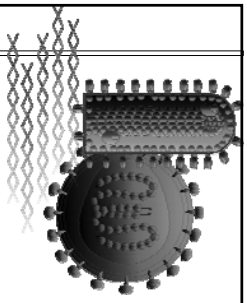
Prion Diseases



Steve Udem, M.D., Ph.D.

VP Wyeth Vaccines Discovery

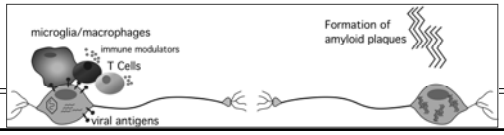
Wyeth Research

Amyloid

- Fibrillar tissue deposits that bind dye (Congo Red)
- Some proteins (amyloidogenic proteins) have greater potential to misfold
- The misfolded protein can induce conformational change in normal proteins causing deposition of insoluble toxic aggregates

Infectious Agents and Slow Degenerative Diseases of the CNS

Viral Diseases	Prion Diseases
Measles (Subacute Sclerosing Panencephalitis)	Scrapie
HIV (HIV-D, HIV dementia)	Mad Cow
HTLV-I Myelopathy	Creutzfeldt-Jakob
JC and BK (Progressive multifocal leukoencephalopathy)	Fatal familial insomnia
	Gerstmann-Straussler
	Scheinker
Rubella panencephalitis	
Rabies	
Canine distemper virus	



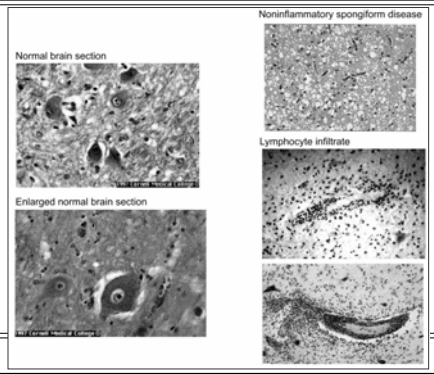
Amyloidosis

A disorder in which insoluble protein fibers are deposited in tissues and organs impairing their function.

- Caused by deposits of homogeneous proteinase-resistant fibrils
- A stable conformational change in normal cellular protein leads to aggregation:

  Soluble ⇒ Insoluble

Brain Histology



Systemic Amyloidoses

Amyloid protein	Precursor	Systemic (S) or localized (L)	Syndrome or involved tissues
AL	Immunoglobulin light chain	S, L	Primary, myeloma-associated
AH	Immunoglobulin heavy chain	S, L	Primary, myeloma-associated
ATTR	Transthyretin	S	Familial, senile systemic
		L?	Tenosynovium
$A\beta_2M$	β_2-microglobulin	S	Haemodialysis
		L?	Joints
AA	(Apo) serum AA	S	Secondary, reactive
AApoAI	Apolipoprotein-AI	S	Familial
			Aortic
AApoAII	Apolipoprotein-AII	S	Familial
AGel	Gelsolin	S	Familial
ALys	Lysozyme	S	Familial
AFib	Fibrinogen α-chain	S	Familial
ACys	Cystatin C	S	Familial
ABri[a]	ABriPP	S	Familial dementia, British
		L?	
Aβ	Aβ-protein precursor (AβPP)	L	Alzheimer's disease, ageing
APrP	Prion protein	L	Spongiform encephalopathies
ACal	(Procalcitonin	L	C-cell thyroid tumours
AIAPP	Islet amyloid polypeptide	L	Islets of Langerhans, insulinomas
AANF	Atrial natriuretic factor	L	Cardiac atria
APro	Prolactin	L	Ageing pituitary, prolactinomas
AIns	Insulin	L	Iatrogenic
AMed	Lactadherin	L	Senile aortic, media
AKer	Kerato-epithelin	L	Cornea, familial
Acibe[r]	tbs	*L*	*Pindborg tumours*
ALac	Lactoferrin	*L*	*Cornea, familial*

From Merlini and Westermark - J. Internal. Med. 2004, 255:159.

Amyloidosis - Examples

- **Systemic**
  - Immunoglobulin light chain - deposits found in kidney, heart, skeletal muscle, nerves. Patients often present with kidney dysfunction. Associated with myeloma.
  - Serum amyloid A - deposits in kidney, liver, spleen. Associated with chonic inflammation (inflammatory arthritis, granulomatous bowel disease, tuberculosis, leprosy…)
- **Hereditary**
  - Transthyretin (prealbumin) - deposits in nervous tissue, gastrointestinal, kidney, heart.
- **Cerebral**
  - Alzheimer's Disease - deposits of A-β peptide in brain (plaques)
  - Prion Diseases - deposits of PrP^{C} protein in brain (plaques)
- **Endocrine**
  - Amylin - Normally packaged with insulin in secretory granules - deposits occur in islets of type 2 diabetes patients (islet amyloid polypeptide, IAPP)

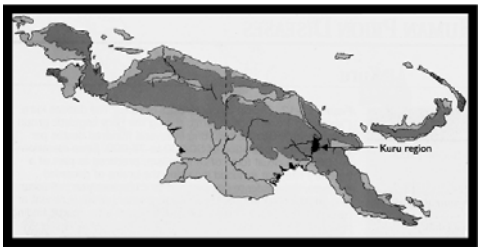
An infectious disease

Kuru

Disease strikes children

Kuru

Papua New Guinea



Kuru

Mother & daughter

Kuru

Walking Sticks

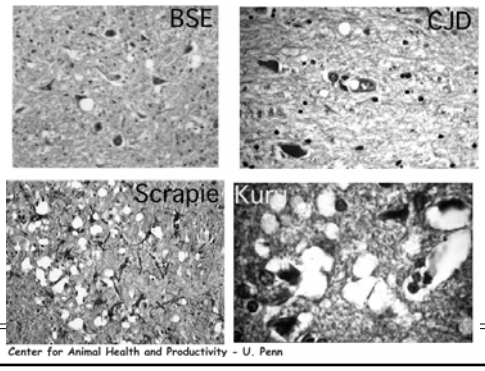
Kuru

Disease affects the Tribe

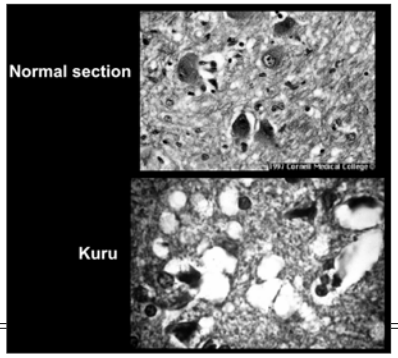
Clinical features of Kuru

Transmission	Autoinoculation/ingestion of infected brain material
Prevalence	Fore linguistic group of Papua New Guinea
Clinical features	Cerebellar ataxia, tremor, movement disorders
	Mental impairment, emotional lability, frontal release signs (snout, suck, root, grasp reflexes)
Course	Fatal 9-24 months after onset

Spongiform Encephalopathies



Spongiform Encephalopathy - Histology



"Slow Viral Diseases" - ?

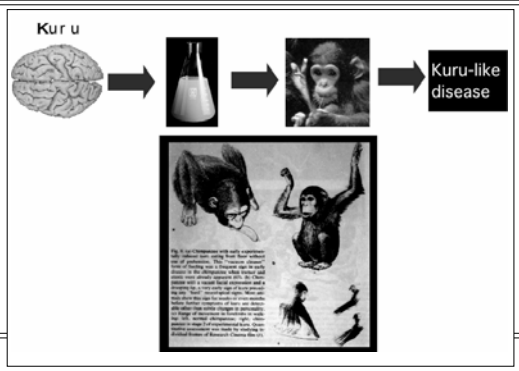
Suggestion that Scrapie is an Infectious Disease

Mid 1930s - vaccine prepared against Louping-ill

- Infectious encephalomyelitis of Sheep
- Viral disease spread by ticks (Flavivirus)
- Formalin-inactivated viral vaccine prepared from sheep brain
- No adverse effects caused by vaccination for 2 years
- Subsequently, some sheep herds developed Scrapie
- Realized that Scrapie was an infectious agent found in some batches of Louping-ill vaccine

Gordon, W.S., PhD. Advances in Veterinary Research. The Veterinary Record; 1946 November 23. Presented at the National Veterinary Medical Association of Great Britain and Ireland Annual Congress, 1946.

Linking Kuru to an Infectious Agent (Gajdusek)



Prion Diseases

Disease	Natural Host	Prion	Pathogenic PrP Isoform
Scrapie	Sheep and goats	Scrapie Prion	$OvPrP^{Sc}$
Transmissible mink encephalopathy (TME)	Mink	TME Prion	$MkPrP^{Sc}$
Chronic wasting disease (CWD)	Deer and elk	CWD Prion	$MdePrP^{Sc}$
Bovine spongiform encephalopathy (BSE)	Cattle	BSE Prion	$BoPrP^{Sc}$
Feline spongiform encephalopathy (FSE)	Cats	FSE Prion	$FePrP^{Sc}$
Exotic ungulate encephalopathy (EUE)	Nyala & greater kudu	EUE Prion	$UngPrP^{Sc}$
Kuru	Humans	Kuru Prion	$HuPrP^{Sc}$
Creutzfeldt-Jakob disease (CJD)	Humans	CJD Prion	$HuPrP^{Sc}$
Gerstmann-Staussler-Scheinker syndrome (GSS)	Humans	GSS Prion	$HuPrP^{Sc}$
Fatal familial insomnia (FFI)	Humans	FFI Prion	$HuPrP^{Sc}$

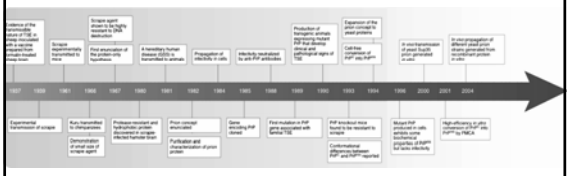
Milestones in Development of the Prion Hypothesis

- Kuru transmitted to Chimps (Gajdusek, Gibbs, Alpers 1966)
- Scrapie agent is resistant to radiation inactivation - suggests an unusual infectious agent that does not contain nucleic acid (Alpers et al., 1967)
- Hypotheses develop for transmission of Scrapie agent (Griffith, 1967)
  - •A protein that induces transcription of its own gene
  - •A protein that acquires a pathogenic conformation
- Creutzfeldt-Jakob Disease transmitted to chimps (Gibbs, Gajdusek et al., 1968)
- Scrapie agent purified - predominately protein; infectivity insensitive to nucleases and other agents that inactivate viruses (Prusiner 1982)

Creutzfeldt-Jakob Disease

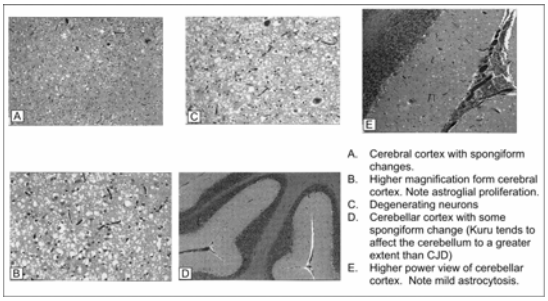
- Most common human TSE - about 1 case/million/yr
- Three forms traditionally recognized
  1. sCJD - sporadic, about 85% of cases
  2. fCJD - familial, about 10% of cases
  3. iCJD - iatrogenic, about 5% of cases
- In 1996 a new variant emerged in the U.K. - vCJD
  - Associated with eating beef infected with BSE agent (Mad Cow)
  - In contrast with traditional forms of CJD, vCJD strikes young adults
  - Crossed species barrier

History of TSE



Soto, Nat. Med. July 2004

Creutzfeldt-Jakob Disease



A. Cerebral cortex with spongiform changes.
B. Higher magnification form cerebral cortex. Note astroglial proliferation.
C. Degenerating neurons
D. Cerebellar cortex with some spongiform change (Kuru tends to affect the cerebellum to a greater extent than CJD)
E. Higher power view of cerebellar cortex. Note mild astrocytosis.

Human Prion Diseases

DISEASE	SYMPTOMS	ACQUSITION	DISTRIBUTION	DISEASE COURSE
Kuru	Loss of coordination followed by dementia	Infection (Cannibilism)	2600cases identified in Papua New Guinea	3 mo - 1 yr
Creutzfeldt-Jakob Disease	Dementia followed by loss of coordination	Usually unknown (Sporadic disease) 15% of cases involve an inherited mutation in the PrP gene Rarely infection through contaminated surgical instrument or organ transplant	Sporadic 1/1,000,000 Inherited 100 extended families identified Infectious 80 cases identified	Usually 1 yr but as short as 1 mo and as long as 10 yrs
Gerstmann-Straussler-Scheinker disease	Loss of coordination followed by dementia	Inheritance of a mutation in the PrP gene	50 extended families identified	2-6 yrs
Fatal familial insomnia	Trouble sleeping and disturbance of the autonomic nervous system. Followed by dementia and loss of coordination	Inheritance of a mutation in the PrP gene	9 extended families identified	About 1 yr

BSE

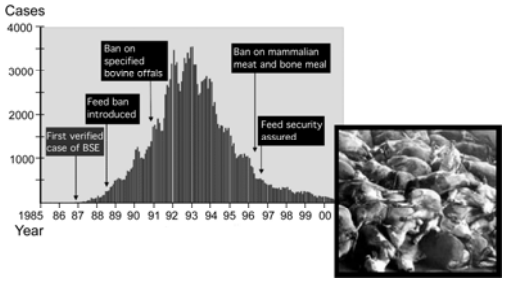
Reported cases of bovine spongiform encephalopathy as of December 2000)(a)

Country	Native cases	Imported cases	Total cases
United Kingdom	180,376(b)	0	180,376
Republic of Ireland	487	12	499
Portugal	446	6	452
Switzerland(c)	363	0	363
Francee	150	1	151
Belgium	18	0	18
Netherlands	6	0	6
Liechtenstein	2	0	2
Denmark	1	1	2
Luxembourg	1	0	1
Germany	3	6	9
Oman	0	2	2
Italy	0	2	2
Spain(d)	0	2	2
Canada	0	1	1
Falklands (UK)	0	1	1
Azores (Portugal)(e)	0	1	1

a Data from Organization of International Epizootics(Paris) and Ministry of Agriculture, Fisheries, and Food (UK).
b Includes 1,287 cases in offshore British islands.
c Includes cases detected by active surveillance with immunologic methods.
d Origin and dates of imported cases are under investigation.
e Case imported from Germany.

table adapted from: *http://www.cdc.gov/ncidod/eid/vol7no1/brown.htm*

BSE Epidemic in UK

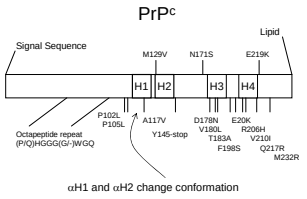


What is Normal PrP^C?

- Glycoprotein ~250 amino acids
- Membrane associated through a C-terminal glycosyphosphatidylinositol (GPI) linkage
- Role in membrane trafficing has been proposed - possibly involved in some endocytic pathways
- Knockout mice develop and behave normally, but perhaps prone to seizures
- Interacts with laminin, which plays a role in cell adhesion and neurite formation
- Also interacts with the laminin receptor resulting in internalization of membrane-bound PrP^C
- Binds Cu^{++} - may have an antioxidant function that promotes neuron survival
- Abundant in brain - also detected in: spleen, lymph node, lung, heart, kidney, skeletal muscle, uterus, adrenal gland, parotid gland, intestine, proventriculus, abomasum and mammary gland.

vCJD in U.S.

Genetic mutations in CJD and other Prion Diseases

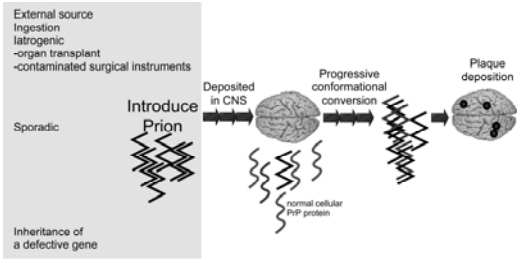


Search for the Agent (Prusiner Lab)

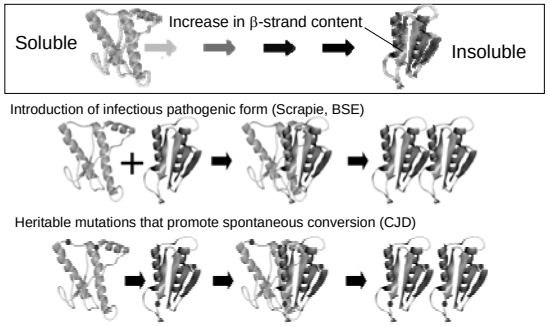
Scrapie

- Extremely small, proteinaceous infectious particle
- Resistant to DNAse and RNAse
- Resistant to limited proteolysis
- Resistant to chemical agents that inactivate conventional viruses

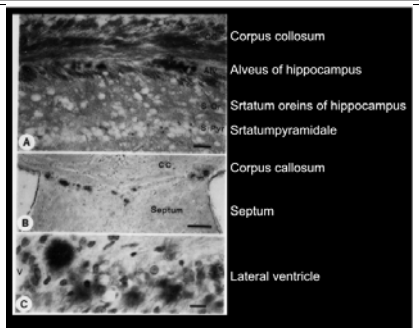
Prion Disease



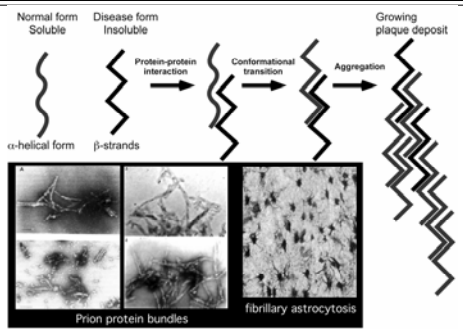
How Does Conformational Conversion Occur?



Plaques Produced by Hamster Prions in Transgenic Mice

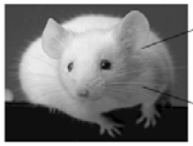


Prion Disease - Amyloid Deposition



Normal PrP^C Required for Disease Progression

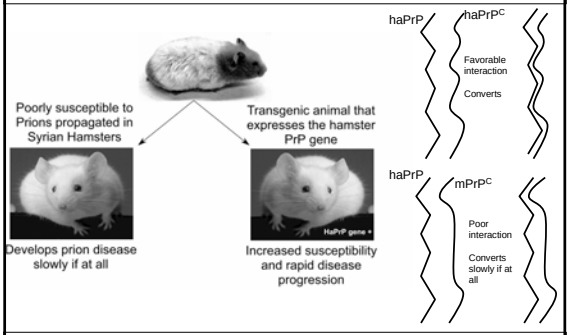
PrP Gene Knockout



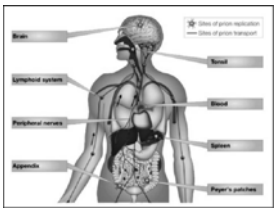
Not susceptible to prion disease

Possibly more prone to seizures

Species Barrier - Conformation Plays a role



Current View of Prion Disease Development



- Prions ingested and absorbed by intestines (Peyers Patches)
- Gains access to lymphoid fluids and blood
- Deposited in lymphoid tissues where it amplifies through conformational conversion
- Amplified prions deposited in brain - perhaps crosses blood-brain barrier or migrates by axonal transport
- Replicates in brain - toxicity resulting in neuronal cell death

Criticisms of the Prion Hypothesis are being Addressed

There are different strains of Prions

•Differ in incubation time, clinical features, and neuropathology

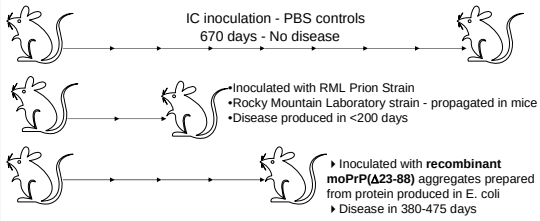
•How are 'Strains' developed without evolution of nucleic acid genomes?

Can conformational transition be observed in vitro?

A recombinant purified Prion has not been shown to induce disease

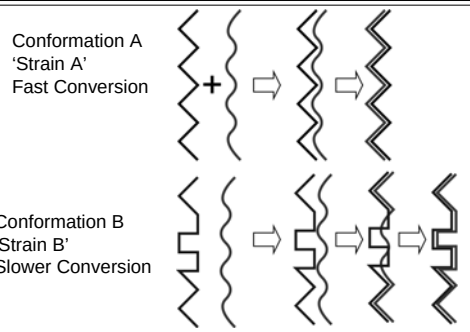
Disease Produced by Synthetic Prions (Prusiner Lab)

- Transgenic mouse model
- Mice express low levels of a deleted form of moPrP (Δ23-88) that aggregates spontaneously
- These mice do not develop disease spontaneously at rate significantly higher than normal mice
- But, they are 'seeded' with a protein that is susceptible to misfolding

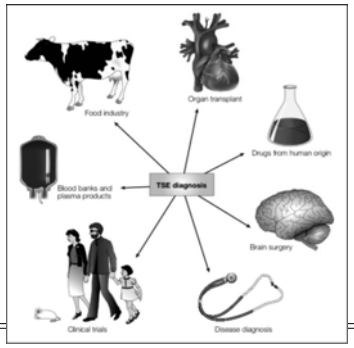


Legname et al., Science (2004) 305:673

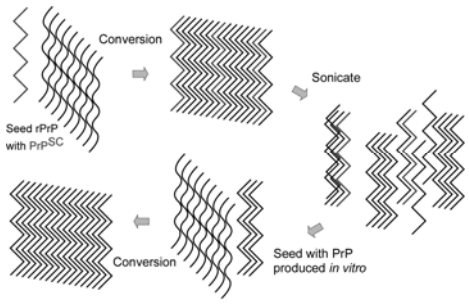
Hypothesis - Conformational Strains



Immediate Need for Prion Diagnostics



Cyclic In Vitro Amplification of a Misfolded Protein



Saborio, Permanne, Soto. Nature 2001

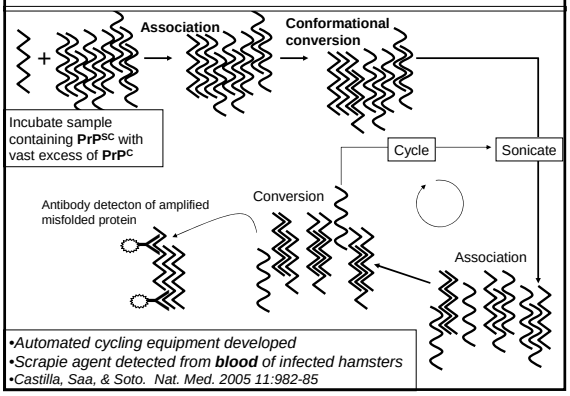
Reading

Soto, C. and Castilla, J. 2004. *The Controversial Protein-Only Hypothesis of Prion Propagation.* Nature Medicine, 10 Supplement: S63-7.

Soto, C. Diagnosing Prion Diseases; *Needs, Challenges, and Hopes.* Nature Reviews, 2:809-13.

Rhodes, R. *Deadly Feast: Tracking the Secrets of a Terrifying New Plague.* Simon & Schuster 1997

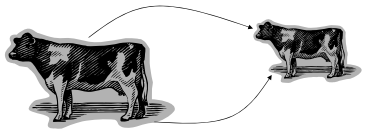
Protein Misfolding Cyclic Amplification (PMCA)



- *Automated cycling equipment developed*
- *Scrapie agent detected from **blood** of infected hamsters*
- *Castilla, Saa, & Soto. Nat. Med. 2005 11:982-85*

Transmission of Prions in Herbivores

Meat processing byproducts in contaminated feed



Natural horizontal transmission?

Prions excreted in urine of infected mice
Seeger et al., Science 310:324-326