10

What About Prions?

Prions are unconventional infectious agents

- Proteinacious infectious particle
- Infectious proteins that cause a group of diseases of the brain and nervous system called transmissible spongiform encephalopathies (TSEs)
- None of the TSEs evoke an immune response
- Prions cause a noninflammatory process that results in vacuolation or spongiosis in the gray matter of the brain

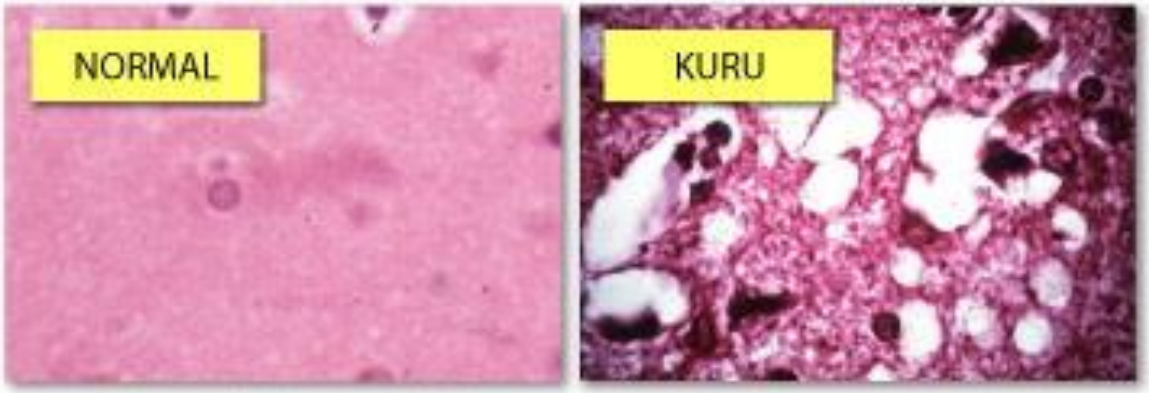


TABLE 19-1 Human Prion Diseases

Disease	Cause	Life Span After Symptoms Occur
Kuru	Cannibalism	3–6 months
Variant Creutzfeldt-Jakob disease (vCJD)	Consumption of contaminated beef (bovine prions)	13–14 months
Creutzfeldt-Jakob disease	Inherited germline mutation in PrP gene, sporadic (unknown cause), or iatrogenic	4–5 months
Gerstmann-Straussler-Scheinker disease (GSS)	Inherited germline mutation in PrP gene	2–6 years
Fatal familial insomnia (FFI)	Inherited germline mutation in PrP gene	12 months

TABLE 19-2 Animal Prion Diseases

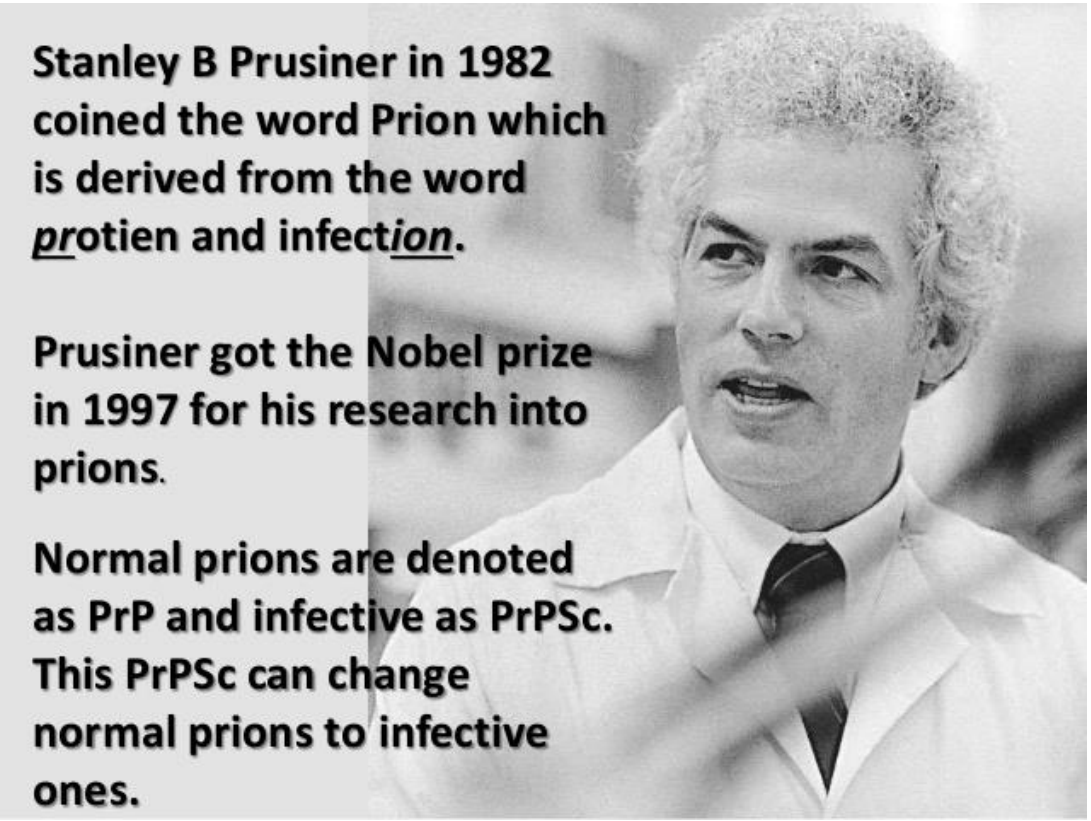
Disease	Animal Host
Scrapie	Sheep and goats
Chronic wasting disease (CWD)	Deer and elk
Feline spongiform encephalopathy (FSE)	Cats
Bovine spongiform encephalopathy (BSE)	Cattle
Exotic ungulate encephalopathy (EUE)	Nyala and Greater Kudu (antelope in South Africa)
Transmissible mink encephalopathy (TME)	Mink

10.1 Kuru and Cannibalism

- Kuru—human TSE
- Mysterious disease affecting large numbers of the South Fore people of Papua New Guinea in the 1950's and 1960's
- Investigated by Vin Zigas, Shirley Lindenbaum and Carleton Gadjusek
- Gadjusek concluded that kuru is transmitted by the ritualistic consumption of the brains of deceased relatives (endocannibalism)
- Gajdusek shared the Nobel Prize for Physiology or Medicine in 1976 for research on the origin and dissemination of TSEs.

10.2 PrP and the “ Protein Only” Hypothesis

- Stanley Prusiner’s team isolated the infectious “prion” agent that caused Kuru.

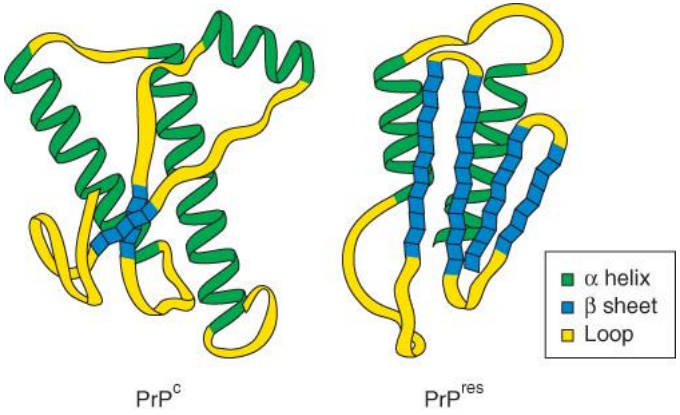


Prion Characteristics

- PrP stands for “proteinaceous infectious particle”
- Prions are highly resistant to routine methods of decontamination
  - Not inactivated by proteases, organic solvents, alkaline cleaners, ultraviolet radiation, ethanol, formaldehyde or extremely high temperatures (e.g. greater than 100 ^{o}C; sterilization for one hour at 121 ^{o}C in an autoclave does not kill prions)
  - Tissues, infectious waste, and instruments used in the processing of prion-contaminated samples are decontaminated in 1 N NaOH or undiluted fresh household bleach followed by autoclaving at 132 ^{o}C for 4.5 hours

Two Distinct Conformations of the Prion Protein

- PrP^C—"cellular" form found throughout the tissues of the body in healthy people and animals
  - PrP^C is sensitive to denaturing agents
- The 'protein only' hypothesis proposes that abnormal, misfolded proteins causes PrP^C to convert to the highly resistant or stable form termed PrP^{res}.
  - Over time, PrP^{res} accumulates into clumps that damage or destroy nerve cells in the brain

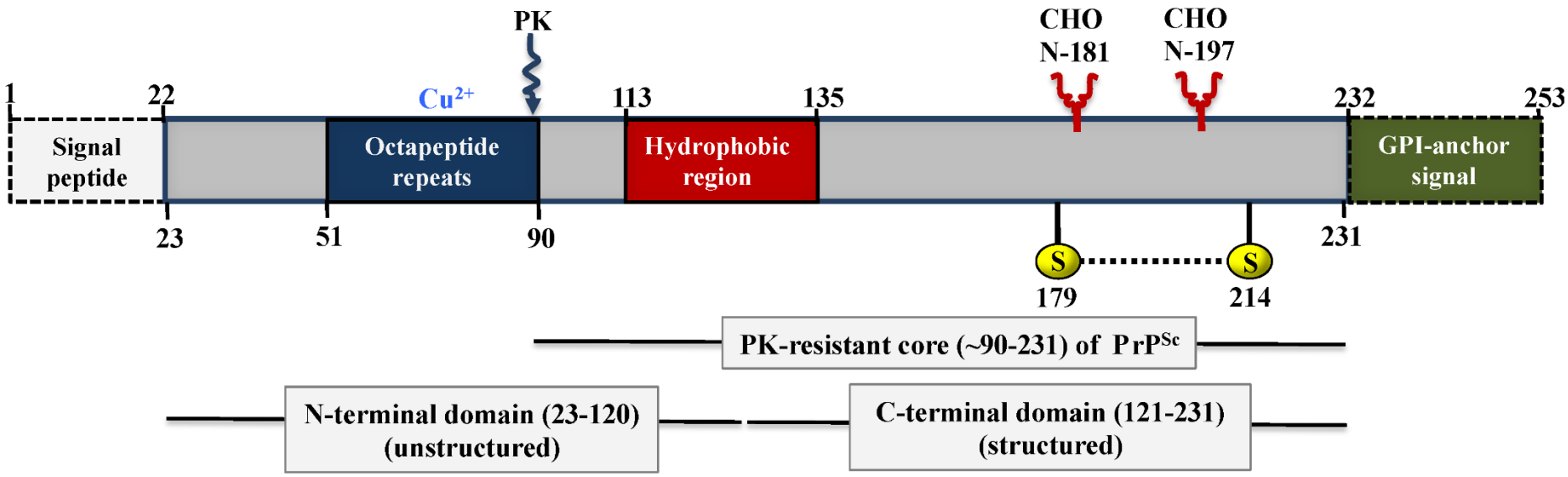


Tertiary Structure of PrP^c and PrP^{res}

PrP^C	PrP^{Res}
sensitive to proteolysis	resistant to proteolysis
soluble	insoluble
associated with cellular membrane	located within the cell as aggregates
mainly α helical structure	mainly β sheet structure

PRNP gene encodes PrP^C

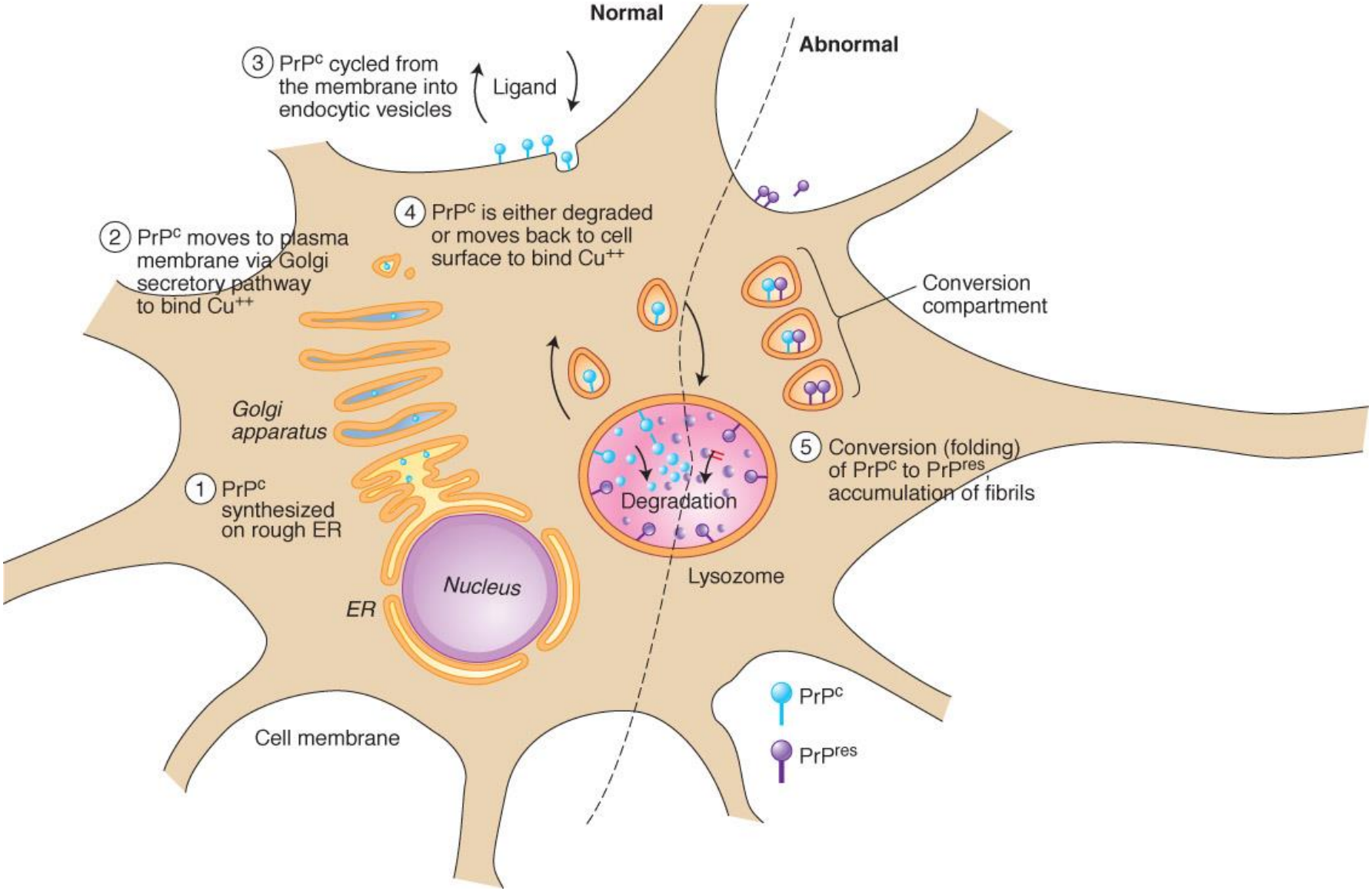
- PRNP gene located on human chromosome 20
- Its 0.76 kb ORF codes for a 253 AA protein, PrP^c.
- It is unique (no other proteins of similar homology in the database)
- PrP^C is targeted via a secretory pathway to the cell surface of neurons and other cell types
- A glycosylinositol phospholipid (GPI) anchors it into the membrane.



PrP^C Function?

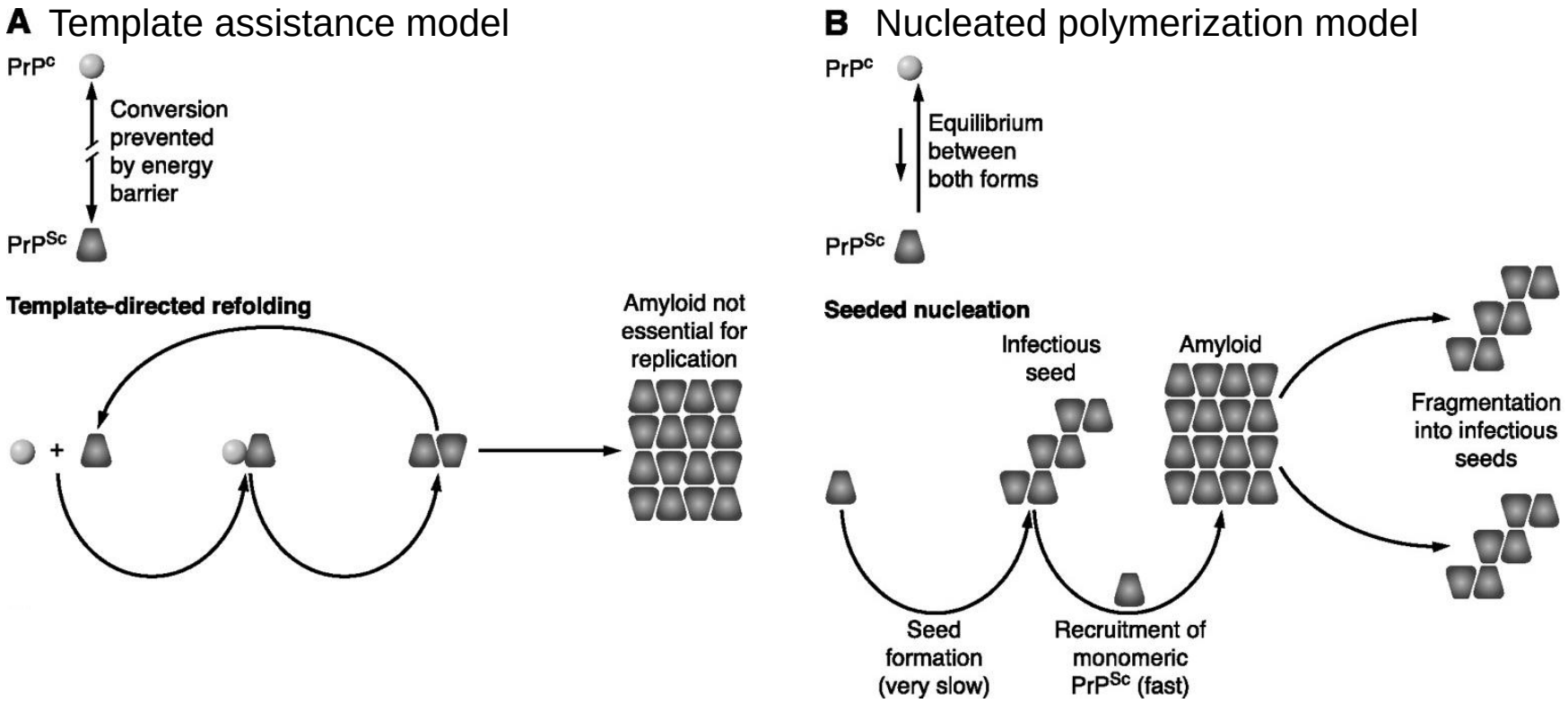
- PrP^C is highly conserved in mammals and expressed predominantly in the brain.
- PrP^c may bind copper and is then cycled back into the cell via endocytic vesicles where they may be degraded in lysosomes.
- Infectious PrP^{res} are resistant to degradation and accumulate, causing neurotoxicity.
- Within the lysozymes, the infectious PrP^{res} may interact with PrP^C, causing the noninfectious form to be converted to the infectious form.
- Exact function is unknown.
- Possible functions are roles in:
  - Signal transduction
  - Cellular differentiation
  - Cell adhesion
  - Copper transport
  - Resistance to the accumulation of destructive free radicals that can result in neuronal death

Hypothetical model showing PrP^c involvement in the secretory pathway of cells



Prion replication

- Two models for the molecular mechanism of prion formation have been proposed



Three Ways that TSEs Can Arise

- Infection
  - diet, vCJD
  - Iatrogenic means (e.g. surgery)
    - Growth hormone injections
    - Corneal transplants
- Inherited
  - Genetic CJD
  - Gerstmann-Straussler-Scheinker Disease (GSS)
  - Fatal Familial Insomnia (FFI)
- Sporadic forms
  - CJD

10.3 Oral Transmission:
How Do "Eaten" Prions Travel to the Brain to Cause Disease? Why Isn't Variant CJD More Common?

- Oral transmission of TSEs is very inefficient compared to intracranial injections
- Prion ingestion leads to infection of the gastrointestinal tract. Neuroinvasion occurs via the splanchnic (내장신경) and vagus(미주신경) nerves present in the abdominal region that extends to the brain.
- New research suggests infectious prions enter the brain via the hypoglossal nerve(설하신경) of the tongue
  - Food products that contain tongue may be a potential source of prion infection for humans

10.4 Other Routes of Transmission
Iatrogenic Transmission, Including Prions in Blood

- Iatrogenic disease—inadvertently caused by a physician or surgeon by a contaminated medical or surgical instrument or diagnostic procedure

Examples of Iatrogenic Transmission of CJD

- Corneal grafts from donors who developed CJD
- Sharing of contaminated deep electroencephalography (EEG) electrodes implanted into brain
- Contaminated neurosurgical instruments
- Receipt of human growth hormone from CJD infected donors
- Patients who received dura mater(경막) grafts from donors who developed CJD

Bloodborne Transmission?

- Blood borne transmission has been suspected for 2 reasons
  1. vCJD can be detected in lymphoid tissues, raising the possibility that it could also be found in circulating lymphocytes present in the blood
  2. Prions may exist in the blood as it travels from the original site of the gut to the brain
  - Experimental studies have shown the transmission of BSE to sheep by blood transfusion from asymptomatic infected sheep to healthy sheep

Blood Transmission Surveillance

- At least 48 individuals who received blood components from 15 donors who later developed variant CJD are being monitored as a precautionary step for their “at risk “ status
- Hemophiliacs in the U. K. have been notified that they are at risk for developing variant CJD.

10.5 Clinical Signs and Symptoms of Variant Creutzfeldt-Jakob Disease (Variant CJD)

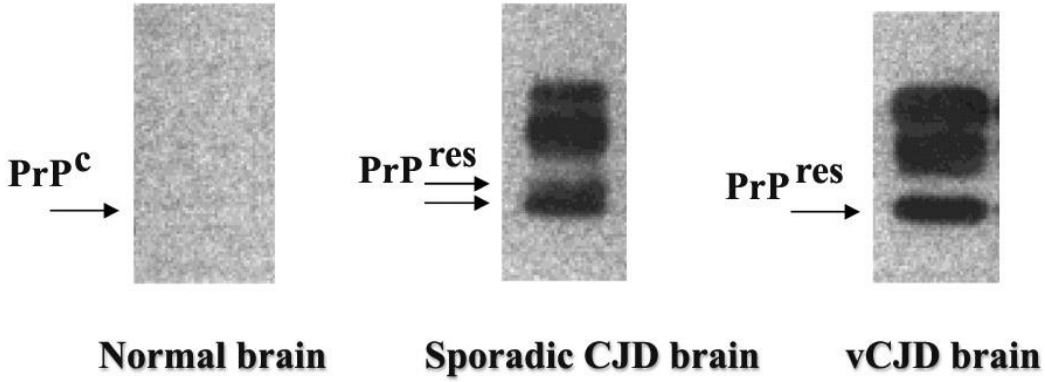
- 50% of variant CJD patients die before the age of 30 - average age of death is 28
- Patients suffering from classic CJD die at an average of 68 years
- Symptoms of Variant CJD
  - Anxiety
  - Memory loss
  - Mood changes
  - Depression
  - Withdrawal(금단)
  - Neurological signs
    - Twitching 근육경련
    - Spasms (jerky movements) 발작
    - Posture and gait abnormalities (motor difficulties)

- Final Symptoms of Variant CJD
  - Loss of speech
  - Stupor 혼미
  - Persistent vegetative state (coma)
  - Death (14 months after symptoms appear)

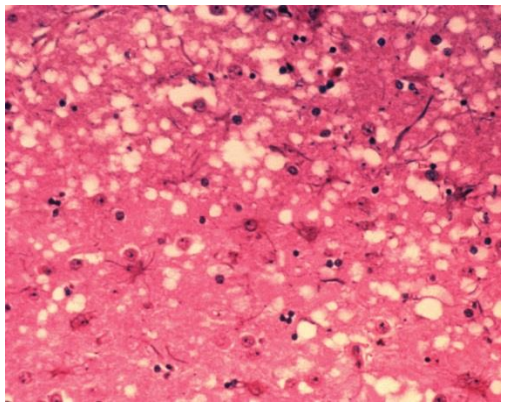
10.6 Diagnosis of Variant CJD

- Most patients referred to a psychiatrist because of behavioral changes
- Definitive diagnosis—prion positive immunostaining of biopsy material from:
  - Tonsil
  - Spleen
  - Lymph nodes
- EEG—looking for slow or negative brain wave activity
- MRI—looking for brain lesions
- CSF—looking for elevated levels of neuronal, astrocytic and glial proteins
  - Elevated levels are a consequence of damage to the blood brain barrier (extensive brain tissue damage)

Gold Standard of Diagnosis is Postmortem Examination of Brain Tissues



Detection of Pr^{Pres} after proteinase K treatment by Western blot analysis.



Spongiosis
Brain tissues showing histopathologic changes found in bovine spongiform encephalopathy.

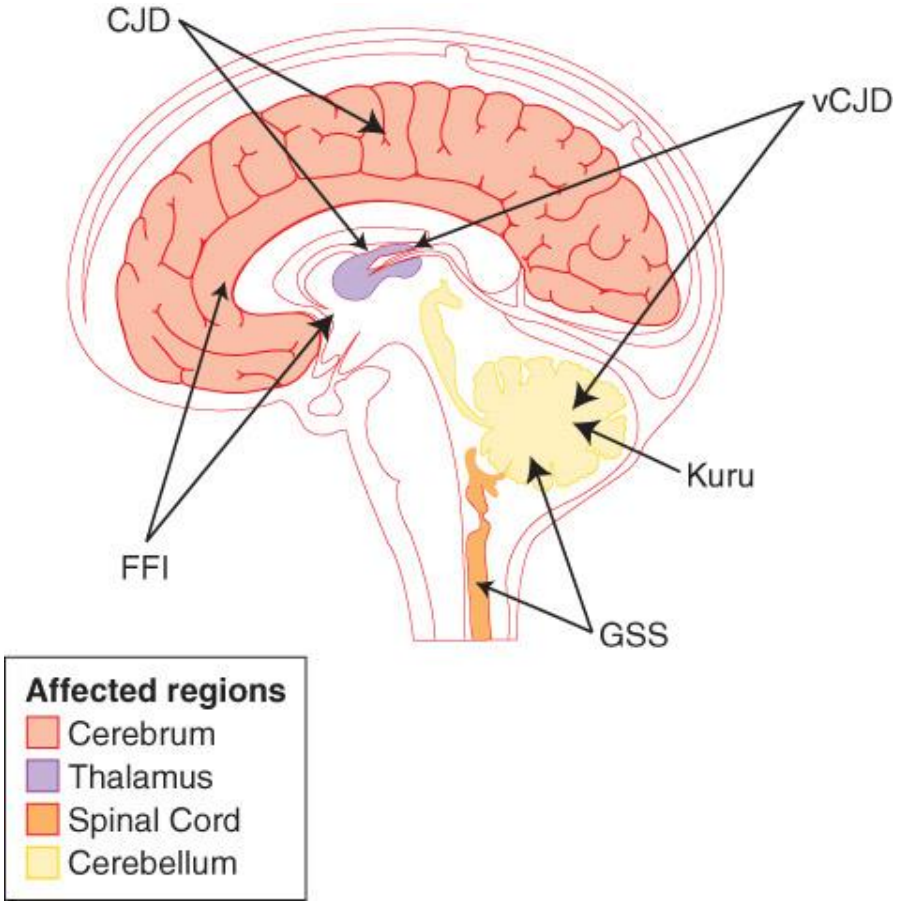
10.7 Pathogenesis of TSEs

- Incubation period of TSEs (with the exception of vCJD) is long (20-56 years)
- South Fore tribe members still getting Kuru some 39-56 years after the cessation of cannibalism

Histological/Brain Changes of TSEs

- Infected brains become spongiform (the brain has vacuoles—clear zones, similar to a sponge)
- Neuronal loss
- Astrocystosis (spread of astrocytes to damaged tissues in the brain)
- Amyloid plaques—formation of PrP^{res} threadlike aggregates

Diagram of the Major Regions of the Human Brain Affected by the Different TSEs



Brain Changes

- Depending upon what region of the brain is affected
  - e.g. memory is affected when the cerebral cortex is infected

- No inflammation or immune defense against prions exists
  - Natural proteins (body does not recognize as foreign antigens)
  - Prion proteins are only harmful when they are converted to PrP^{res}

10.8 Genetic Research and the Function of PrP^C

- Knockout mice that do not express PrP^C are resistant to scrapie infection
- Do changes in copper metabolism cause TSEs? (assuming prions are involved in copper metabolism)
- *PRNP* gene lacking octarepeat copper binding motif was introduced into knock-out mice.
  - Mice became susceptible to scrapie infection
  - Copper binding is not important in pathogenesis?
  - Other copper binding sites outside of the octarepeat region which may play a more important role in copper metabolism?

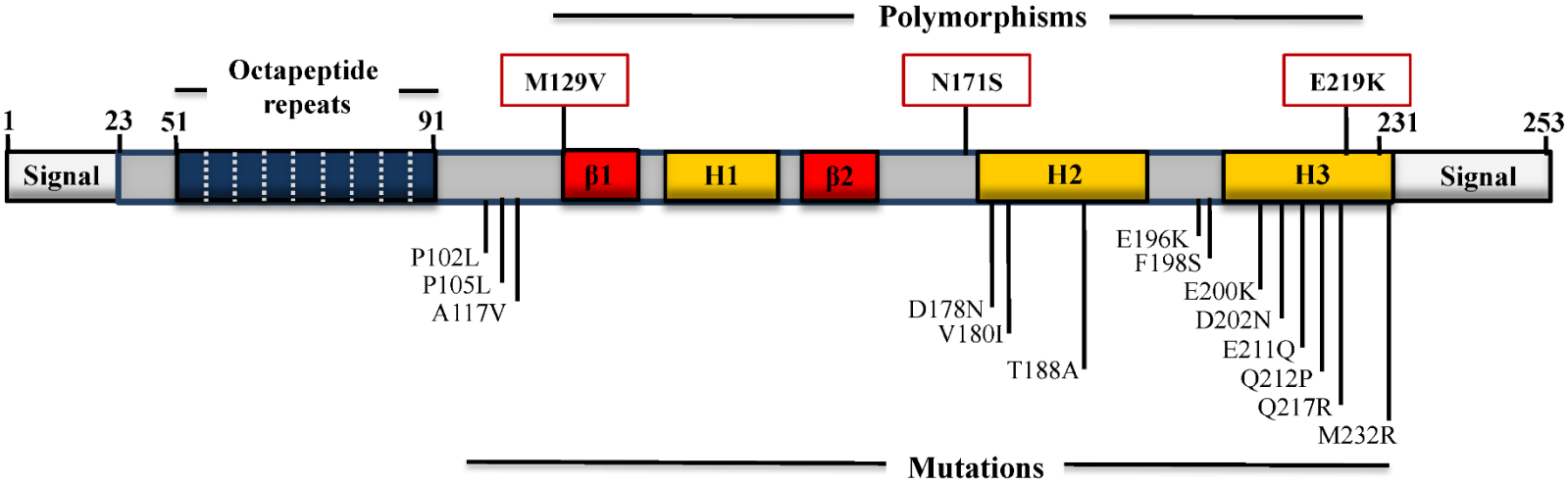
Other Copper Experiments

- Radioactive copper added to cells expressing PRNP gene
  - Radioactive copper bound to the PrP^C

- PRNP-expressing cells were more resistant to copper toxicity (free copper radical) and oxidative stress compared to cells that did not express PRNP
- Does PrP^C play a role in cellular scavenging mechanisms?
- The loss of PrP^C function may change in copper balance in the brain and cause oxidative damage throughout the brain.

Human Genetics: Codon 129

- 50 known mutations in the PRNP gene
- *PRNP* codon 129 appears to act as a genetic susceptibility factor (codes for methionine or valine at position 129 of the PrP^{C}).
- All people suffering from vCJD acquired through consuming prion-contaminated beef products were homozygous methionine at codon 129



1 to 9 Octapeptide repeat inserts
[P(H/Q)GGG(-/G)TGQ]

10.9 Steps Toward Treatment and Vaccination

- No drug therapies available.
- Treatment is supportive
- No vaccine available
  - PrP^{C} antibodies injected into the brains of mice cause neurotoxicity

10.10 Species Barrier
Bovine Spongiform Encephalopathy (BSE) and Variant Creutzfeldt-Jakob Disease (Variant CJD)

- Transmissibility among species is easy
- Transmission can occur between different species
- Origin of BSE unclear
  - Accepted hypothesis is that BSE came from cattle ingesting scrapie-contaminated bone meal derived from sheep offal fed to young calves

Scrapie

- Scrapie was considered a rare disease of sheep that did not cause disease in humans
- Scrapie has been a disease of sheep and goats in Great Britain, Western Europe for more than 250 years
- First case in the U.S. was in 1947 (Michigan flock of sheep)
- Australia and New Zealand are still scrapie-free

BSE

- First diagnosed in 1985
- Dairy farmer in England notices several cows with abnormal behavior
  - Unsteady gait,
  - Aggressiveness,
  - Kicking during milking
- The term “raging” or mad cow disease was coined
- Other signs of BSE
  - Difficulty in rising from a lying position
  - Itching
  - Heightened sensory perception
  - Anorexia 식욕부진
  - Excessive licking
  - Decreased milk production
- Symptoms last for 2-6 months before the animal dies