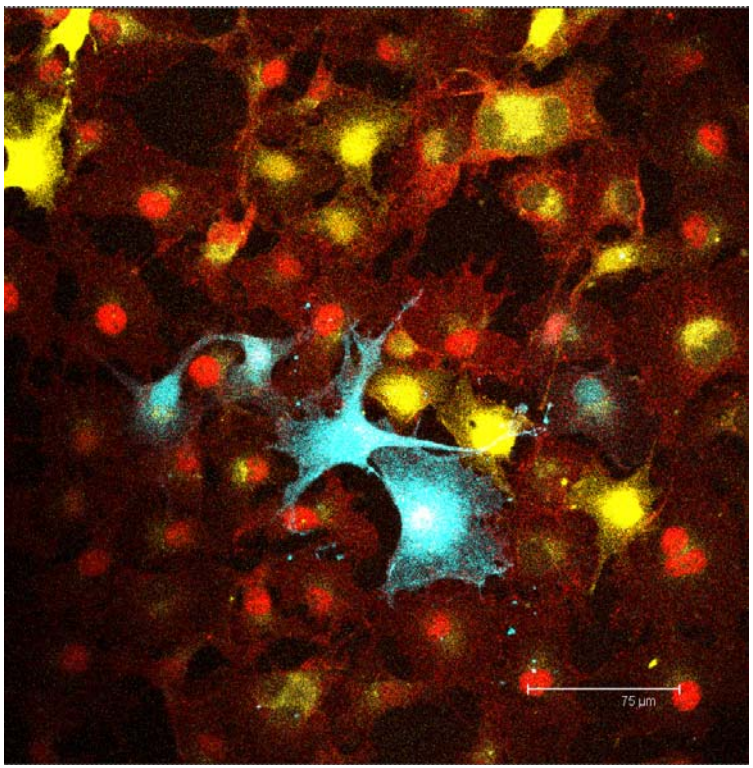
PRION DISEASES

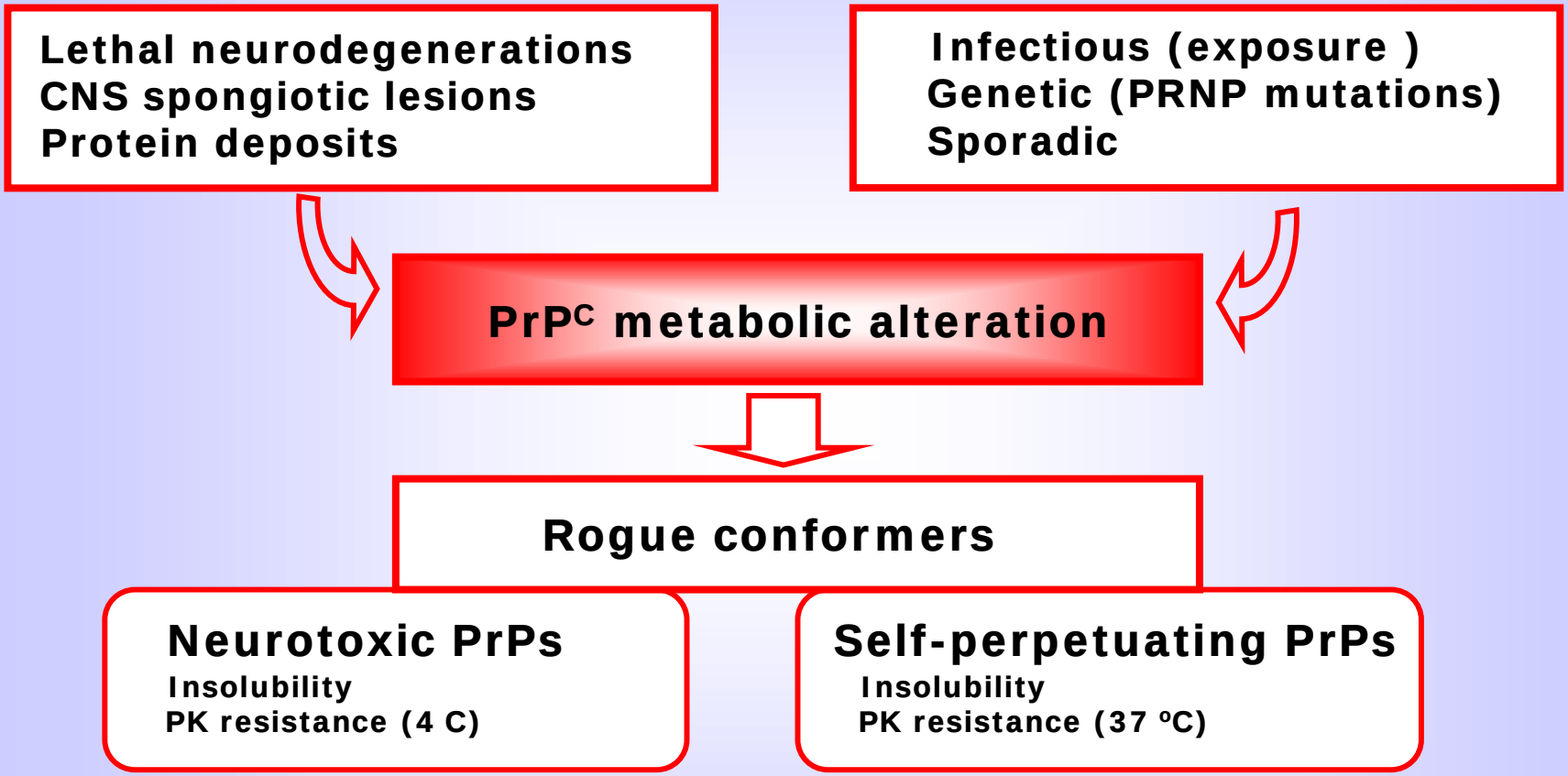
Overview and Therapeutic Approaches



María Gasset
IQFR-CSIC

VII-SEQT, Sitges 2006

PRION DISEASES or PrP-RELATED DISORDERS



Gain / Loss of function

Therapeutical approaches: Prophylaxis vs novel targets

Preclinical
(periphery)

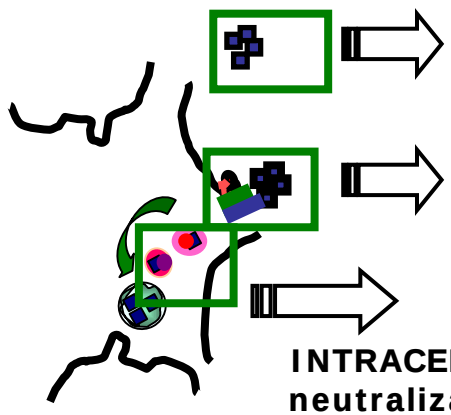
NOVEL TARGETS
Covalent multiplicity
Conformational multiplicity

Diagnostic markers
(PrPDis, surrogates)

Disease progression

Clinical
(CNS damage)

Compassionate Intervention



EXTRACELLULAR:
Dissociating agents,
Succide ligands

CELL SURFACE: NC-PrP receptors, Secreated PrP, MAbs, Raft disruption

INTRACELLULAR : pH neutralization, targeting

I. COVALENT MULTIPLICITY

PrP^C is not one but a four-member family

Single gene

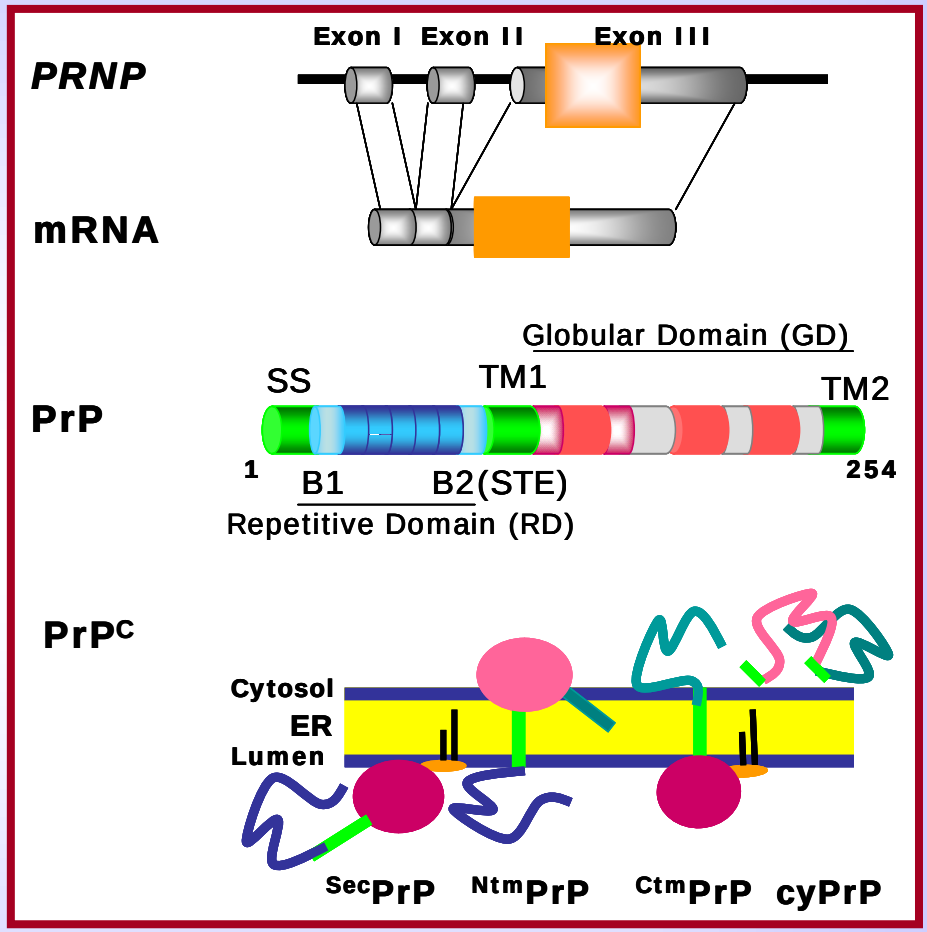
Single transcript

Single chain

Four basic forms

Intracelular / cell surface
Soluble, Integrated, Anchored

Multiple targeting
Multiple folding
Moonlighting



Disease

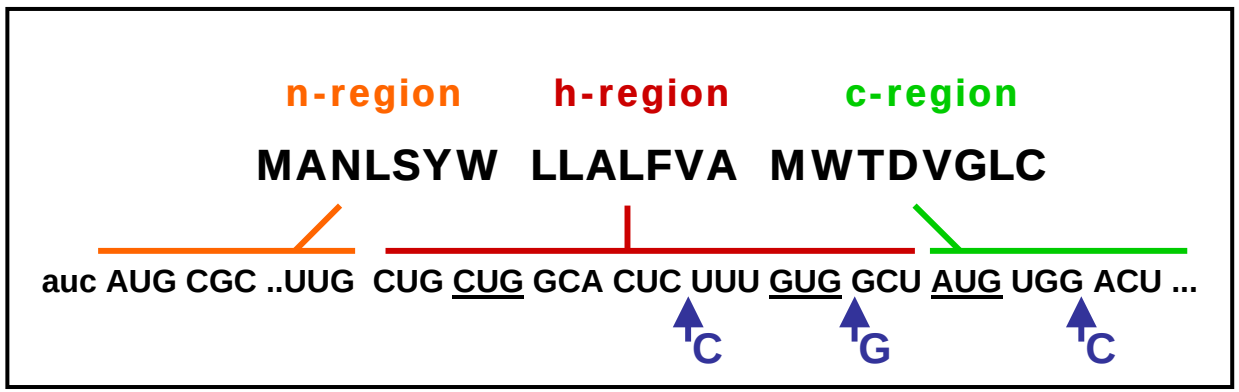
Propagative

Toxic

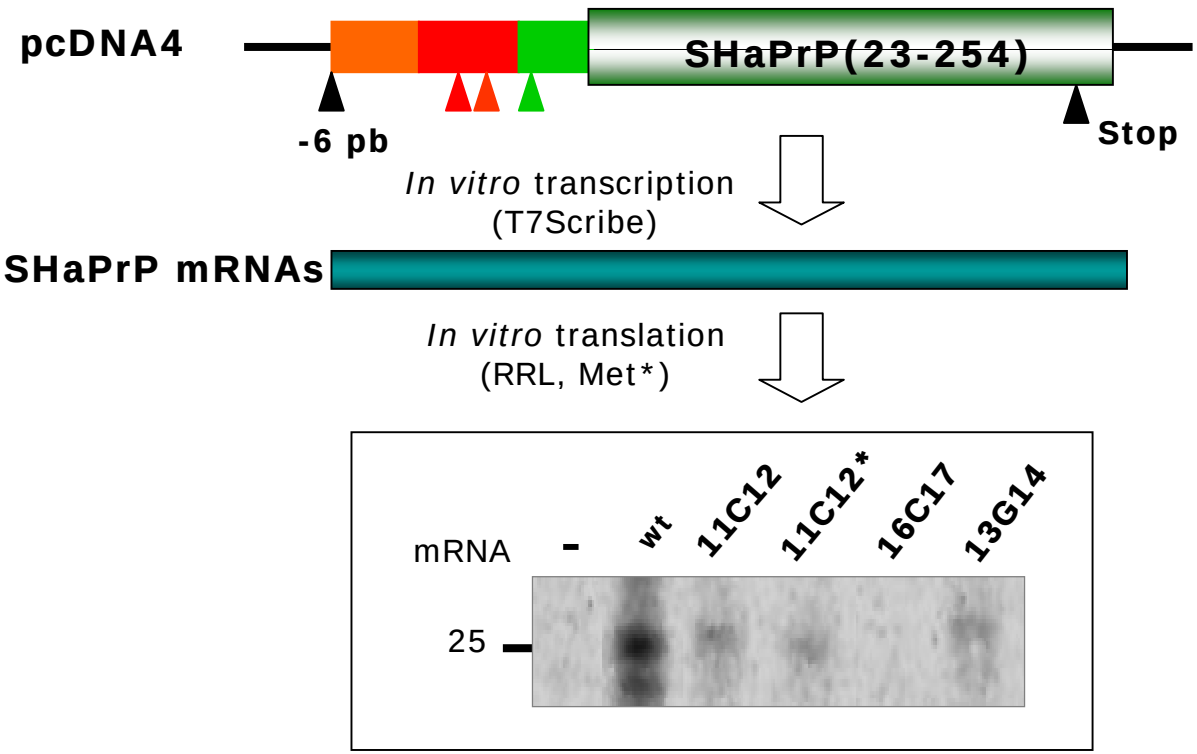
Toxic

Deciphering PrP signal sequence code for CyPrP generation

Off-secretory route (de novo, retrotranslocation)
Intracellular (aggresomes, microtubules, etc)
Toxic (proteasome -)
Ataxic lethal phenotype



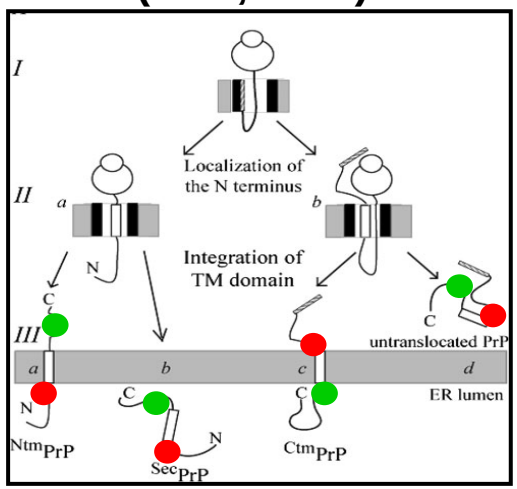
Does SHaPrP signal sequence coding region contain a start site?



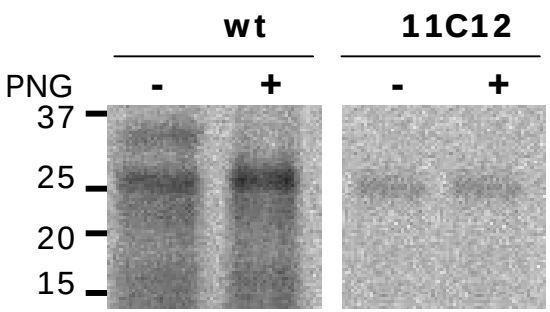
- AUG coding for M15 permits translation initiation
- Translation from M15 accounts for 10-15% of total

Does PrP(Δ1-14) truly seggregate outside the secretory route?

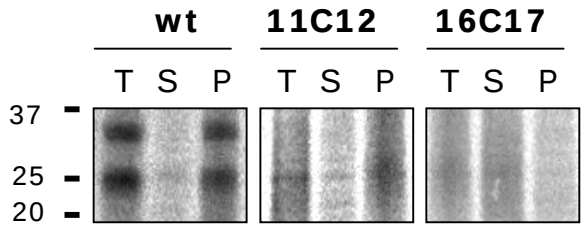
Cell-free synthetic pool (RRL, PMC)



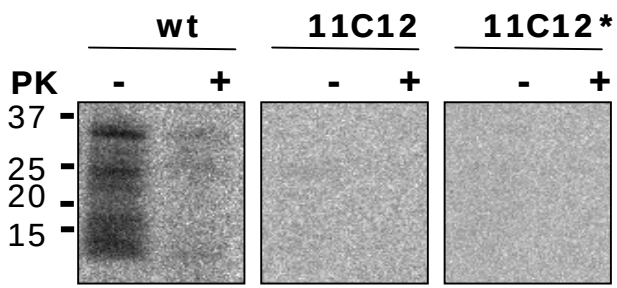
I. No glycosilated forms



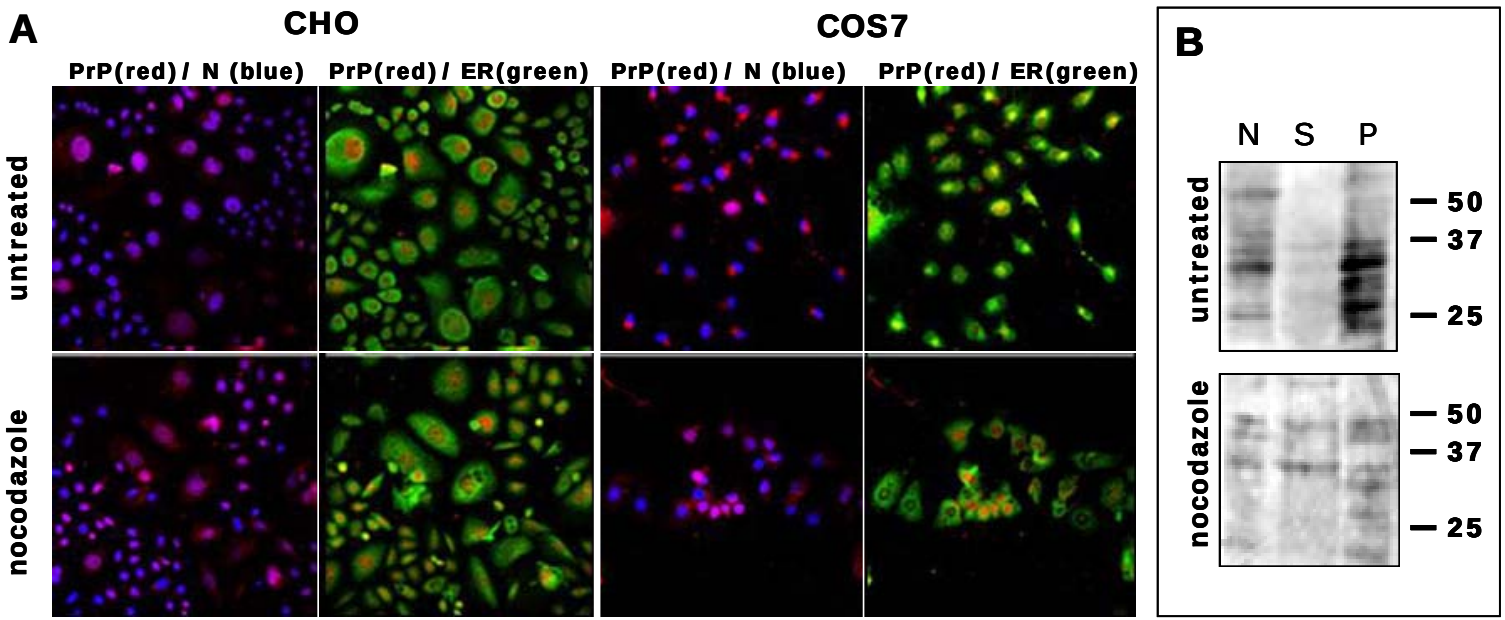
III. Intrinsic insolubility



II. No membrane bound forms

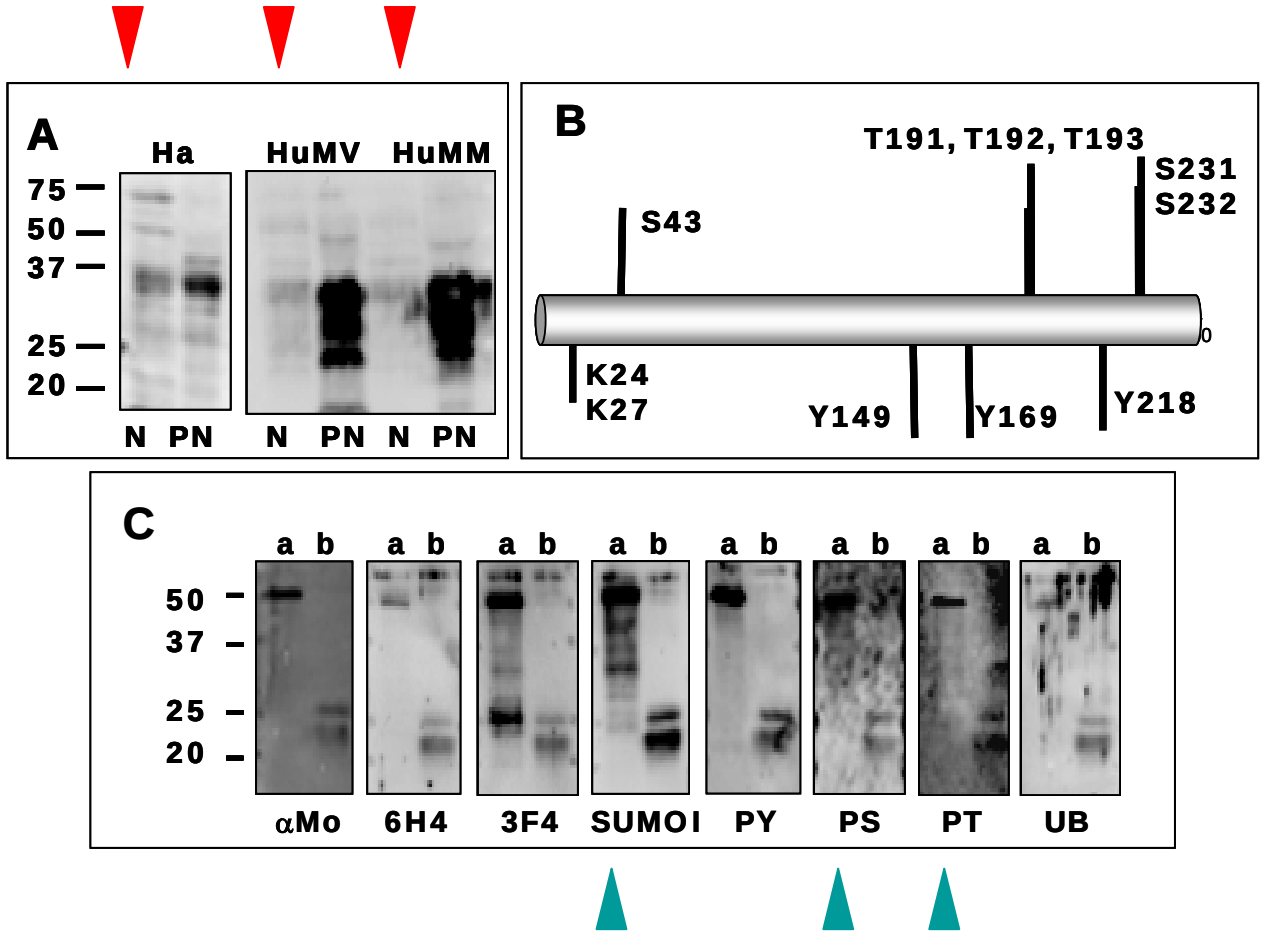


Does 11C12 translation occur in a cell context ?



In stably transfectants, SHaPrP(Δ1-14) is found both in the nucleus and in the cytoplasm, the relative distribution depending on the cell type and on microtubule integrity.

Is nuclear PrP detected in brains and what is the origin of its size?



Is there a specific phenotype linked to SHaPrP(Δ1-14) expression?

COS-7	G_0/G_1	S	G_2
SHaPrPwt	62.5 ± 0.5	16.2 ± 0.5	21.7 ± 0.5
SHaPrP (16C17)	61.4 ± 0.4	16.9 ± 0.4	21.7 ± 0.4
SHaPrP(11C12)	83.6 ± 0.6	14.2 ± 0.6	2.2 ± 0.6
SHaPrP(11C12)*	61.0 ± 0.7	15.4 ± 0.7	23.6 ± 0.8

Expressing of SHaPrP(11C12) but not that of SHaPrP(11C12)*, mimicking cy-PrP models, undergo cell cycle catrastophe by a G0/G1-phase arrest.

CONCLUSIONS

I. PrP covalent multiplicity

- **PrP^C is a complex protein family involving at least two distinct isoforms resulting from the consitutive or inducible alternate translation initiation (AUG, ACA, ACG).**

- **PrP isoform resulting from AUG_2 (cy-PrP or PrP(Δ1-14)), segregates outside the secretory route, displays a nucleocytoplasmic location and its expression is specifically linked to a G0/G1 cell cycle arrest that if prolonged causes cell death.**

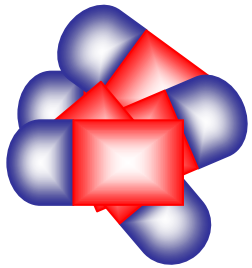
II. CONFORMATIONAL MULTIPLICITY

PrP conformational maleability: Limits of the α/β dimorphism

PrP^{C} 33-35 kDa

Post-translational

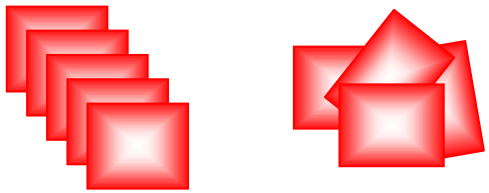
PrP^{Sc} 33-35 kDa



Amorphous aggregates

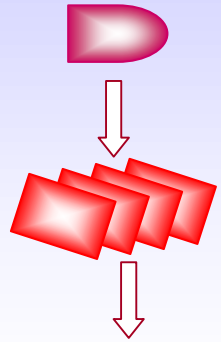
PK digestion (SK)

PrP^{Sc} 27-30 kDa



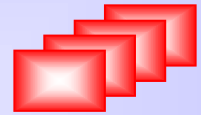
Rods (amyloid)

Spheres (non amyloid)



Tg(89-254)

N-Brain homogenate



PrP^{Sc}-sample

PMCA

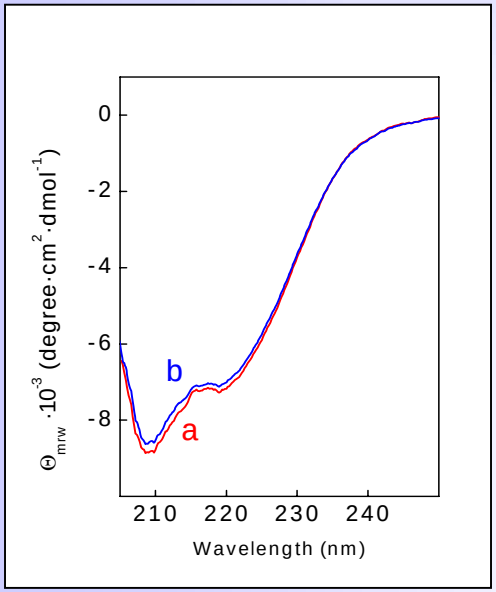
Disease (wt animal)

Not all prions are PK resistant
Not all prions are insoluble
Some PrP deposits lack infectivity

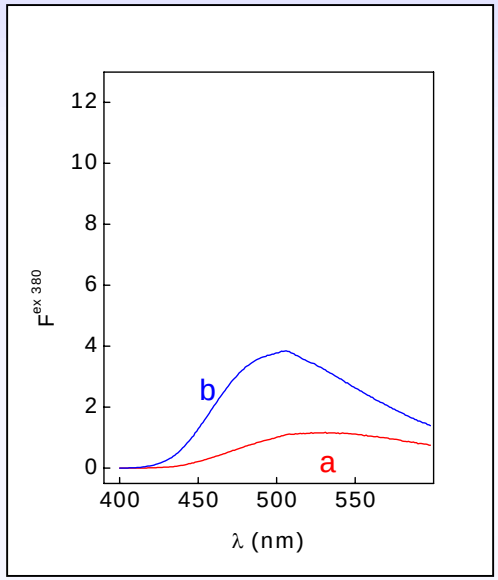
Conformational versatility of SHaPrP(23-232)

PHYSIOLOGICAL PERTURBATION: pH 4.5, 0.19 M GdnCl

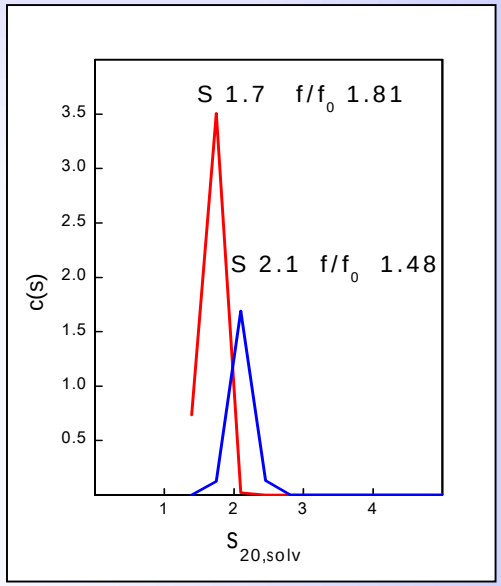
far-UV CD



ANS binding

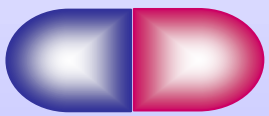


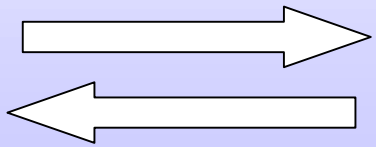
Sedimentation velocity



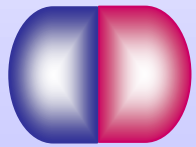
No α changes **Hydrophobic region exposure** **More-compact monomer**

α-SHaPrP(23-232)





SHaPrP*(23-232)



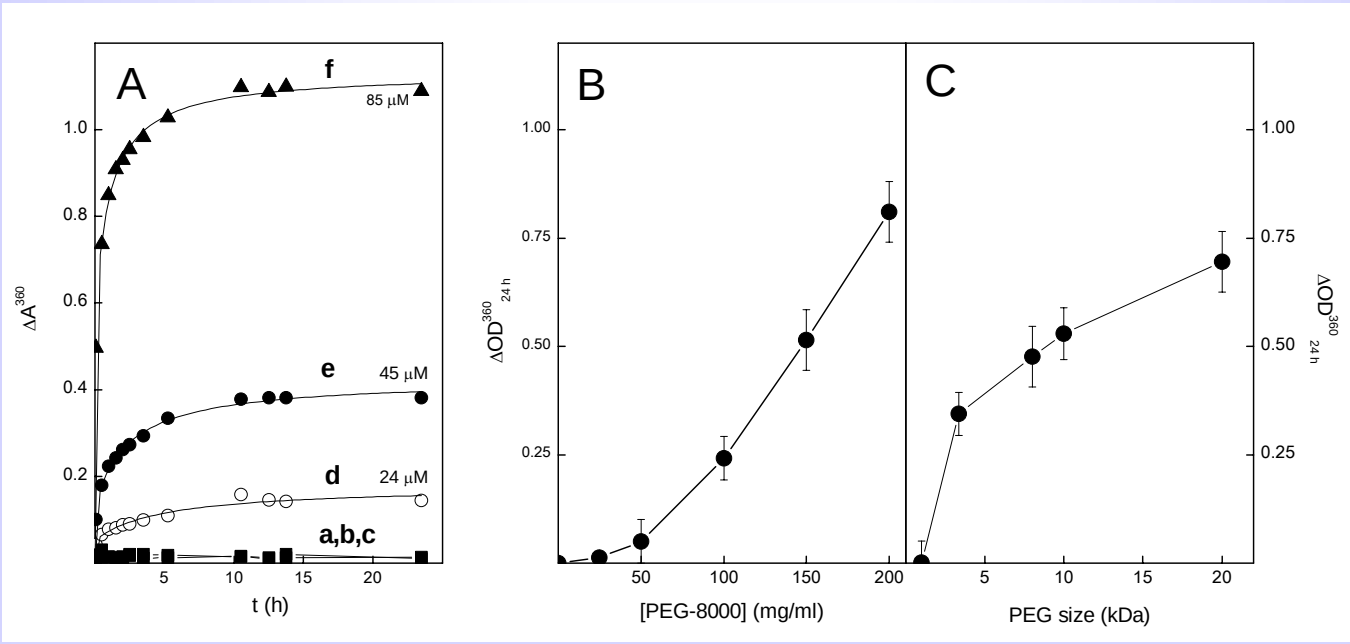
SHaPrP*(23-232) is an aggregation competent state

Crowded environments (inert polymers)

Aggregation

Increased turbidity

Insoluble polymers



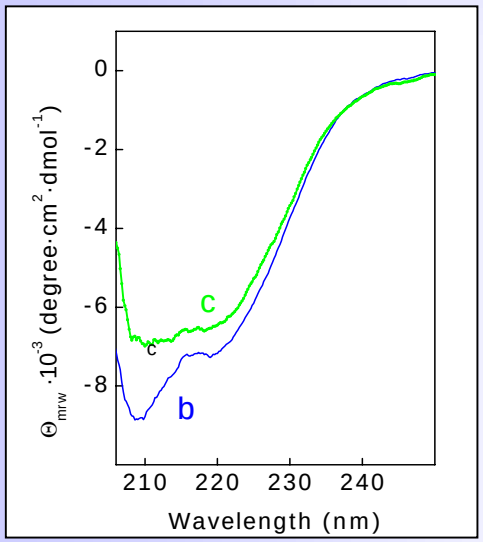
Protein concentration

Crowder concentration

Crowder size

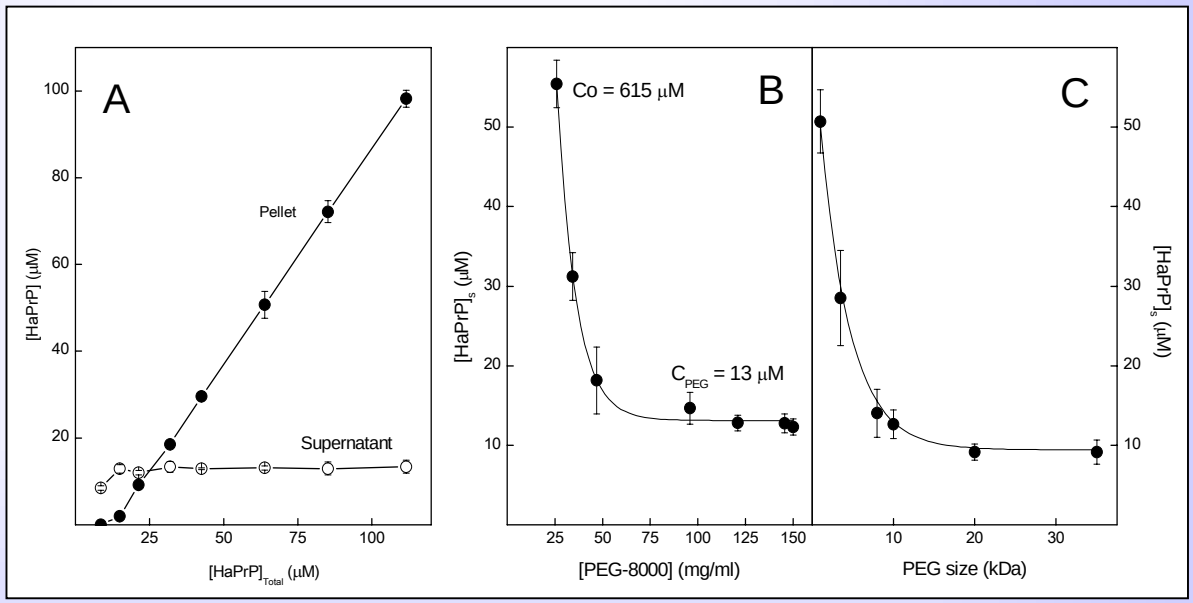
SHaPrP* polymerization mechanism

Decreased α



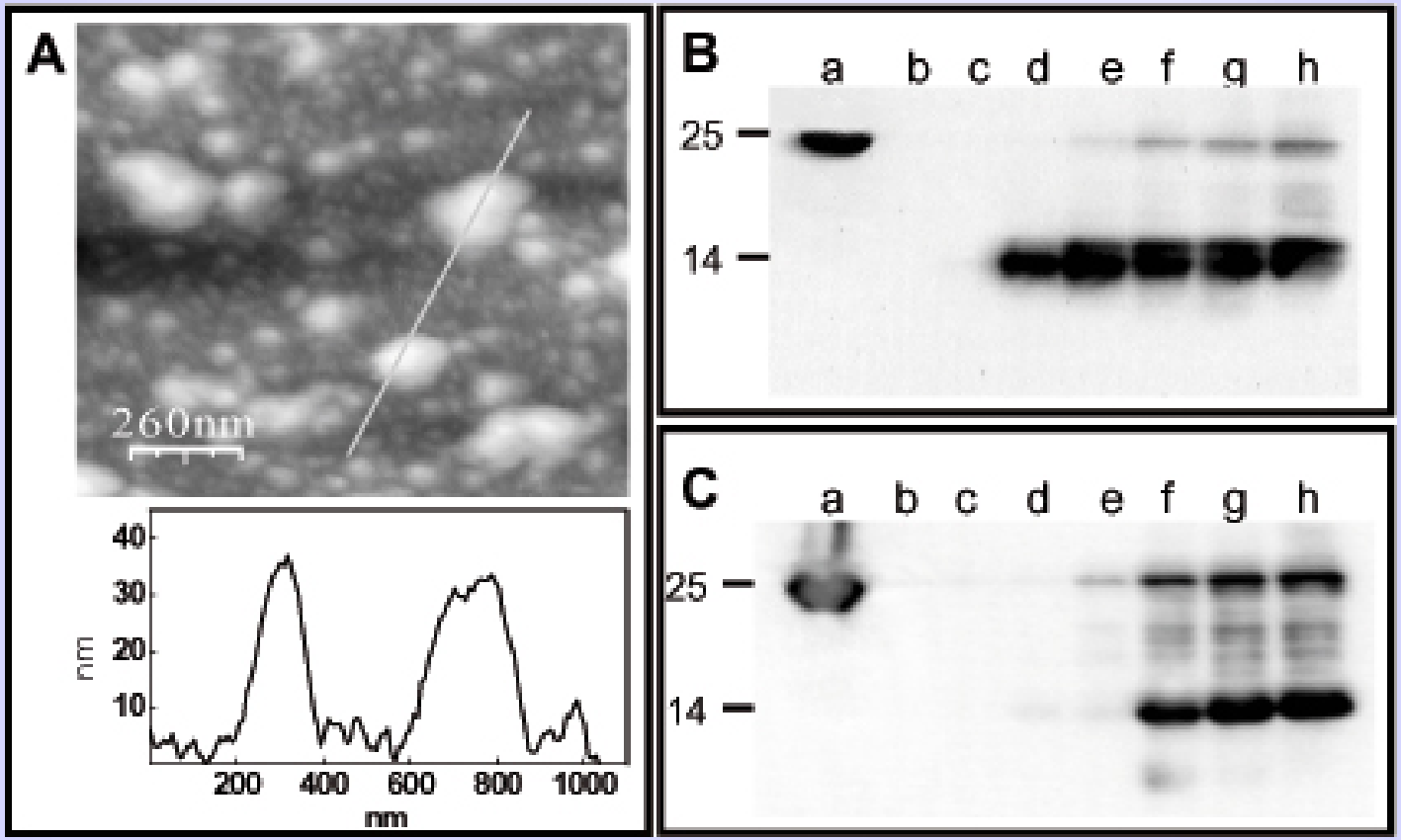
Conformational-seeded polymerization

Cc for polymerization



Cc varies with crowder size and concentration

SHaPrP* polymers compare with PrP^{Sc} 33-35 polymers: insoluble, β-enriched, amorphous, PK resistant



SHaPrP* polymers lack infectivity

Ligand-free and bound rSHaPrP*(23-232) polymers lack prion infectivity probed by inoculation in normal hamster.

Inoculum	Passage	Incubation Period (days)	n/no
Normal brain homogenate	First (ipx1)	>600	0/5
Scrapie brain homogenate	First (ipx1)	120	5/5
rSHaPrP*(23-232)	First (ipx3)	>600	0/5
rSHaPrP*(23-232):Cu(II)	First (ipx3)	>600	0/5
rSHaPrP*(23-232):HS	First (ipx3)	>600	0/5
rSHaPrP*(23-232):Cu(II), HS	First (ipx3)	>600	0/5
Normal brain homogenate	Second (ic)	>360	0/5
Scrapie brain homogenate	Second (ic)	90	5/5
rSHaPrP*(23-232)	Second (ic)	>360	0/5
rSHaPrP*(23-232):Cu(II)	Second (ic)	>360	0/5
rSHaPrP*(23-232):HS	Second (ic)	>360	0/5
rSHaPrP*(23-232):Cu(II),HS	Second (ic)	>360	0/5

1st passage:
no signs, no PrP^{Sc} detection

2nd passage:
no signs, no PrPSc detection,
no PrP^{C} changes (solubility, levels)
(subclinical detection, transmission)

1st passage+EAE
no signs

CONCLUSIONS

I. PrP covalent multiplicity

- **PrP^C is a complex protein family involving at least two distinct isoforms resulting from the consitutive or inducible alternate translation initiation (AUG, ACA, ACG).**

- **PrP isoform resulting from AUG_2 (cy-PrP or PrP(Δ1-14)), segregates outside the secretory route, displays a nucleocytoplasmic location and its expression is specifically linked to a G0/G1 cell cycle arrest that if prolonged causes cell death.**

II. PrP conformational multiplicity

- **PrP chain exists as a complex mixture of α monomers and β-polymers under a close-to-physiological environment provided by crowders. Presence of β-forms does not imply existence of infectivity**

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