Prion Diseases - Basic Science

Dr. David Westaway
Centre for Prions
and Protein Folding Diseases,

University of Alberta
david.westaway@ualberta.ca
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
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 led to the discovery of two important proteins, PrP$^\text{Sc}$ and PrP$^\text{C}$

These proteins are actually “isoforms”
Better Prion Assays

Measuring Prion Infectivity

- Sheep: 2-3 Years
- Goats: 2-3 Years
- Monkeys: 2-3 Years
- Mice: 1 Year
- Hamsters: 0 Animal Years
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.
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:


PrP\textsuperscript{Sc} derives from the host

open reading frame of an intronless Prnp gene

PrP\textsubscript{C}

Expression, maturation

PrP\textsubscript{Sc}

“Conversion”

Proteinase K digestion

PrP 27-30
Arriving at the “conformational hypothesis” (1)

- PrP<sup>Sc</sup> and PrP<sup>C</sup> have closely related amino acid sequences (1985).
- The PrP gene has a single uninterrupted coding exon (1986).
- Low resolution structural analysis reveal PrP<sup>C</sup> is α-helical (1992).
Arriving at the “conformational hypothesis” (2)

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

α–helix

β–sheet
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”

<table>
<thead>
<tr>
<th>Parent Protein</th>
<th>Amyloid</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrP</td>
<td>Plaques</td>
<td>Prion</td>
</tr>
<tr>
<td>APP</td>
<td>Aβ</td>
<td>Alzheimer’s</td>
</tr>
</tbody>
</table>

Congo red staining of prion plaques
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 form infectious for ordinary mice, with lipids and non-coding RNA as additives (2010)
Protein X might act here to unfold PrP\(^{C}\)
Heterodimer hypothesis (Prusiner)

- A large energy barrier prevents spontaneous conversion of PrP\textsuperscript{C} to PrP\textsuperscript{Sc}.
- PrP\textsuperscript{C} is unfolded by a hypothetical molecular chaperone called protein X. Identity of protein X is unknown.
- The replication intermediate is a PrP\textsuperscript{C} /PrP\textsuperscript{Sc} heterodimer (60 kDa).
“Seeding Hypothesis” of Lansbury and Caughey: seeded growth of monomeric subunits into a multimer
The diagram illustrates the formation of multimers and fibrils over time. The x-axis represents time, with a 'lag time' indicated before the formation of a 'nucleus'. The amount of monomer decreases as fibril formation occurs, leading to the formation of multimers. The concentration $C_R$ is indicated on the right side, showing the equilibrium point.
Remove, dilute and re-seed
Visualizing the “seeding hypothesis”
Facets of the “Seeding” hypothesis

• There is only a small energy barrier between PrP<sub>C</sub> and PrP<sub>Sc</sub> but spontaneous conversion is prevented by a kinetic barrier: conversion is too slow.
• Once a pre-formed seed of PrP<sub>Sc</sub> multimers is made the long lag period is avoided and PrP<sub>C</sub> to PrP<sub>Sc</sub> conversion takes place rapidly of the surface of the multimeric PrP<sub>Sc</sub>.
• 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. This 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.
<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Infectious</td>
<td>Kuru was transmitted among New Guinea natives by ritualistic cannibalism. Iatrogenic Creutzfeldt-Jakob disease caused by growth hormone derived from human pituitaries.</td>
</tr>
<tr>
<td>2. Sporadic</td>
<td>Creutzfeldt-Jakob disease occurs at one per million population across the earth.</td>
</tr>
<tr>
<td>3. Familial</td>
<td>Gerstmann-Straussler-Scheinker syndrome, familial Creutzfeldt-Jakob disease and fatal familial insomnia occur in families where 50% of the members are afflicted.</td>
</tr>
</tbody>
</table>
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.

In Gerstmann-Straussler syndrome (GSS), a Pro -> Leu substitution at codon 102 PrP was found.

The C -> T mutation in codon 102 creates a Dde I restriction site.
Inheritance of a prion mutation tracks with disease in GSS families
Human \textit{PRNP} variants

Compilation from Beck \textit{et al}, Human Mutation, e1551-1563, 2010

\textbf{A}

Pathogenic mutations
Non-pathogenic genetic variation

P102L
D178N
+9 ORs

Compilation from Beck \textit{et al}, Human Mutation, e1551-1563, 2010

\textbf{B}
Sporadic prion disease

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

```
PrP\textsuperscript{C} \rightarrow PrP-U ? \rightarrow PrP\textsuperscript{Sc}
```
<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Diseases</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Infectious</td>
<td>Kuru and iatrogenic</td>
<td>Transmission</td>
</tr>
<tr>
<td></td>
<td>Creutzfeldt-Jakob disease</td>
<td></td>
</tr>
<tr>
<td>2. Sporadic</td>
<td>Creutzfeldt-Jakob disease</td>
<td>Somatic mutation or spontaneous</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$PrP^c \rightarrow PrP^{Sc}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Overexpression?)</td>
</tr>
<tr>
<td>3. Inherited</td>
<td>Gerstmann-Straussler-Scheinker syndrome, familial</td>
<td>Germline mutation</td>
</tr>
<tr>
<td></td>
<td>Creutzfeldt-Jakob disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and fatal familial insomnia</td>
<td></td>
</tr>
</tbody>
</table>
In prion disease, ‘All Roads Lead to Rome’
Some reading


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

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\textsuperscript{C} depletion) is predicted in the second theory (panel b).

Reading Assignments

• Sustained translational repression by eIF2a-P mediates prion neurodegeneration


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

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)