Unusual infectious agents

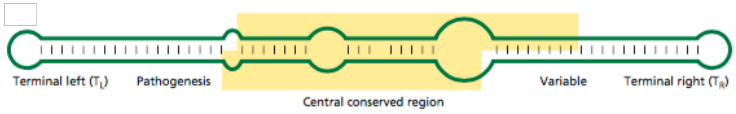
Lecture 24
Biology 3310/4310
Virology
Spring 2017

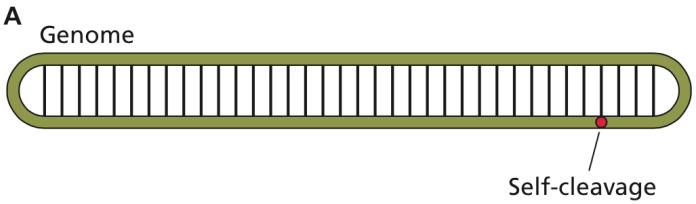
So come up to the lab and see what's on the slab
- Dr. Frank-N-Furter
The Rocky Horror Picture Show

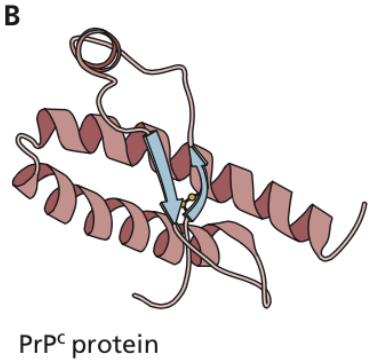
A fundamental question

What is the minimum genome size needed to sustain an infectious agent?

Could an infectious agent exist without ANY genome?

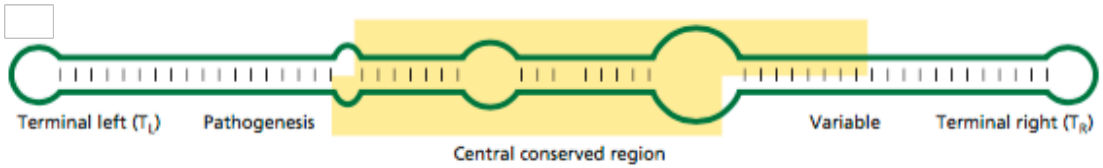






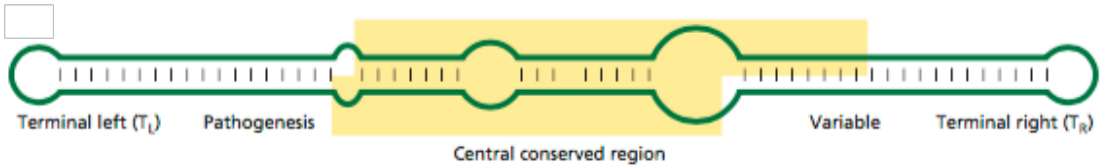
Viroids, satellites, and prions provide answers

Viroids



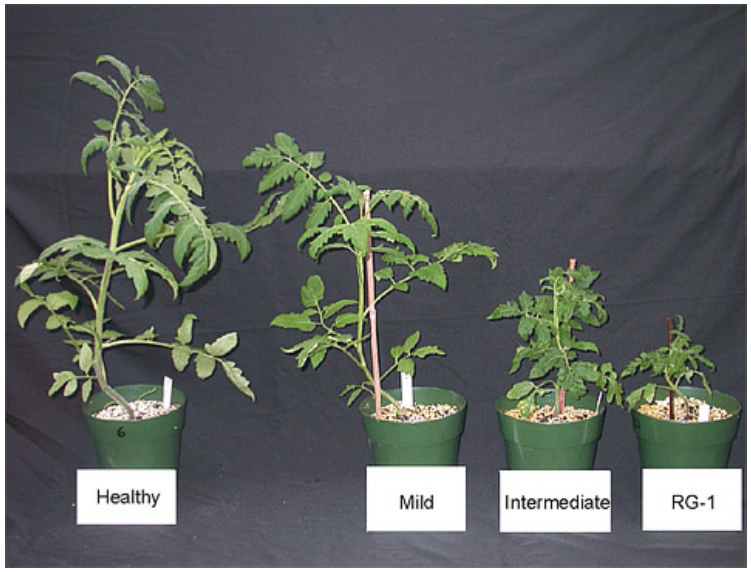
- Circular ssRNA
- No protein coding regions
- No protective coat, yet migrate from host to host (no receptors required)
- Replicate when introduced into plants
- Families *Pospiviroidae* (replicate in nucleus) and *Avsunviroidae* (replicate in chloroplasts)

Viroids



- Circular ssRNAs, 120 - 475 nt
- RNA displays extensive internal base-pairing, appears as 50 nm rod in EM
- Some are ribozymes
  - *Activity essential for replication*
- Distinction from the virus life style
  - *Viruses are parasites of host translation machinery*
  - *Viroids are parasites of host transcription machinery*

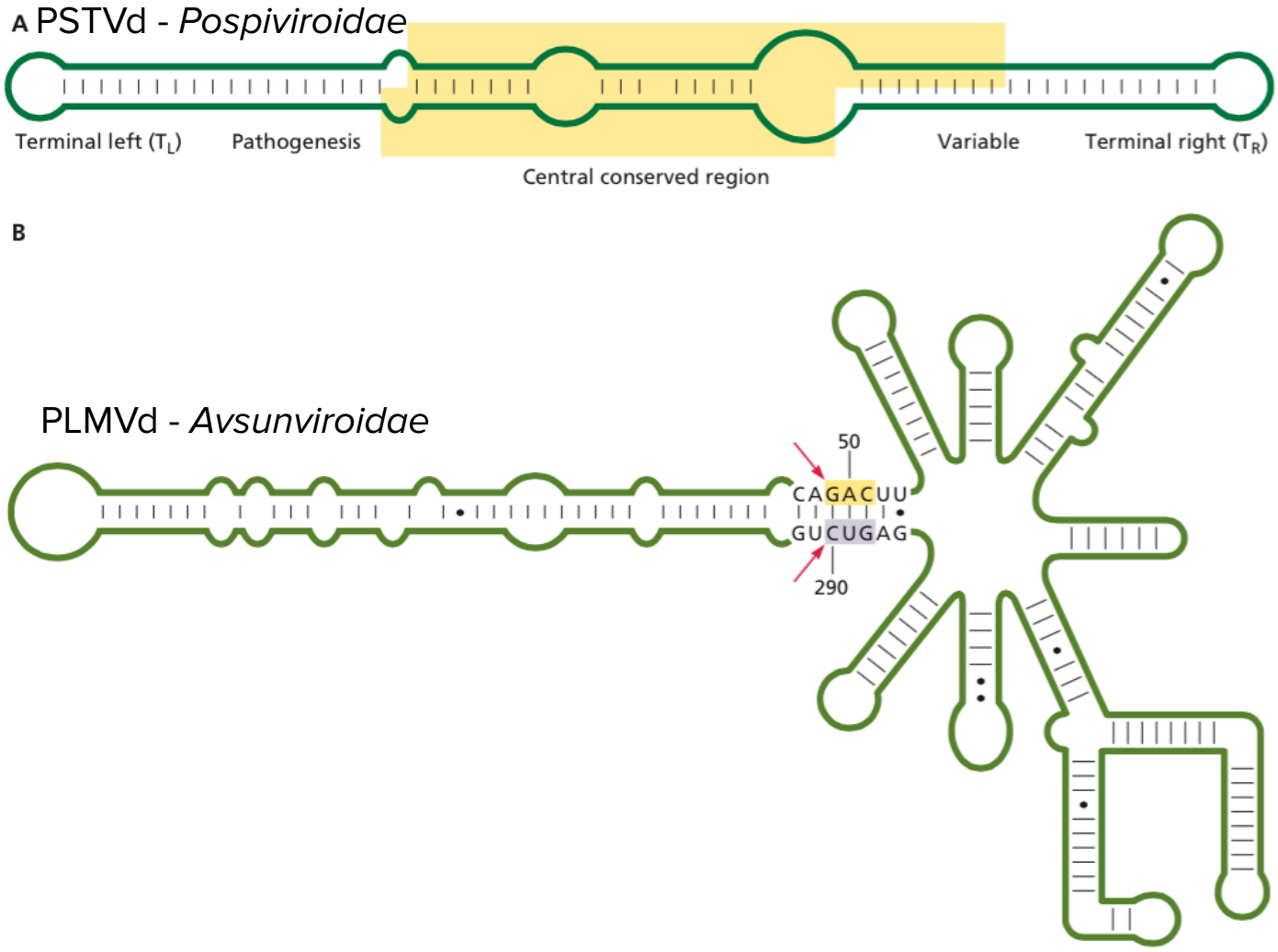
- Potato spindle tuber viroid (PSTVd) discovered 1967
  - *Prototype for smallest known nucleic acid-based agents of infectious disease*
  - *359 nucleotides*
- Some are benign, others cause economically important diseases of crop plants



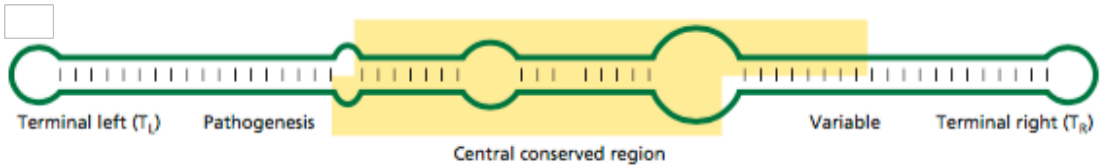
Some of my favorite viroids

- Cadang-Cadang coconut viroid
  - *CCCVd causes lethal disease of coconut palms*
  - *Pina colada drinkers are sad*
- Hop latent viroid
  - *HLVd - no symptoms in the hop plant*
  - *Beer lovers are relieved*
- Apple scar skin viroid
  - *ASSVd - mild symptoms; apples look bad, taste good*
  - *Picky consumers don't buy these apples*

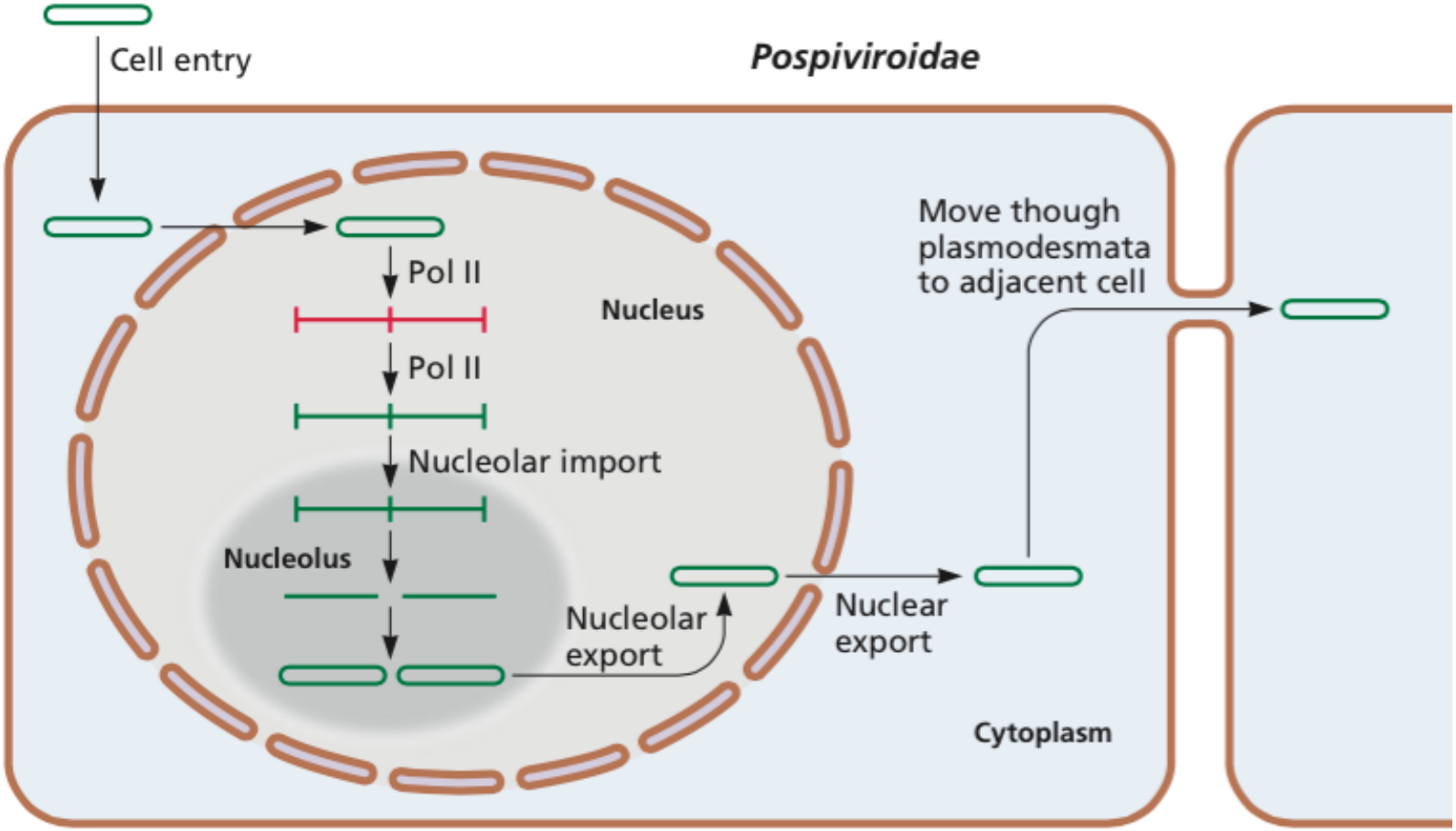
Functional regions of viroid RNA

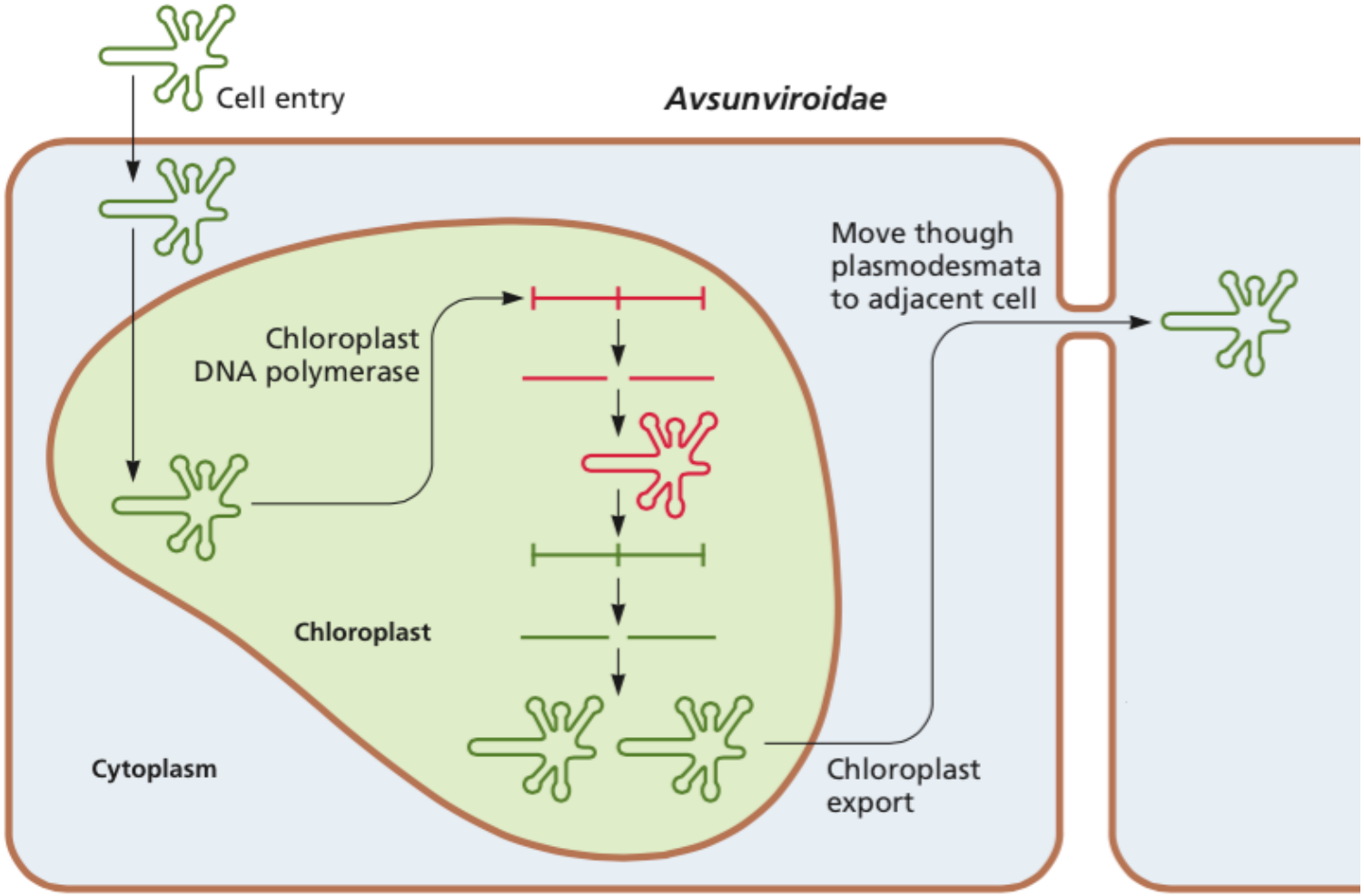


How do viroids replicate?



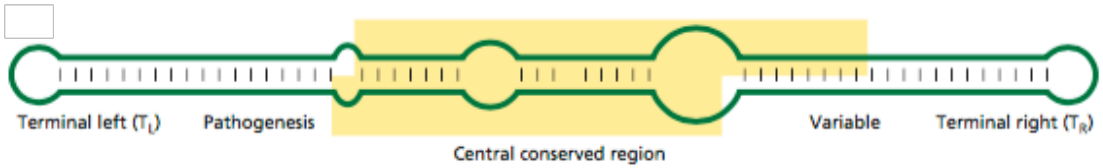
- Concatamers of viroid RNA produced by host RNA polymerase II
- One group of viroids forms ‘hammerhead ribozyme’
  - *A ribozyme: autocatalytic, self cleaving RNA*
  - *Ability of RNA to catalyze a reaction in the absence of protein discovered 1981*
  - *Used to cleave multimeric structures produced during replication*
- Other viroids cleaved by host nuclear enzymes


Cell entry
Pospiviroidae
Pol II
Nucleus
Pol II
Nucleolar import
Nucleolus
Nucleolar export
Nuclear export
Move though plasmodesmata to adjacent cell
Cytoplasm



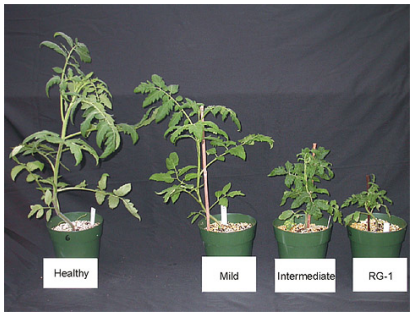
Hammerhead ribozyme

Origin of viroids

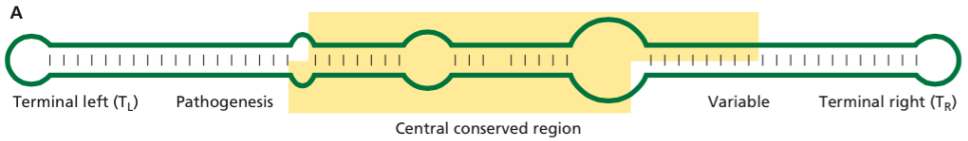


- Originated in the 20th century by chance transfer from wild plants used in breeding modern crops
- Worldwide use of genetically identical plant breeding lines (monoculture)
- Mechanical transmission by contaminated farm machinery, equipment, hands, plant to plant

How do viroids cause disease?



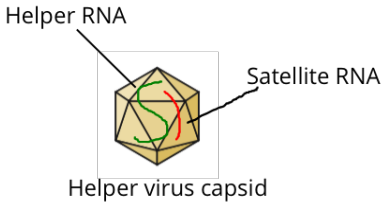
- Small 21-24 nt RNAs (siRNAs) derived from viroid RNAs in plants may guide RNA silencing of host genes and induce disease
- Symptom development correlates with production of small RNAs
- Many siRNAs map to pathogenicity-modulating domain of viroid



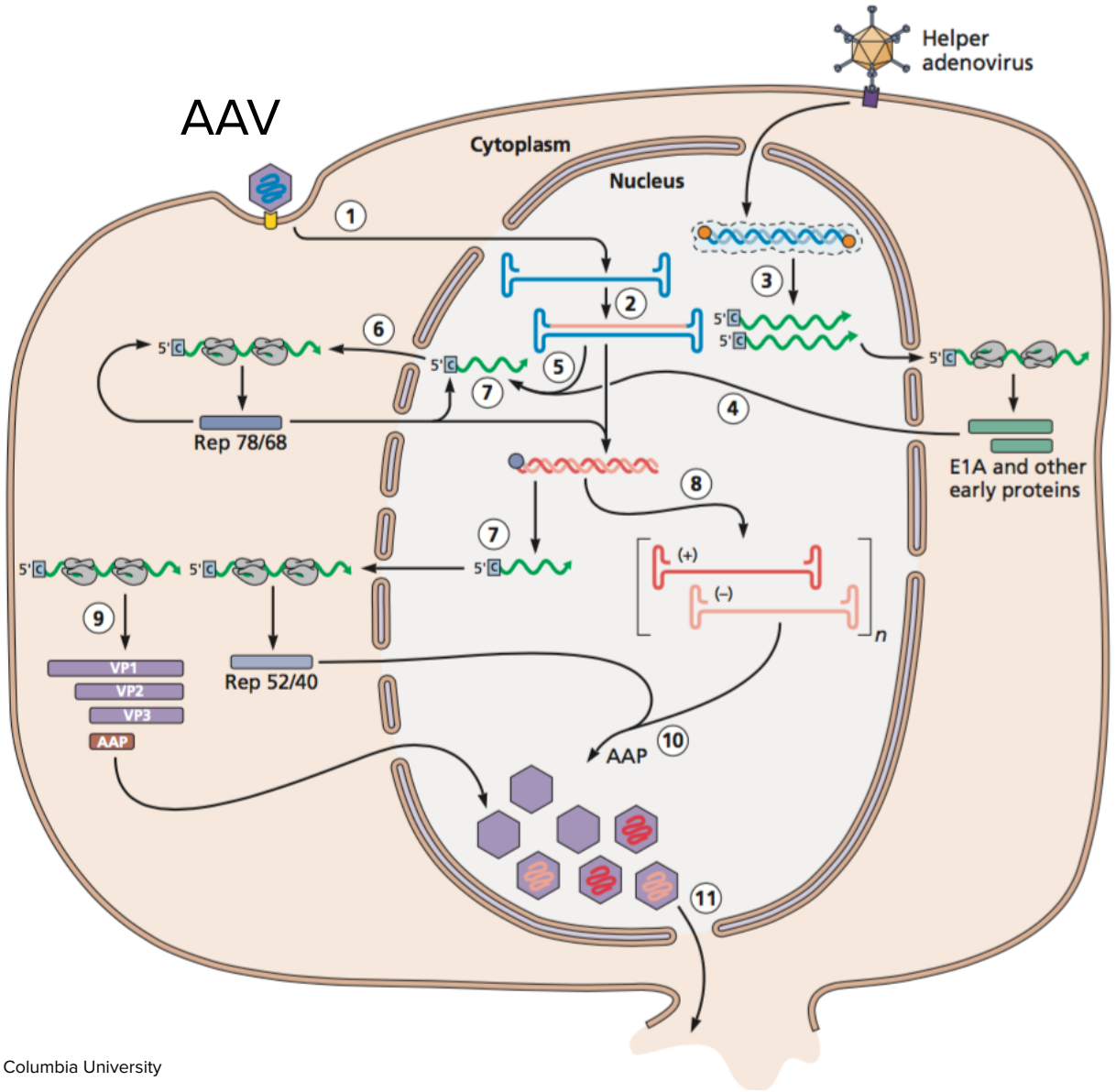
Principles of Virology, ASM Press

Satellites

- ssRNA, DNA, cRNA genomes
- Depend on helper virus for propagation
- Lack genes required for replication
- **Satellite viruses**: Encode structural proteins that encapsidate the genome (form distinct particles)
- **Satellite RNAs**: Packaged by helper virus proteins, also rely on helper for replication. May or may not encode protein.



AAV


Helper adenovirus
Cytoplasm
Nucleus
1
2
3
4
5
6
7
8
9
10
11
Rep 78/68
E1A and other early proteins
Rep 52/40
VP1
VP2
VP3
AAP
AAP
(+)
(–)
n

Satellites

- In plants, satellites cause distinct disease symptoms not seen with the helper virus alone (necrosis, chlorosis)
- Satellites are not defective viruses derived from the helper
  - *Genomes have no homology with helper*

Satellite viruses

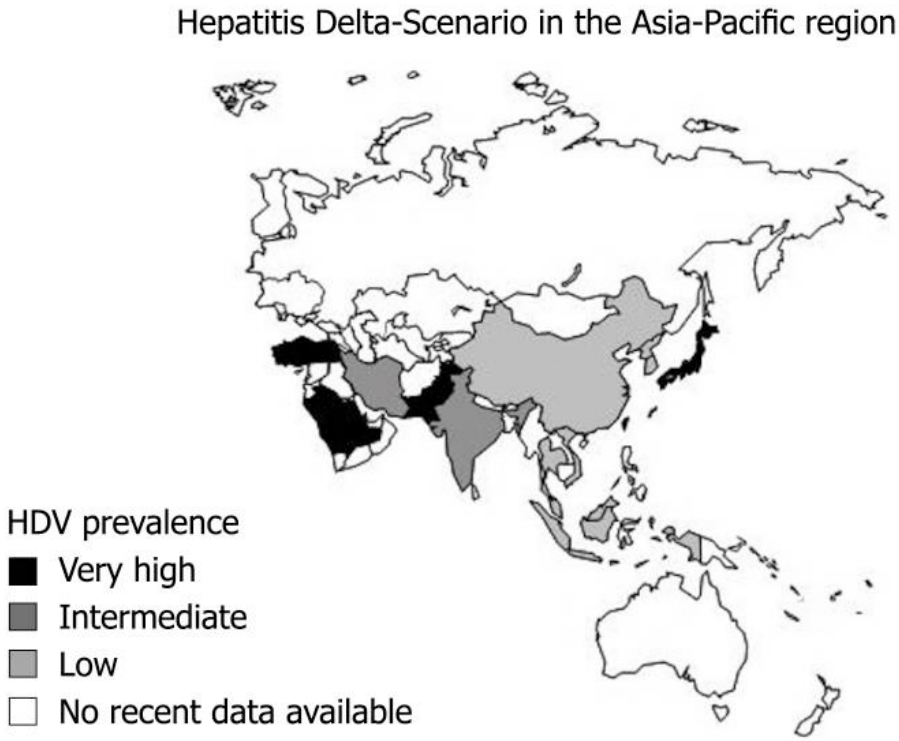
Helper/satellite virus	Nucleic acid	Particle nm	Genome nt	Capsid protein kDa	Host
Adenovirus or herpesvirus/ adeno-associated virus	ssDNA	20-24	4700	87, 73, 62	Vertebrate
Chronic bee paralysis virus/ CBPV satellite	ssRNA	17	1100	15	Animal
Tobacco necrosis virus/TNV satellite	ssRNA	17	1239	22	Plant

Hepatitis delta virus

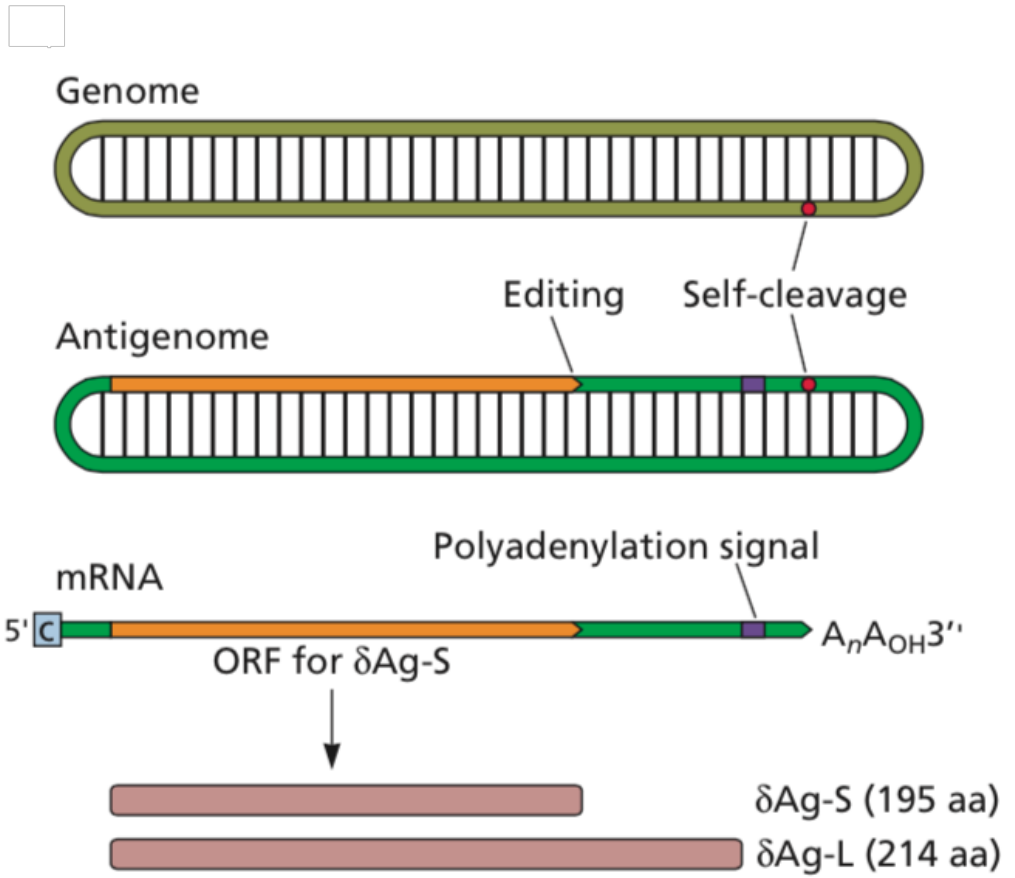
- Properties of viroid and satellite
- Helper virus is hepatitis B virus
- Increases severity of HBV liver disease

HDV global distribution

- 18 million people HDV infected; 5% of 350 million carriers of HBV
- Declining in Europe, Asia-Pacific region highly prevalent



HDV genome

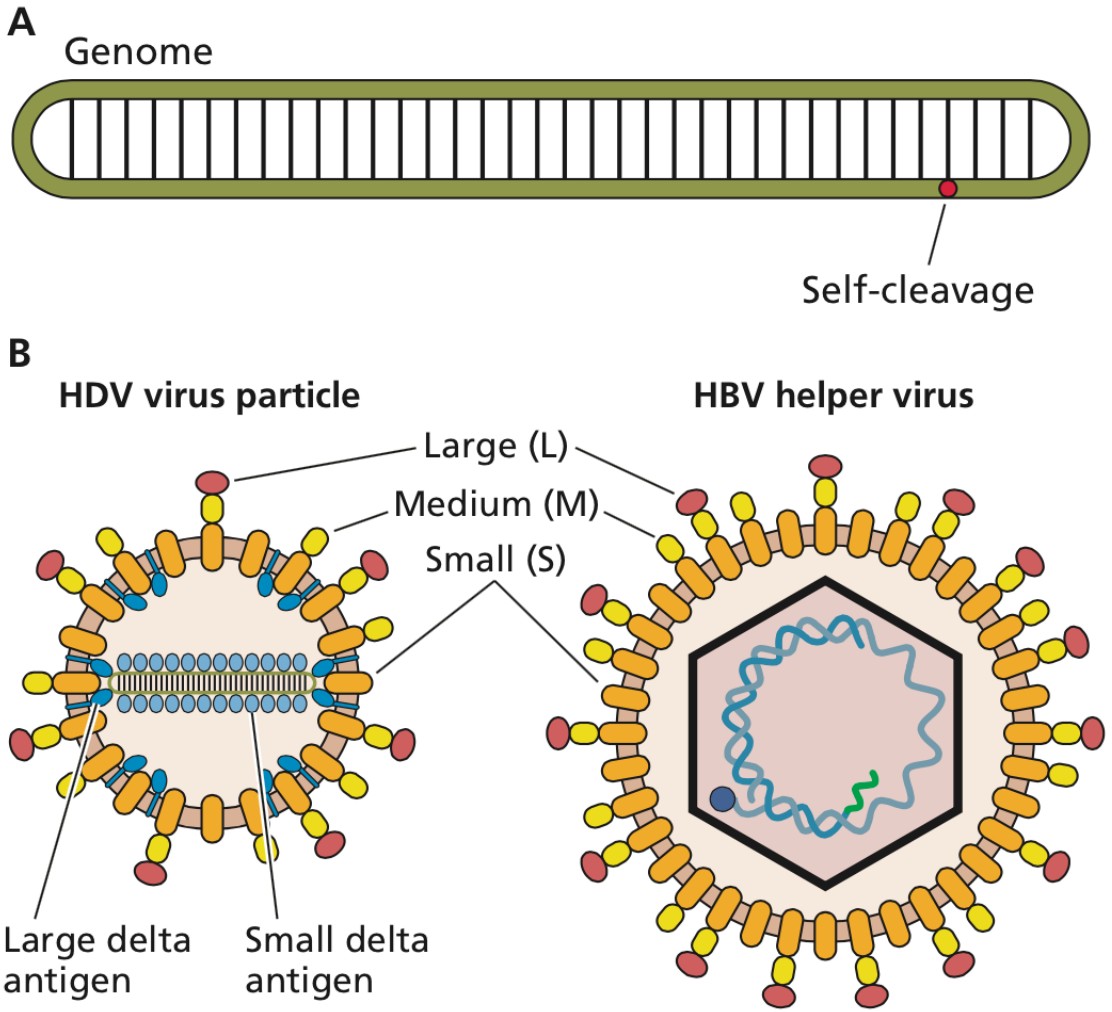


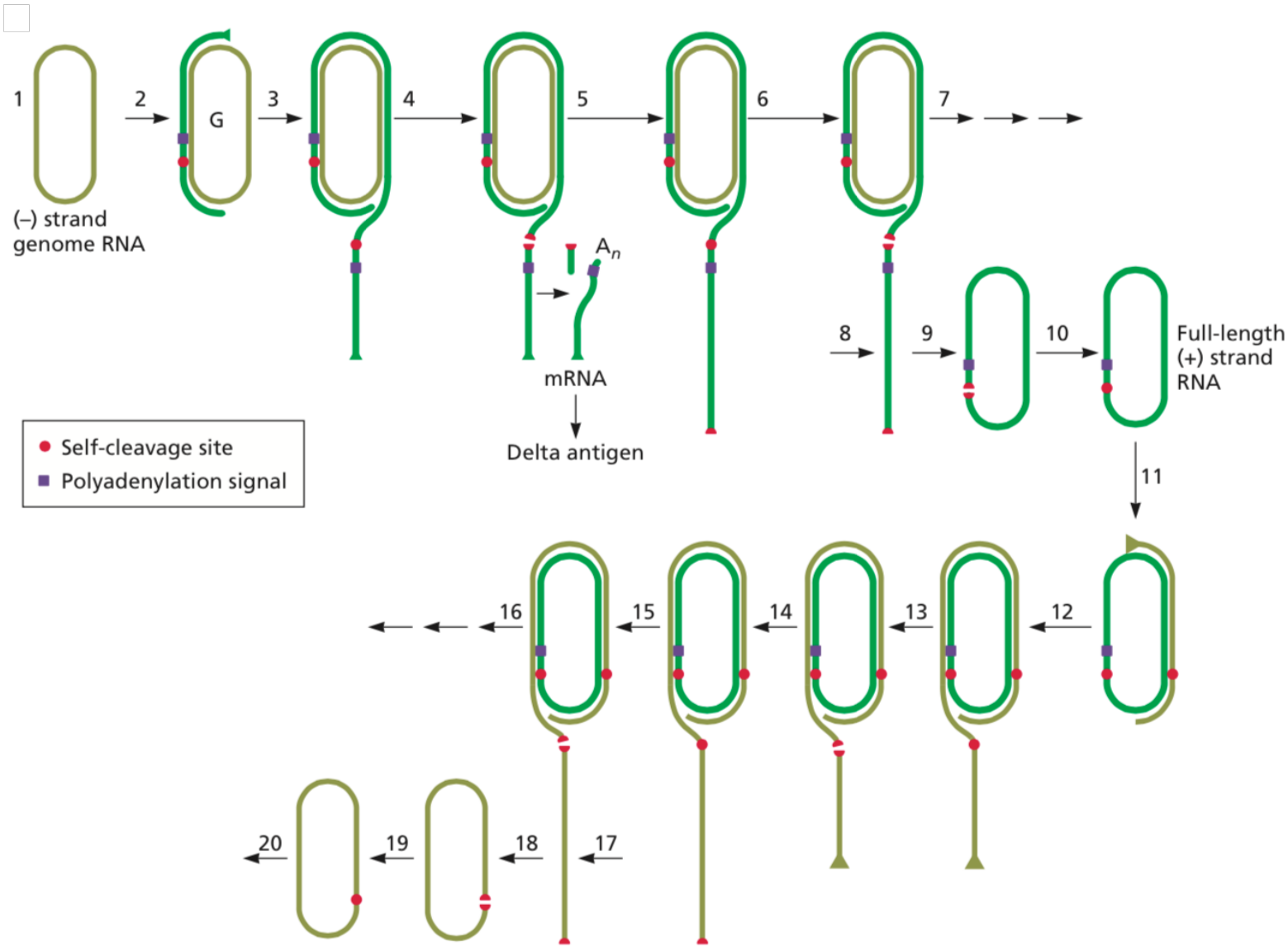
1.7 kb

ribozyme

Principles of Virology, ASM Press

HDV and HBV


A
Genome
Self-cleavage
B
HDV virus particle
HBV helper virus
Large (L)
Medium (M)
Small (S)
Large delta antigen
Small delta antigen

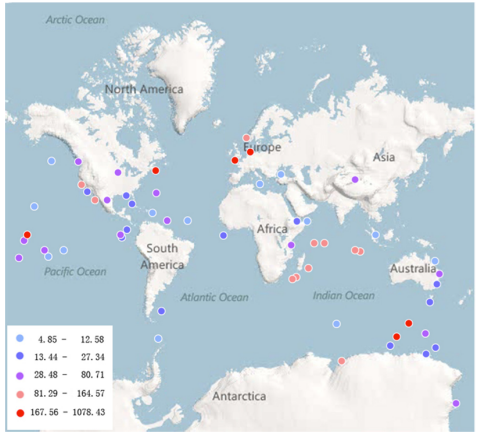

1
2
G
3
4
5
6
7
(–) strand
genome RNA
A_n
mRNA
Delta antigen
8
9
10
Full-length
(+) strand
RNA
11
12
13
14
15
16
17
18
19
20
Self-cleavage site
Polyadenylation signal

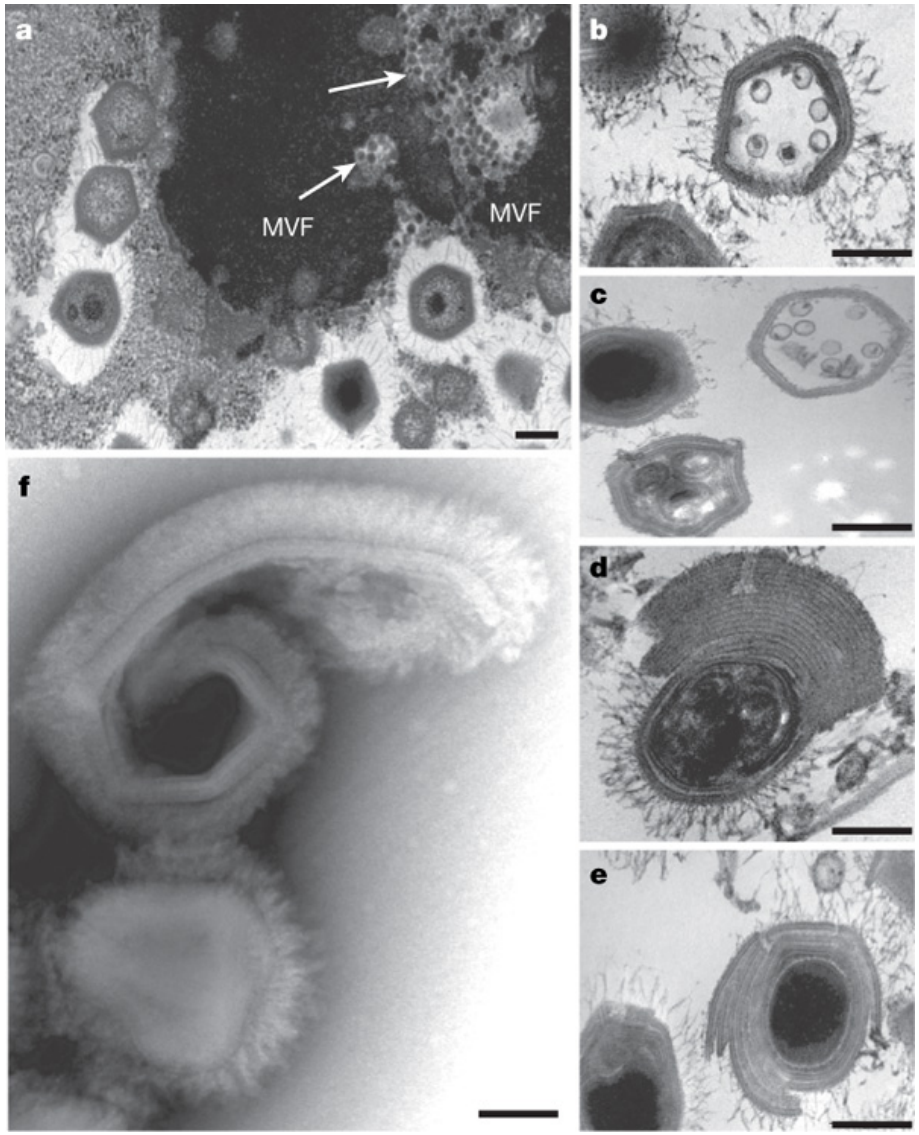
Virophages

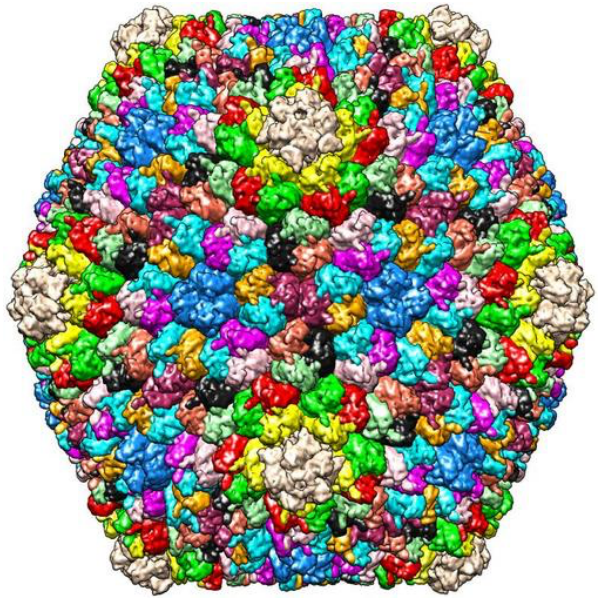
- Derived from bacteriophage (*phagein* = Greek, to eat), means ‘virus eater’
- Circular dsDNA viruses, icosahedral
- Replicate only in cells infected with a giant virus
- Interfere with helper virus replication

Virophages

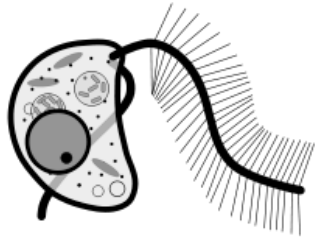
Virophage	Location	Host		Genome	
		Virus	Eukaryote	Size (bp)	No. of ORFs
Sputnik	A cooling tower in Paris, France	*Acanthamoeba polyphaga* mimivirus	*A. polyphaga*	18,343	21
Mavirus	Coastal waters of Texas	*Cafeteria roenbergensis* virus	Marine phagotrophic flagellate (*C. roenbergensis*)	19,063	20
OLV	Organic Lake, a hypersaline meromictic lake in Antarctica	Large DNA viruses	Prasinophytes (phototrophic algae)	26,421	26
Sputnik 2	Contact lens fluid of a patient with keratitis, France	Lentille virus	*A. polyphaga*	18,338	20
YSLV1	Yellowstone Lake	Phycodna- or mimiviruses?	Microalgae?	27,849	26
YSLV2	Yellowstone Lake	Phycodna- or mimiviruses?	Microalgae?	23,184	21
YSLV3	Yellowstone Lake	Phycodna- or mimiviruses?	Microalgae?	27,050	23
YSLV4	Yellowstone Lake	Phycodna- or mimiviruses?	Microalgae?	28,306	34
ALM	Ace Lake in Antarctica	mimiviruses?	Phagotrophic protozoan?	17,767	22




a
MVF
MVF
b
c
d
e
f



Virophages



- Mavirus - virophage of giant virus of *Cafeteria roenbergensis*, a marine phagotropic flagellate
- Organic Lake virophage of phycodnaviruses that infect algae
- Gene exchangers?
- Impact on ocean ecology?

Are virophages satellites?

Like many autonomous viruses, they depend on transcriptional machinery, except in their case it is from another virus, not a host cell

Prions: Infectious proteins, no nucleic acid

- Prions in the news
  - *BSE, mad cow disease, CJD, scrapie, kuru, chronic wasting disease of deer and elk*
  - *1997 Nobel Prize in Medicine - Stanley Prusiner*

May 8, 1984
THE DOCTOR'S WORLD

THE DOCTOR'S WORLD; THE MYSTERY OF BALANCHINE'S DEATH IS SOLVED

By LAWRENCE K. ALTMAN, M.D.

IN a laboratory at Columbia-Presbyterian Medical Center a

few months ago, a pathologist leaned over a microscope and

peered at an illuminated slice of brain tissue about 10 microns thick. In the center of the minute specimen of brain cells was a pink circle, known as a kuru plaque, one sign of a strange family of diseases known as slow viruses.

Those brain cells were George Balanchine's, and therein lay the solution to the mystery of his death last year at 79 years of age. The once-athletic choreographer died after a period of several months that had been awash in pathos. He could hardly move, let alone dance, and he could hardly think, let alone choreograph.

Certainly, he was suffering from some degenerative neurological disorder. But what was it?

In the weeks following his death, pathologists determined that Mr. Balanchine had suffered from one of the world's most unusual diseases - Creutzfeldt-Jakob disease. It is categorized in the group of so-called slow virus diseases because of its extremely long incubation period.

http://www.nytimes.com/1984/05/08/science/the-doctor-s-world-the-mystery-of-balanchine-s-death-is-solved.html

Transmissible spongiform encephalopathies

- Encephalopathy - disease of the brain
- Fatal neurodegenerative disorders of mammals
- Thousands of humans diagnosed each year, 1% arise by infection
- By 2002, 120 humans had contracted Creutzfeld-Jacob disease, from consumption of meat from animals with BSE

Transmissible spongiform encephalopathies

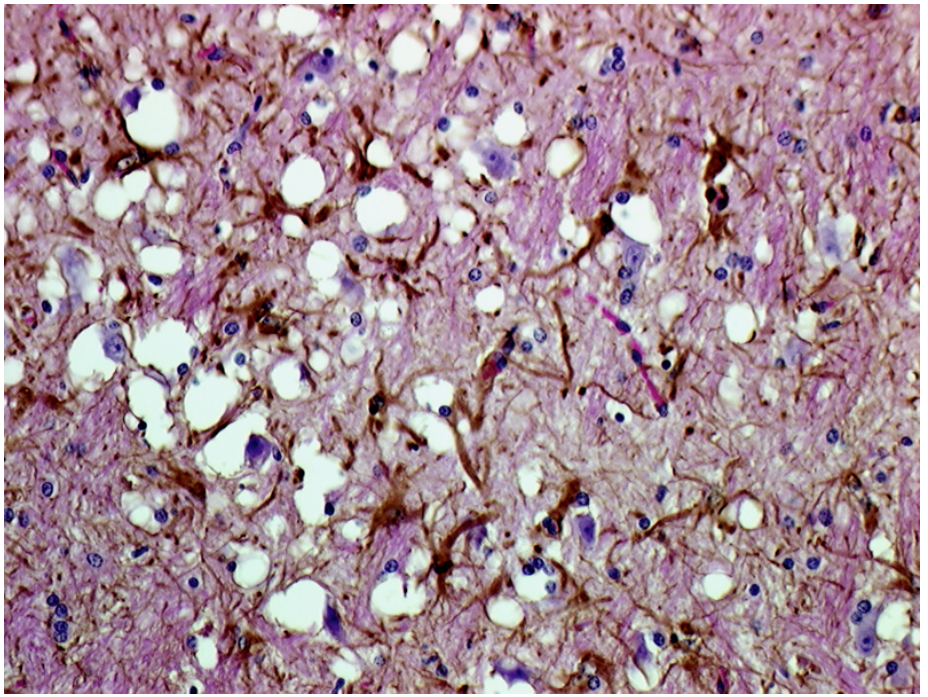
- TSE diseases of animals
  - Bovine spongiform encephalopathy (BSE) (“mad cow disease”)
  - Chronic wasting disease (CWD) (deer, elk, moose)
  - Exotic ungulate encephalopathy (EUE) (nyala and greater kudu)
  - Feline spongiform encephalopathy (FSE) (domestic and great cats)
  - Scrapie in sheep and goats
  - Transmissible mink encephalopathy (TME)

Transmissible spongiform encephalopathies

- TSE diseases of humans
  - Creutzfeldt-Jakob disease (CJD)
  - Fatal familial insomnia (FFI)
  - Gerstmann-Sträussler syndrome (GSS)
  - Kuru
  - Variant CJD disease (vCJD)

Spongiform

- Infected brain has sponge-like holes throughout
- Severe psychomotor dysfunction
- Symptoms depend on which part of the brain is damaged
- Each disease has a characteristic symptomatology and pathology

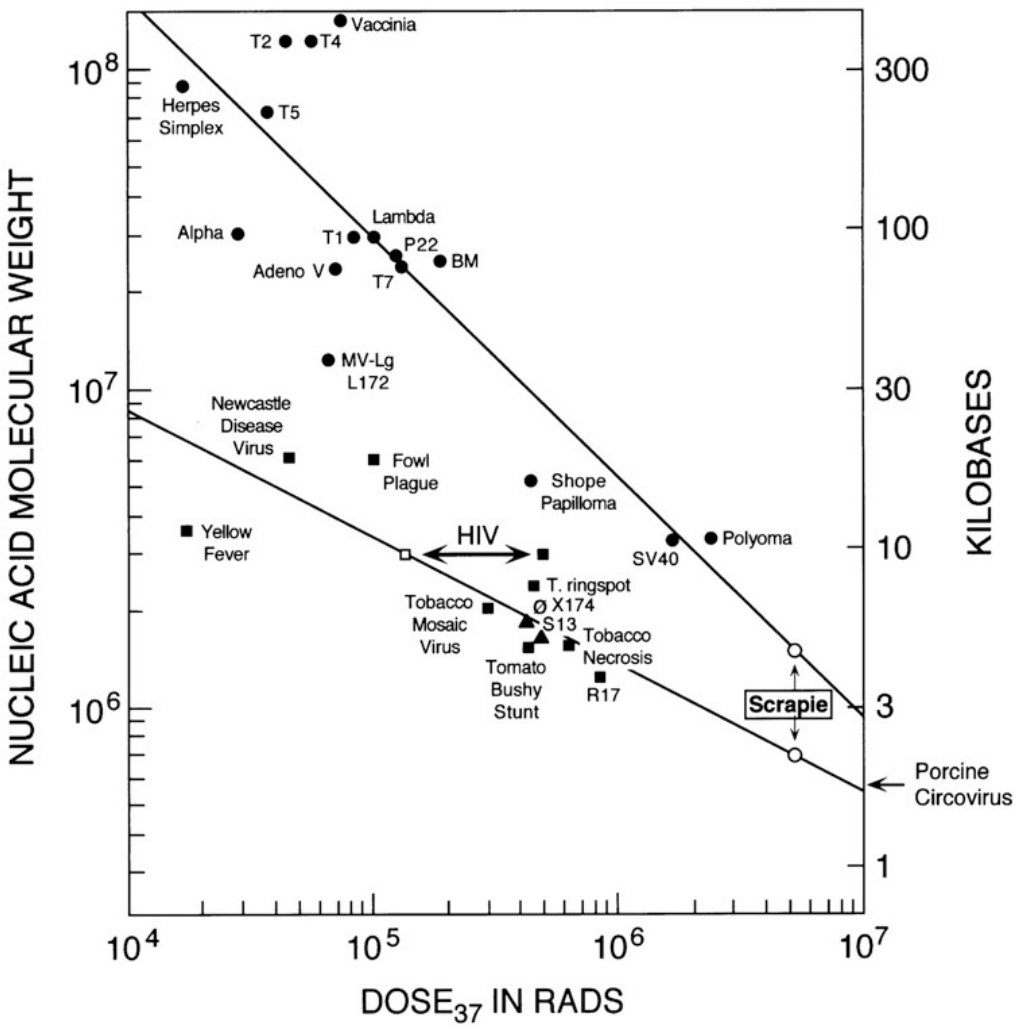


Scrapie

- First TSE recognized
- Infected sheep rub on fences
- Motor disturbances, uncontrollable trembling (*tremblant du mouton*), paralysis, weight loss, death 4-6 weeks
- Recognized as disease of European sheep for over 250 years
- Endemic in some countries: UK, 1% of sheep/yr

Scrapie

- Sheep farmers found that animals could transmit scrapie to healthy herds: infectious agent
- 1939: infectivity from sheep brains shown to pass through filters which pass only viruses
- Agent is highly resistant to UV, ionizing radiation, formaldehyde
- Believed not to contain nucleic acid; clearly not typical infectious agents



Nature 308:658, 1984

TSEs

- Animal and human TSEs exhibit same histochemical abnormalities
  - *Defect in plasma membrane formation*
  - *Vacuolation of neurons, astrocytes, oligodendrocytes*
  - *Loss of neurons in gray matter of brain*
  - *Spongiform appearance*
  - *Accumulation of glial fibrillary acidic protein in clumps*
  - *Amyloidosis in brain; fibrils of amyloid precursor protein*

TSE pathogenesis

- Agent detected by injection of organ homogenates into susceptible species
- Cerebellar ataxia, dementia, death after many months or years
- Agent first accumulates in lymphoreticular and secretory organs, then spreads to the CNS
- In CNS, pathology includes astrocytosis, vacuolization (spongiform), loss of neurons
- No inflammatory, antibody, or cellular response

TSEs

- Undetected before symptoms develop
- Untreatable; no way to alleviate symptoms
- Invariably fatal

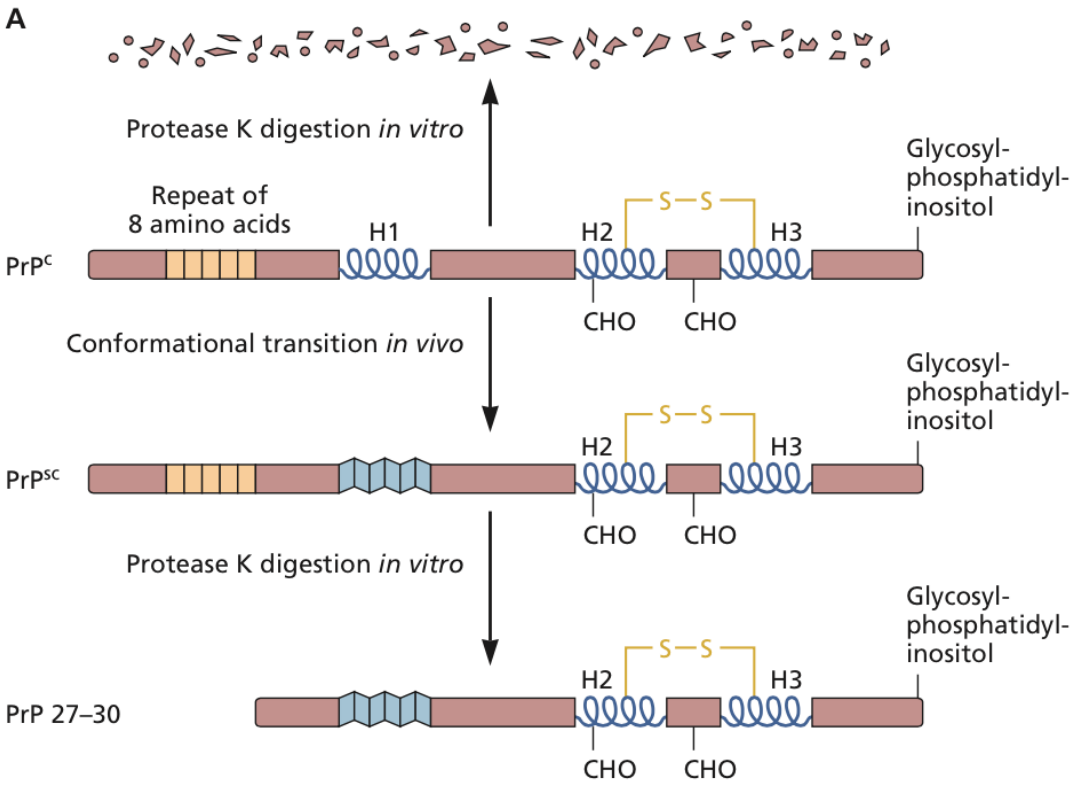
Prions

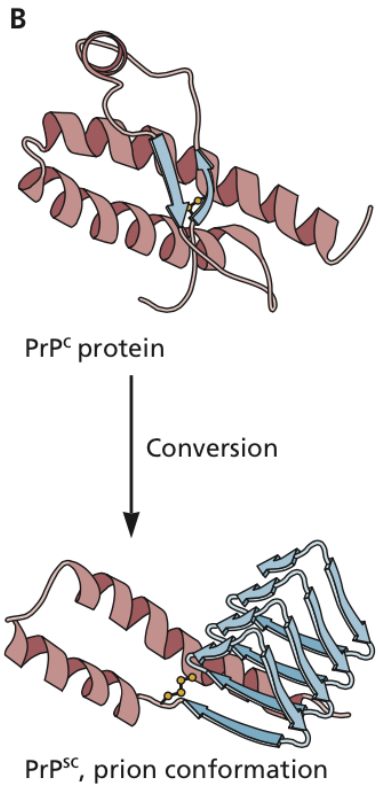
- 1967 Griffith suggested that TSE agents were protein
- 1981 Prusiner identified infectious protein complexes in scrapie brain, purified protein, transmitted to animals
- Called the agent a *prion*, (*pr*oteinaceous and *in*fectious particle)
- Encoded by *prnp* gene, essential for pathogenesis of TSEs

Current view of prions

- Pathogenic prion is a conformational isoform of a normal host protein, PrP^{c}
- PrP^{c} is found predominantly on the outer surface of neurons, GPI anchor
- The abnormal conformer, when introduced into the organism, causes conversion of normal PrP^{c} into the pathogenic conformation (PrP^{sc} for scrapie)

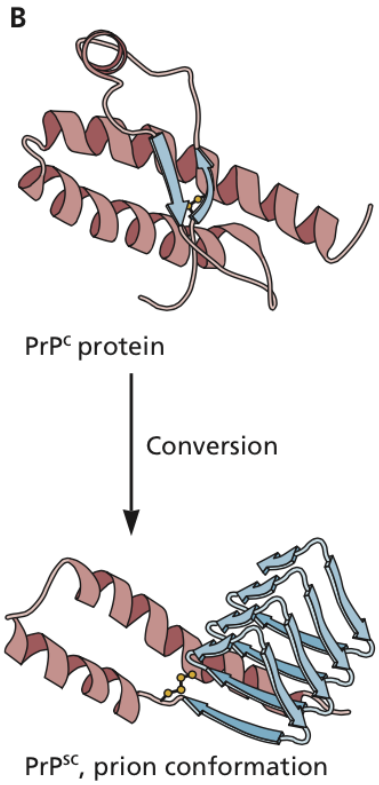
PrP^c to PrP^{sc}



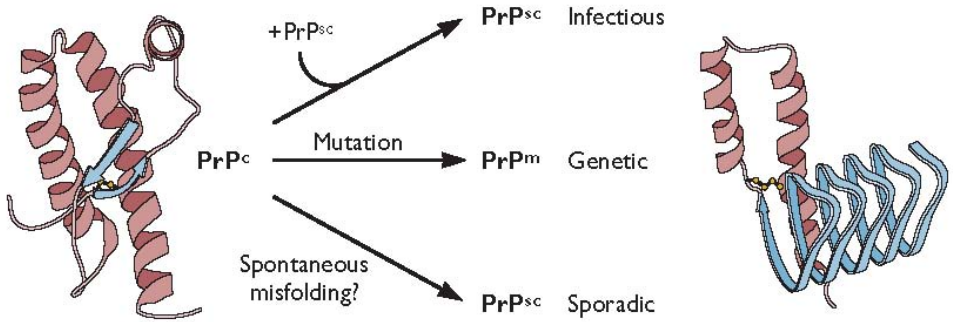


Prion hypothesis

- Mice lacking both copies of *prnp* are resistant to infection
- PrP^{sc} can be introduced ('infection') or produced by rare mutations in *prnp*
- PrP^{sc} accumulates in CNS, leading to symptoms

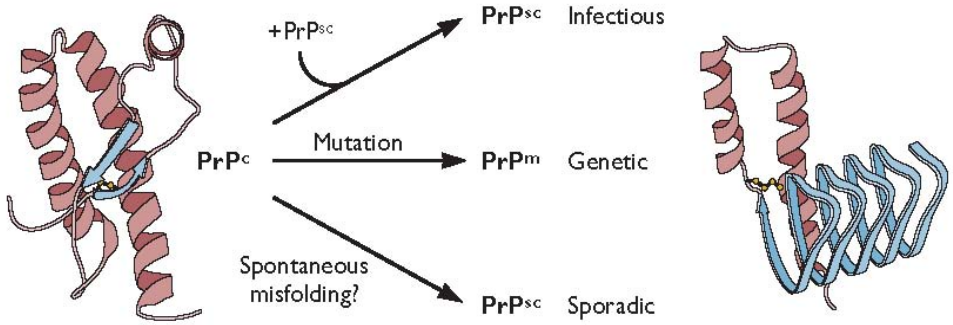


Three types of spongiform encephalopathies



- *Infectious* or transmissible spongiform encephalopathy
- *Familial* (genetic) spongiform encephalopathy
- *Sporadic* spongiform encephalopathy
- All three diseases can be transmitted experimentally to animals by inoculation or ingestion of infected tissue

Human TSE

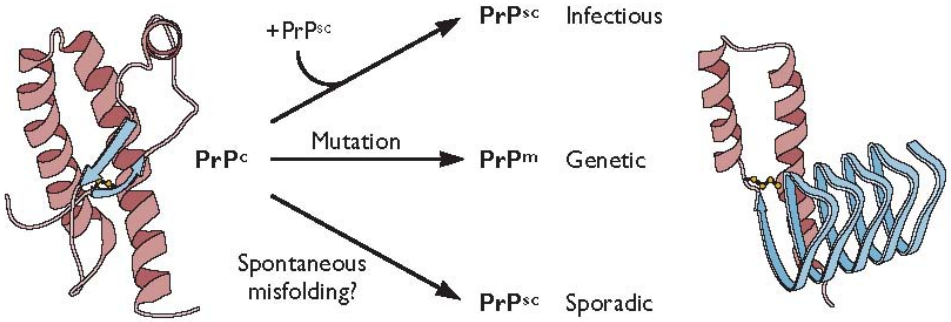


- Infectious or transmissible
  - Kuru
  - Iatrogenic spread by transplantation of infected corneas, hormones, transfusion from patients with CJD
  - BSE: feeding infected animals to cattle
  - Variant CJD (new human disease): eating BSE beef

Human TSE

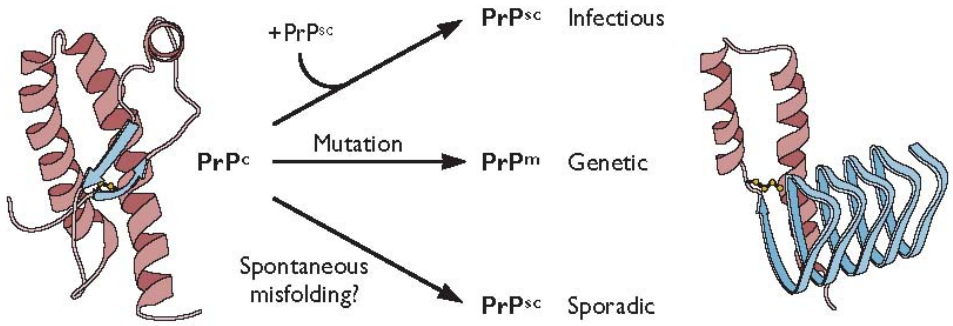
- Kuru: fatal encephalopathy found in Fore people of New Guinea
- 30 year incubation period
- Found by Carleton Gajdusek to spread among women and children through ritual cannabalism of brains of deceased relatives
- When cannabalism ceased, so did kuru

Sporadic CJD



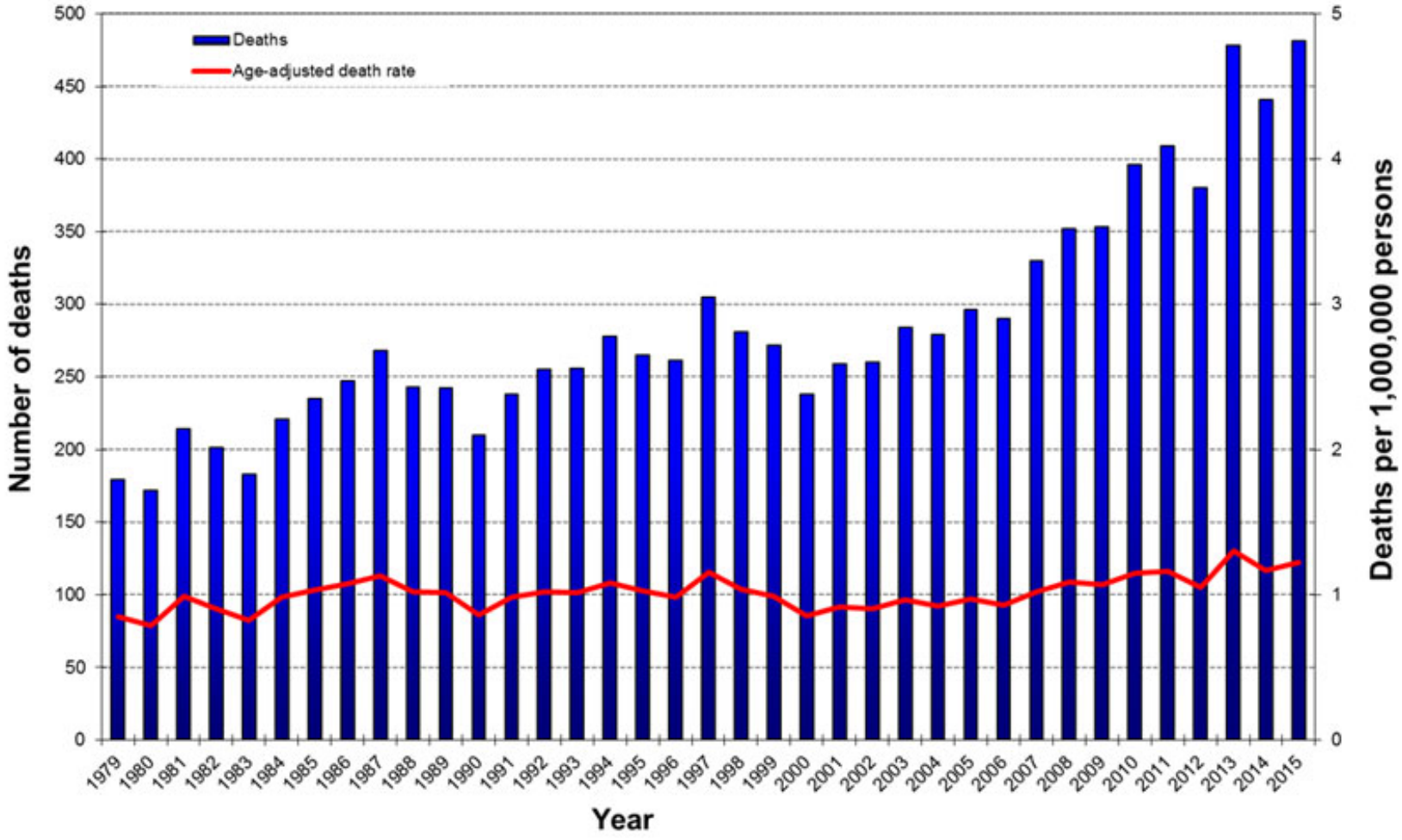
- Affects 1-2/1 million humans worldwide, 50 -70 years of age; 65% of TSE
- Disease appears with no warning or epidemiological indications
- Patients have normal *prnp* genes
- Can be transmitted to others leading to CJD
- Kuru may have been established in New Guinea by eating brain of person with sporadic CJD

Familial spongiform encephalopathy



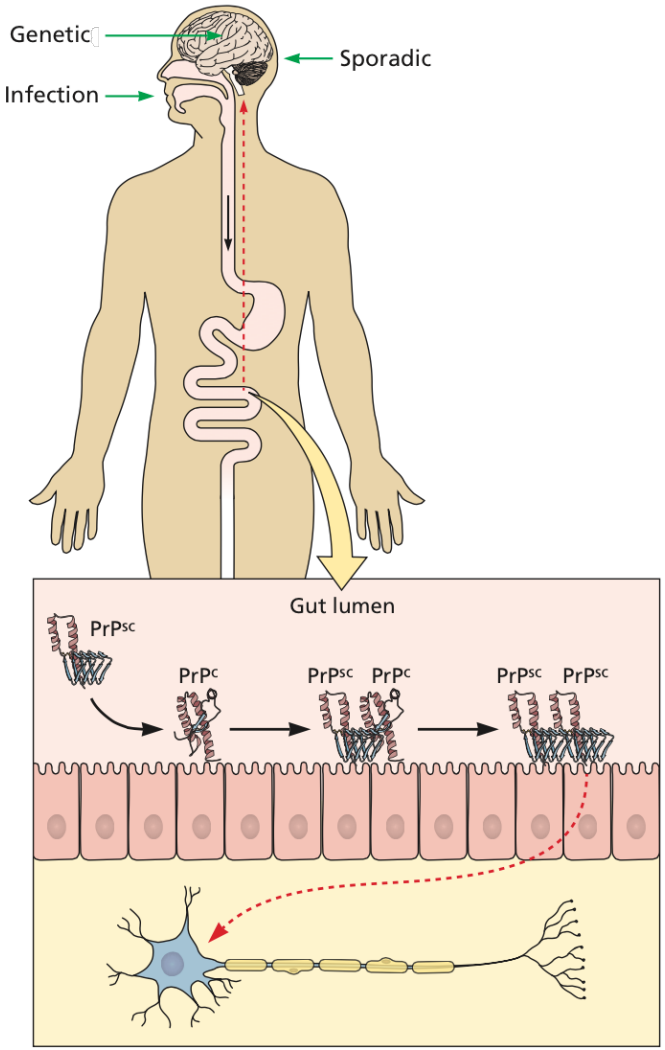
- An inherited disease
- Autosomal dominant mutation in *prnp* gene
- Organs, corneas, blood products from people afflicted with sporadic CJD can be infectious, transmit CJD to others

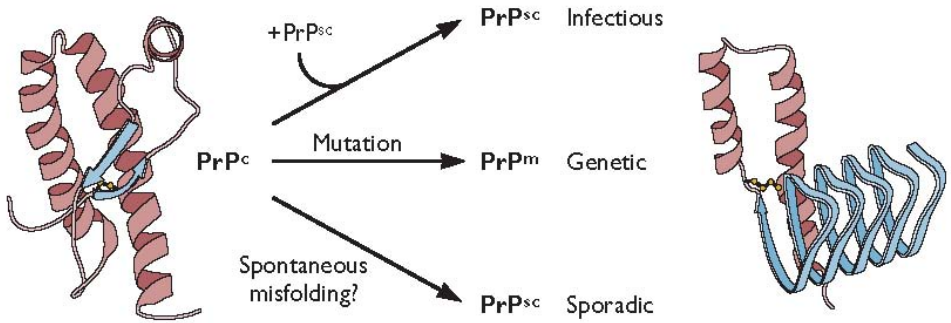
CJD deaths, US, 1979-2015



https://www.cdc.gov/prions/cjd/occurrence-transmission.html

Spongiform encephalopathies and prion protein





Principles of Virology, ASM Press

Forced cannibalism spreads BSE

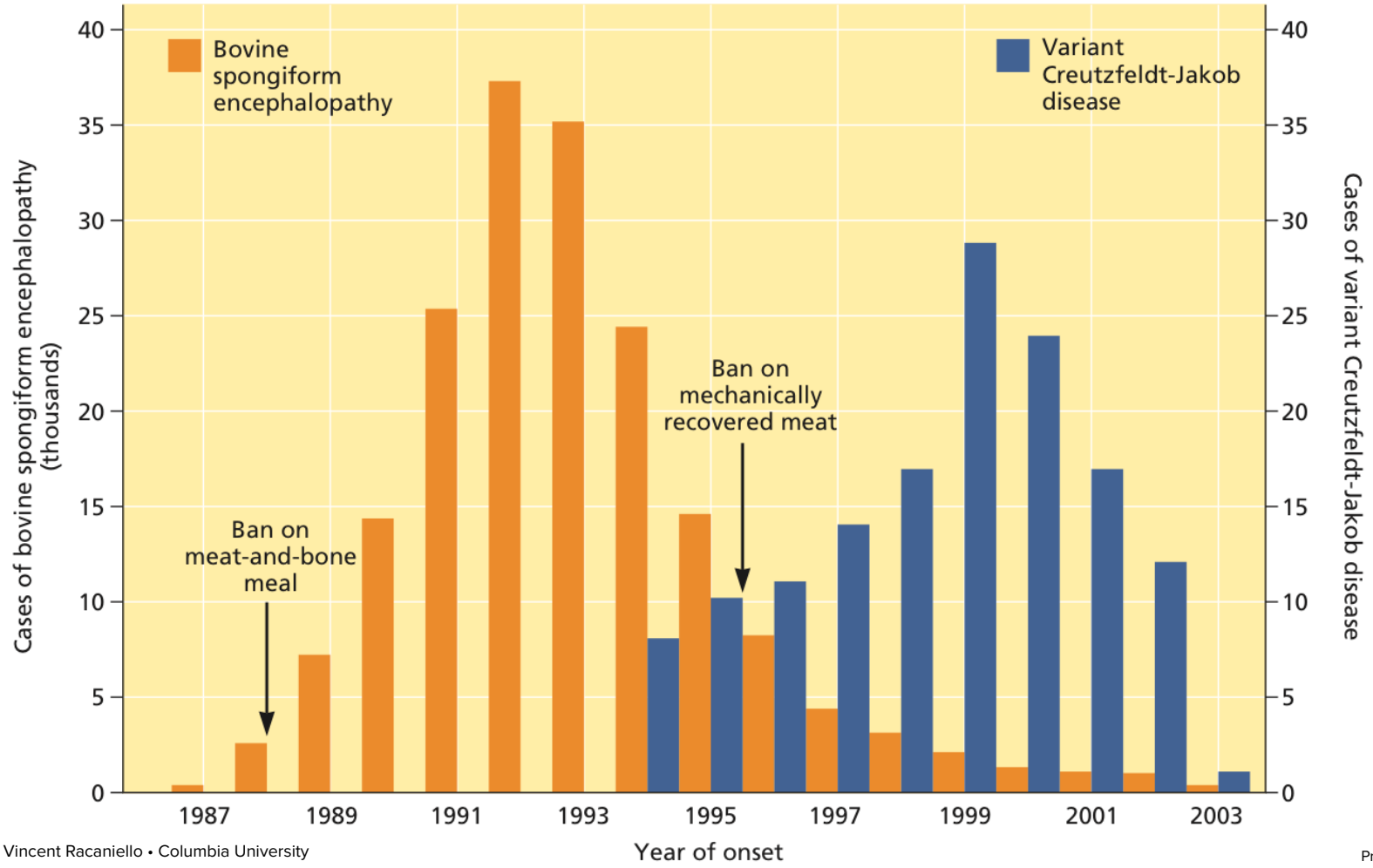
Epidemic spread of bovine spongiform encephalopathy (BSE, mad cow disease) among British cattle was a form of cannibalism

Forced cannibalism spreads BSE

- Resulted from the practice of feeding processed animal byproducts (including sheep with scrapie) to cattle as protein supplements
- In the 1970s method of preparing MBM changed, allowed scrapie proteins to survive and pass into cows
- Mad cow disease stopped when animal byproduct feeding stopped
- Strong evidence that consumption of BSE-infected beef transmits bovine TSE to humans
- Variant CJD (new disease): eating BSE beef

1-2 million cattle were infected with prions

Incubation time 5 yr, slaughtered 2-3 yr

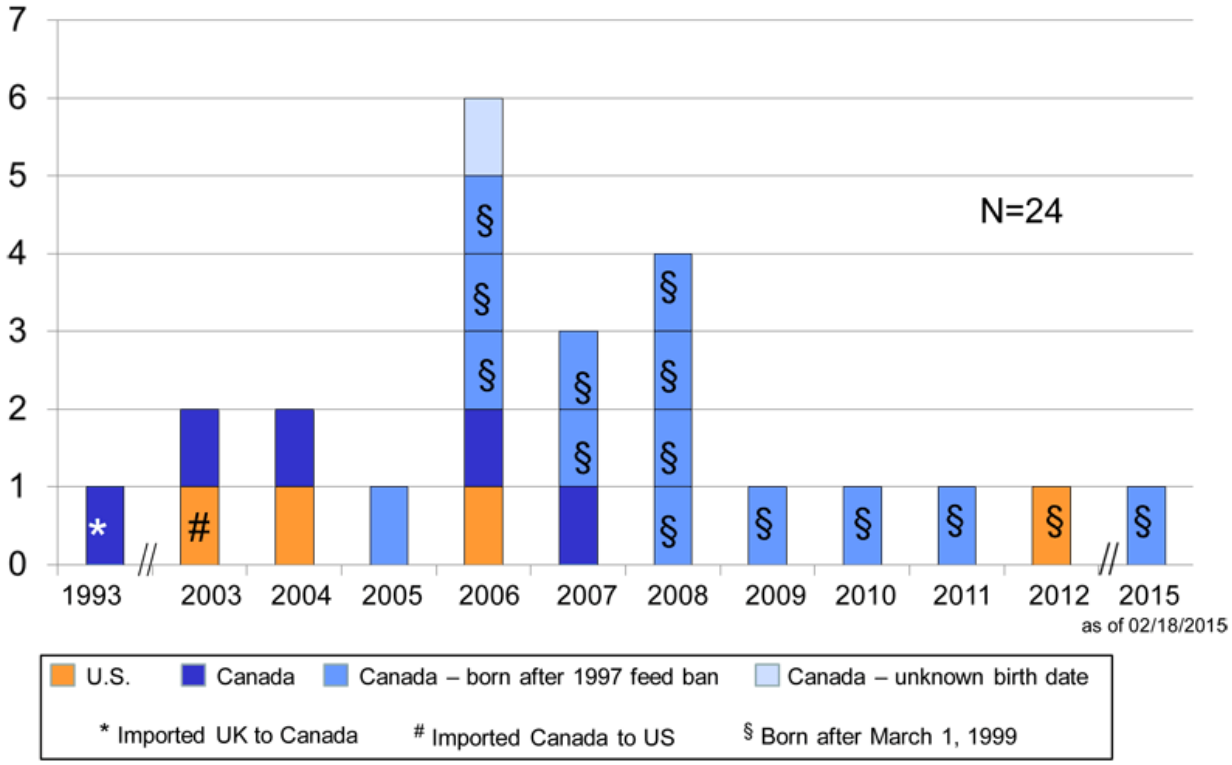


Principles of Virology, ASM Press

Prions in the food supply

- New cases of BSE in cattle still occur
- Most are likely to be sporadic
- Efforts are aimed at protecting the food supply, but in US and Canada <2% of slaughtered cattle are tested
- Diagnostic tests have been developed
- Screening for drugs that block accumulation of prions in cultured cells

BSE Cases in North America, by Year and Country of Death, 1993 – Feb 2015

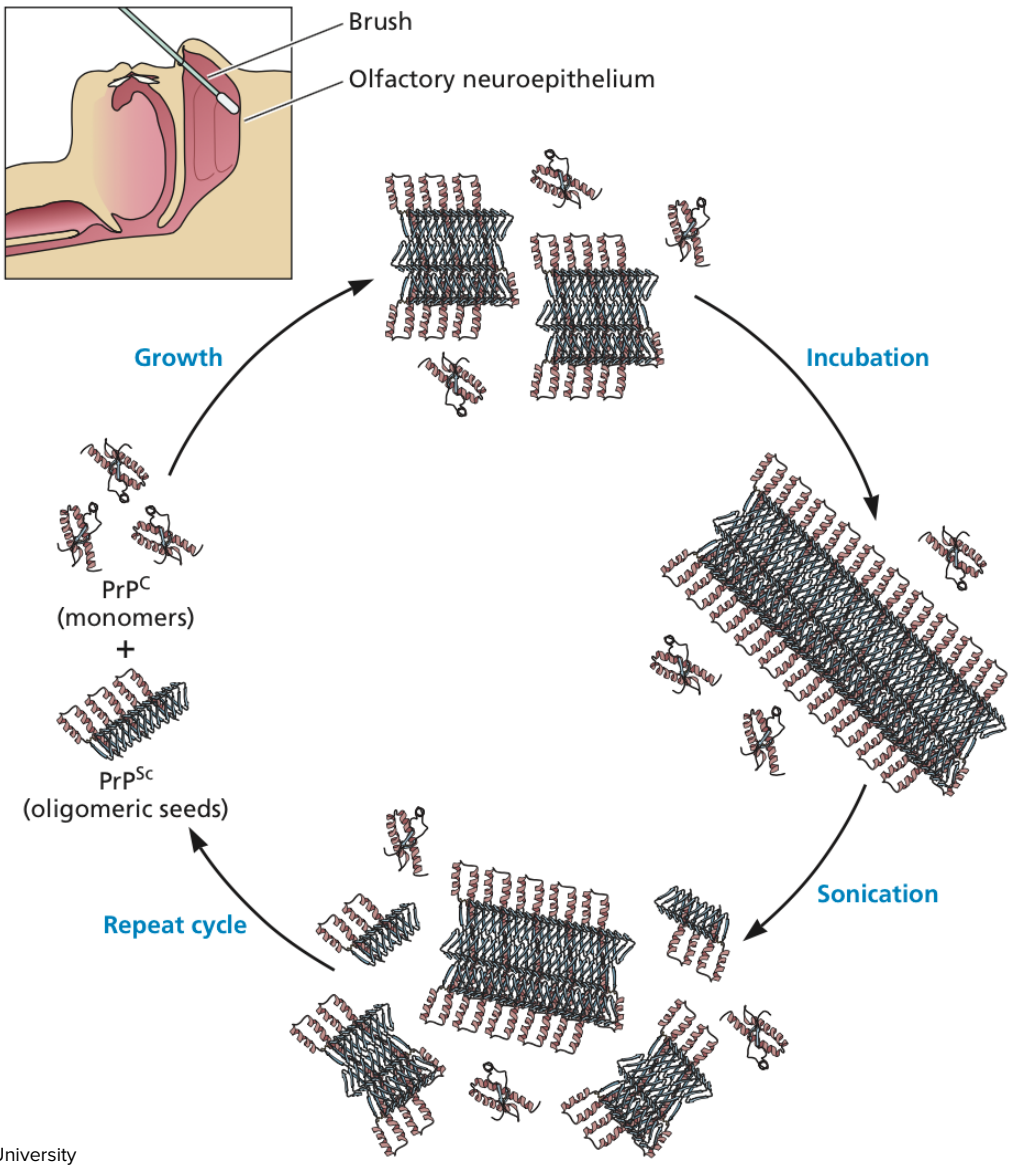


Case of prion disease from contaminated beef

http://www.virology.ws/2015/10/01/a-case-of-prion-disease-acquired-from-contaminated-beef/

Prion contamination in the emergency room

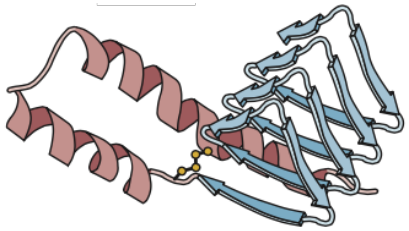
http://www.virology.ws/2015/10/08/prion-contamination-in-the-emergency-room/


Brush
Olfactory neuroepithelium
Incubation
Sonication
Repeat cycle
Growth
PrP^C
(monomers)
+
PrP^Sc
(oligomeric seeds)

Prion species barrier

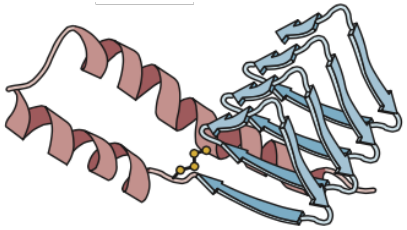
- Inoculation of diseased brain material into same species reproduces disease
- Inoculation into different species is inefficient
- Sequences of PrP^{sc} in inoculum and PrP^{c} in host should be isologous
- Barrier to interspecies transmission is in the sequence of PrP protein

Prion species barrier



No disease

Hamster PrP^{sc}

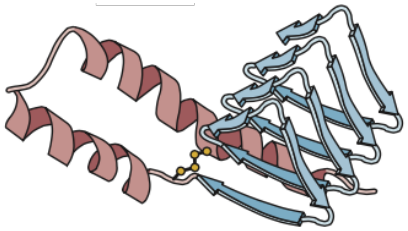


Disease

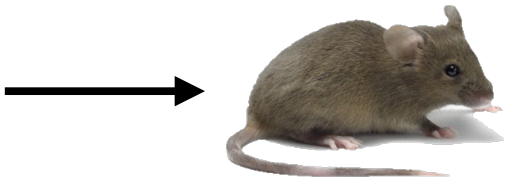
Hamster PrP^{sc}

transgenic for hamster *prnp*

Prion species barrier



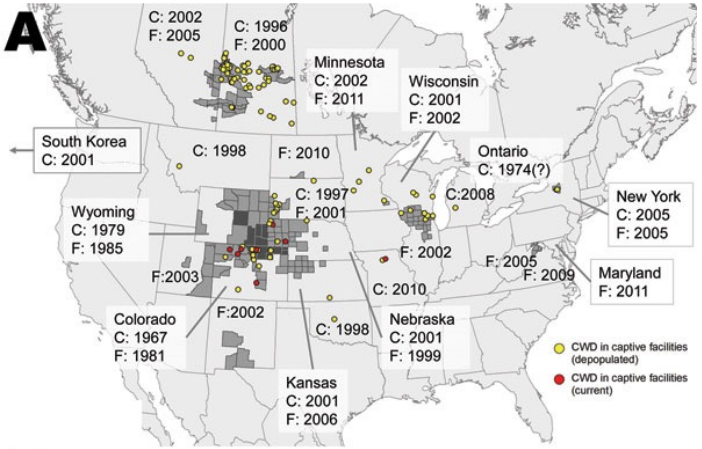
Bovine PrP^{sc}

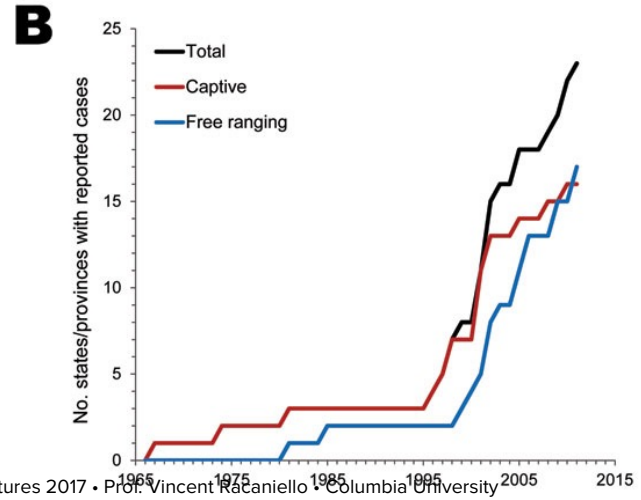


Disease

- BSE PrP^{sc} has broad host range, infects many meat eating mammals including humans
- Clearly some prions overcome the influence of primary sequence on host range
- This is why BSE is a concern

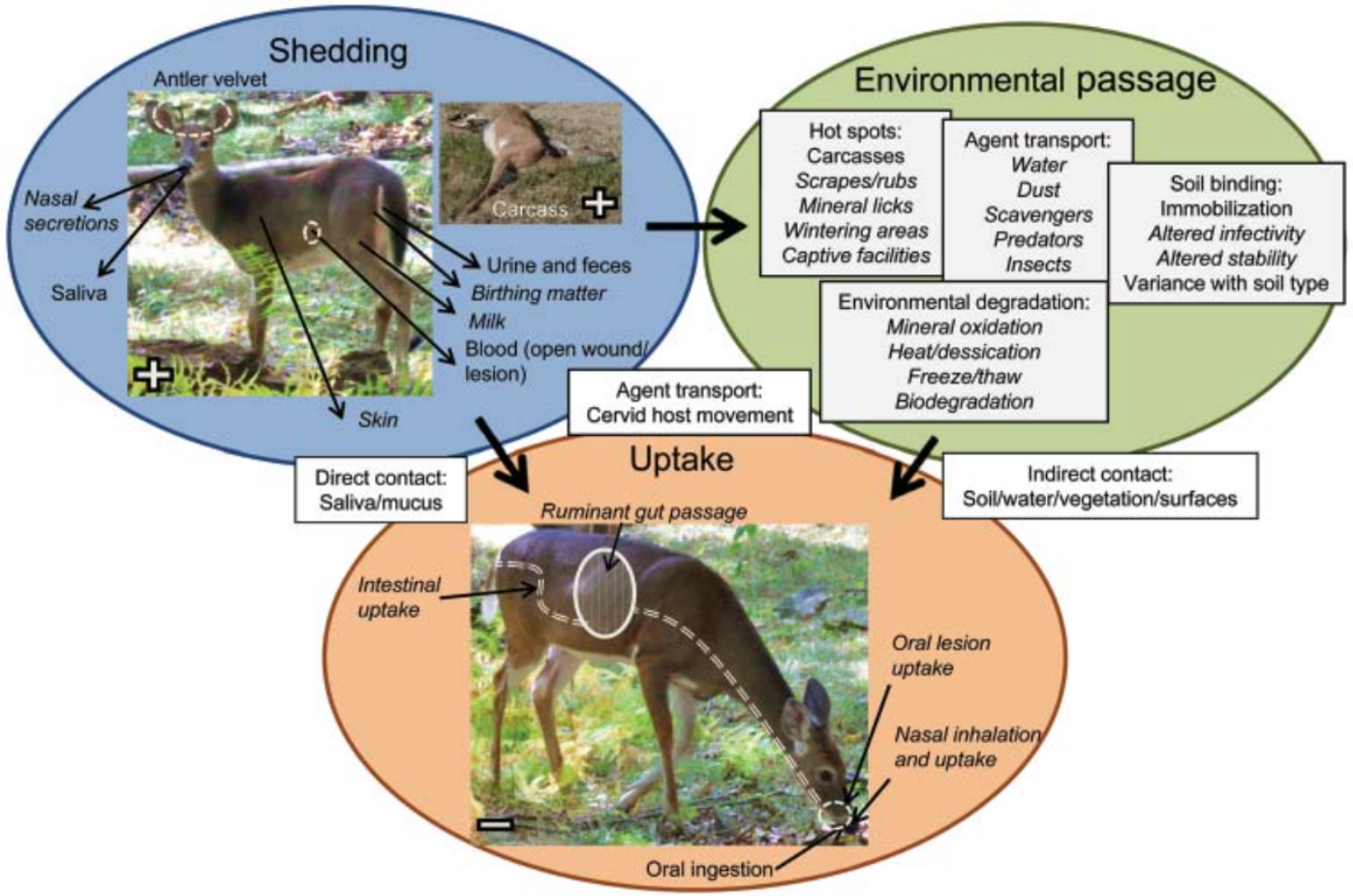
Chronic wasting disease - TSE of deer, elk, moose



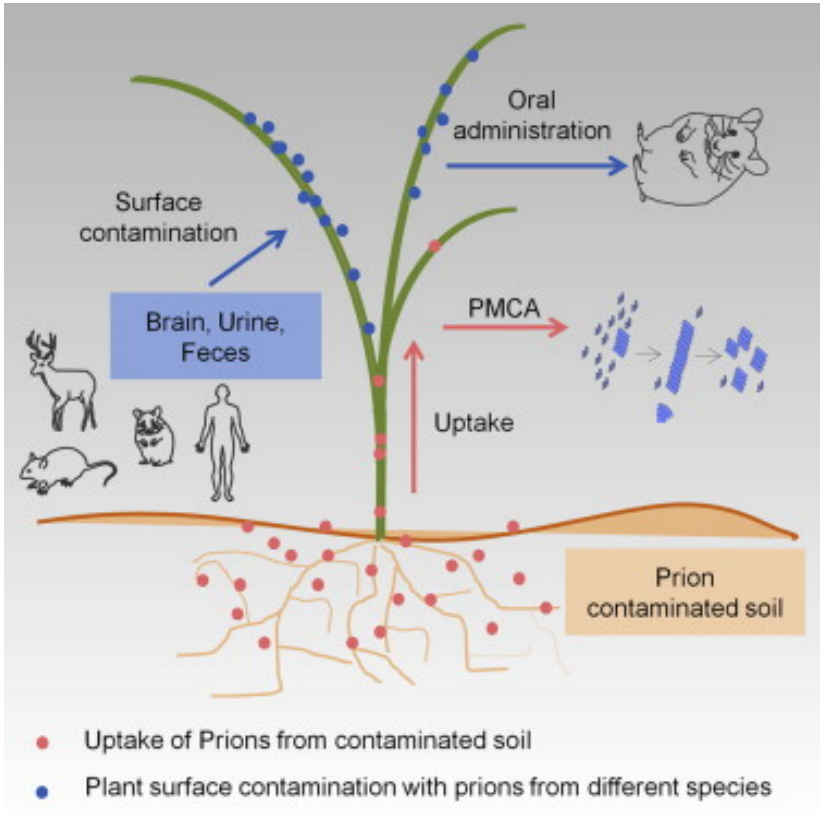


- In standing herds up to 90% of mule deer and 60% of elk are positive
- Incidence in wild cervids as high as 15%

Chronic wasting disease

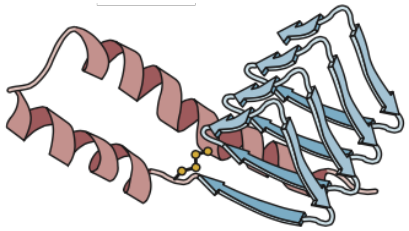


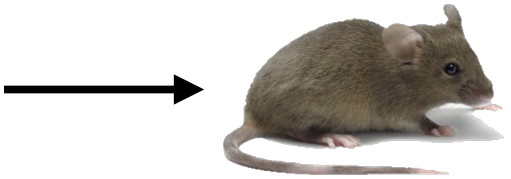
Grass plants bind, retain, take up, and transport infectious prions



http://www.sciencedirect.com/science/article/pii/S2211124715004374

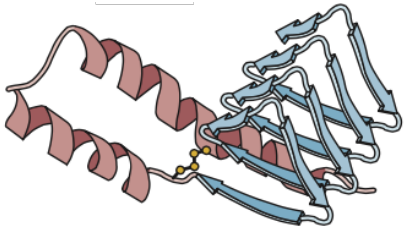
Chronic wasting disease

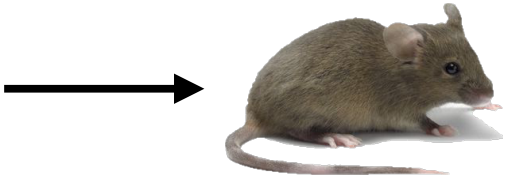




No disease

Cervid PrP^{sc}



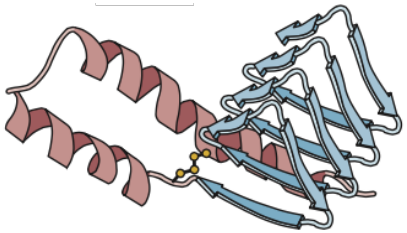


Disease

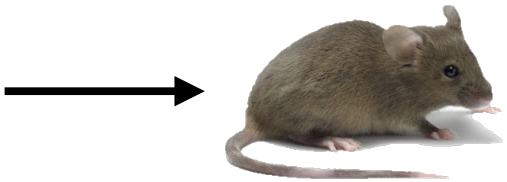
Cervid PrP^{sc}

transgenic for cervid *prnp*

Chronic wasting disease



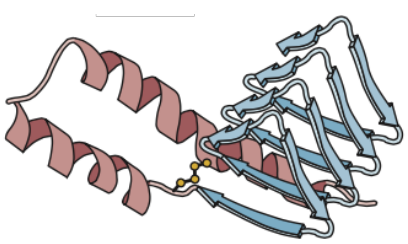
Cervid PrP^{sc}



Transgenic for human *prnp*

No disease

Chronic wasting disease



Deer PrP^{sc}

IC

Disease

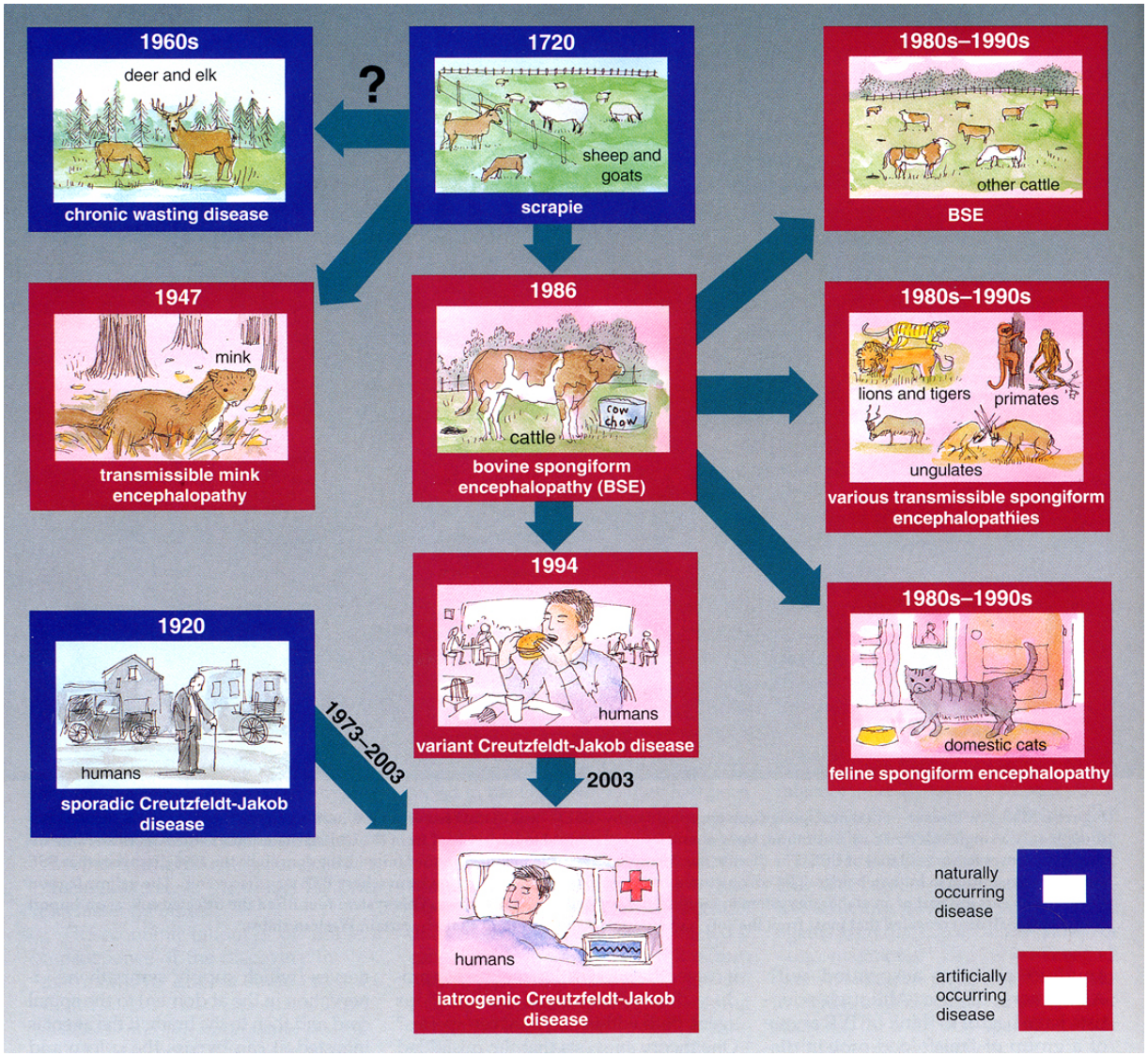
Could CWD prions transmit to cattle grazing in contaminated pastures?

Hunters beware!

http://www.cwd-info.org/

- Do not shoot, handle, or consume any animal that is acting abnormally or appears to be sick
- Wear latex or rubber gloves when field dressing deer or elk
- Bone out meat. Don’t saw through bone, avoid cutting through brain or spinal cord
- Minimize handlng of brain and spinal tissues
- Wash hands and instruments thoroughly after field dressing
- Don’t eat brain, spinal cord, eyes, spleen, tonsils, lymph nodes
- Don’t eat meat from animal that tests positive

Or sporadic?


1960s
deer and elk
chronic wasting disease
?
1720
sheep and goats
scrapie
1980s–1990s
other cattle
BSE
1947
mink
transmissible mink encephalopathy
1986
cow chow
cattle
bovine spongiform encephalopathy (BSE)
1980s–1990s
lions and tigers
primates
ungulates
various transmissible spongiform encephalopathies
1994
humans
variant Creutzfeldt-Jakob disease
1980s–1990s
domestic cats
feline spongiform encephalopathy
1920
humans
sporadic Creutzfeldt-Jakob disease
1973–2003
2003
humans
iatrogenic Creutzfeldt-Jakob disease
naturally occurring disease
artificially occurring disease