The Agent: Prions as Emerging Infectious Particles

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Topics Covered

- Prions
  - Prion paradigm shift
  - Prion composition and structure
  - Prion replication
- Prions as emerging infectious particles
  - Prion diseases
  - Emerging prion diseases
Kuhn’s Scientific Revolutions

• Paradigm-based normal science
• Crisis provoked by anomalous observations
• Revolution
  – Competition between new and old paradigms
  – New paradigm displaces old
• Normal science under a new paradigm
  – Transition is discontinuous
  – New or revised field is repopulated with believers
VIROLOGY / MICROBIOLOGY PARADIGM

Prion Revolution

TSE VIROLOGY

CRISES

VIRUS PARTICLES NOT SEEN

NUCLEIC ACID NOT FOUND

RESISTANCE TO RADIATION

Protein Hypothesis

Membrane Hypothesis

Polysaccharide Hypothesis

Virino Hypothesis
CRISIS

RESISTANCE TO RADIATION

TSE VIROLOGY

VIROLOGY / MICROBIOLOGY PARADIGM

VIRUS PARTICLES NOT SEEN

Protein Hypothesis

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Virino Hypothesis
Virology / Microbiology Paradigm

CRISIS

Virus Particles Not Seen

Resistence to Radiation

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Virino Hypothesis

TSE Virology

Crisis
Crisis

Virology / Microbiology Paradigm

TSE Virology

Resistance to Radiation

Protein Hypothesis

Virino Hypothesis

Normal Science

Under Prion Paradigm
Prion Paradigm Shift

- Prion paradigm displaced virus paradigm
  - Prion paradigm is better at explaining the existing data
  - Prion paradigm is not necessarily correct
- Shift to prion paradigm takes time
  - Young scientists or those from other fields tend to adopt the prion paradigm
  - Established scientists tend to resist the prion paradigm
  - Crisis began ~1965; gained wide acceptance ~1990’s
  - Transition to new paradigm depends on the individual
Prion (Protein) Hypothesis

- Prions are infectious agents composed of a modified host protein, PrP^Sc
- A non-host nucleic acid is not a component
- PrP^Sc is derived from the cellular form, PrP^C
- PrP^Sc and PrP^C share the same primary structure
- PrP^Sc and PrP^C have different conformations
Unusual Properties of Prions
Early Studies

- Resistant to UV and ionizing radiations
- Resistant to various chemical and enzymatic treatments
- Heterogeneous in size and density
- No unitary structure identified by EM
Unusual Properties of Prions
Later Studies

- Scrapie-associated fibrils (SAF) identified by EM
- Abnormal host protein (PrP\textsuperscript{Sc}) purifies with infectivity
- Degrading PrP\textsuperscript{Sc} destroys infectivity
- Denaturing PrP\textsuperscript{Sc} destroys infectivity
- Purified preps lack specific nucleic acid
PrP$^{Sc}$ and Prions

- PrP$^{Sc}$ and infectious prions copurify
- PrP$^{Sc}$ is the only macromolecule consistently identified in purified prion preparations
- No prion-specific nucleic acid has been identified (except host PrP gene & mRNA)
- Prions are unaffected by many treatments that degrade nucleic acids
PrP\textsuperscript{Sc} and Prions (2)

- PrP\textsuperscript{Sc} and prions resist degradation by proteases
- Prolonged digestion degrades PrP\textsuperscript{Sc} and destroys infectivity
  - Kinetics of degradation of PrP correlate with prion inactivation
- Denaturing PrP\textsuperscript{Sc} destroys infectivity
  - Physical treatments
  - Chemical treatments
PrP^Sc and Prions (3)

- PrP^Sc concentration and prion infectivity correlate in various preparations
- PrP^Sc binds to PrP^C \textit{in vitro} and can change its conformation
- Mice that don’t have the PrP gene (Prnp\(^{0/0}\)) cannot be infected by prions
- Neurons that do not produce PrP^C are not damaged by prion infection
## Properties of PrPC and PrPSc

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PrPC</th>
<th>PrPSc</th>
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</thead>
<tbody>
<tr>
<td>Normal brain</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Diseased brain</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Infectious</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Concentration (µg/g)</td>
<td>20 – 40</td>
<td>60 – 160</td>
</tr>
<tr>
<td>Fibrils in vivo</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Amyloid plaques</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Soluble in Sarkosyl</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Resists proteolysis</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>


PrP detected by Western blotting
Purified PrP<sup>Sc</sup> is Infectious

**Sample**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Specific Activity (LD&lt;sub&gt;50&lt;/sub&gt;/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrP&lt;sup&gt;Sc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lanes 1 &amp; 2*</td>
<td>6 x 10&lt;sup&gt;10&lt;/sup&gt;</td>
</tr>
<tr>
<td>(Lane 2 contains 4 times as much PrP as lane 1)</td>
<td></td>
</tr>
<tr>
<td>Trypsin fragment of PrP&lt;sup&gt;Sc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lane 3*</td>
<td>3 x 10&lt;sup&gt;11&lt;/sup&gt;</td>
</tr>
</tbody>
</table>


*PrP detected by silver staining*
Prion Replication
Prion Replication
Prion Aggregation Dynamics
Prion Structural Models

PrP<sup>C</sup> \(\xrightarrow{\text{PK + detergent}}\) PrP<sup>Sc</sup> \(\xleftarrow{\text{PrP<sup>Sc</sup> binding & conversion}}\) PrP 27-30

NMR Structure

Electron Crystallography Structural Model


PrP<sup>C</sup> Metabolism in a Normal Cell

Possible Sites of PrP$^{Sc}$ Action

Summary

• Prions are composed of an abnormal host protein, PrP\textsuperscript{Sc}
• Conformation of PrP\textsuperscript{Sc} is different from host PrP\textsuperscript{C}
• Prion-specific nucleic acid molecules have not been identified
• Protein-only hypothesis provides the best explanation for data (to date)
Prion Disease Etiologies

• Familial
  – More than 25 mutations in *PRNP* gene
  – Autosomal dominant mutations
  – Some cases are infectious (transmitted experimentally)

• Infectious
  – Transmissible vs. contagious
  – Biologically distinct strains
  – Infectious particle contains PrP<sup>Sc</sup> from wild-type gene

• Sporadic
  – No demonstrable link to familial or infectious causes
Species Variation and Mutations


Disease-causing mutations

All vCJD victims are M129

Disease susceptibility loci
Infectious Prion Diseases

**Animal Diseases**
- Scrapie
- BSE
- CWD
- TME
- FSE
- Exotic ungulate encephalopathy

**Human Diseases**
- Variant CJD (vCJD)
- Iatrogenic CJD (iCJD)
- kuru
- sCJD as source for
  - iCJD
  - kuru
  - ???
Emerging Prion Diseases

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Interspecies Transmission of Prions
Prions as Agents of Emerging Disease

**Good News**
- Long incubation times
  - Time to react
- Not highly contagious
- Prions show restricted tissue distribution
- Species barriers

**Bad News**
- Long incubation times
  - Undetected reservoirs
- Invariably fatal
- Difficult to inactivate
- Persist in environment
- Difficult to detect
- High prion titers in CNS
Revolution

“I have shown the existence of at least three classes of replication mechanisms and that, therefore, the occurrence of a protein agent would not necessarily be embarrassing although it would be most interesting.”

Revolution (2)

“This shows that there is no reason to fear that the existence of a protein agent would cause the whole theoretical structure of molecular biology to come tumbling down.”
