Contains ~1 billion neurons (nerve cells)
Each contains ~1 billion proteins
Proteins:
linear chains of amino acids

- Enzymes (catalyze chemical reactions, metabolism)
- Structural, mechanical elements of organisms
- Regulators & mediators of most physiological processes

Prion Protein (PrP)
Protein Misfolding Diseases

- Not transmissible(?)
  - Alzheimer’s
  - type II diabetes
  - Parkinson’s
  - Huntington’s
  - amyotrophic lateral sclerosis
  - sickle cell anemia
  - cystic fibrosis
  - secondary amyloidoses
  - spinocerebellar ataxias
  - etc.

- Transmissible
  - TSE (prion) diseases

Abnormal amyloid deposits in brain slice

Chiti F, Dobson CM. 2006.
Annu. Rev. Biochem. 75:333–66
Transmissible spongiform encephalopathies (prion) diseases
Slow, fatal, untreatable, transmissible neurodegenerative diseases

BSE
(mad cow disease)

chronic wasting disease
(CWD)

scrapie

Creutzfeldt-Jakob disease (CJD), GSS, Kuru
Human TSE (prion) diseases

- **SPORADIC:**
  - Creutzfeldt-Jacob disease (sCJD)
  - 1 case per 2 million people annually worldwide
  - accounts for 95% of human TSE
  - no known prion protein mutations
  - probably spontaneous disease

- **FAMILIAL:**
  - familial CJD
  - Gerstmann-Sträussler-Scheinker syndrome
  - fatal familial insomnia
  - prion protein mutations

- **INFECTIOUS:**
  - kuru
  - iatrogenic CJD (from medical mistakes)
    - neurosurgery, dura mater and corneal transplants, growth hormone
  - variant CJD (from BSE-infected cattle)
TSE risks in perspective

**CJD:**
- ~1 case per million per year worldwide; rate virtually unaffected by exposure to TSE-infected hosts
- Human-to-human transmission is usually inefficient
- *But* blood transfusion-based vCJD transmissions raise new concerns.

**BSE:**
- Attack rate in humans is low; ~224 human vCJD cases from millions of exposures. BSE incidence (cattle) is ~0.01% of peak.

**Sheep scrapie:**
- No known transmission to humans; risk is hypothetical.

**CWD:**
### Annual Causes of Death in the United States (2000)

<table>
<thead>
<tr>
<th>Cause</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart disease</td>
<td>710,000</td>
</tr>
<tr>
<td>Cancer</td>
<td>553,000</td>
</tr>
<tr>
<td>Tobacco</td>
<td>435,000</td>
</tr>
<tr>
<td>Poor Diet and Physical Inactivity</td>
<td>365,000</td>
</tr>
<tr>
<td>Alcohol</td>
<td>85,000</td>
</tr>
<tr>
<td>Microbial Agents</td>
<td>75,000</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>49,558</td>
</tr>
<tr>
<td>Motor Vehicle Crashes</td>
<td>43,000</td>
</tr>
<tr>
<td>Adverse Reactions To Prescription Drugs</td>
<td>32,000</td>
</tr>
<tr>
<td>Suicide</td>
<td>30,622</td>
</tr>
<tr>
<td>Incidents Involving Firearms</td>
<td>29,000</td>
</tr>
<tr>
<td>Homicide</td>
<td>20,308</td>
</tr>
<tr>
<td>Illicit Use of Drugs</td>
<td>17,000</td>
</tr>
<tr>
<td>TSEs (total)</td>
<td>~250</td>
</tr>
<tr>
<td>TSE (from BSE or CWD infections)</td>
<td>0</td>
</tr>
</tbody>
</table>
TSE prions: a strange new class of infectious agent

- Little immune response by host
- No genes of its own
- Hard to decontaminate

An infectious protein

Purified prion amyloid
How infectious proteins (prion) reproduce: an abnormal form of a host protein

Input: 1 infectious unit
Output: 1-100 billion infectious units!
Abnormal prion protein amyloid (infectious, deadly)

Normal prion protein (harmless)

stack of 8 molecules

Mike Dolan
Bradley Groveman
Computer simulation of infectious prion structure
Computer simulation of infectious prion structure

Understanding prion structure and replication

- Designing drugs/therapeutics
  - blockers of replication
  - blockers of spread
  - blockers of toxicity

- Vaccine development
  - encouraging the immune system to recognize and eliminate pathological prion protein
Isolated prions

replication & spread

Spread of prions throughout the brain

Mouse brains full of prions (brown)
PrP\textsuperscript{CJD} formation and potential toxic mechanisms in prion-infected cell

Caughey & Baron, Nature (2006)
Drug discovery:
High-throughput screening of anti-scrapie compounds

Scrapie-infected cells in 96-well plate → Grow for 4 days with drug → Measure scrapie prion protein accumulation by “dot blot”

~250 compounds
47 new inhibitors

Dr. Yraima Cordeiro
Federal University of Rio de Janeiro, Brazil
Challenges in CJD therapeutics development

- Early diagnosis
- Stopping further damage to brain
  - Blocking continued prion protein misfolding
  - Understanding toxic mechanisms
- Reversing damage
  - Rebuilding damaged circuits
  - Replacing dead or dysfunctional cells
    - Stem cells?

Preferred drugs/therapeutic agents

- Good access to, or production within, the central nervous system
  - Cross blood-brain barrier
- Few side effects
  - Tolerable long-term
Diagnostic challenges of TSE/prion diseases

- Definitive diagnostic tests
  - preclinical
  - early clinical

- Rapid, sensitive assays for infectivity
  - diagnosis
  - detecting contamination:
    - blood
    - transplanted organs
    - foods, feeds, dietary supplements
    - other agricultural products
    - biotechnology products
    - pharmaceuticals
    - medical devices
    - cosmetics
    - environment

Current diagnosis of sCJD

Probable CJD
- Clinical features (remarkably heterogeneous)
- EEG
- MRI
- 14-3-3 protein test (cerebrospinal fluid)

Definite CJD
- $\text{PrP}^{\text{CJD}}$ deposition in brain tissue

Living patients

Post-mortem

In progress:

Definitive tests based on detecting $\text{PrP}^{\text{CJD}}$ in living patients
### Need multiple tests:

- primary & confirmatory
- improve specificity, minimize false positives
- cope with wide range of sample types and applications

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PMCA</td>
<td>Seeded conversion of brain PrP&lt;sup&gt;沈&lt;/sup&gt;; Sonicated; western blot</td>
<td>0.1 ag (hamster 263K et al.)</td>
<td>10&lt;sup&gt;-13&lt;/sup&gt; (hamster 263K et al.)</td>
<td>≤16 d</td>
<td>Brain, blood, feces, urine, spleen, milk, oral secretions, liver</td>
<td>Propagates infectivity; Sonication difficult to standardize</td>
<td>2, 3, 24–39</td>
</tr>
<tr>
<td>ASA</td>
<td>Seeded fibrilization of rec PrP&lt;sup&gt;沈&lt;/sup&gt;; Shaken; Multiwell ThT detection</td>
<td>1 fg (hamster Sc237 et al.)</td>
<td>10&lt;sup&gt;-9&lt;/sup&gt; (hamster Sc237, sCJD et al.)</td>
<td>~1 d</td>
<td>Brain</td>
<td>Relies on decreased lag phase relative to spontaneous fibrilization</td>
<td>0, 43</td>
</tr>
<tr>
<td>S-QuIC</td>
<td>Seeded fibrilization of rec PrP&lt;sup&gt;沈&lt;/sup&gt;; Shaken; western blot</td>
<td>0.1–100 fg (hamster 263K et al.)</td>
<td>10&lt;sup&gt;-10&lt;/sup&gt; (hamster 263K)</td>
<td>1.2 d</td>
<td>Brain, CSF</td>
<td>spontaneous fibrilization minimized</td>
<td>5, 7</td>
</tr>
<tr>
<td>RT-QuIC</td>
<td>Seeded fibrilization of rec PrP&lt;sup&gt;沈&lt;/sup&gt;; Shaken; Multiwell ThT detection</td>
<td>1 fg (hamster 263K et al.)</td>
<td>10&lt;sup&gt;-7–10&lt;/sup&gt; (hamster 263K, sCJD, vCJD)</td>
<td>~2 d</td>
<td>Brain, CSF, nasal fluids</td>
<td>No infectivity propagation; spontaneous fibrilization minimized; CJD diagnosis</td>
<td>6, 12, 15, 16, 18</td>
</tr>
<tr>
<td>eQuIC</td>
<td>Immunoprecip. + enhanced RT-QuIC; Multiwell ThT detection</td>
<td>1 ag (vCJD)</td>
<td>10&lt;sup&gt;-14&lt;/sup&gt; (sCJD, vCJD)</td>
<td>2–3 d</td>
<td>Blood plasma, brain</td>
<td>Captures seeding activity from inhibitory samples</td>
<td>8</td>
</tr>
<tr>
<td>Edgeworth</td>
<td>Steel bead capture + ELISA</td>
<td>10&lt;sup&gt;-10&lt;/sup&gt; (vCJD)</td>
<td>~2 d</td>
<td>Blood, brain</td>
<td>Captures PrP&lt;sup&gt;沈&lt;/sup&gt; from inhibitory samples; e.g., vCJD blood samples</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>
Plate-based fluorescence detection of prion-seeded PrP amyloid
(Real-time Quaking-Induced Conversion: RT-QuIC)

- **Sample** + Normal PrP protein + Amyloid stain

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-3}$-10^{-8}$</td>
<td>0-45</td>
</tr>
<tr>
<td>$10^{-9}$</td>
<td>0-45</td>
</tr>
<tr>
<td>$10^{-10}$</td>
<td>0-45</td>
</tr>
<tr>
<td>$10^{-4}$-10^{-7}$</td>
<td>0-45</td>
</tr>
</tbody>
</table>

96-well plate

Shaking fluorescence plate reader

ThT fluorescence (sum of 8 replicate wells)

- **Scrapie brain**
- **Normal brain**

Infectious PrP seed + Normal PrP

PrP amyloid with fluorescent stain

- Extremely sensitive
  - up to 1 billion-fold amplification!
- Quantitative
- Disease specific
- Much faster and cheaper than similarly sensitive tests

J. Wilham  C. Orrú
Real-time QuIC assays for prions

• Demonstrated applications:
  • human vCJD, sCJD, gCJD
  • sheep scrapie
  • deer/elk CWD

• Accessible diagnostic specimens

  Cerebral spinal fluid (humans, hamsters, sheep)
  • best diagnostic CSF test for human sCJD
    ➢ 80-90% sensitivity (% sCJD giving positive test)
    ➢ 99-100% specificity (% non-sCJD giving negative test)

  Nasal brushings or fluids (humans, hamsters, deer)
  • gentle, non-invasive sampling
  • improved CJD diagnosis
  • source of prion shedding

  Blood plasma (humans, hamsters)
  • basis for blood test for prions
    • easier diagnosis?
    • screening the blood supply?
Nasal brushing procedure for collecting diagnostic specimens for RT-QuIC

Discrimination of sCJD from non-CJD patients
- 100% sensitivity (% sCJD giving positive test)
- 100% specificity (% non-CJD giving negative test)

Christina Orrú, Matilde Bongianni, Gianluigi Zanusso
Potential applications:

- Early definitive diagnosis, especially human CJD
- Routine screening of human blood supply for prions
- Surveillance: CWD in cervids, BSE in cattle, scrapie in sheep
- Validation of prion-free status of livestock, game farms
- Meat testing
- Medical device testing
- Environmental samples (water, soils, pastures)
- TSE research
- Extend strategy to allow early diagnosis of other diseases
  - e.g., Parkinson’s, Alzheimer’s, Type II diabetes
Prion-like characteristics of other misfolded proteins???
(e.g. in Alzheimer’s, Parkinson’s, Lou Gherig’s, diabetes)

• Transmissibility?
• Pathogenic mechanisms:

Prion-like spread of pathology from localized site? vs Multicentric initiation of non-spreading pathology?
Prion Diseases: into the Future

• Prevention
  • Risk reduction
  • Vaccines against pathological forms of prion protein

• Diagnostics
  • Next generation tests for PrP\(^{\text{CJD}}\) improve prospects for early, definitive, non-invasive diagnoses.

• Therapeutics
  • Prospects are improved by early diagnosis.
  • New blockers of prion propagation & spread.
    • small molecules
    • proteins
    • vaccines
  • New insights into protein quality control processes may suggest ways to promote elimination of toxic forms of prion protein.
  • New toxic mechanisms (therapeutic targets) are being identified.
  • Stem cell and other emerging technologies may improve repair of brain damage.

  • Much remains to be done...

• Prion-like characteristics of other protein-misfolding diseases???
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