Prion Diseases: toward further reduction of animal experimentation


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Transmissible Spongiform Encephalopathies (TSE) Prion Diseases

* Fatal Neurodegenerative diseases
  - Distroy the central nervous system
  - Induce locomotor, behavioral and sensory disorders

* Long Incubation period and clinically silent
  5 years in Cow, 2 years in Sheep,
  up to 40 years in human

* Absence of immune response

* Transmissibles

* Nature of the causal agent: Non Conventiennal Transmissible Agent
Prion diseases affect Animals and Human

- **Scrapie in sheep and goats** (since 1730)

- **Bovine Spongiform Encephalopathy: BSE** (since 1985)

- **Creutzfeldt-Jakob Diseases CJD** (1920-1921)
  sporadic, genetic, iatrogenic, or contagious (variant CJD)

- **Chronic Wasting Disease in Cervids**
  (mainly USA, Canada and South Korea (since 1960))
  1st cases of CWD in Europe, Norway 2016
Nature of the Infectious agent:
The Prion Protein that exists in two forms, the normal and abnormal one.
The normal cellular Prion Protein PrP$^C$

### Biological Functions:
- Cellular signalisation
- Synaptic transmission
- Cell adhesion (zebrafish)
- Copper Homeostasis
- Sleep Cycle
- Oxydatif Stress (Neuroprotector)
- Neuron myelinisation

**But unnecessary Protein**
No major phenotype in PrP knock-out (PrP$^{0/0}$) animals
Prion: The Protein Only Hypothesis (S. Prusiner 1982)
Double-face Protein

- Fatal Neurodegenerative Protein misfolding disease

Normal Prion Protein \( \text{PrP}^C \)
Pathological Prion protein \( \text{PrP}^\text{Sc} \)
\( \text{Sc}: \) Scrapie

Auto-replicative

Partial Resistance to Proteolysis
Prion Strains: A hot question at the heart of TSE studies

• In the same host, several Prion strains propagate with distinct phenotypic traits
• Prion strains differ by their own biological and physicochemical properties

The Phenotypic differences reflect diversity of PrPSc Conformations/Assemblies
How to study Prions in the Lab?

1- Cell Culture models

<table>
<thead>
<tr>
<th>Non Infected</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal PrPc: green</td>
<td>Abnormal PrPSc: green</td>
</tr>
<tr>
<td>Nuclei: blue</td>
<td>Nuclei: in blue</td>
</tr>
</tbody>
</table>

2- Purified recombinant PrP:
Biophysical and structural studies

3- In vitro Prion Amplification:
*PMCA: Protein Misfolding Cyclic Amplification
*QuiC: Quacking Induced Conversion
**PMCA: Protein Misfolding Cyclic Amplification**

*(Adapted from Saborio Permanne et Soto 2001)*

1 Round de PMCA (24-48h)

- **PrP<sup>Sc</sup>** Neoformed
- **PrP<sup>C</sup>** Brain Lysate
- **PMCA**:
  - Incubation 30 min
  - Sonication 30s
  - Incubation
  - Sonication

*1 cycle*
Brain and Cell based miniaturized bead-PMCA (mb-PMCA)

Main PMCA Improvements:
❖ Decrease of sample volume by 3 folds
❖ Adaptation to 96 well microplate format
❖ Use of cultured Cell lysate

Sample to be sected (Inoculum)

PrPSc

Incubation

PrPc

Sonication

10 fold serial Dilutions

Brain Lysate

Cultured Cell Lysate

Produit PMCA

Teflon beads (Baskakov Lab)

Transgenic healthy mice

Brain and Cell based miniaturized bead-PMCA (mb-PMCA)

Moudjou et al., Mbio 2013
Lacroux et al., Plos Pathogens 2014
Chapuis et al., Acta Neuropath Com 2016
Igel-Egalon et al., Plos Pathogens 2017...

INRA SCIENCE & IMPACT
Rabbit Kidney epithelial cell line (RK13) transfected to express PrPc of different species

Hum5 cells: Human PrPC

Ham2 cells: Hamster PrPC

P2FJ6 cells: Ovine PrPC
Example of prion amplification using both Cell based- and Brain based mb-PMCA

Scrapie Prion

Human Variant Creutzfeldt-Jakob

Moudjou et al., Mbio 2013
Infectivity Titration:

$10^{-1}$ ....... 6 mice
$10^{-2}$ ....... 6 mice
$10^{-3}$ ....... 6 mice
$10^{-4}$ ....... 6 mice
$10^{-5}$ ....... 6 mice
$10^{-6}$ ....... 6 mice

To titrate one fraction: 36 mice

If titration of 1/2 samples: need 450 mice
If titration of 1/3 samples: need 300 mice

Biochemical fractionation of brain Prion particles assemblies using Sedimentation Velocity Gradient technique

Prions

Top:
Small particles

Bottom:
Large particles

Example of mb-PMCA impact on the reduction of animal bioassays

Tixador et al., Plos Pathogens 2010
Laferrière et al., Plos Pathogens 2013
The Brain mb-PMCA will use only one mouse brain that replace 300-400 mice that would be necessary for the Bioassay

With Cell based-mb-PMCA, 0 mouse brain is needed for Scrapie prion. At worst, half PrP\(^0/0\) brain will be necessary for other prion strain (human, hamster)

PMCA Data obtained in 48 hours instead of several months with the Bioassay
Brain-PMCA and Cell-based PMCA : Conclusions

➢ Highly efficient methods for fundamental and applied studies

➢ Applied to animal and human health
  * Anti-Prion drugs screening,
  * Validation of decontamination procedures,
  * Human Blood diagnosis of vCJD…
    UK prevalence of vCJD 1/2000 UK (based on retrospective appendix analysis)

➢ Brain and Cell based mb-PMCA constitute a method that will participate to further reduce and even replace animal use in some Prion disease studies
Perspectives

- Amplify with high efficiency other prion strains using only cultured cell lysate as substrate

- Extrapolate the mb-PMCA procedure to the amplification of some misfolded proteins involved in other neurodegenerative diseases such as Alzheimer and Parkinson diseases that start to be considered as a:

Prion-Like Diseases
(Protein Misfolding Diseases)
So How was the ESB?

Oh the Empire State Building, It gave me a Headache and Vertigo!
BSE and variant CJD Crisis: Epidemiological aspects

**Global Distribution of Iatrogenic CJDs**

- **Dura matter graft:** 228
- **Surgical Instruments:** 4
- **Cornea transplants:** 2
- **Growth Hormone:** 226
- **Gonadotropines:** 4
- **Blood transfusion:** 4 (UK)

178 cases in UK
27 in France
First case of Chronic Wasting Disease in Europe in a Norwegian free-ranging reindeer


*Highly transmissible disease for which the expansion could be incontrolable.
*Zoonotic risk

Active Surveillance
Example of scrapie prion amplification using both Cell based- and Brain based mb-PMCA

Scrapie Prion

PMCA Amplicons (48h) are as infectious as the brain of infected mice at terminal stage (2 months)

Moudjou et al., Mbio 2013
The normal cellular Prion Protein PrP<sub>C</sub>

### Biological Functions:
- ✓ Cellular signalisation
- ✓ Synaptic transmission
- ✓ Cell adhesion (zebrafish)
- ✓ Copper Homeostasis
- ✓ Sleep Cycle
- ✓ Oxidative Stress (Neuroprotector)
- ✓ Neuron myelinisation

**But unnecessary Protein**

**Primary Sequence**

```
MVKSHIGSWI LVLFVAMWSD VGLCKKRPKP GGGWNTGGSR YPGQGSPGGN
51
RYPPQGGGWGWQPHGGGWQPQPH HGGWQPHGG GGGWGQPHGG GGGWGQPHGG GGGWGQPHGG
QWNKPSKPKT NMXHVAAGAA AGAVVGGGLGG YMLGSVMSRP LIHGFDNYED
101
RYYRENMYRY PNQVYYPFVD QYSNQNNFVH DCVNTVKQH TVTHTTKGEN
151
FTETDIKIME RVVEQMCITQ YQRESQAYYQ RGASVILFSS PPDVVILLISFL
201
IFLIVG
```

- Bi-glycosylated
- Mono-glycosylated
- Non-glycosylated

Electrophoretic Profile
Prion Stains: A hot question at the heart of TSE studies

- In the same host, several Prion strains propagate with distinct phenotypic traits
- Prion strains differ by their own biological and physicochemical properties

The Phenotypic differences reflect diversity of PrPSc Conformations/Assemblies
➢ Ovine PrP<sub>Sc</sub> (VRQ) can store >18 stable and distinct SSD
Caractéristiques histopathologiques des Prions :
La Triade de Hadlow

* Mort des neurones
** Spongiosose (Formation de « trous » dans le cerveau)
*** Astrocytose et Gliose
Pour la souche de tremblante rapide, les assemblages de petite taille dans le cerveau sont les plus infectieux...

... et ont un pouvoir replicatif plus important \textit{in vitro} (mb-PMCA)
Cinétique d’accumulation de la PrP\textsuperscript{Sc} et de l’infectivité des Prions dans le cerveau de souris

\begin{itemize}
  \item PrP\textsuperscript{Sc}
  \item Infectivité du cerveau
  \item % abnormal PrP\textsuperscript{Sc} ± SEM
  \item Signes cliniques

\end{itemize}

\begin{itemize}
  \item LAN21K Fast
  \item LAN19K
  \item BSEov
  \item Nor98

\end{itemize}

V.B, Non publié

(Nakaoke et al., 2000)
➢ Ovine PrP<sub>Sc</sub> (VRQ) can store >18 stable and distinct SSD
Prion cross-species barrier is controlled by structural fit between PrP$^C$ and the infecting prion strain.
Modèle de Conversion et de Propagation des Prions (nucléation / polymérisation)

Noyaux infectant : quelques molécules de PrPSc

PrPC Normale

Conversion

élongation

Fragmentation

Propagatioln

Adaptée de Philippe Tixador