Targeting the Functional Activity of PrP$\text{C}$ as a Novel Strategy for Drug Discovery in Prion Diseases

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One protein, two forms: PrP\text{C} and PrP\text{Sc}

PrP\text{C}  \rightarrow  PrP\text{Sc}

\downarrow

PrP\text{Sc}

\downarrow

Infectivity & Pathology

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PrP Sc alone seems sufficient to generate infectivity, but not toxicity!

Normal host prion protein necessary for scrapie-induced neurotoxicity

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Depleting Neuronal PrP in Prion Infection Prevents Disease and Reverses Spongiosis

Giovanna Mallucci, Andrew Dickinson, Jacqueline Linehan, Peter-Christian Klöhn, Sebastian Brandner, John Collinge*

Prion propagation and toxicity in vivo occur in two distinct mechanistic phases

Malin K. Sandberg†, Huda Al-Doujaily†, Bernadette Sharps†, Anthony R. Clarke† & John Collinge†
PrP<sub>C</sub> and PrP<sub>Sc</sub>: partners in crime?

The normal function of PrP<sub>C</sub> could be corrupted by PrP<sub>Sc</sub> (or some other aberrant PrP form) to generate toxicity!

PrP<sub>Sc</sub> does not necessarily represent the right pharmacological target for treating prion diseases!

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What is the normal function of PrP<sub>C</sub>?

<table>
<thead>
<tr>
<th>Candidate Interactor</th>
<th>Candidate Function</th>
<th>Identification Method</th>
<th>Localization</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Grb2</td>
<td>Signal transduction (adaptor protein)</td>
<td>Yeast two-hybrid; co-immunoprecipitation</td>
<td>Cytoplasm</td>
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<td>Synapsin 1b</td>
<td>Synaptic receptor, co-transport</td>
<td>Yeast two-hybrid; co-immunoprecipitation</td>
<td>Cytoplasm (synaptic vesicles)</td>
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<td>TREK-1</td>
<td>Two pore K&lt;sup&gt;+&lt;/sup&gt; ion channel</td>
<td>Immunoprecipitation</td>
<td>Plasma membrane (transmembrane)</td>
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<td>Tubulin</td>
<td>Microtubule subunit</td>
<td>Cross-linking</td>
<td>Cytoplasm (cytoskeleton)</td>
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<td>receptor-interacting</td>
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<td>MAGE homologue)</td>
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<td>STI-1 (stress-inducible protein 1)</td>
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<td>Cytoplasm; Plasma membrane?</td>
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<td>Plasma membrane (transmembrane and GPI-anchored forms)</td>
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<td>Caveolin-1</td>
<td>Caveolar coat</td>
<td>Co-immunoprecipitation</td>
<td>Plasma membrane (hairpin loop)</td>
<td>[102]</td>
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</table>
Prion toxicity is not observed in cultured cells

**Transfected cell lines**
- Cell transfection with mutant forms of PrP

**Primary Cultures**
- Tg mice expressing mutant forms of PrP
  - 7 days pups

**PrP<sup>Sc</sup>-infected cells**
- Cell infection with PrP<sup>Sc</sup>

→ Very mild (or no) toxicity

→ Infectivity in absence of toxicity!
Understanding prion neurotoxicity: Open questions

• What are the toxic species in prion diseases?

• Is an aberrant activity of PrP$^\text{C}$ responsible for toxicity in prion diseases?

• What is the normal function of PrP$^\text{C}$?

• Why cultured cells cannot reproduce prion toxicity?
PrP function and toxicity: two sides of the same coin
An artificial PrP mutant deleted for the central region (ΔCR) is highly neurotoxic in absence of infectivity
PrP ΔCR induces an abnormal channel activity at the cell membrane.
A screenable phenotype for PrP ΔCR: The Drug-Based Cell Assay (DBCA)

PrP ΔCR induces hypersensitivity to several antibiotics, including Zeocin, G418 and Hygromicin

% Cell Viability

PrP WT
PrP ΔCR

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Is the activity of \textbf{PrP} \textit{ΔCR} relevant for prion diseases?
Anti-prion compounds inhibit the activity of PrP $\Delta$CR

Pharmacological chaperone for the structured domain of human prion protein

Andrew J. Nicoll$^a$, Clare R. Trevitt$^b$, M. Howard Tattum$^b$, Emmanuel Risse$^b$, Emma Quarterman$^a$, Amaury Avila Ibarra$^b$, Connor Wright$^b$, Graham S. Jackson$^b$, Richard B. Sessions$^b$, Mark Farrow$^b$, Jonathan P. Walther$^b$, Anthony R. Clarke$^{a,b}$, and John Collinge$^{a,b,2}$

Channel Activity

Fe(III)-TMPyP

DBCA

PrP WT

PrP $\Delta$CR

% Cell Viability

- TMPyP

+ TMPyP
• PrP $\Delta$CR activity is related to the normal function of PrP$^C$
• This mutant provides robust screenable phenotypes in cell cultures
• $\Delta$CR-dependent phenotypes in cells are inhibited by anti-prion compounds

Screening for inhibitors of PrP $\Delta$CR activity may lead to the discovery of new anti-prion compounds
Targeting the activity of PrP ΔCR to discover novel anti-prion compounds
(in collaboration with Harvard Neurodiscovery Center)

Zeocin or G418

WT or ΔCR cells

384-well plates pre-coated with compounds from the LDDN library of small molecules

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Drug discovery Flow Chart: Pre-clinical studies

- **In vitro screening of 70,000 small molecules by DBCA** (LDDN, Harvard Medical School)

- **In silico analyses** to identify high affinity ligands for PrP<sup>c</sup> (Barreca and Iraci, University of Perugia)

- Best hits will be ranked for their ability of stabilizing PrP<sup>c</sup> by molecular dynamics

- Binding to PrP<sup>c</sup> validated *in vitro* by SPR and NMR (McKnight, Boston University; Gobbi, Mario Negri Institute)

- Secondary screenings:
  1. Channel activity
  2. Test effect on PrP localization (EGFP-PrP Assay)
  3. PMCA
  4. Zebrafish-based assay (Malaga Trillo, UniKonstanz)

- Chemical Optimization

- Bioassay in Tg(ΔCR) and PrP<sup>Sc</sup>-infected mice

- Obtain preliminary efficacy, toxicity and pharmacokinetic information

78 compounds were found positive by DBCA and selected for further analyses
David Harris
(Department Head)

Tania Massignan
(Cell assays)

Brian Fluharty
(αβ studies)

Isaac Solomon
(Channel activity)

Natasha Katry
(N2a-PK1 assay)

Paula Saa
(PMCA)

Jessie Turnbaugh
(Studies in Tg mice)

Greg Bever
(PrP mutants)

Rick Bowman
(Lab manager)

Jorge De Castro
(Mouse colonies)

James McKnight
(Dep. Biophysics, NMR analyses)

Marco Gobbi
Matteo Stravalaci
(SPR studies)

Letizia Barreca
Nunzio Iraci
(In silico analyses)

Marcie Glicksman
Kathleen Seib
Greg Cuny
(In vitro screening)

Edward Malaga Trillo
(Zebrafish assay)