Prion Diseases - Basic Science

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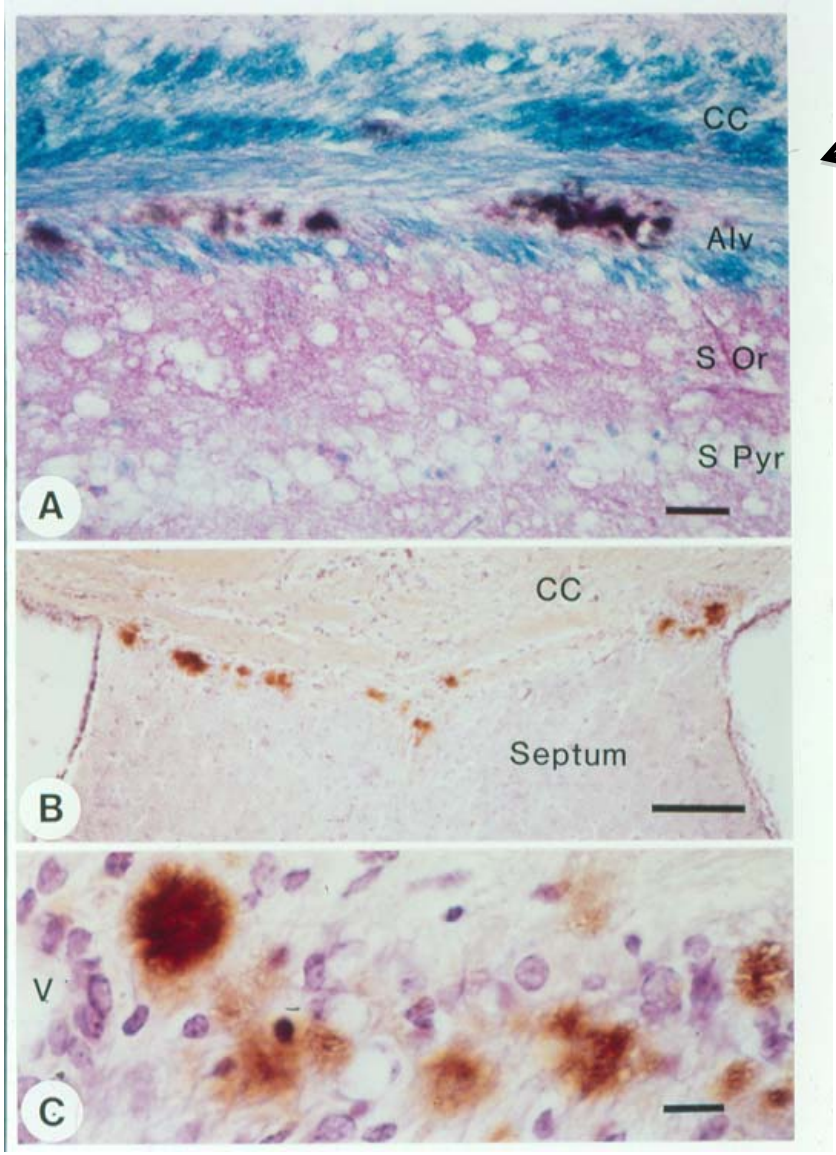
Part I: The causative agent and patterns of manifestation



Spongiform change and amyloid in a scrapie-infected brain.

Slow and "unstoppable" disease progression

Diseases to be discussed:
Scrapie, CWD, BSE, CJD, GSS, FFI, kuru

The spectrum of prion diseases includes three patterns of manifestation

- Infectious (kuru, iatrogenic CJD, vCJD experimental disease
- Familial (autosomal dominant genetic). g-CJD, GSS, FFI
- Sporadic (sporadic CJD, sporadic fatal insomnia).

How can this possibly be?

Are diseases like scrapie infectious or genetic?

HB “James” Parry, Univ Oxford: a genetic disease controlled by the recessive “s” gene

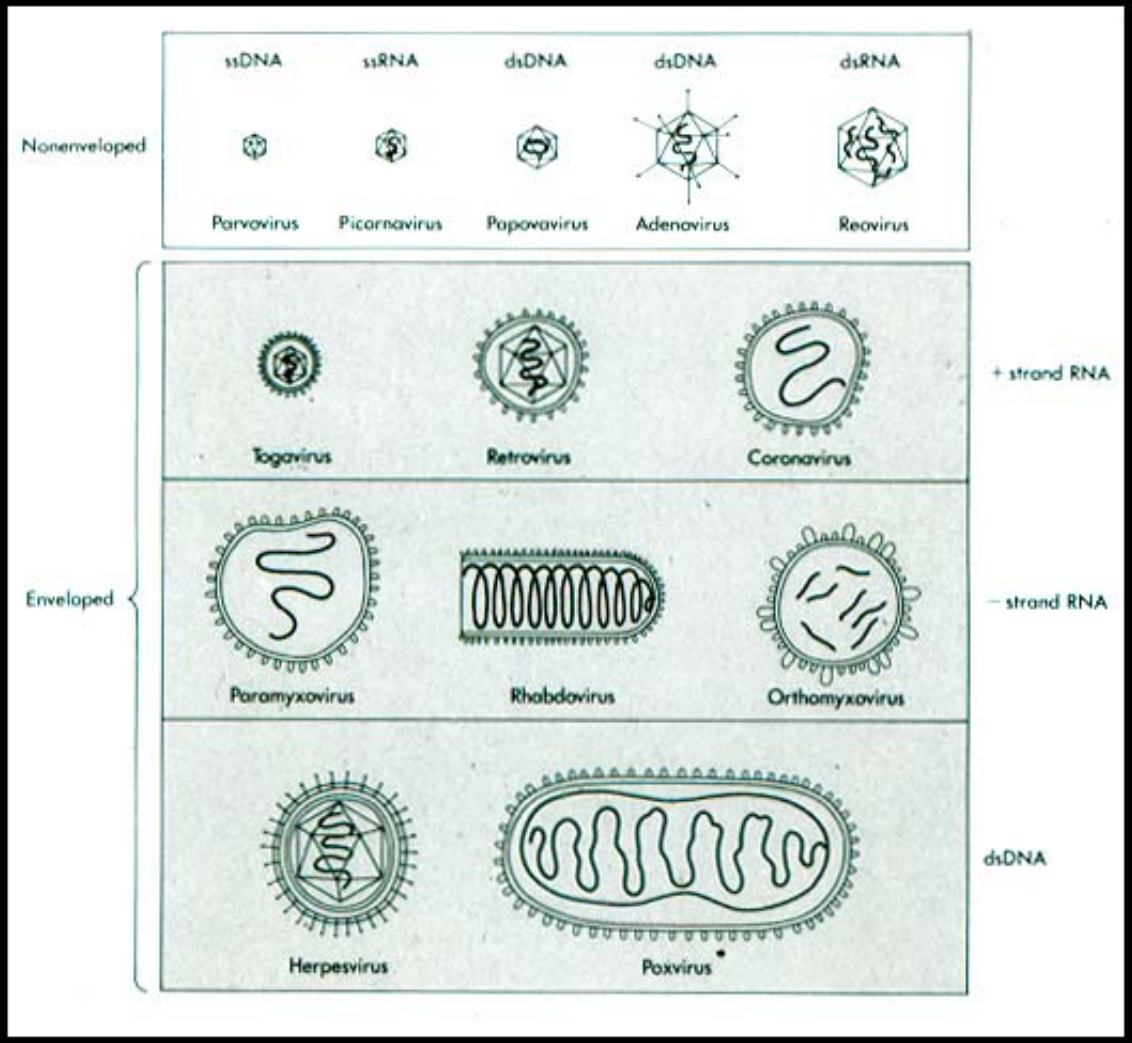
Alan Dickinson, Univ Edinburgh: a naturally infectious disease

The search for a genomic nucleic acid in the scrapie agent

Studies in the 1960’ and 1970’s when prions were sometimes referred to as “unconventional slow viruses”

Notes on nomenclature:

Unconventional slow virus – meaningless
TSE - inaccurate


ssDNA
ssRNA
dsDNA
dsDNA
dsRNA
Nonenveloped
Parvovirus
Picornavirus
Papovavirus
Adenovirus
Reovirus
Enveloped
Togavirus
Retrovirus
Coronavirus
+ strand RNA
Paramyxovirus
Rhabdovirus
Orthomyxovirus
− strand RNA
Herpesvirus
Poxvirus
dsDNA

Inactivation of the scrapie agent

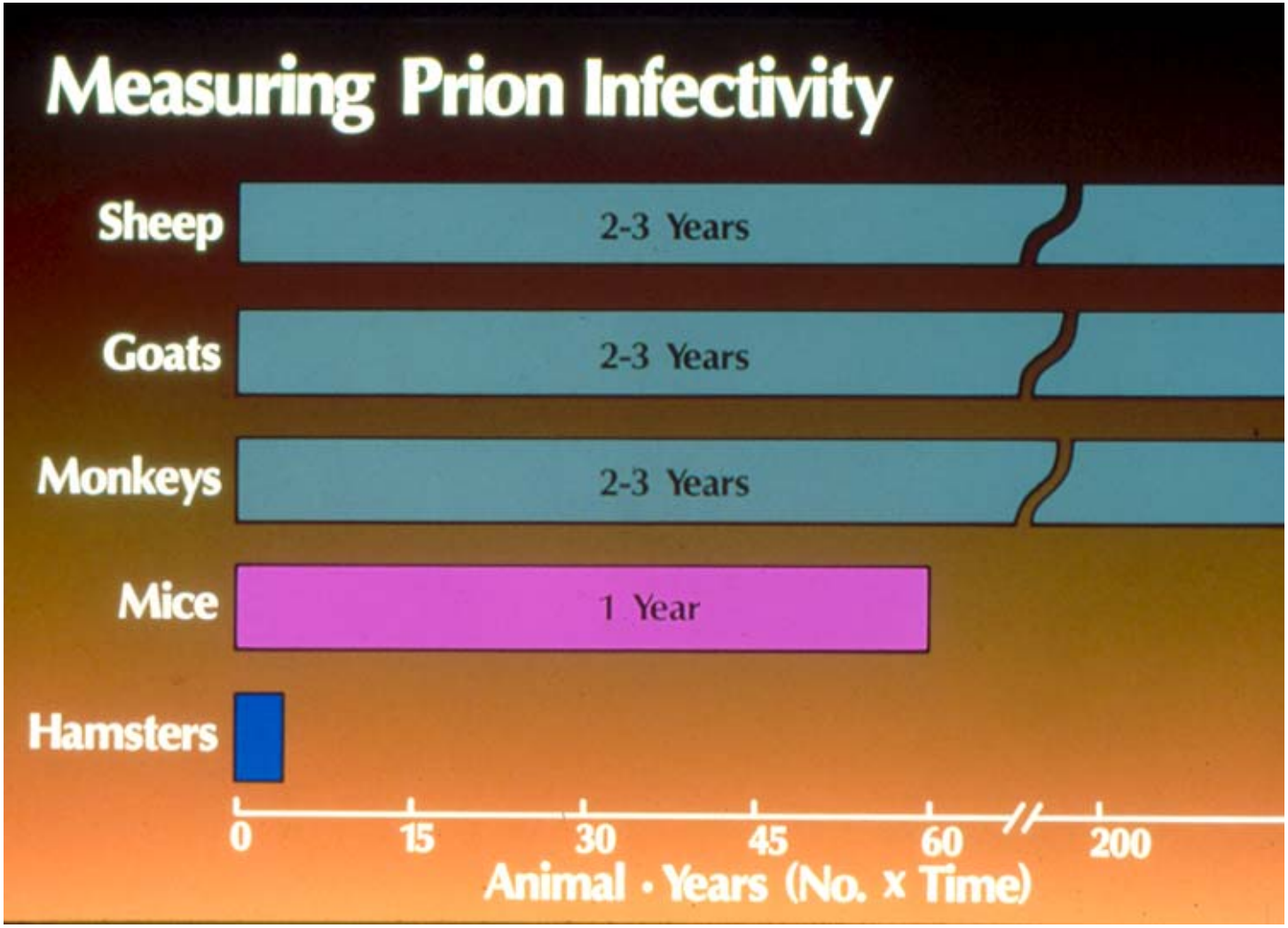
- Ionizing radiation target size indicates an infectious particle of 55 kDa.
- UV irradiation indicates that if there is a double-stranded DNA genome it would have a size of about 40 base-pairs.
- Scrapie agent was resistant to agents that destroy or modify nucleic acids including psoralen, DNAses, RNAses, divalent cations.

Purification of the scrapie agent

Purification of the scrapie agent led to the discovery of two important proteins, PrP^{Sc} and PrP^{C}

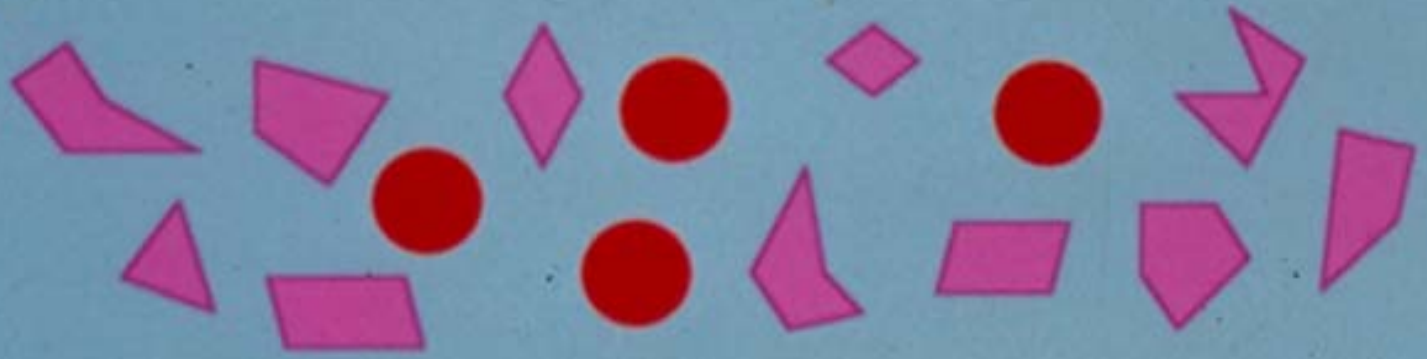
These proteins are actually “isoforms”

Better Prion Assays



To learn the composition of the scrapie agent, we undertook its purification or isolation.

Infected hamster brains were used as the source of scrapie agent.



Scrapie agent needed to be separated from contaminants.

Initial purification studies required nearly eight years (1974 - 1982)

Pure preparations of the scrapie agent led to many discoveries:

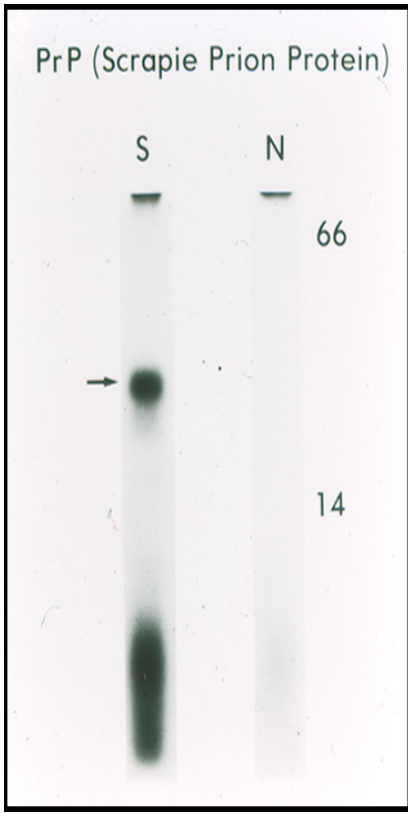
1. Scrapie agent infectivity is destroyed by procedures that modify proteins.

2. Scrapie infectivity is resistant to procedures that modify nucleic acids.

3. These unusual properties distinguish the scrapie agent from both viroids and most viruses.

4. Based on these properties the " prion " was introduced.

Discovery of the prion protein in 1982



- A protease-resistant protein can be visualized in highly purified (~2000-5000 x enriched) preparations of scrapie infectivity
- This protein is called PrP27-30, because of its size (in kDa).
- These preparations were made using detergent insolubility and sucrose gradient fractionations and PK digestion.

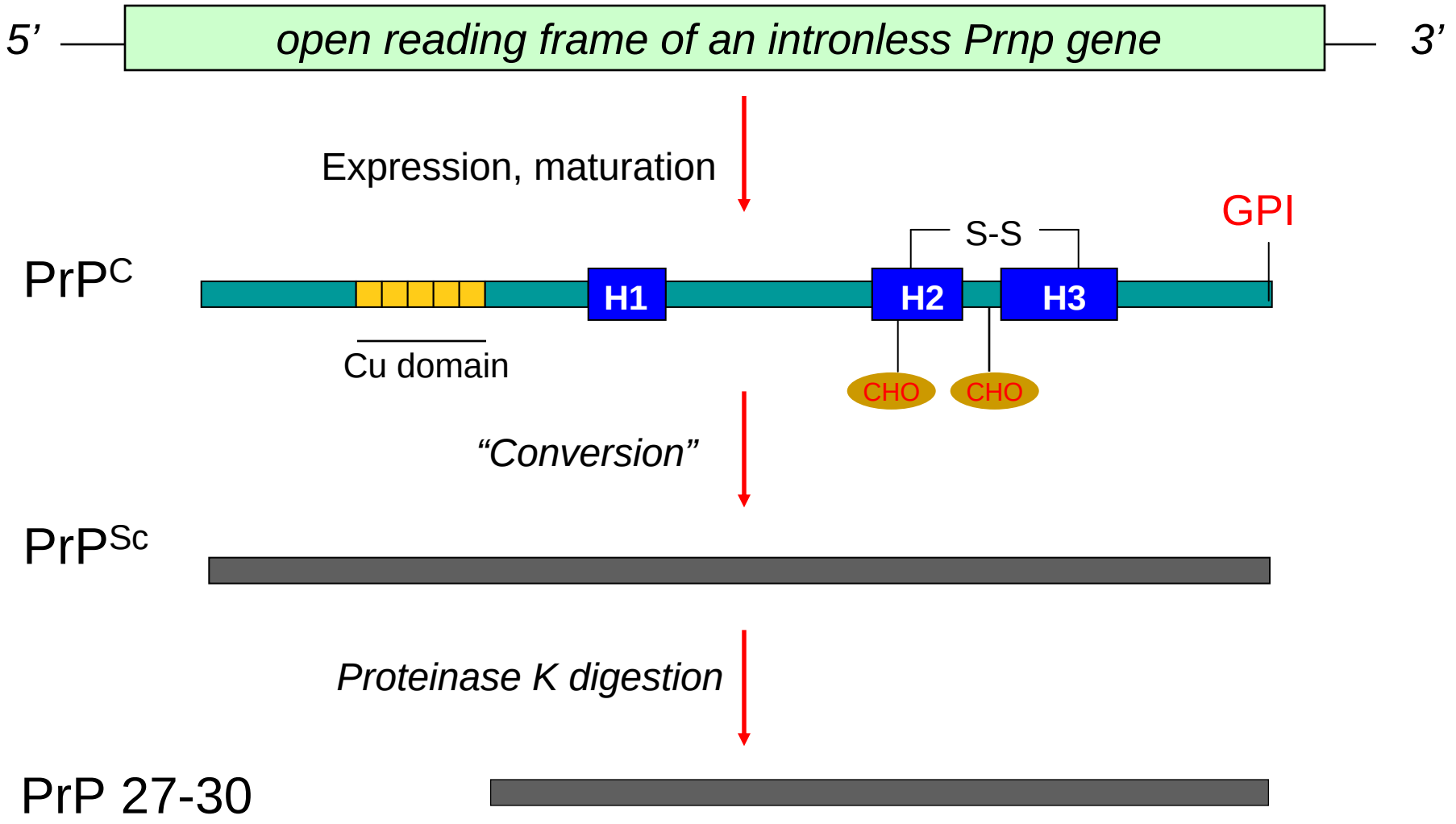
Prions are made of proteins with naturally-occurring amino acids

- N-terminal sequencing of highly infectious scrapie prion preparations gives:

G-Q-G-G-G-T-H-N-Q-W-N-K-P-S-K

X-X-X-T-H-N-W-X-K-P

PrP^{Sc} derives from the host



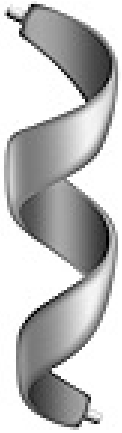
Arriving at the “conformational hypothesis” (1)

- PrP^{Sc} and PrP^{C} have closely related amino acid sequences (1985).
- The PrP gene has a single uninterrupted coding exon (1986).
- Low resolution structural analysis reveal PrP^{C} is α-helical (1992).

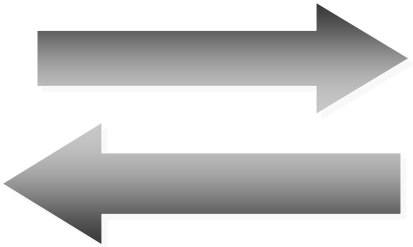
Arriving at the “conformational hypothesis” (2)

- Low resolution structural analysis reveal PrP^{Sc} is enriched in β-sheet (1992).
- PrP^{Sc} amyloid deposits in scrapie-infected hamsters stain with Congo Red dye (1985).
- Amino acid analysis of proteolytic fragments of PrP^{Sc} (arising from *in vitro* digestion of purified material) reveal no differences from the predicted sequence of PrP^{C} (1993).

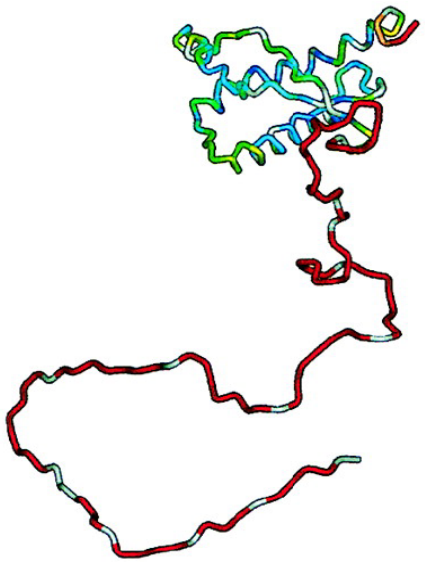
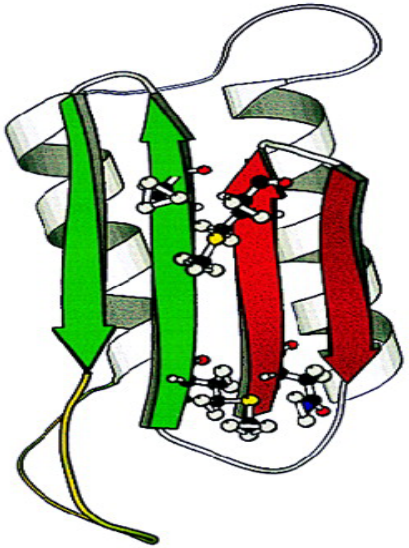
α-helices and β-sheets



α–helix

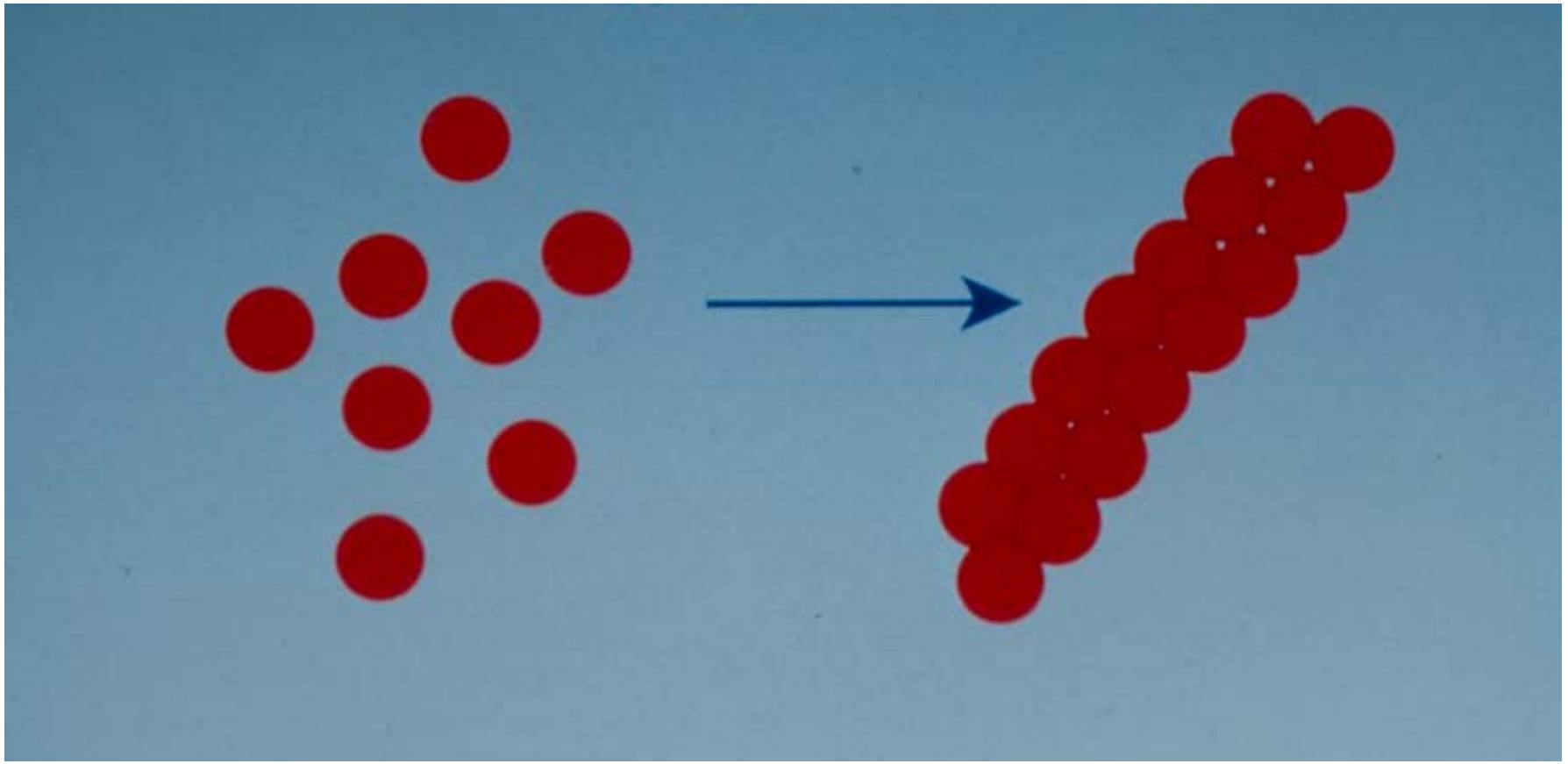


β–sheet

	PrP^{C}	PrP^{Sc}
Structure		
α-helix	~43%	~34%
β-sheet	~3%	~43%
Solubility	+	-
Protease resistance	-	+

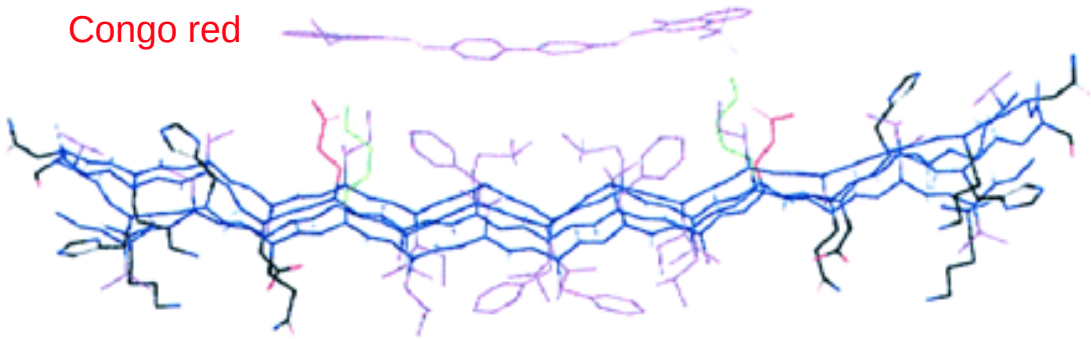
- *Genetic and cell biological experiments are in favour of the prion hypothesis too*.

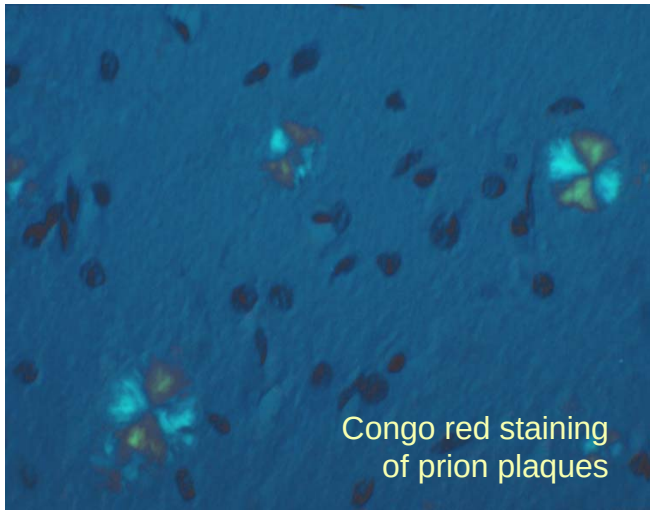
Prions can aggregate to form amyloid



Aggregated β-sheet proteins form clumps called “amyloid”

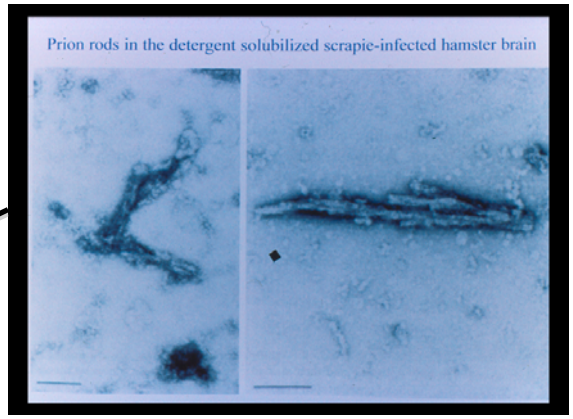
Parent Protein	Amyloid	Disease
PrP	Plaques	Prion
APP	Aβ	Alzheimer’s

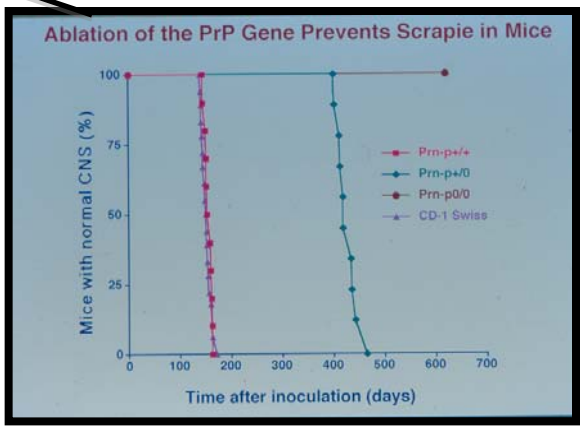


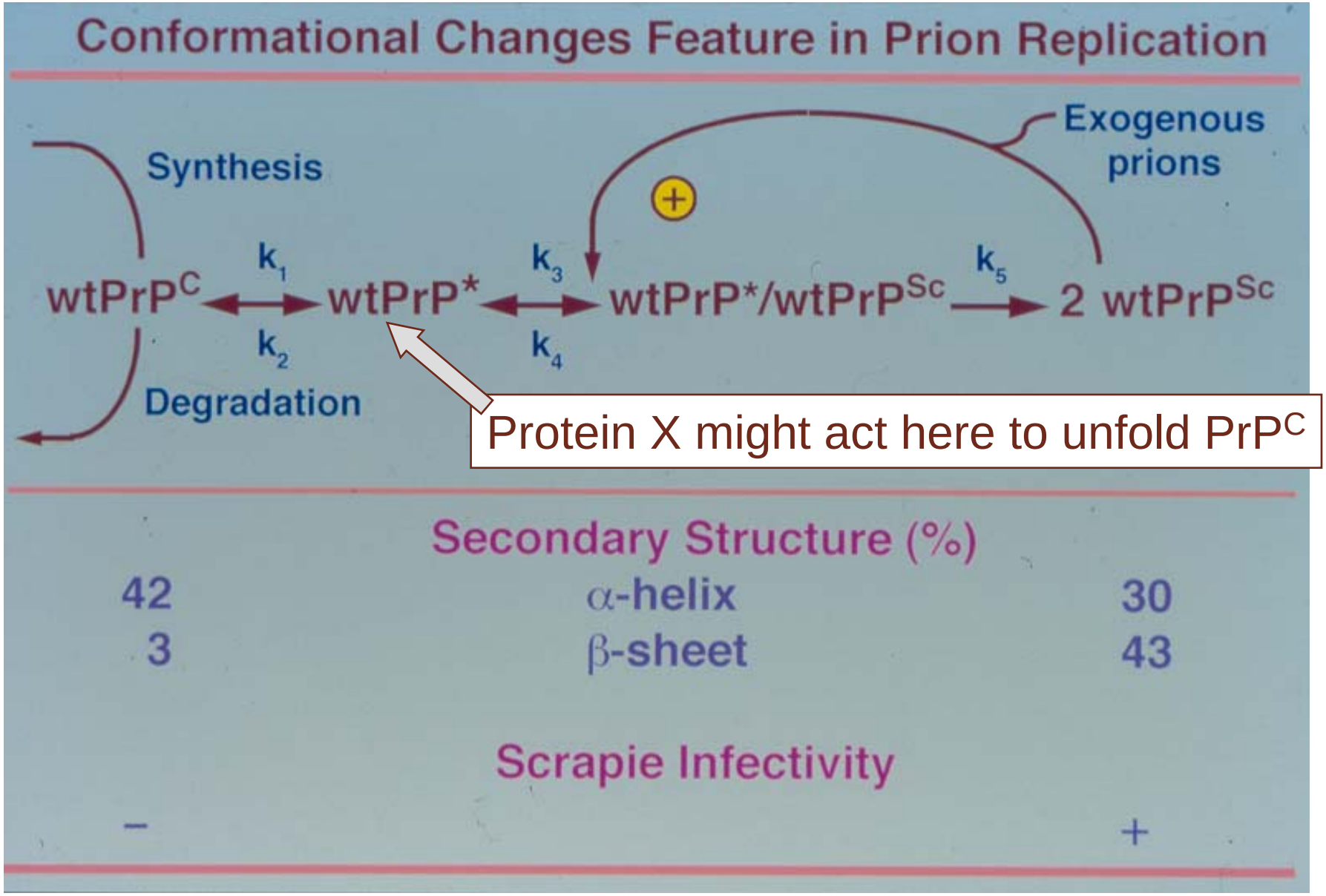


The “protein-only” hypothesis for the composition of the scrapie agent

- Failure to detect a nucleic acid genome in purified preparations
- Failure to detect uniform particles by EM
- PrP k/o mice are resistant to infection
- Yeast ‘prion like’ traits are transmitted by a protein (1994).
- Pure recombinant PrP can be re-folded (to a form infectious for transgenic mice) in the absence of other co-factors (2004)
- Pure recombinant PrP can be re-folded to a from infectious fro ordinary mice, with lipids and non-coding RNA as additives (2010)

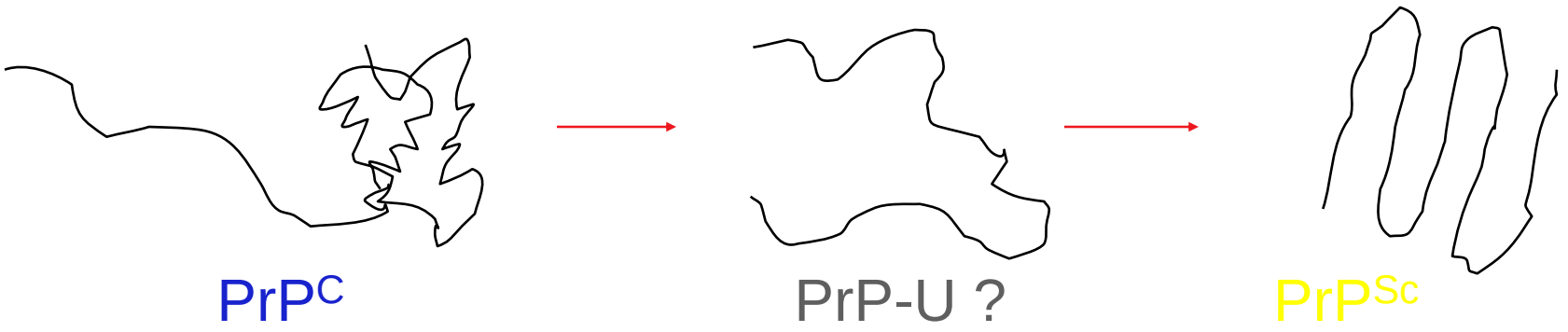




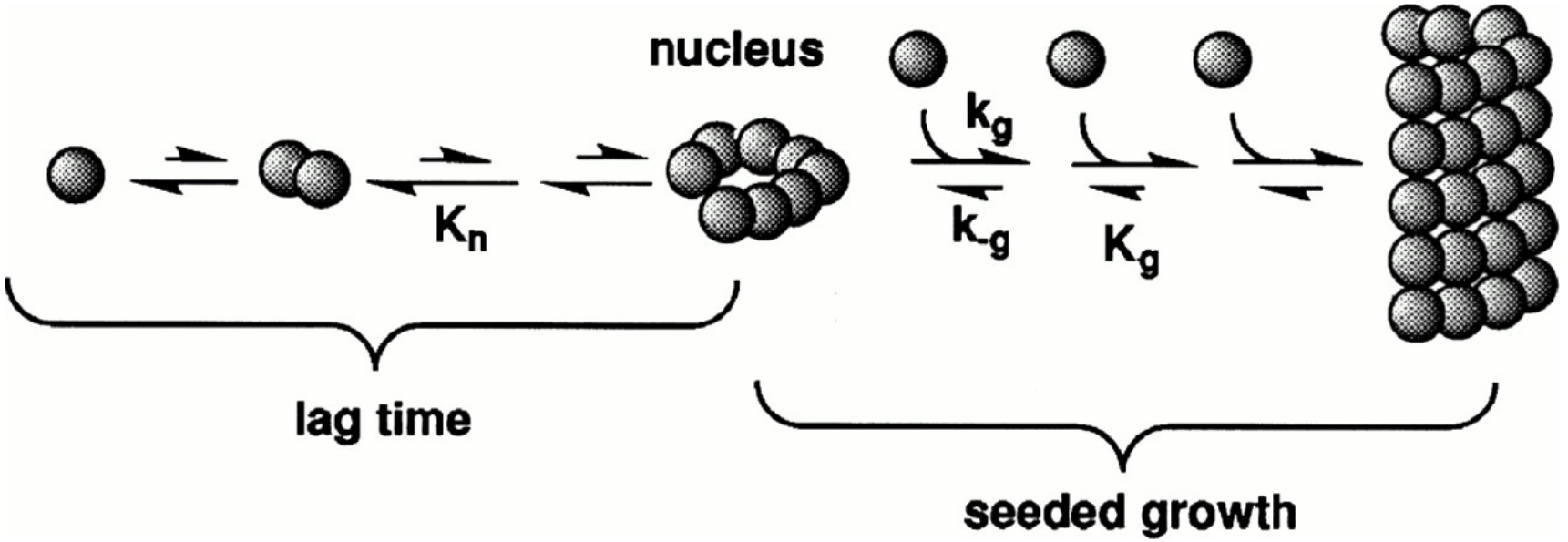

Conformational Changes Feature in Prion Replication
Synthesis
Exogenous prions
wtPrP^C
k1
k2
wtPrP*
k3
k4
wtPrP*/wtPrP^Sc
k5
2 wtPrP^Sc
Degradation
Protein X might act here to unfold PrP^C
Secondary Structure (%)
42
α-helix
30
3
β-sheet
43
Scrapie Infectivity
–
+

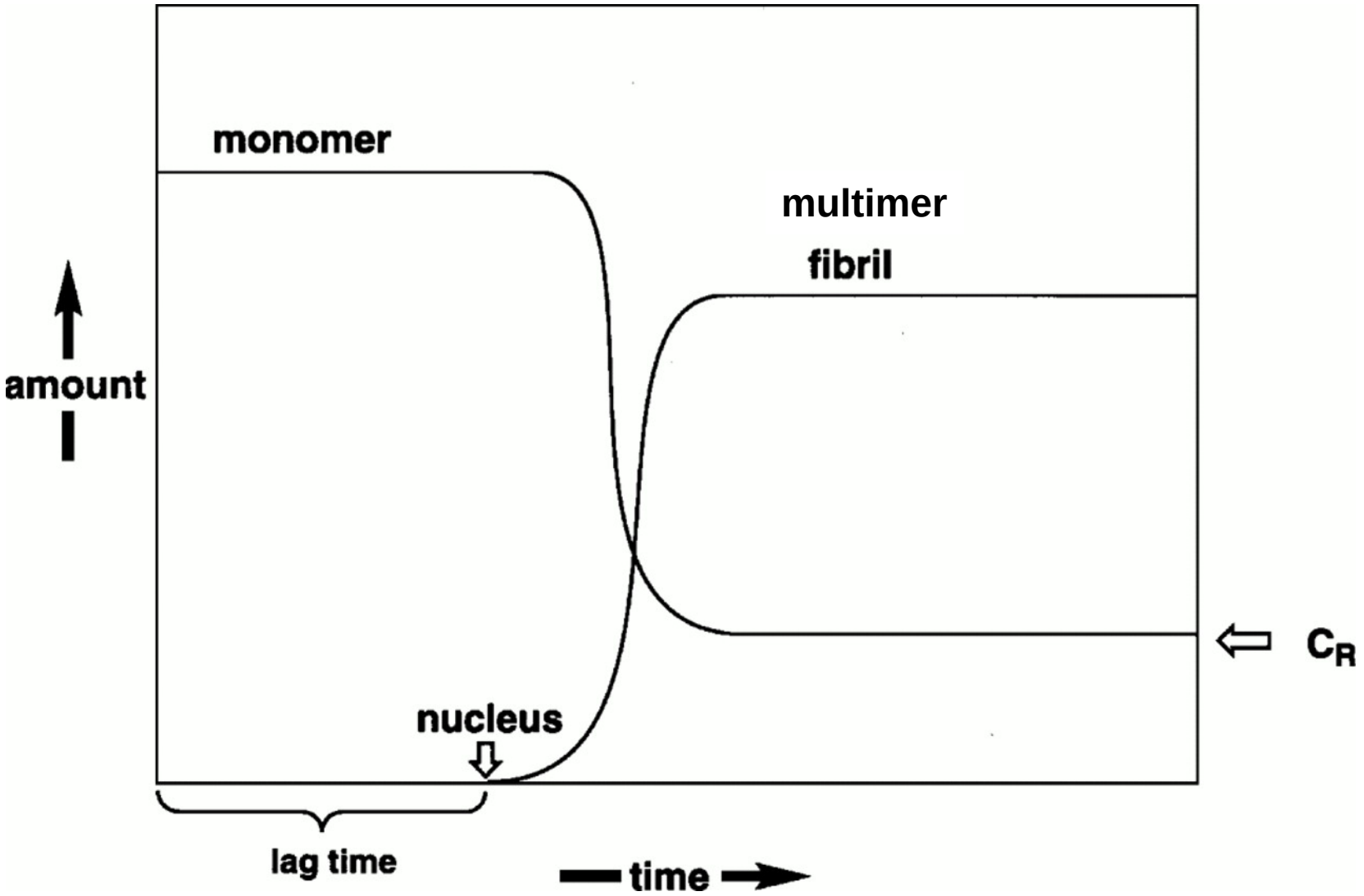
Heterodimer hypothesis (Prusiner)

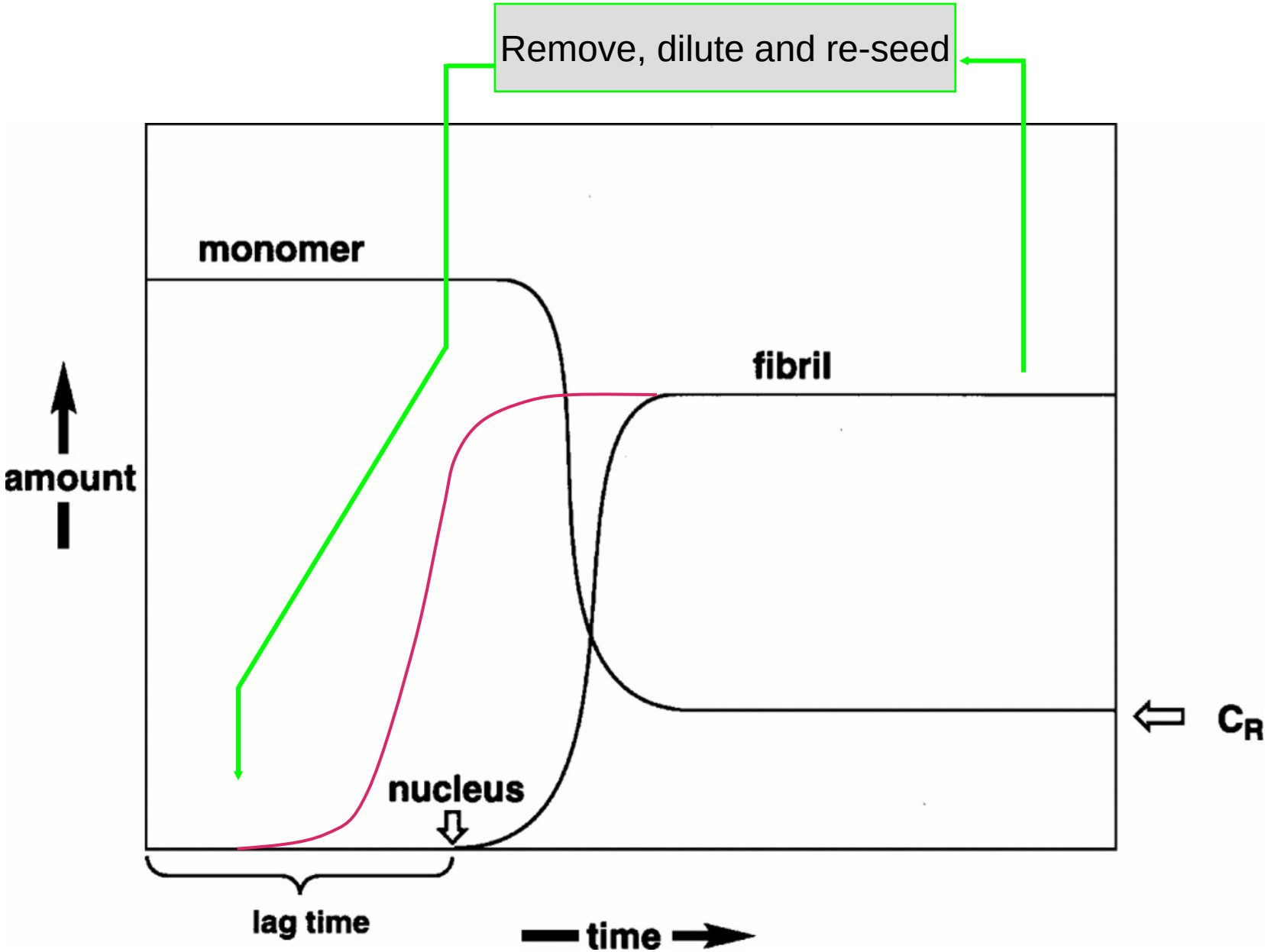
- A large energy barrier prevents spontaneous conversion of PrP^{C} to PrP^{Sc}.
- PrP^{C} is unfolded by a hypothetical molecular chaperone called protein X. Identity of protein X is unknown
- The replication intermediate is a PrP^{C} /PrP^{Sc} heterodimer (60 kDa).



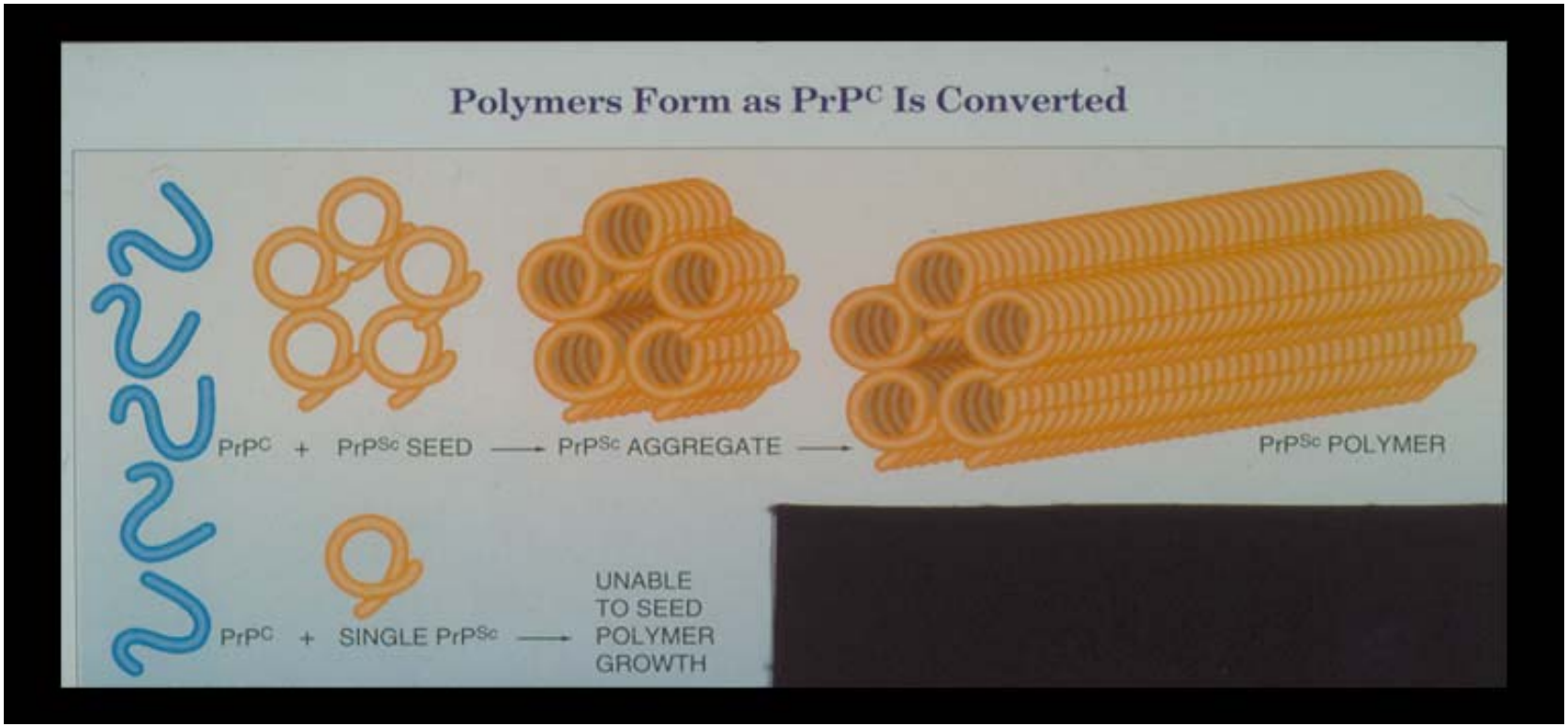
“Seeding Hypothesis” of Lansbury and Caughey: seeded growth of monomeric subunits into a multimer




monomer
multimer
fibril
amount
C_R
nucleus
lag time
time


Remove, dilute and re-seed
monomer
fibril
amount
nucleus
c_R
lag time
time

Visualizing the “seeding hypothesis”



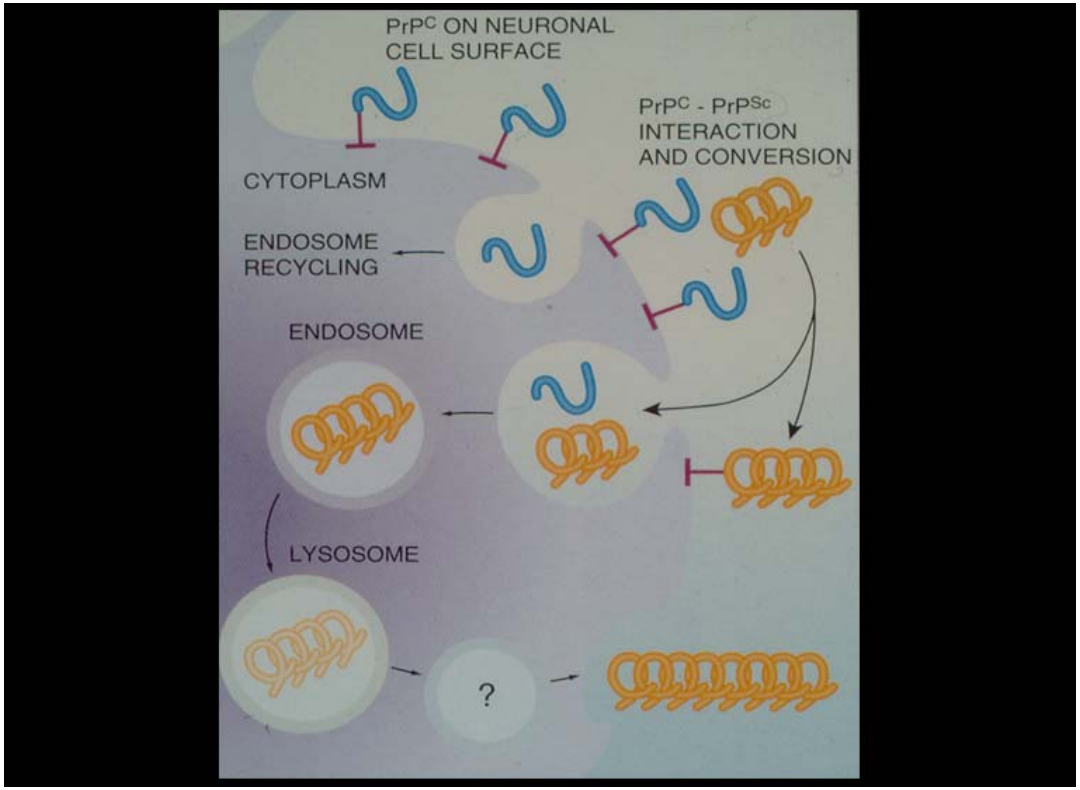
Facets of the “Seeding” hypothesis

- There is only a small energy barrier between PrP^{C} and PrP^{Sc} but spontaneous conversion is prevented by a kinetic barrier: conversion is too slow.
- Once a pre-formed seed of PrP^{Sc} multimers is made the long lag period is avoided and PrP^{C} to PrP^{Sc} conversion takes place rapidly of the surface of the multimeric PrP^{Sc}.
- As multimers get bigger they fragment and thus can create multiple “new” seeds.
- Replication intermediates are big.

All labs *do* agree that “conversion” takes place on the cell-surface or in an early endosomal compartment

-distinction from viruses

- Implies a molecular chaperone outside the cell might modulate re-folding?

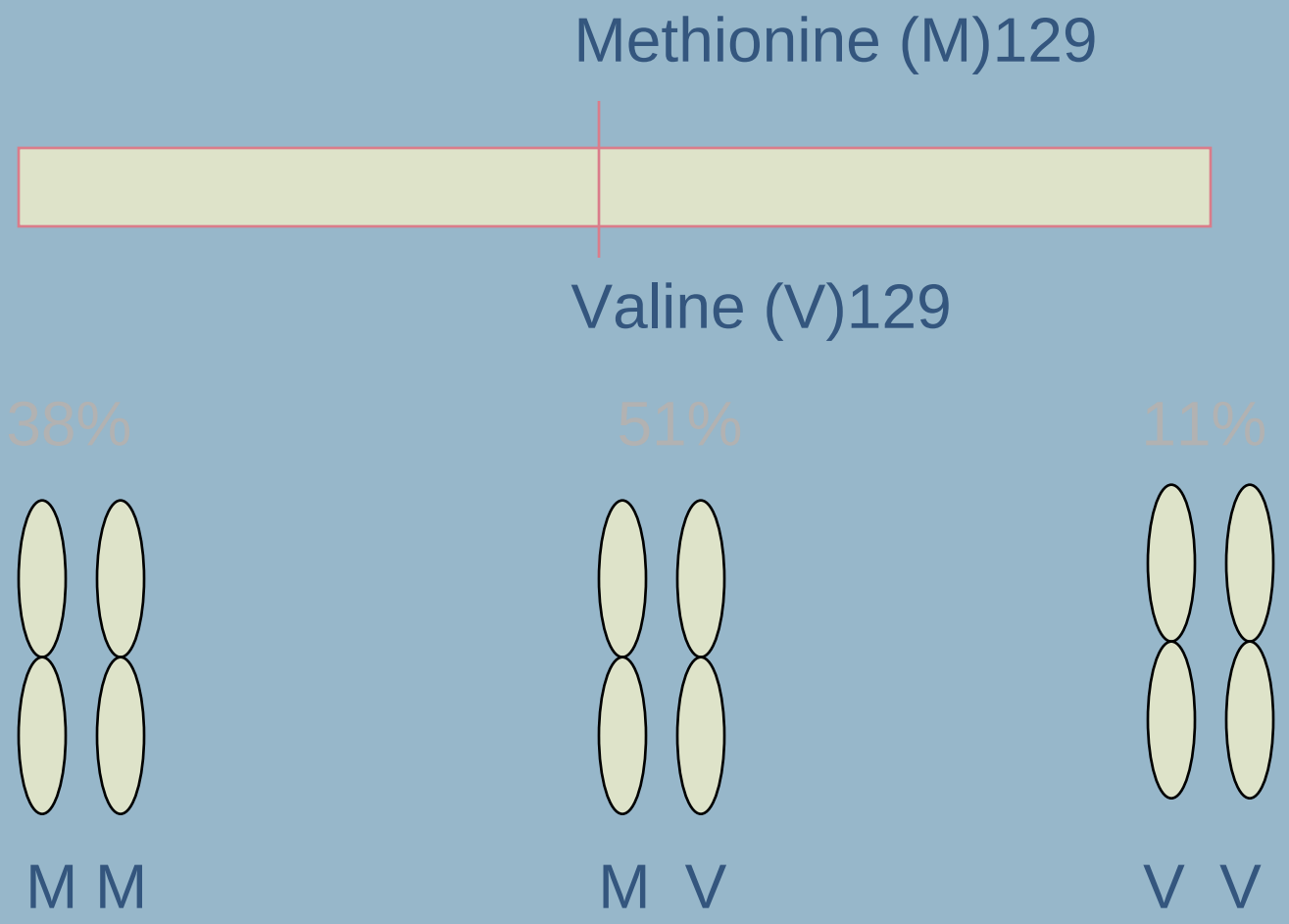


The Host genetics of Prion Disease: variant prion proteins modulate disease susceptibility

- Mouse PrP gene mutations
- Sheep PrP gene mutations
- Human PrP gene mutations
- Deer PrP gene mutations

Missense mutation substitute one amino acid for another

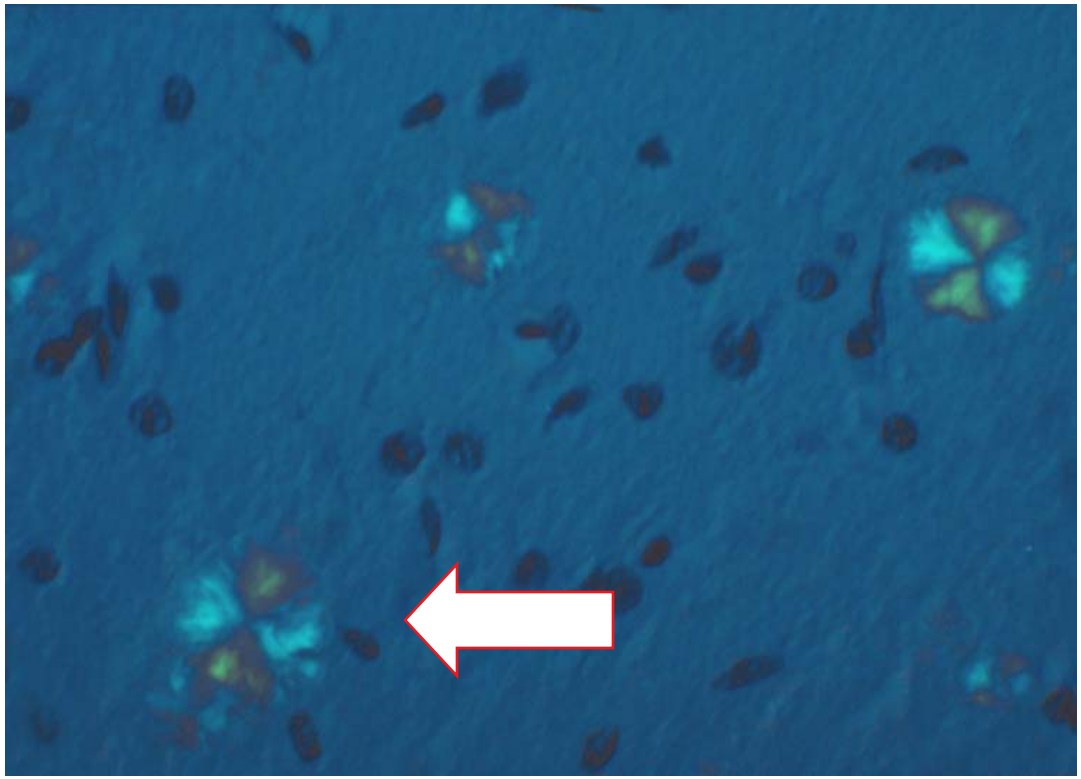
“Net” genotype for polymorphism affects outcome of infectious, sporadic or familial prion diseases



Human Prion Diseases

Manifestation	Diseases
1. Infectious	Kuru was transmitted among New Guinea natives by ritualistic cannibalism. Iatrogenic Creutzfeldt-Jakob disease caused by growth hormone derived from human pituitaries.
2. Sporadic	Creutzfeldt-Jakob disease occurs at one per million population across the earth.
3. Familial	Gerstmann-Straussler-Scheinker syndrome, familial Creutzfeldt-Jakob disease and fatal familial insomnia occur in families where 50% of the members are afflicted.

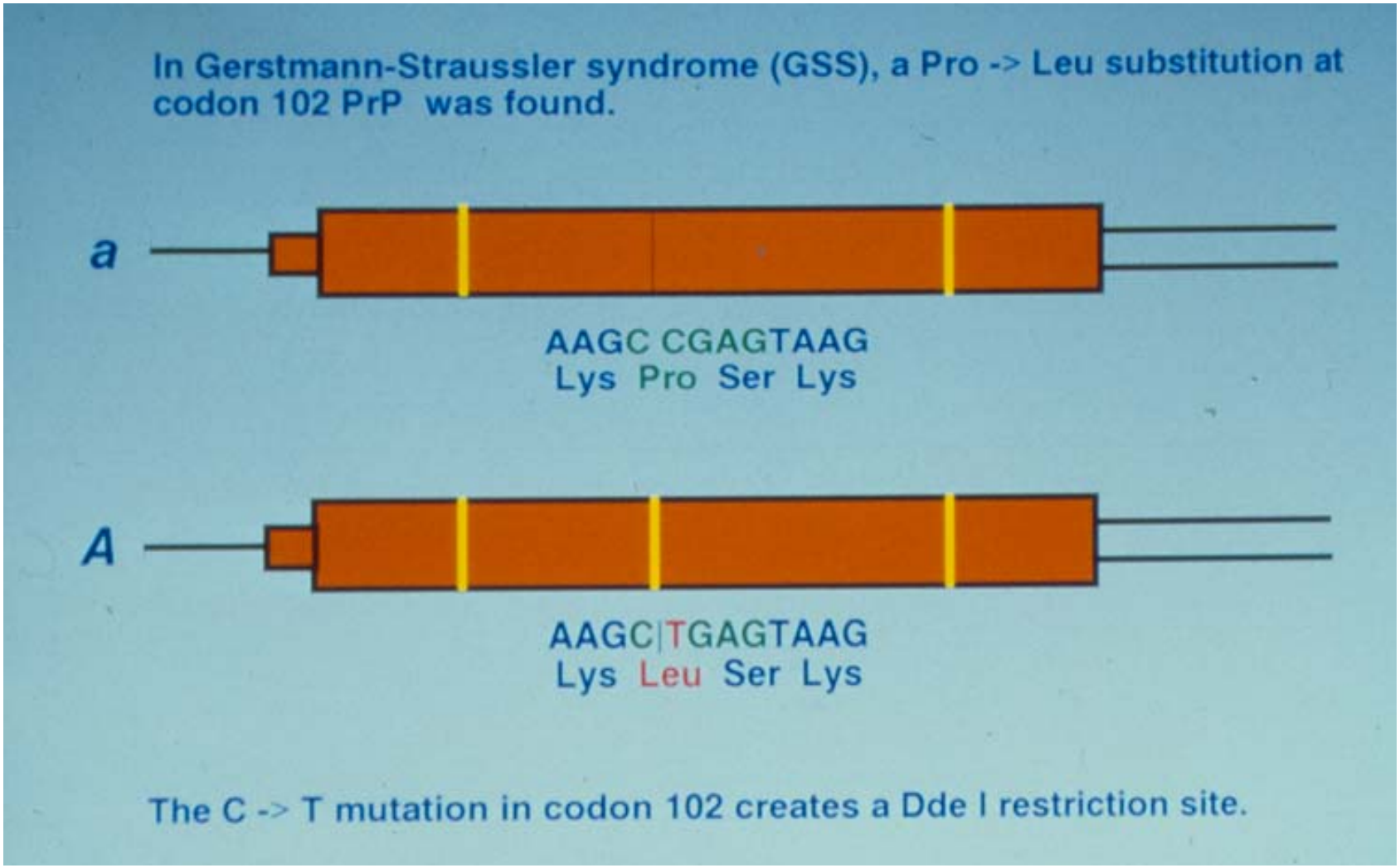
Birefringent amyloid plaques in a prion disease (GSS)



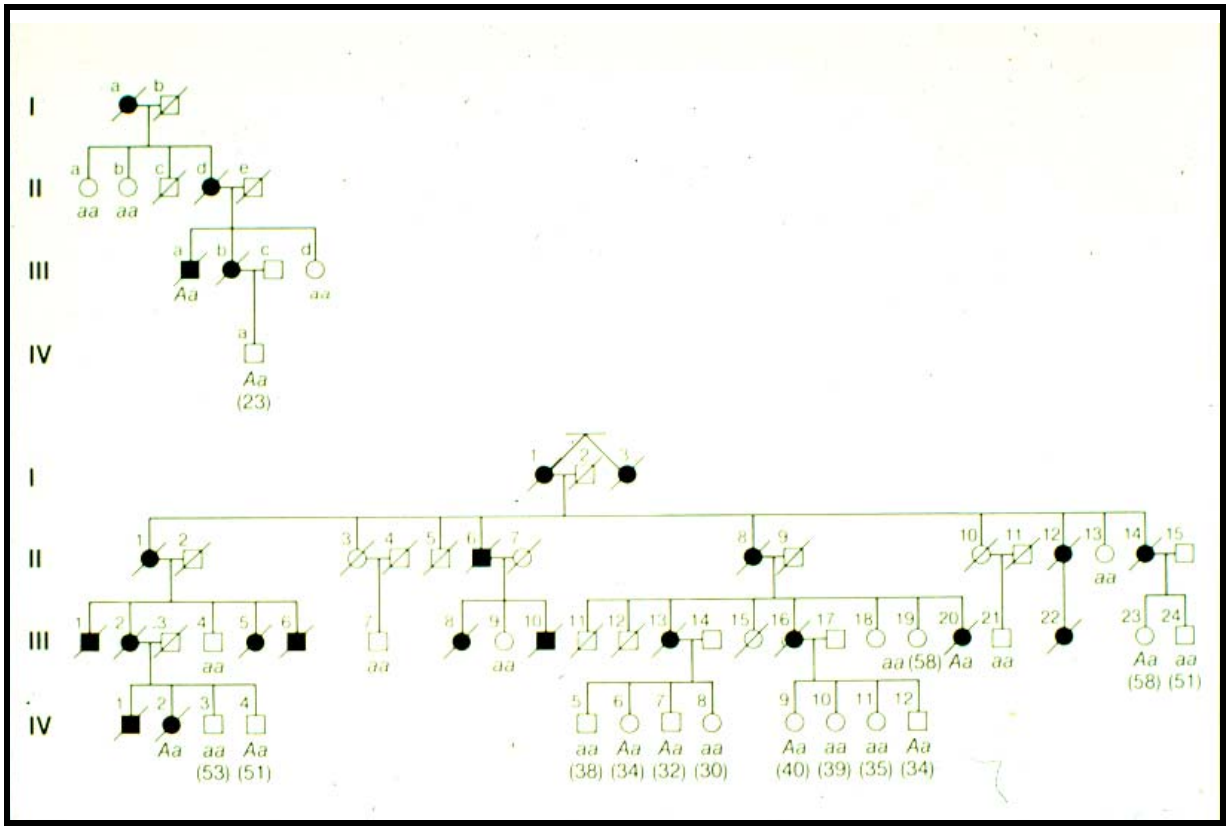
Congo Red staining of Maltese-cross shaped GSS amyloid plaques.

Plaques can also be stained with thioflavin S, or with PrP-directed antibodies

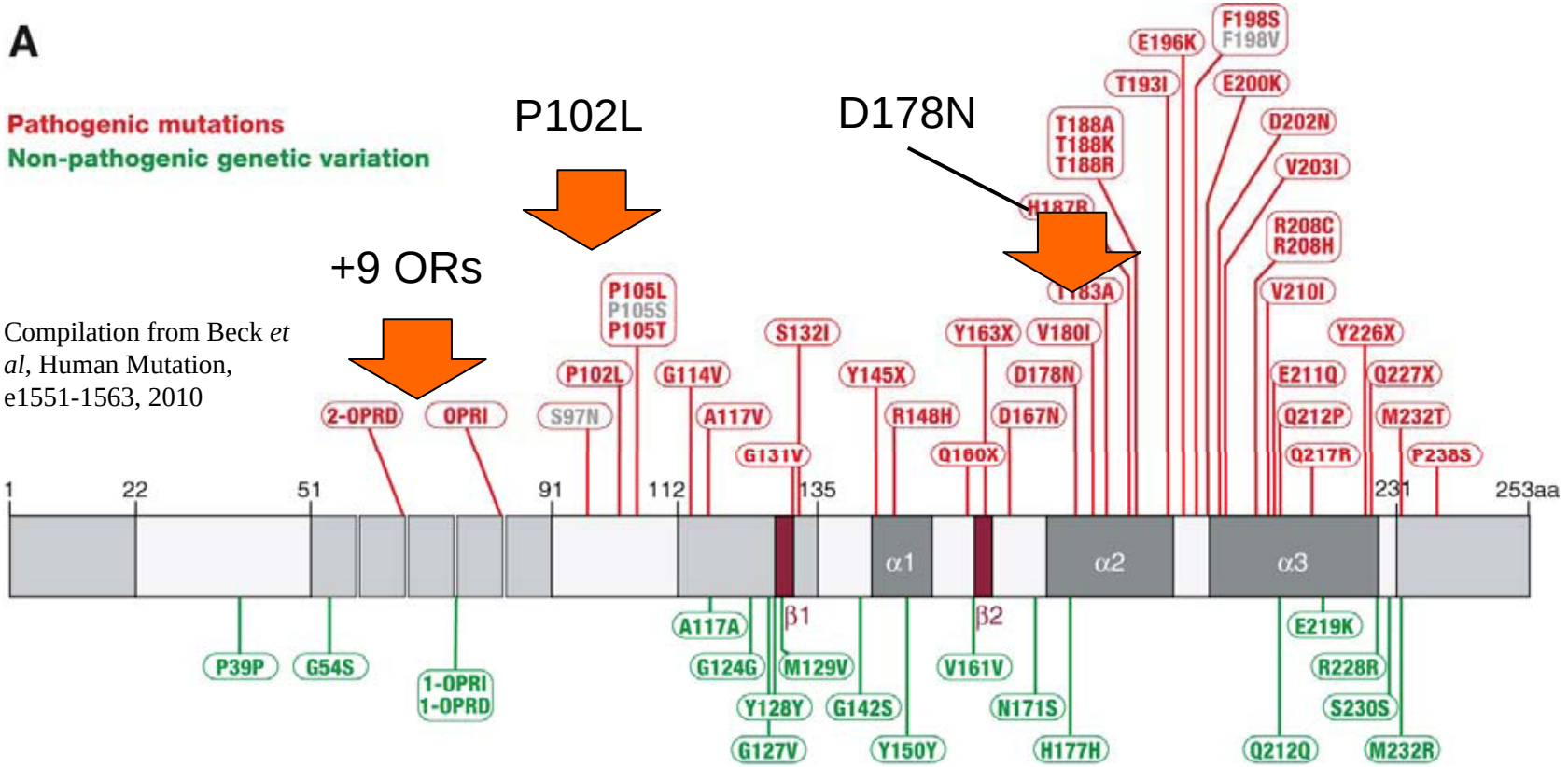
The first prion gene mutation was found in GSS



Inheritance of a prion mutation tracks with disease in GSS families

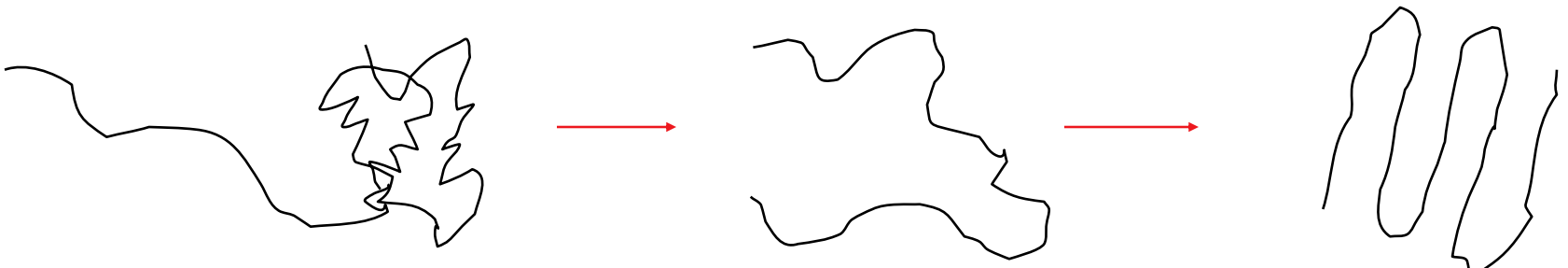


Human *PRNP* variants



Sporadic prion disease

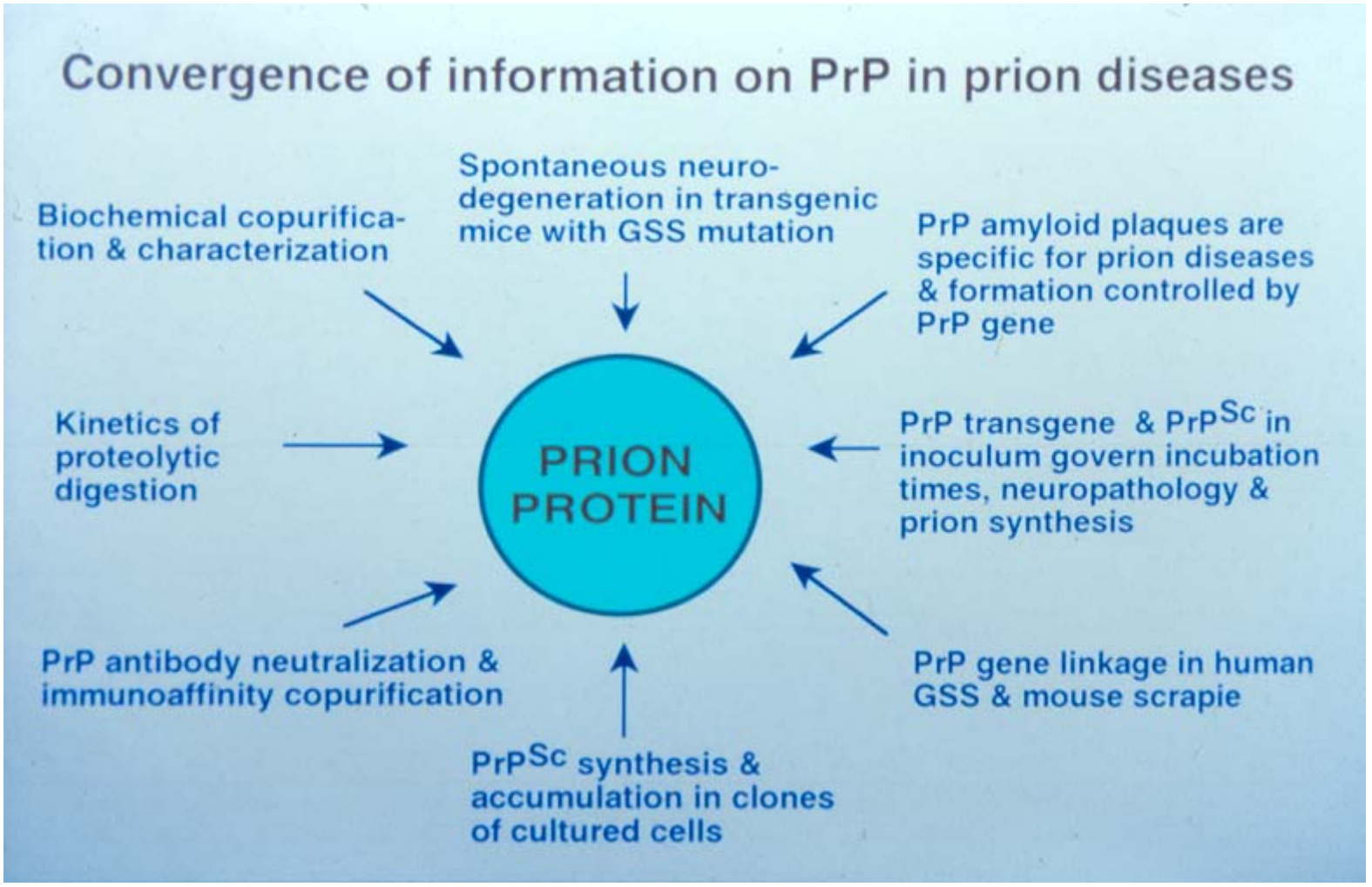
- No families, no clusters to indicate infectious spread : disease “appears out of nowhere”
- Due to spontaneous misfolding of PrP^{C} or infection from a cryptic animal reservoir
- Sporadic disease (infectivity) has been modeled *in vitro* by use of PMCA with extended cycles and by introducing metal wires into cell cultures



Human Prion Diseases

Manifestation	Diseases	Mechanism
1. Infectious	Kuru and iatrogenic Creutzfeldt-Jakob disease	Transmission
2. Sporadic	Creutzfeldt-Jakob disease	Somatic mutation or spontaneous PrP^{C} ---> PrP^{Sc} (Overexpression?)
3. Inherited	Gerstmann-Straussler-Scheinker syndrome, familial Creutzfeldt-Jakob disease and fatal familial insomnia	Germline mutation

In prion disease, ‘All Roads Lead to Rome’



Some reading

- **Prion Biology and Diseases (second edition):** Ed, Prusiner S.B. Cold Spring Harbor Laboratory press, Cold Spring Harbor New York, 2004

- **The prion's elusive reason for being.** Aguzzi A, Baumann F, Bremer J. Annu Rev Neurosci. 2008;31:439-77.

- **Discovering DNA encodes Heredity and Prions are Infectious Proteins.** Prusiner SB, McCarty M. Annu Rev Genet. 2006;40:25-45.

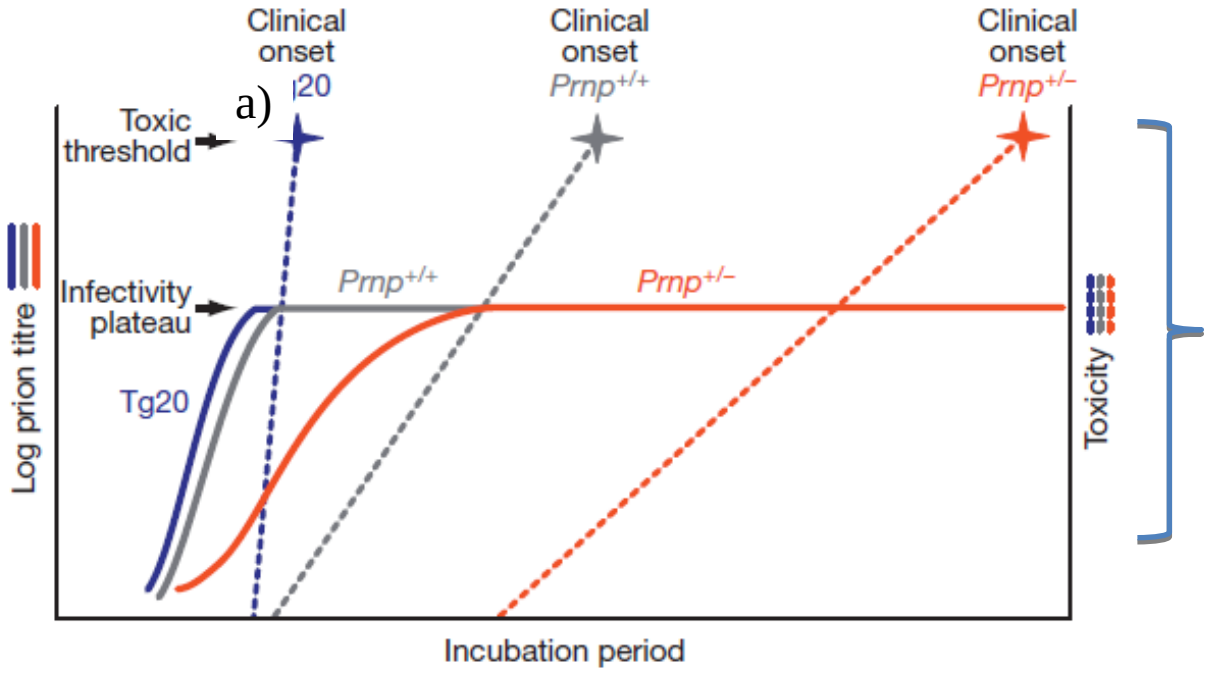
- **A general model of prion strains and their pathogenicity.** Collinge J, Clarke AR. Science. 2007 Nov 9;318(5852):930-6.

Assignment - What is going on in the “silent” and symptomatic phases of prion disease ?

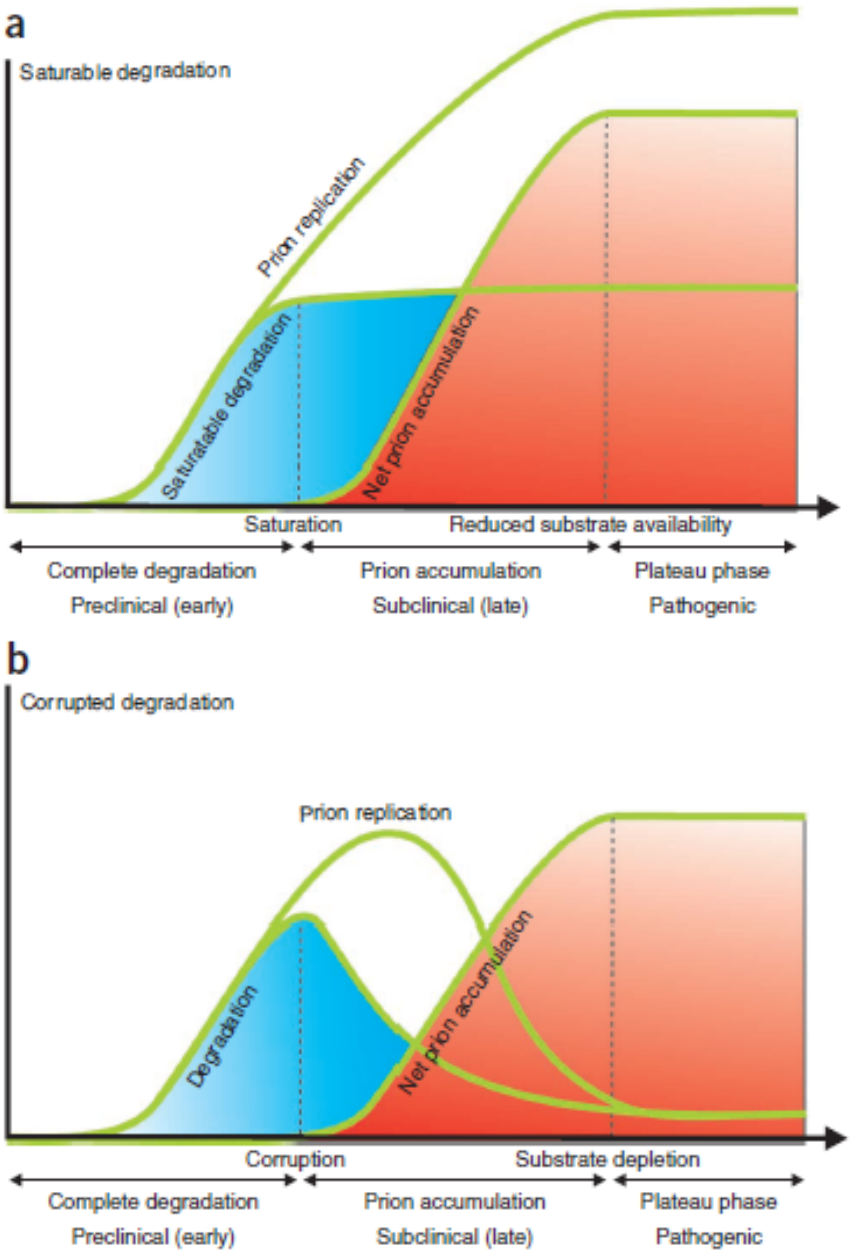
General thoughts

- Which events might be important insofar as they might be reliable disease markers?
- Which events might be important insofar as they might be disease targets for small molecule therapy ?

Theories to explain events in the subclinical phase of prion disease



Theory from Sandberg *et al* Nature 470:540-542, 2011 positing accumulation of a theoretical toxic form of PrP above a threshold



Theories to explain events in the subclinical phase of prion disease

Two theories from the Aguzzi lab to account for the properties of subclinical prion disease. Note that substrate depletion (i.e. PrP^C depletion) is predicted in the second theory (panel b).

Source: Aguzzi and Falsig, Nature Neuroscience 15:936-939, 2012

Reading Assignments

- **Sustained translational repression by eIF2a-P mediates prion neurodegeneration**

  Moreno et al, Nature 485 (7399): 507-511, 2012

- **Disease-associated prion protein oligomers inhibit the 26S proteasome.**

  Kristiansen et al, Molecular Cell 26 (2): 175-188, 2007

Project assignment, group 1

- Critically appraise the paper from the Mallucci group and present a 20 min Powerpoint show to illustrate your critique.
- Also read the paper from the Tabrizi group and the background “review” papers from Collinge and Aguzzi
- At the end of the slide show provide an opinion as to which mechanism (proteasome or unfolded protein response) might be more important and why, or, if they are the about the same, why are they equally important? (1 slide)
- Do the two papers “cover the whole waterfront”, or are there significant gaps where the research might yet advance? (1 slide)

Project assignment, group 2

- Critically appraise the paper from the Tabrizi group and present a 20 min Powerpoint show to illustrate your critique.
- Also read the paper from the Mallucci group and the background “review” papers from Collinge and Aguzzi
- At the end of the slide show provide an opinion as to which mechanism (proteasome or unfolded protein response) might be more important and why, or, if they are the about the same, why are they equally important? (1 slide)
- Do the two papers “cover the whole waterfront”, or are there significant gaps where the research might yet advance? (1 slide)