



VIROLOGY LIVE

WITH VINCENT RACANIELLO

Unusual infectious agents

Session 24

Virology Live

Fall 2021

So come up to the lab and see what's on the slab

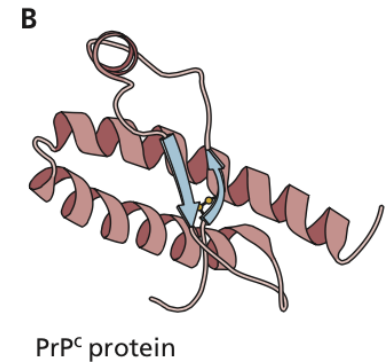
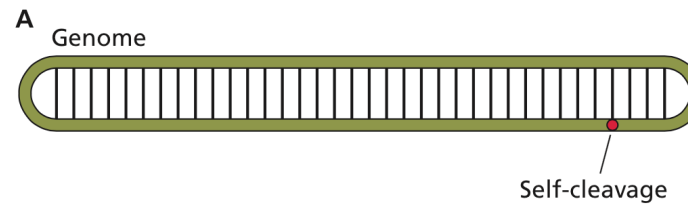
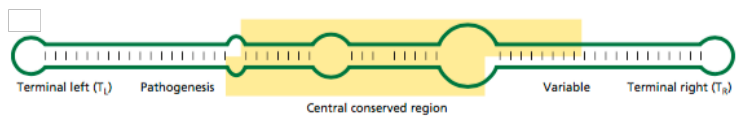
- DR. FRANK-N-FURTER

The Rocky Horror Picture Show

A fundamental question

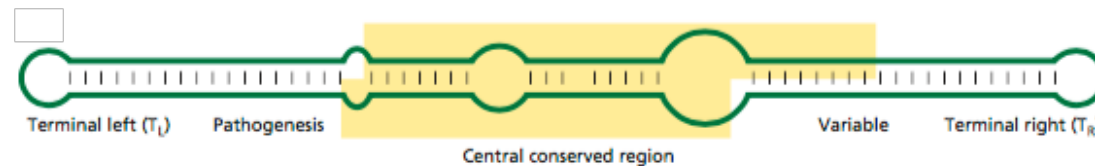
What is the minimum genome size needed to sustain an infectious agent?

Could an infectious agent exist without ANY genome?



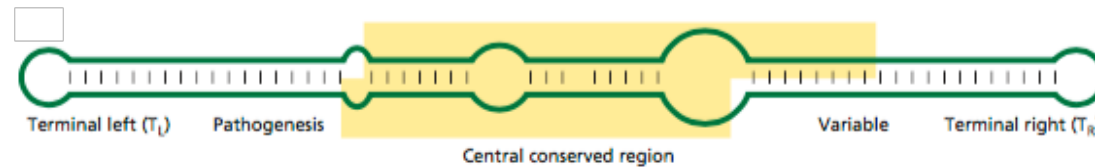
Viroids, satellites, and prions provide answers

Viroids



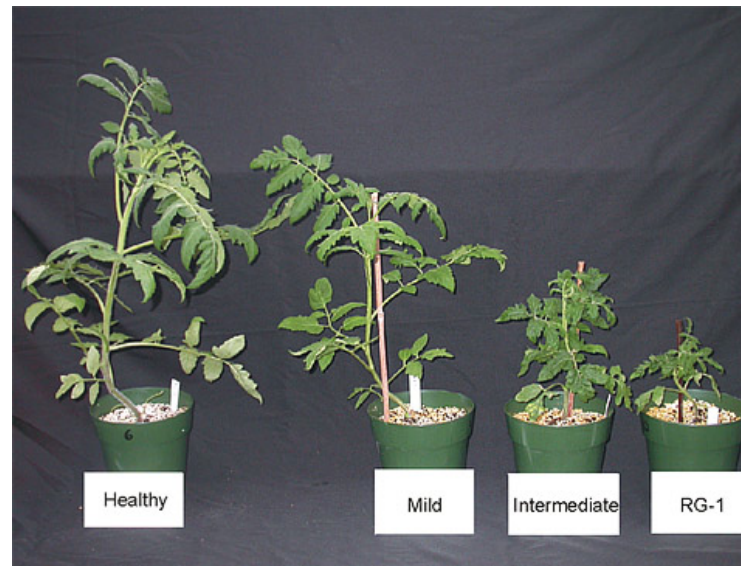
- Circular ssRNA, 120-475 nt
- RNA displays extensive internal base-pairing, appears as 50 nm rod in EM
- No protein coding regions
- No protective coat, yet migrate from host to host (no receptors required)
- Replicate when introduced into plants
- Families *Pospiviroidae* (replicate in nucleus) and *Avsunviroidae* (replicate in chloroplasts)

Viroids



- 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



*Production of potato seed stocks

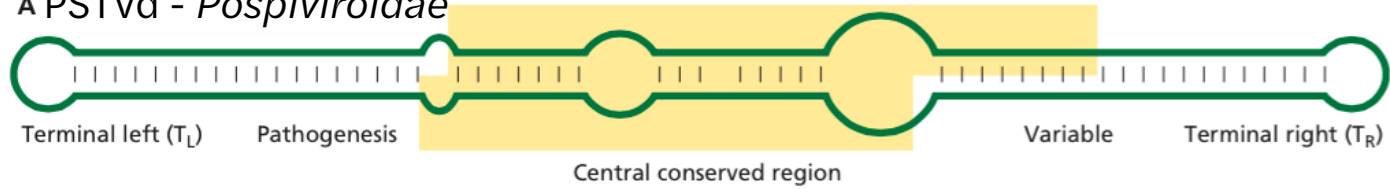
Some of my favorite viroids

- 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



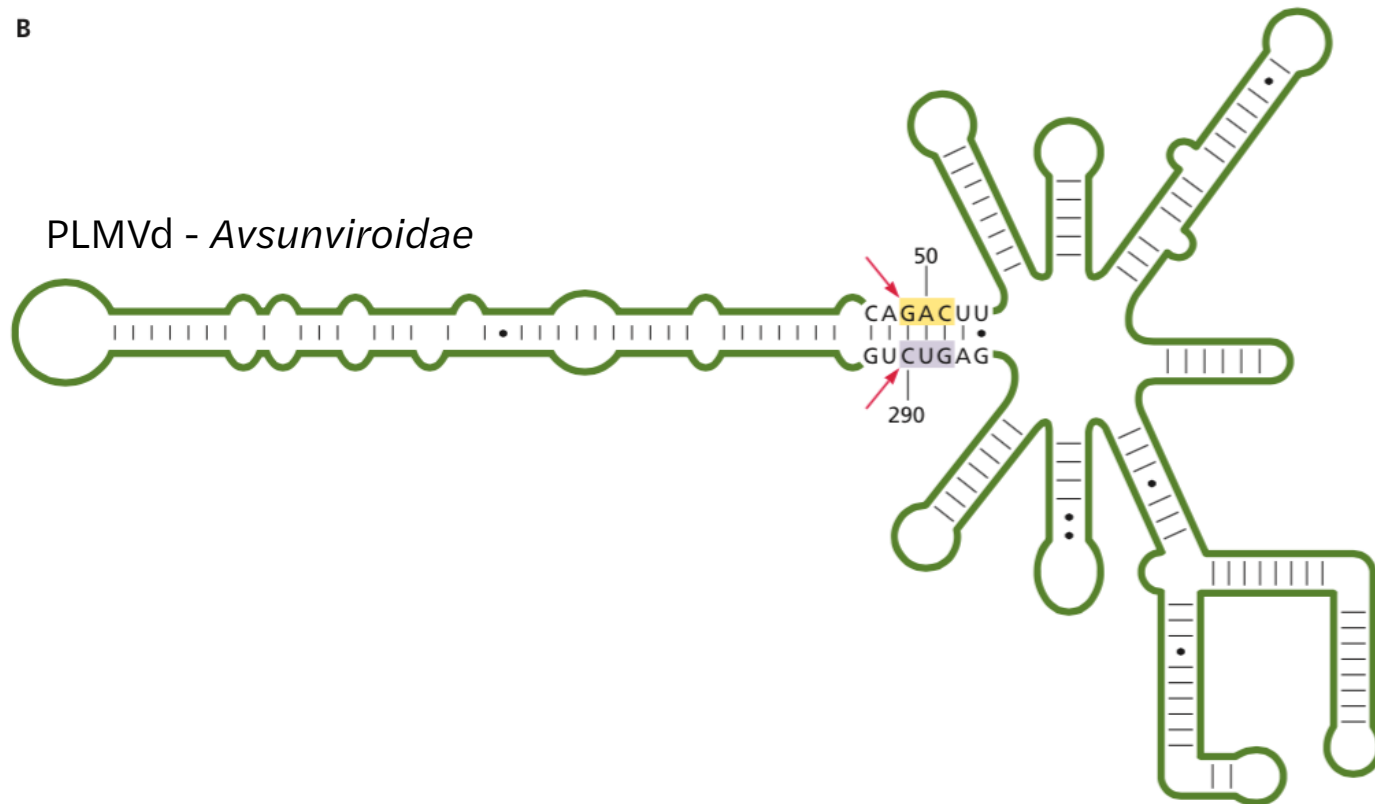
Functional regions of viroid RNA

A PSTVd - *Pospiviroidae*

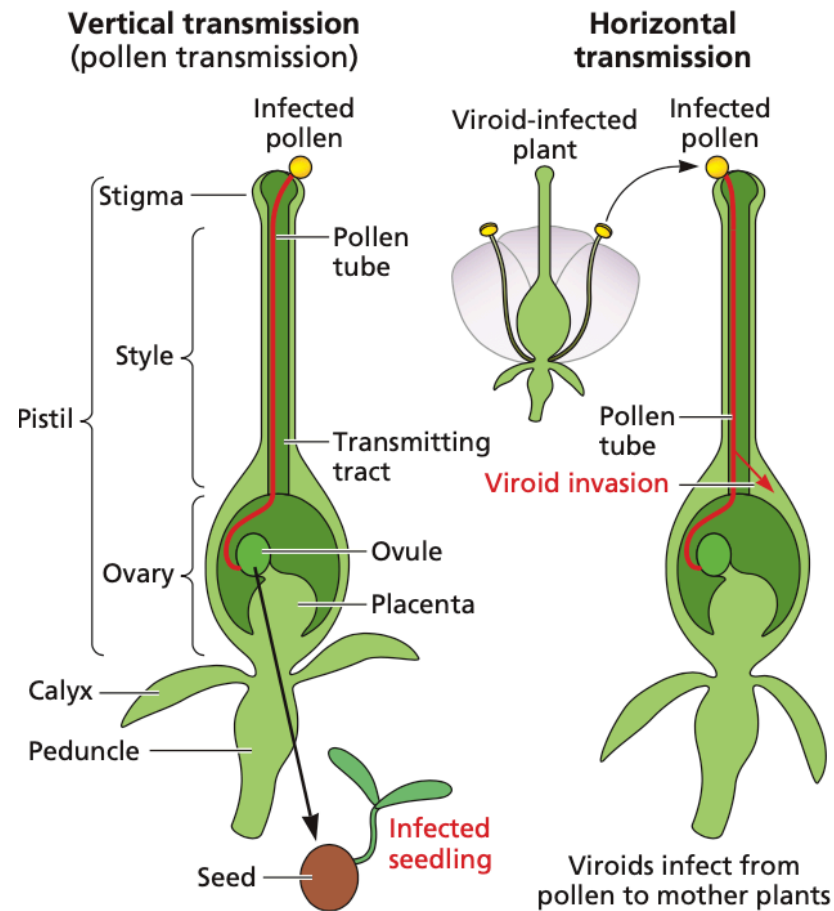


B

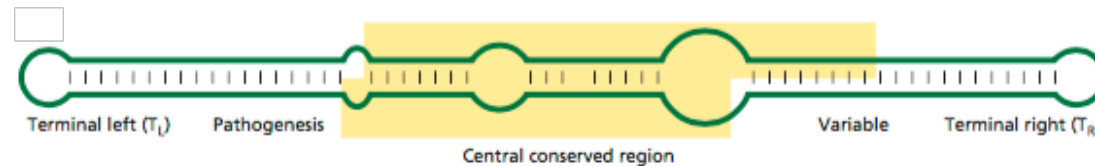
PLMVd - *Avsunviroidae*



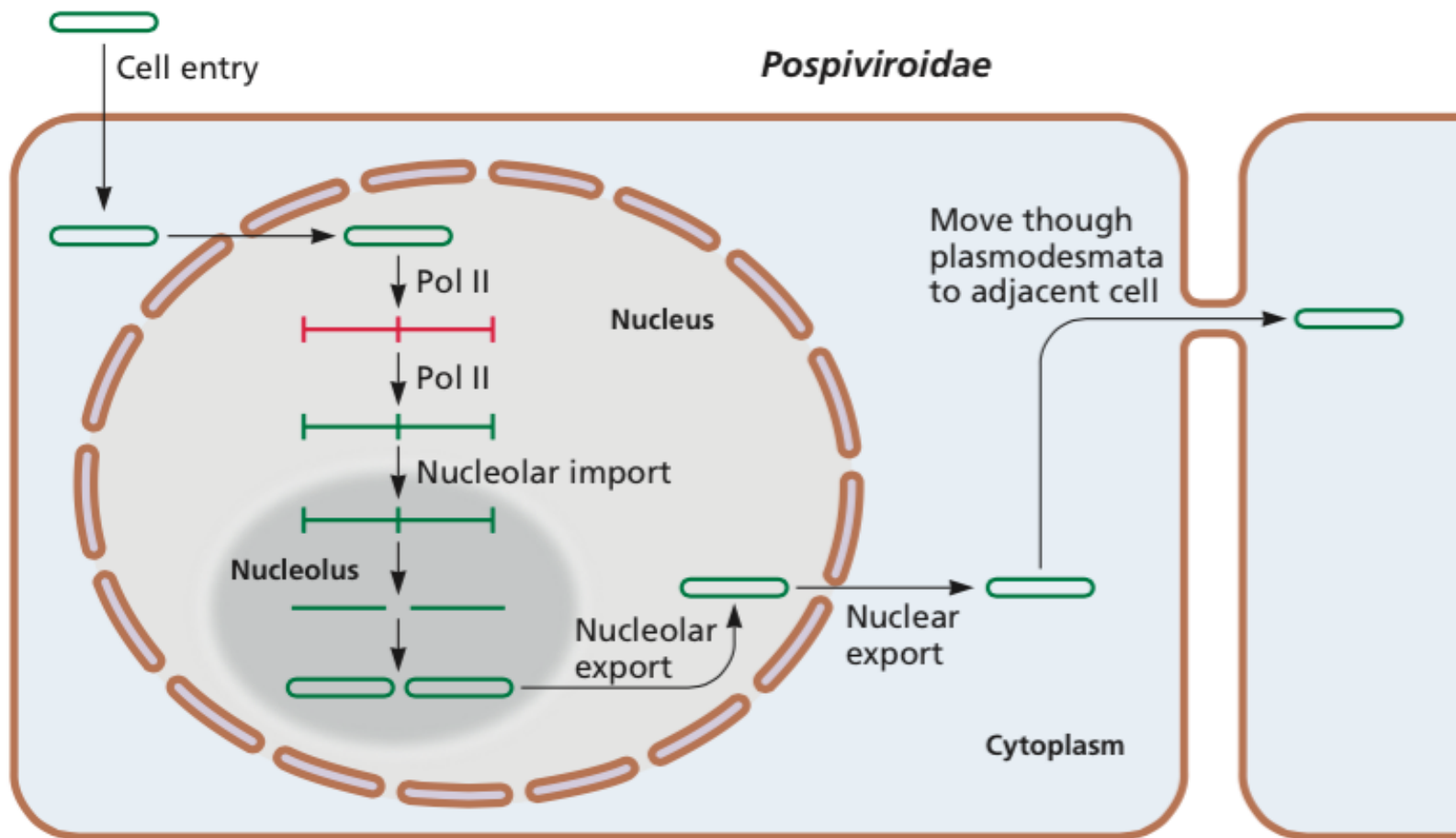
Vertical and horizontal transmission of viroids in plants via pollen

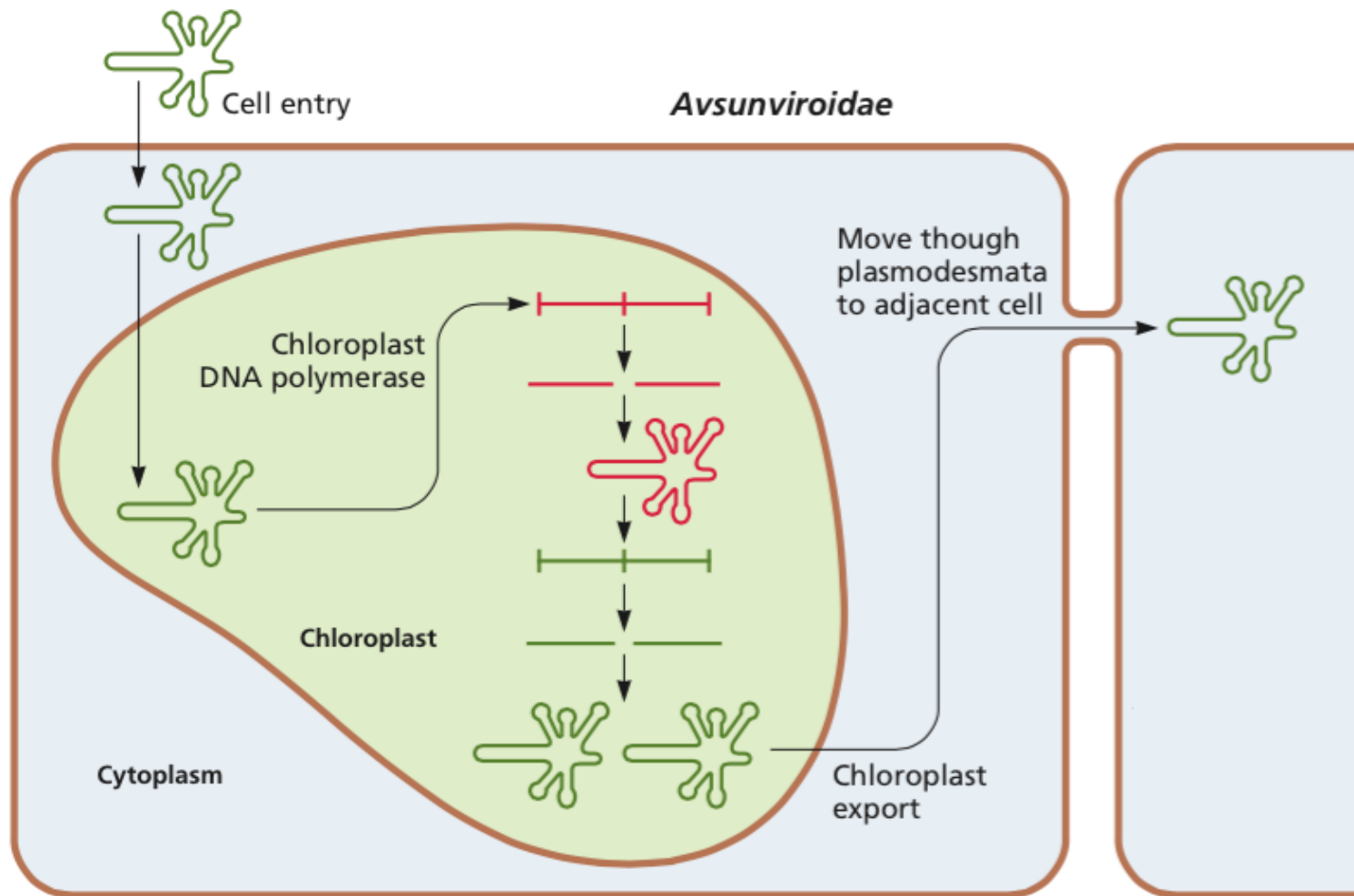


How do viroids replicate?



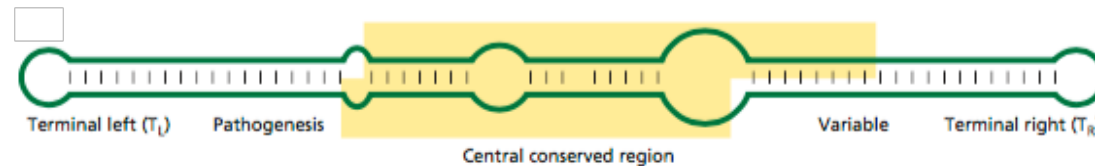
- Concatamers of viroid RNA produced by host RNA polymerase II
- One group of viroids forms 'hammerhead ribozyme'
 - *A ribozyme: autocatalytic, self cleaving RNA*
 - *Ability of RNA to catalyze a reaction in the absence of protein discovered 1981*
 - *Used to cleave multimeric structures produced during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





Hammerhead ribozyme

Origin of viroids

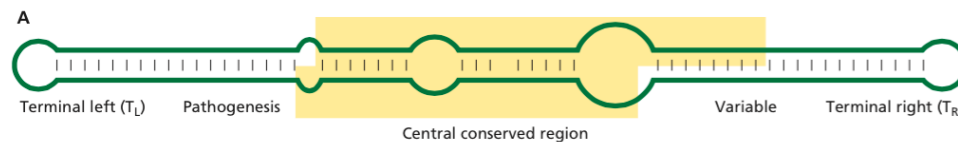


- Discovered in the 20th century by chance transfer from wild plants used in breeding modern crops
- Relics of an RNA world?
- Worldwide use of genetically identical plant breeding lines (monoculture)
- Mechanical transmission by contaminated farm machinery, equipment, hands, plant to plant

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



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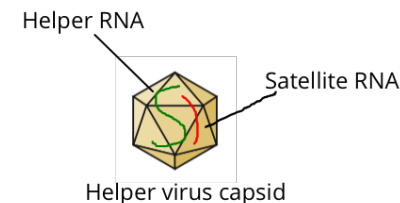
**b.socrative.com/login/student
room number: virus**

Which of the following is not a property of viroids?

- A. Some contain a ribozyme
- B. Replicated by host RNA polymerase
- C. Metastable capsid
- D. Circular, rod-like
- E. None of the above

Satellites

- Depend on helper virus for propagation
- ssRNA, DNA, cRNA genomes
- 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.



Satellite RNAs

Class 1 Satellite RNAs



Linear RNA, 220–1,500 bases
One ORF encoding nonstructural protein

Class 2 Satellite RNAs

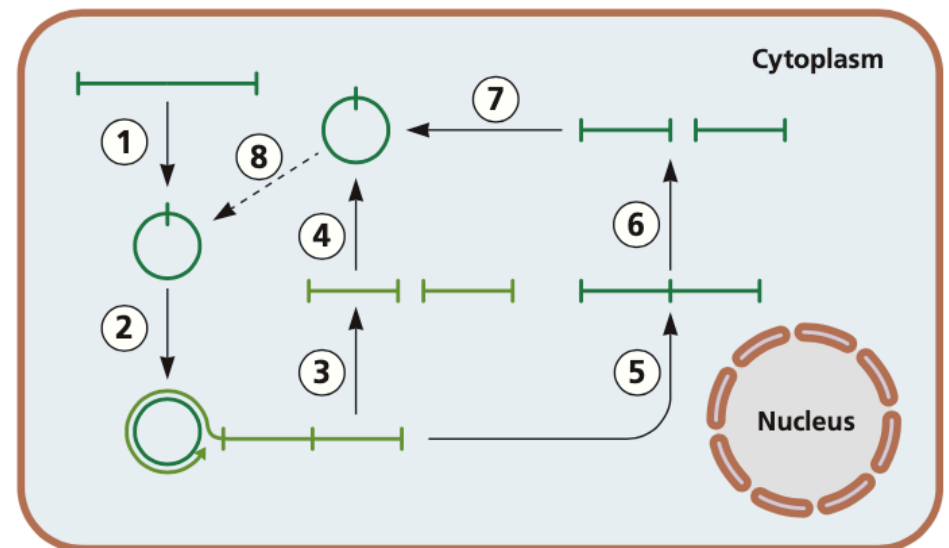


Linear RNA, <700 bases
Do not encode protein

Class 3 Satellite RNAs

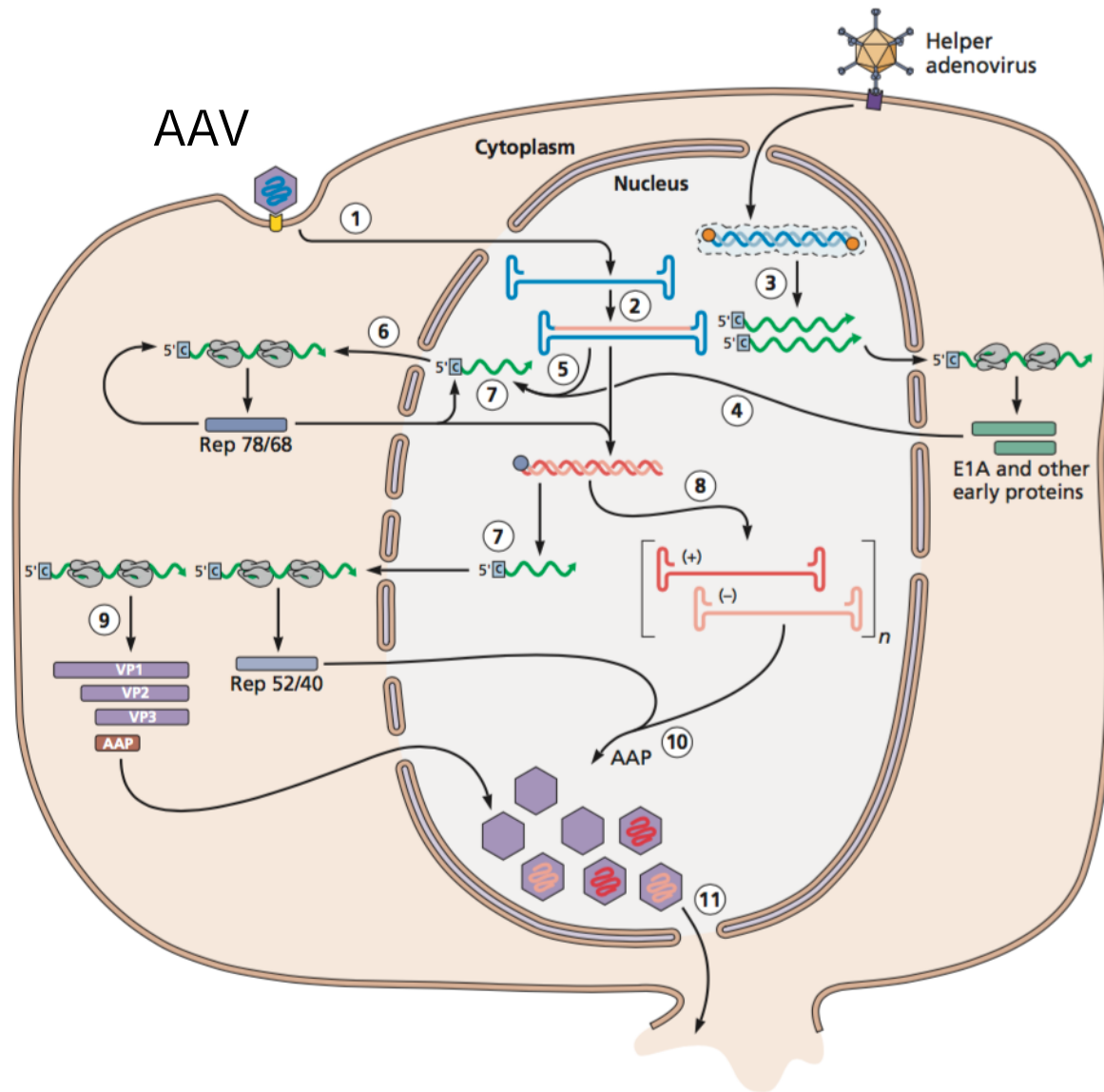


Circular RNA, 350–400 bases
Do not encode protein



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



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*

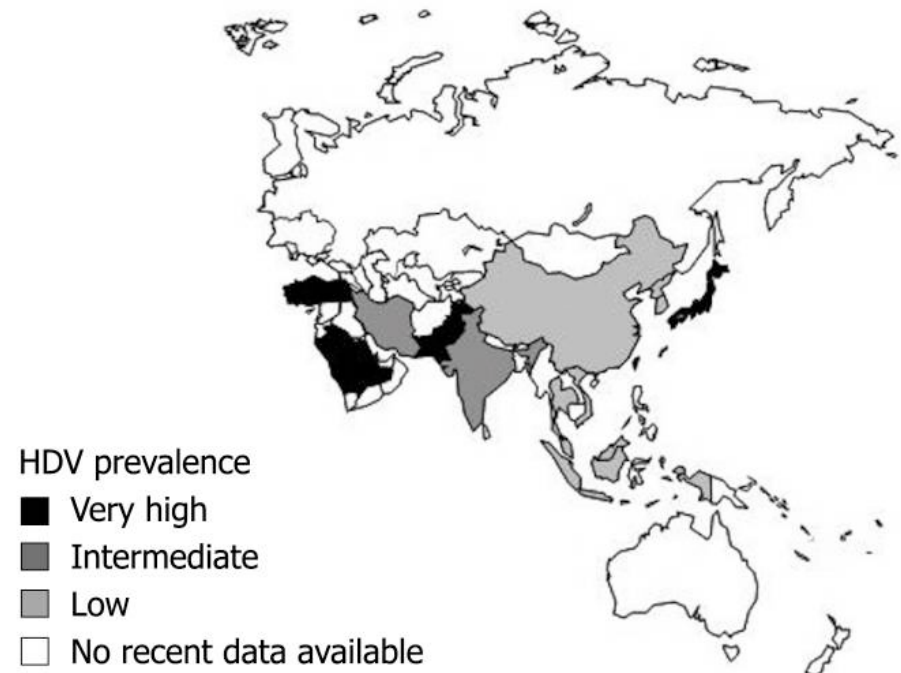
Hepatitis delta virus

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

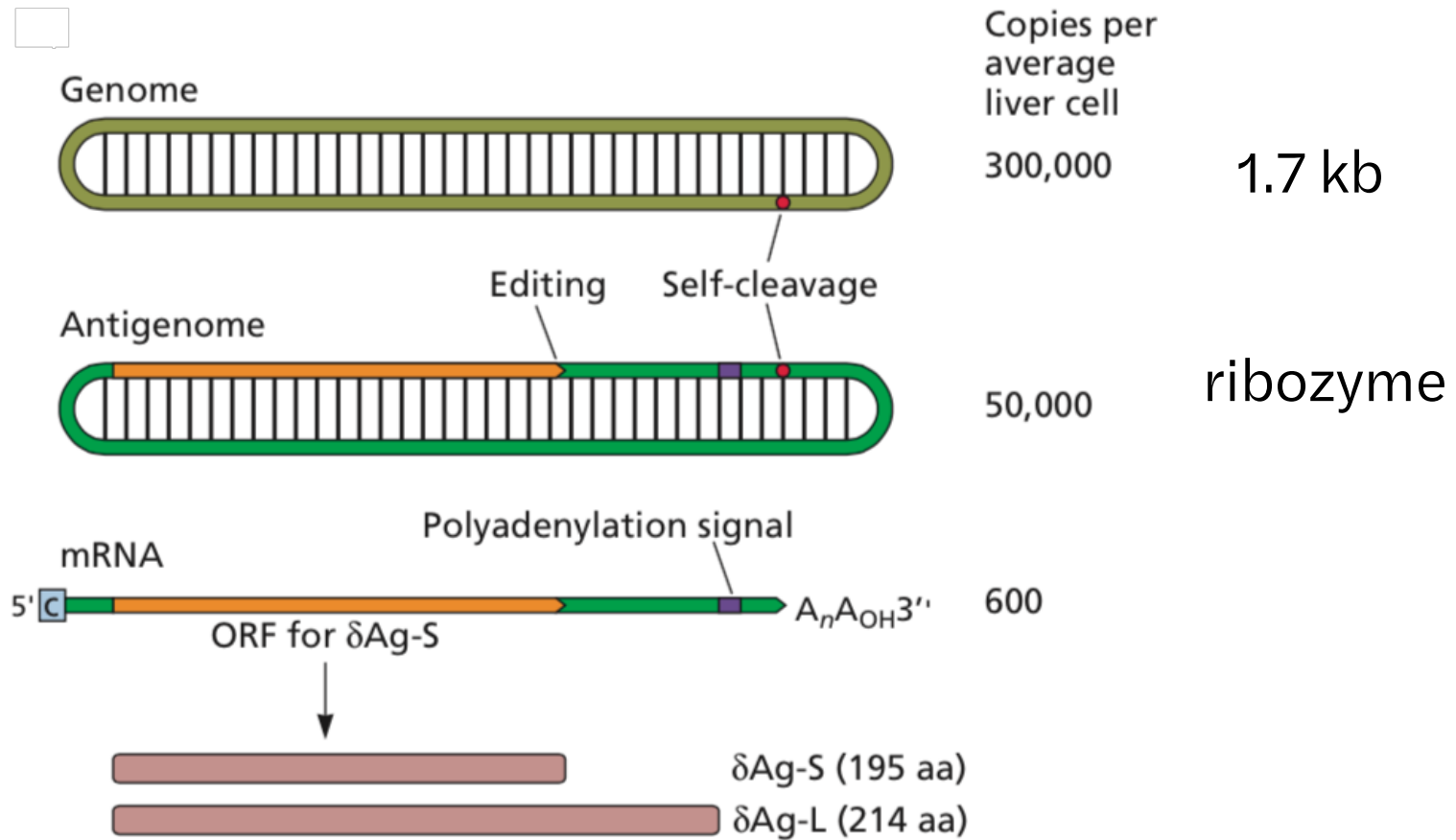
HDV global distribution

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

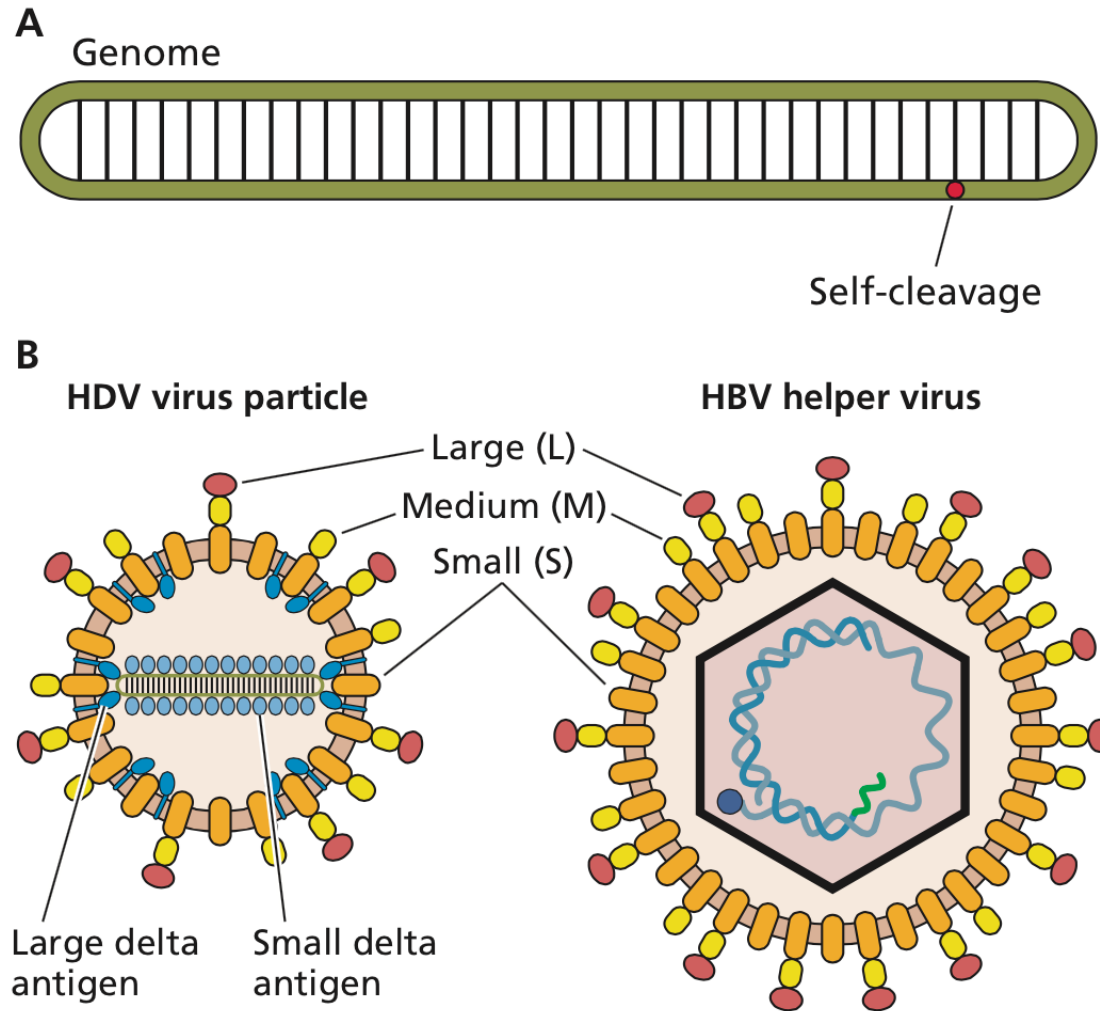
Hepatitis Delta-Scenario in the Asia-Pacific region

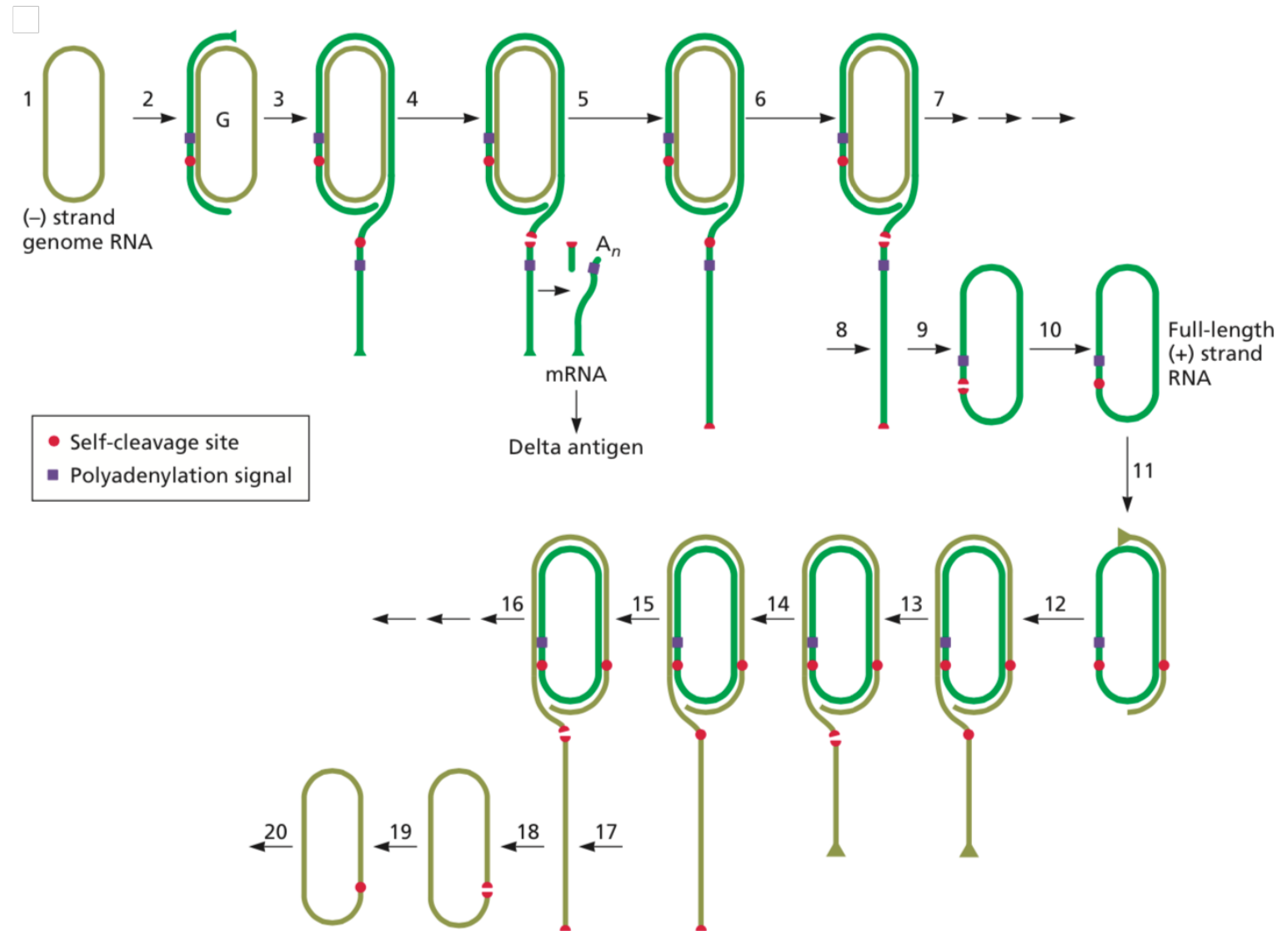


HDV genome

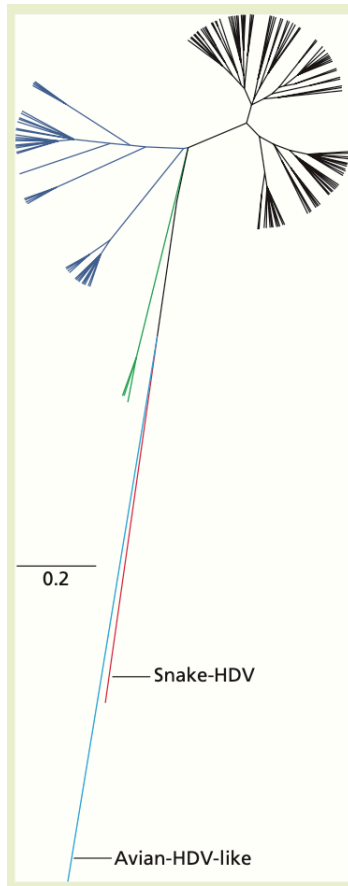


HDV and HBV





Hepatitis delta-like viruses in birds and snakes



- Discovered in RNA transcripts of healthy birds and sick snakes
- No hepatitis B-like virus detected
- Viruses other than HBV can help HDV in cell culture (HCV, VSV, dengue virus, WNV)

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Which of the following statements about satellites is correct?

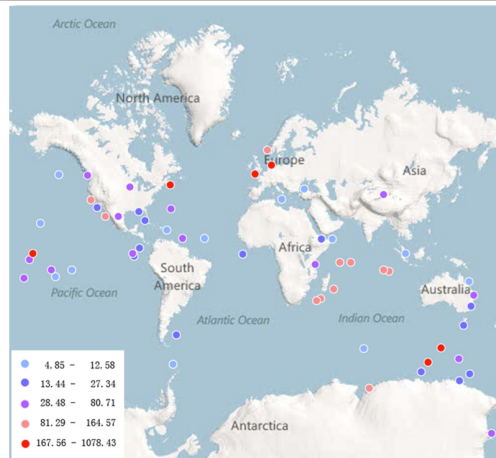
- A. There are no human satellites
- B. They are defective viruses derived from the helper
- C. Like viroids, they do not encode protein
- D. In plants, they cause symptoms distinct from helper virus
- E. All of the above are correct

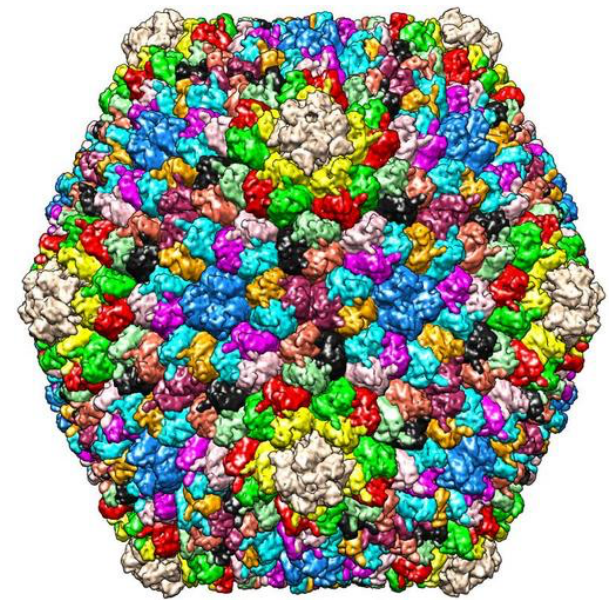
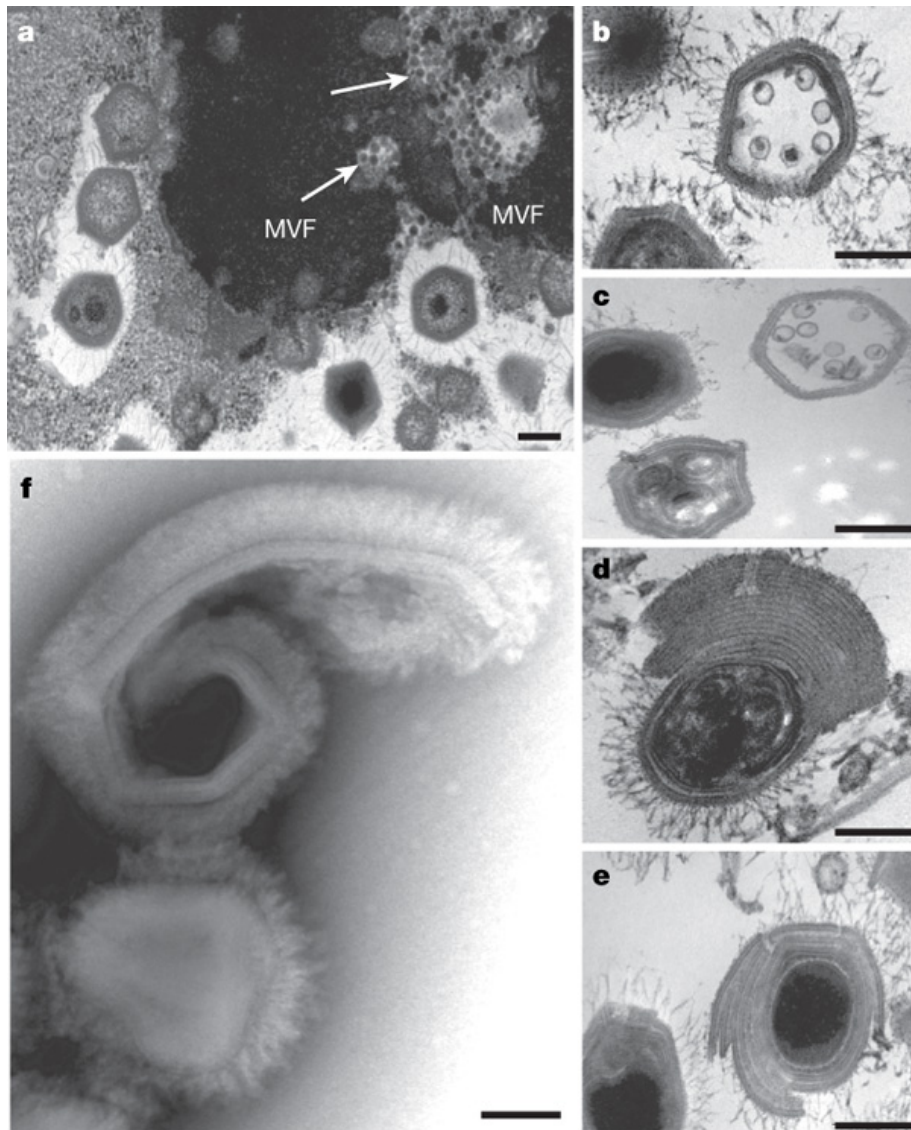
Virophages

- Derived from bacteriophage (*phagein* = Greek, to eat), means ‘virus eater’
- Circular dsDNA viruses, icosahedral
- Replicate only in *cells* infected with a giant virus (*not* within another virus!)
- Interfere with helper virus replication

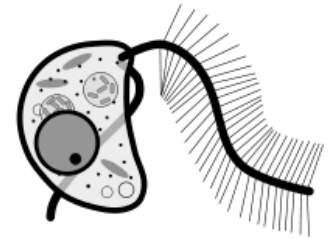
Virophages

Virophage	Location	Host		Genome	
		Virus	Eukaryote	Size (bp)	No. of ORFs
Sputnik	A cooling tower in Paris, France	<i>Acanthamoeba polyphaga</i> mimivirus	<i>A. polyphaga</i>	18,343	21
Mavirus	Coastal waters of Texas	<i>Cafeteria roenbergensis</i> virus	Marine phagotrophic flagellate (<i>C. roenbergensis</i>)	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	<i>A. polyphaga</i>	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





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)

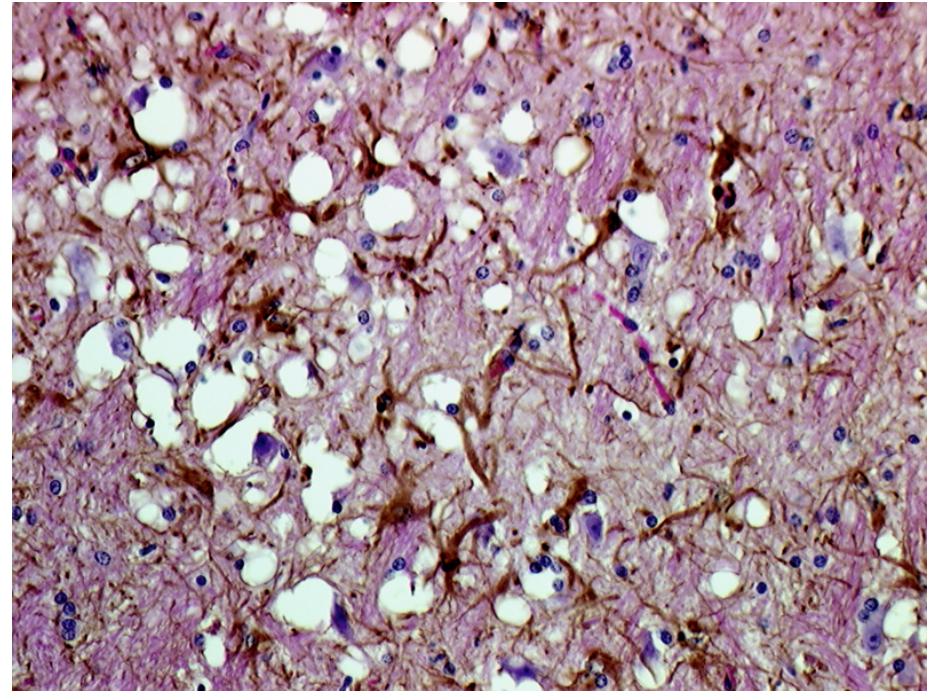


Transmissible spongiform encephalopathies

- 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

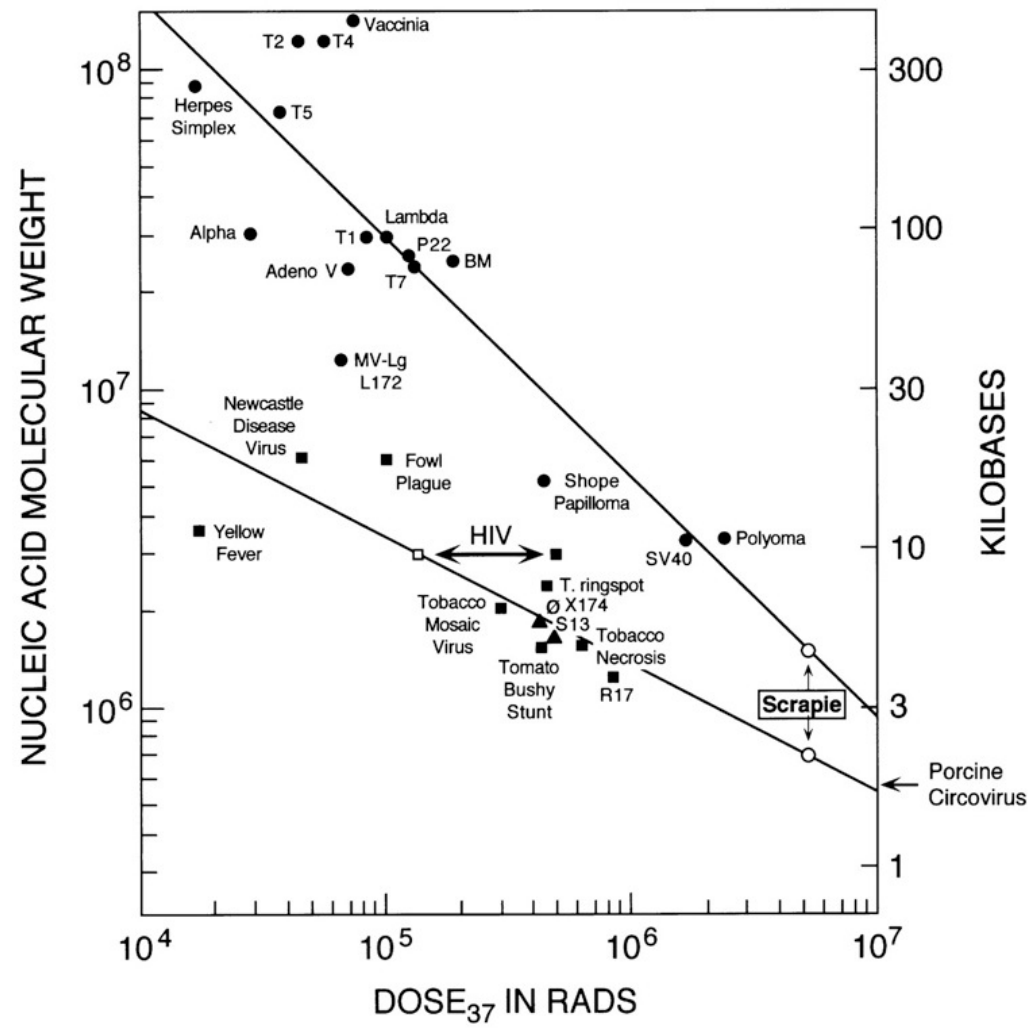
- First TSE recognized
- Infected sheep rub on fences
- Motor disturbances, uncontrollable trembling (*tremblant du mouton*), paralysis, weight loss, death 4-6 weeks
- Recognized as disease of European sheep for over 250 years
- Endemic in some countries: UK, 1% of sheep/yr



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



TSEs

- Animal and human TSEs exhibit similar 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 misfolded prion 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 astrogliosis, vacuolization (spongiform), loss of neurons
- No inflammatory, antibody, or cellular response

TSEs

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

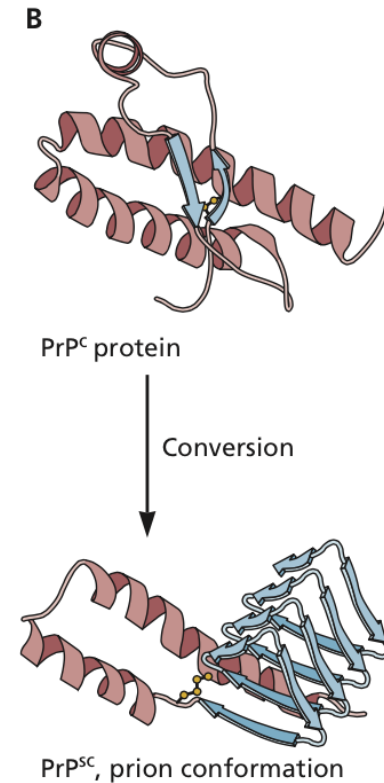
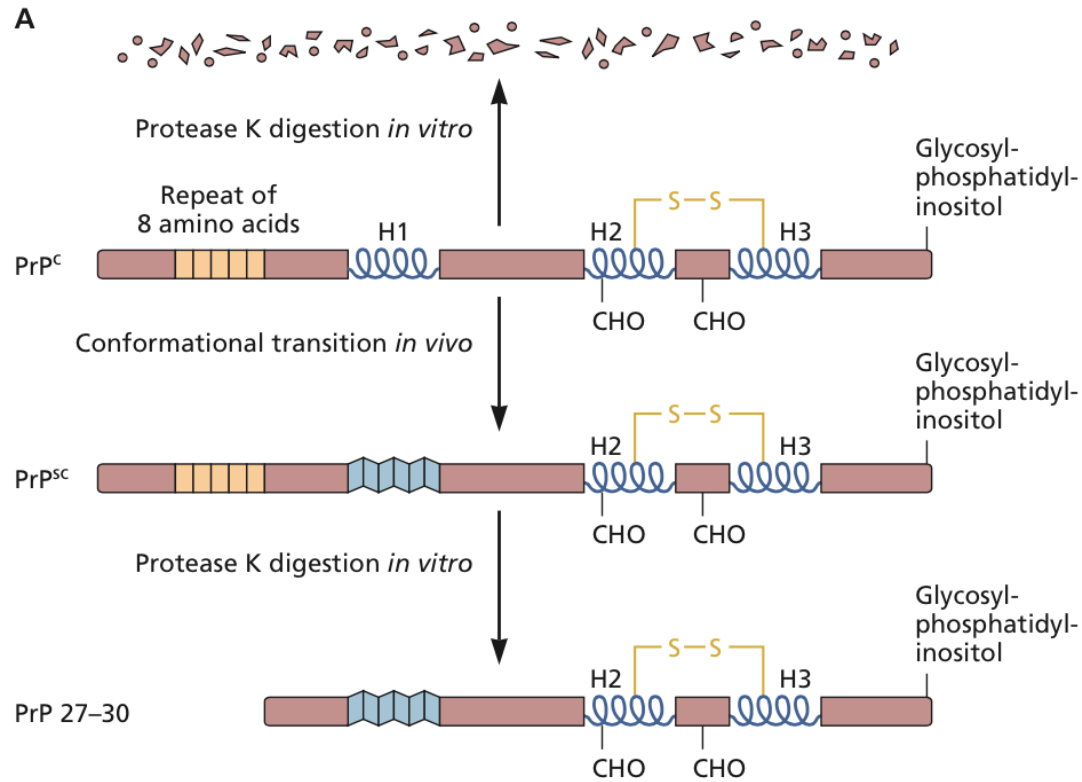
Prions

- 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*, (*proteinaceous* and *infectious* particle)
- Encoded by *prnp* gene, essential for pathogenesis of TSEs

Current view of prions

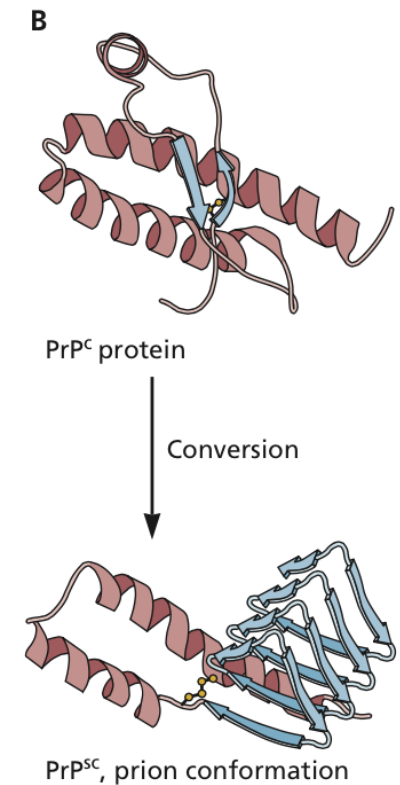
- Pathogenic prion is a conformational isoform of a normal host protein, PrP^c
- PrP^c is found predominantly on the outer surface of neurons, GPI anchor
- The abnormal conformer, when introduced into the organism, causes conversion of normal PrP^c into the pathogenic conformation (PrP^{sc} for scrapie)

PrP^c to PrP^{sc}



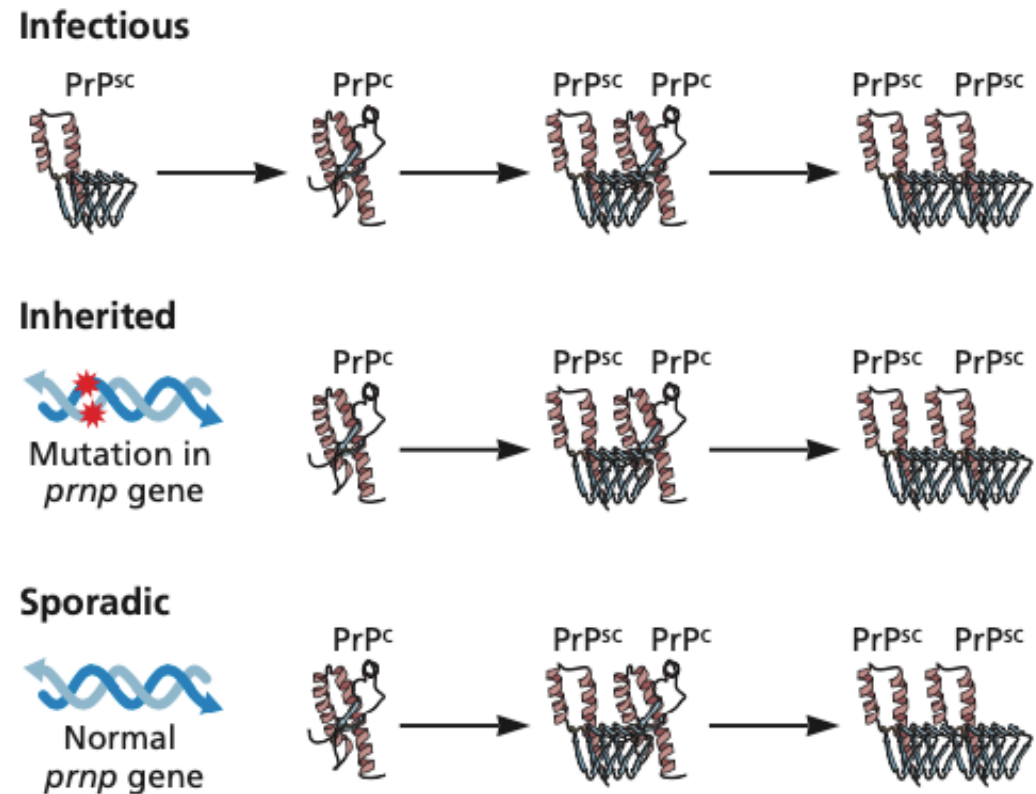
Prion hypothesis

- 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

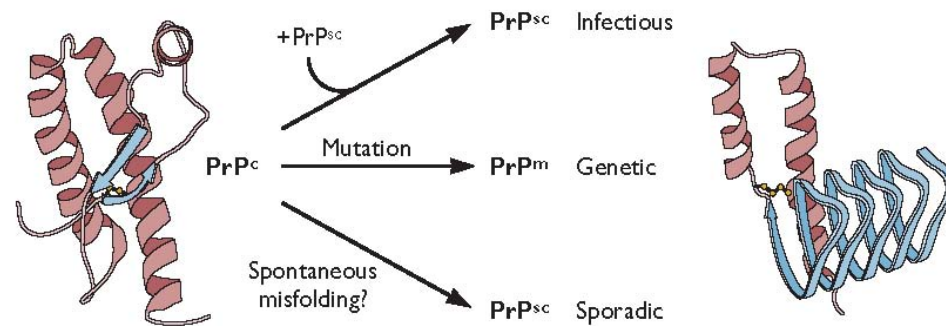


Three types of spongiform encephalopathies

- *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

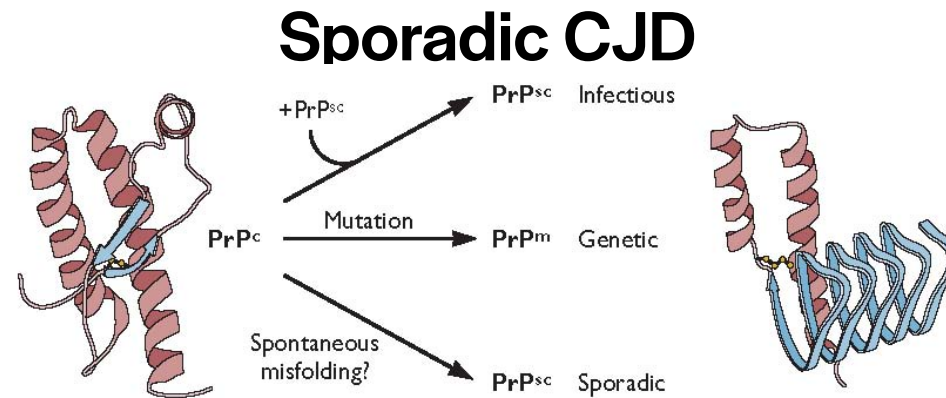


- Infectious or transmissible
 - Kuru
 - Iatrogenic spread by transplantation of infected corneas, hormones, transfusion from patients with CJD
 - BSE: feeding infected animals to cattle
 - Variant CJD (new human disease): eating BSE beef

Human TSE

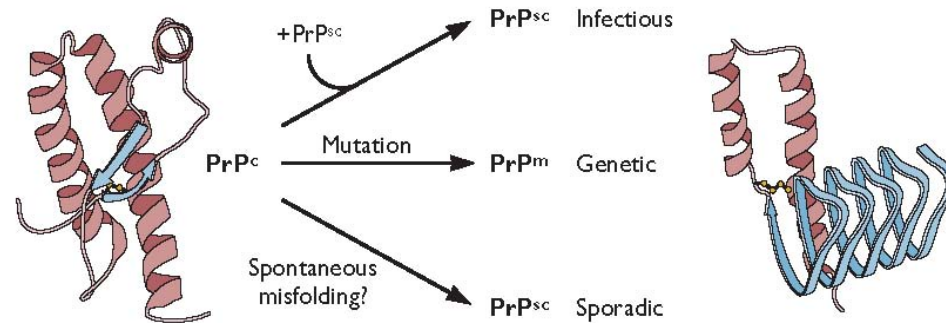


- Kuru: fatal encephalopathy found in Fore people of New Guinea
- 30 year incubation period
- Found by Carleton Gajdusek to spread among women and children through ritual cannibalism of brains of deceased relatives
- When cannibalism ceased, so did kuru



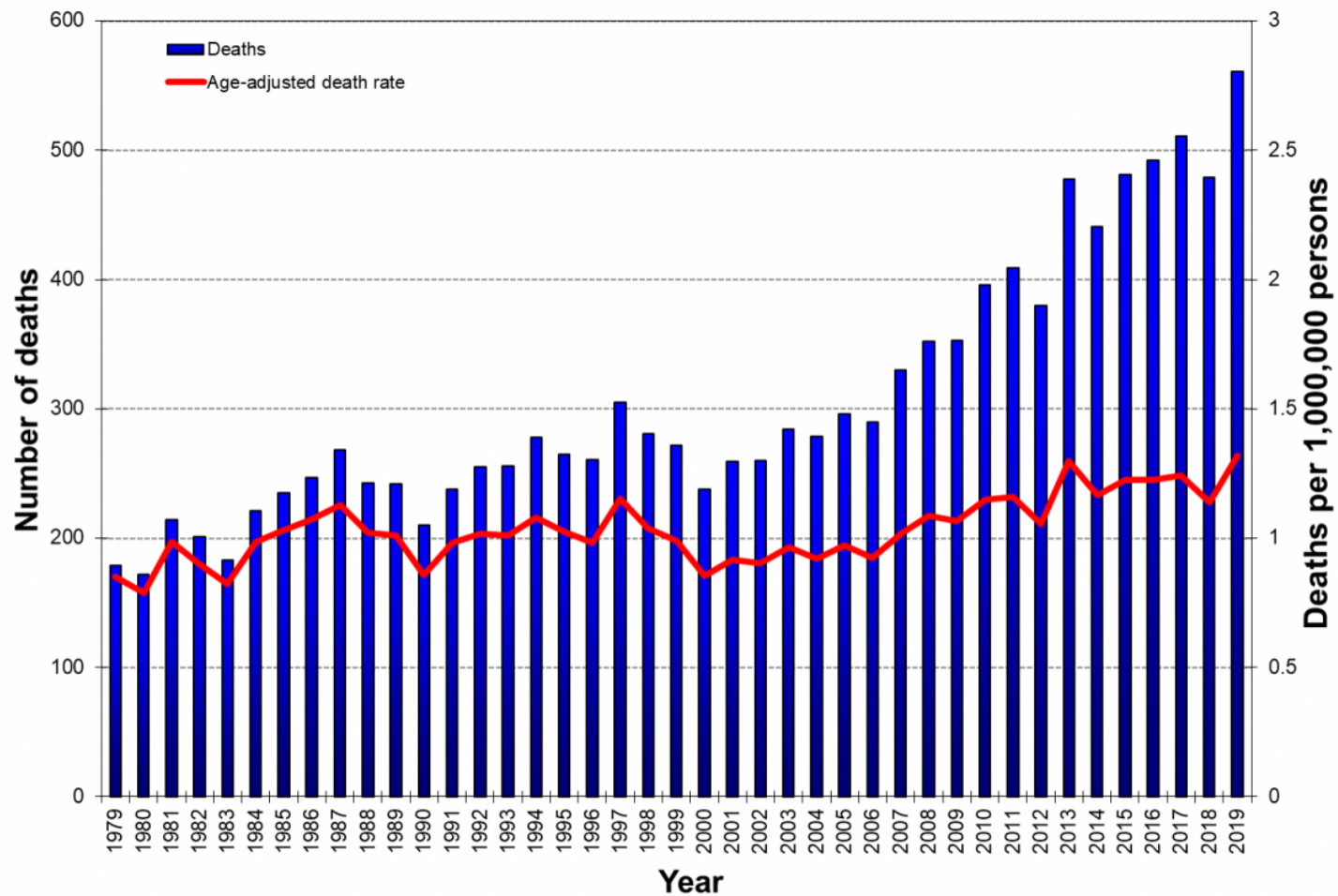
- Affects 1-2/1 million humans worldwide, 50 -70 years of age; 65% of TSE
- Disease appears with no warning or epidemiological indications
- Patients have normal *prnp* genes
- Can be transmitted to others leading to CJD
- Kuru may have been established in New Guinea by eating brain of person with sporadic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

Familial spongiform encephalopathy

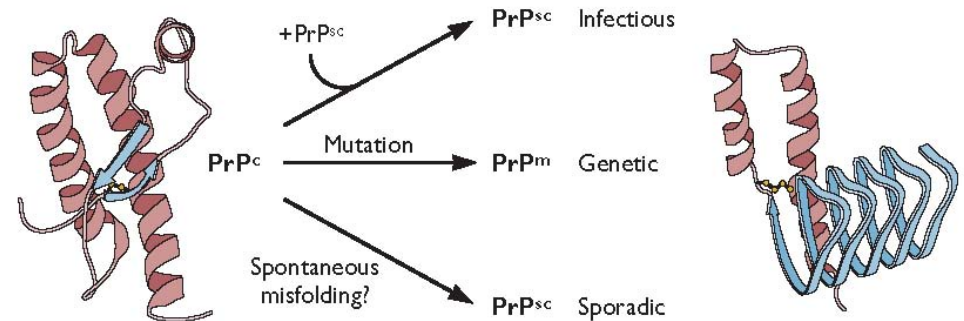
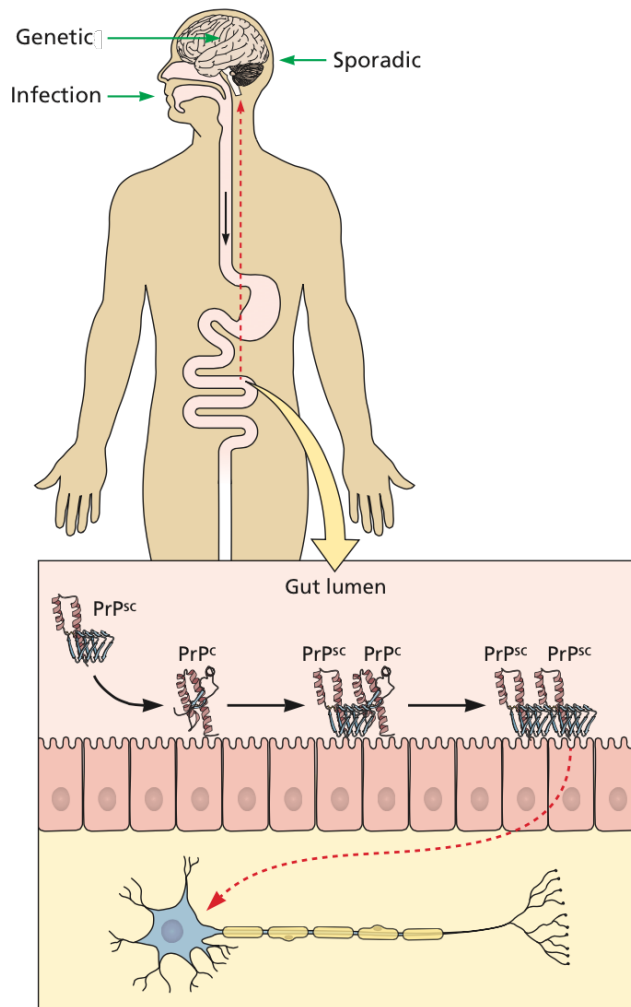


- An inherited disease
- Autosomal dominant mutation in *prnp* gene
- Organs, corneas, blood products from people afflicted with sporadic CJD can be infectious, transmit CJD to others

CJD deaths, US, 1979-2015



Spongiform encephalopathies and prion protein



Forced cannibalism spreads BSE

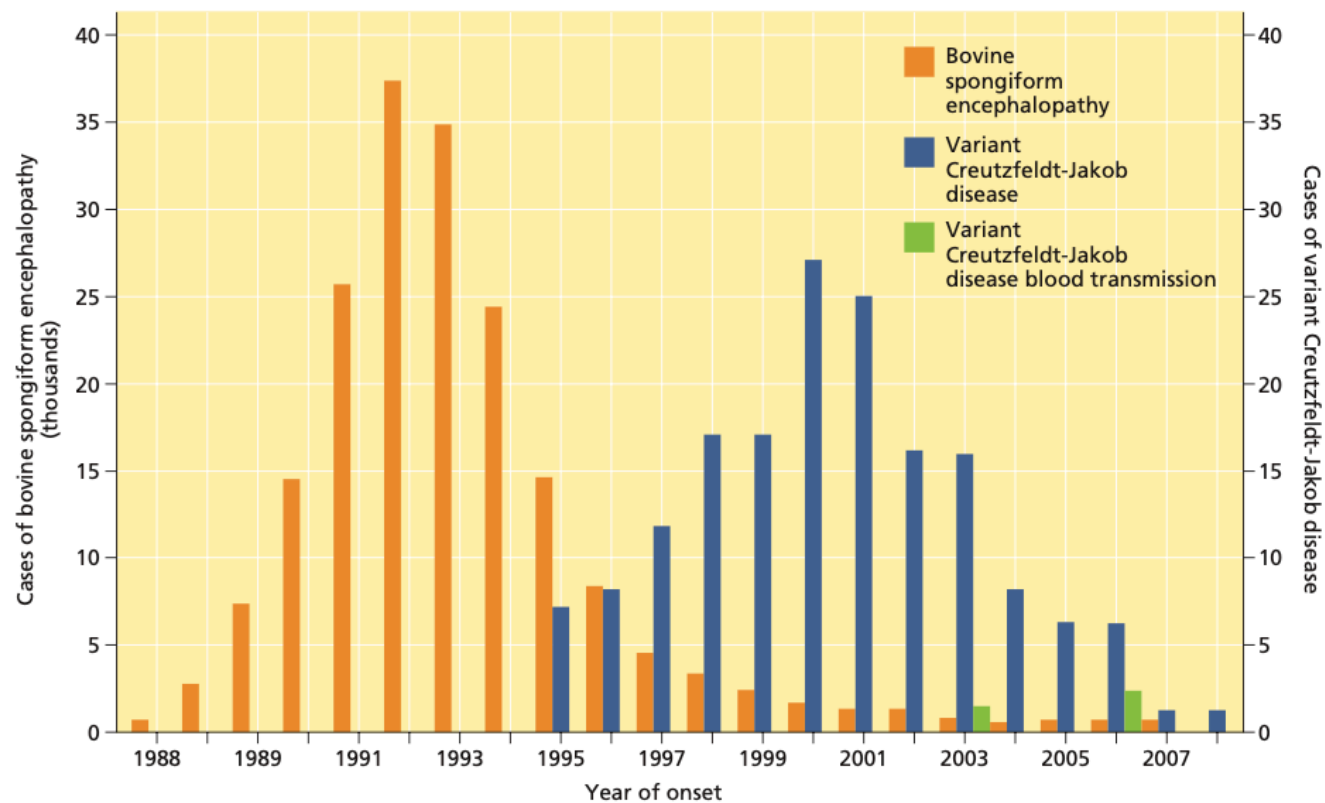
Epidemic spread of bovine spongiform encephalopathy (BSE, mad cow disease) among British cattle was a form of cannibalism



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

BSE in cattle and vCJD in humans



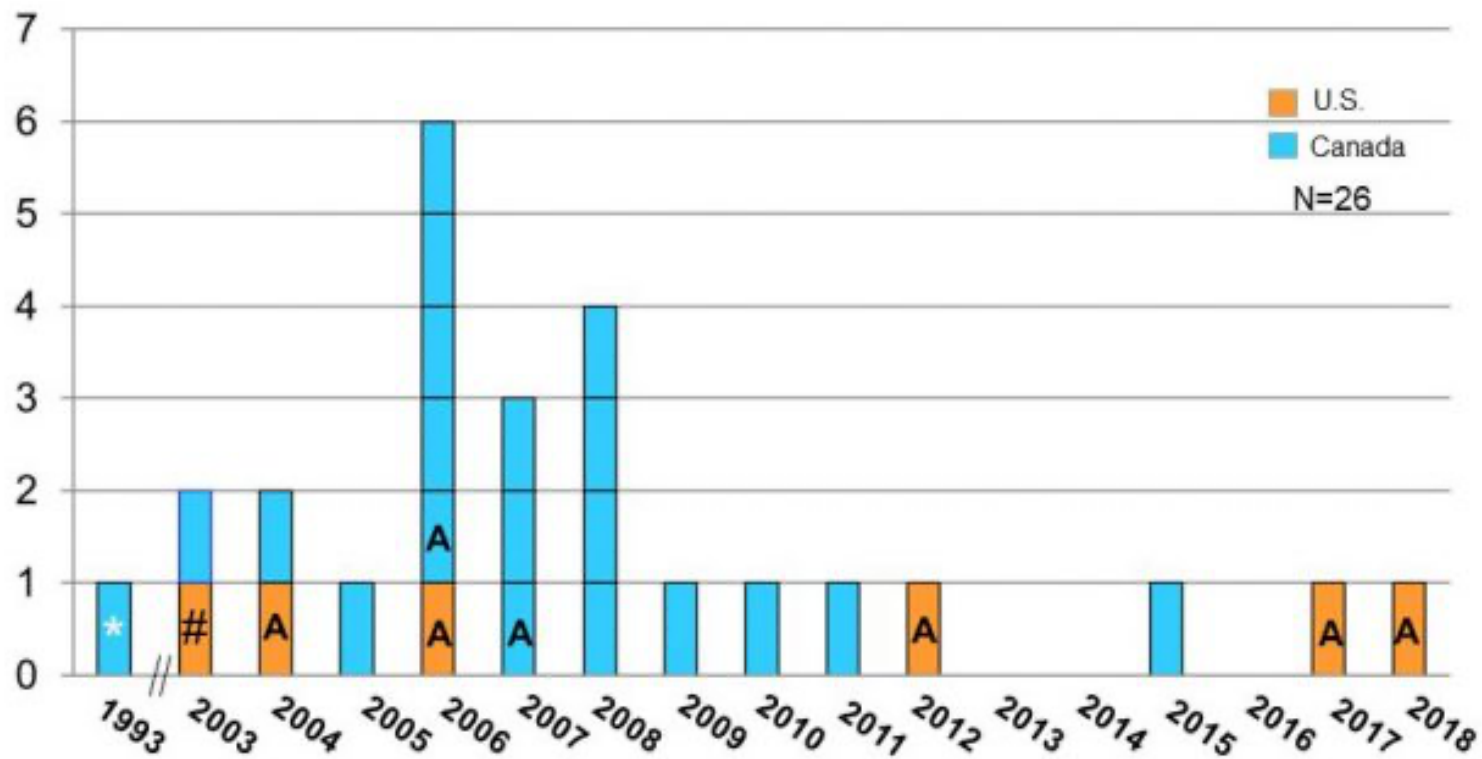
1-2 million cattle were infected with prions

Incubation time 5 yr, slaughtered 2-3 yr

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, 1993-2018



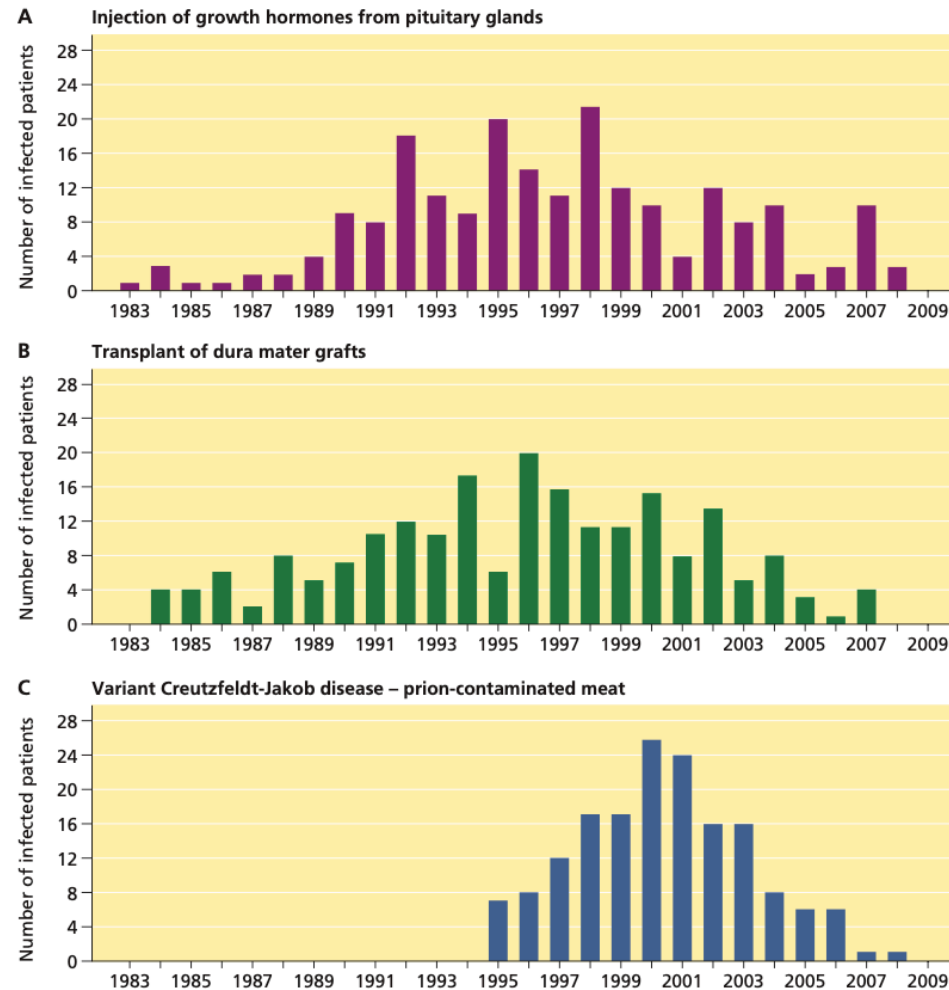
Case of prion disease from contaminated beef

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

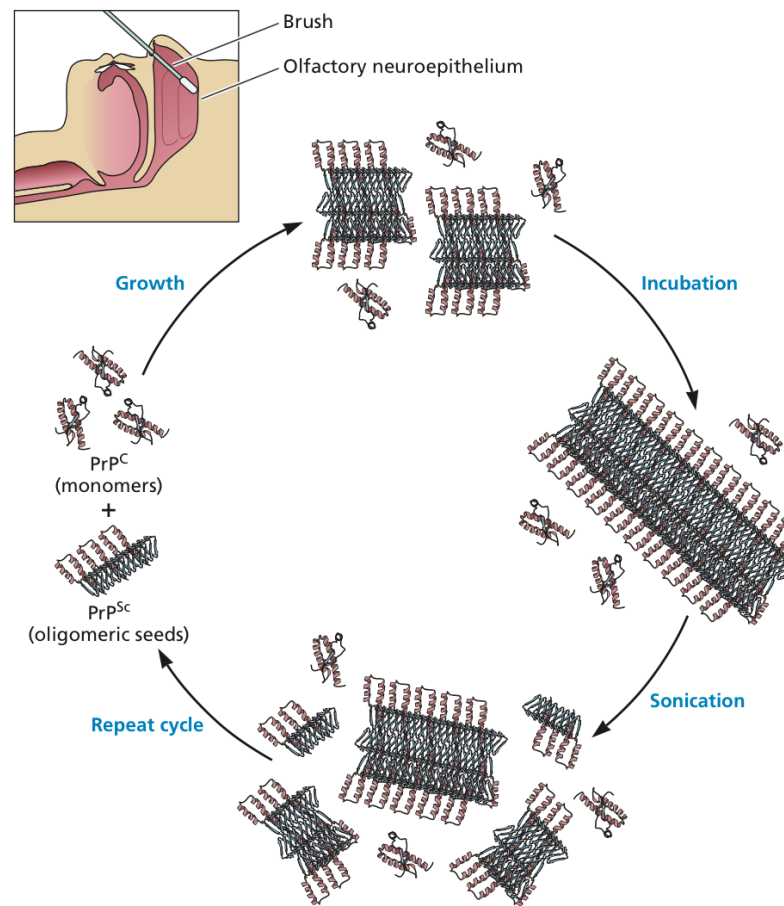
Prion contamination in the emergency room

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

Three epidemics of Crutzfeldt-Jakob disease



Detection of CJD prions in nasal brushings and urine

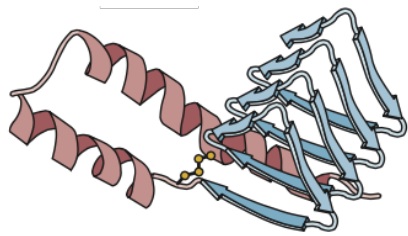


Prion species barrier



- 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

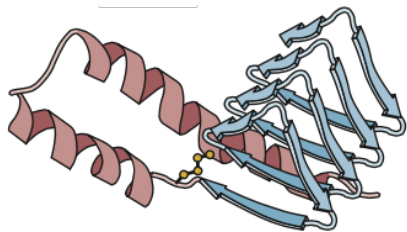
Prion species barrier



Hamster PrP^{Sc}



No disease



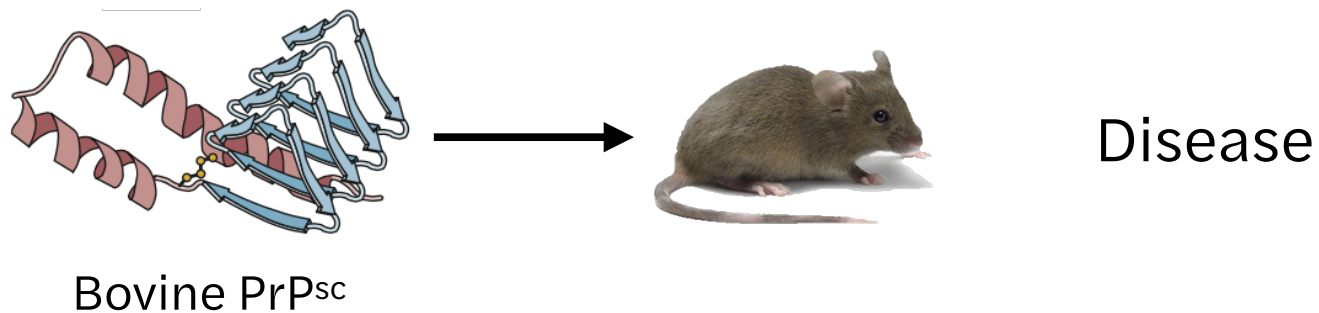
Hamster PrP^{Sc}



transgenic for
hamster *prnp*

Disease

Prion species barrier



- 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

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Which of the following observations illustrate the prion species barrier?

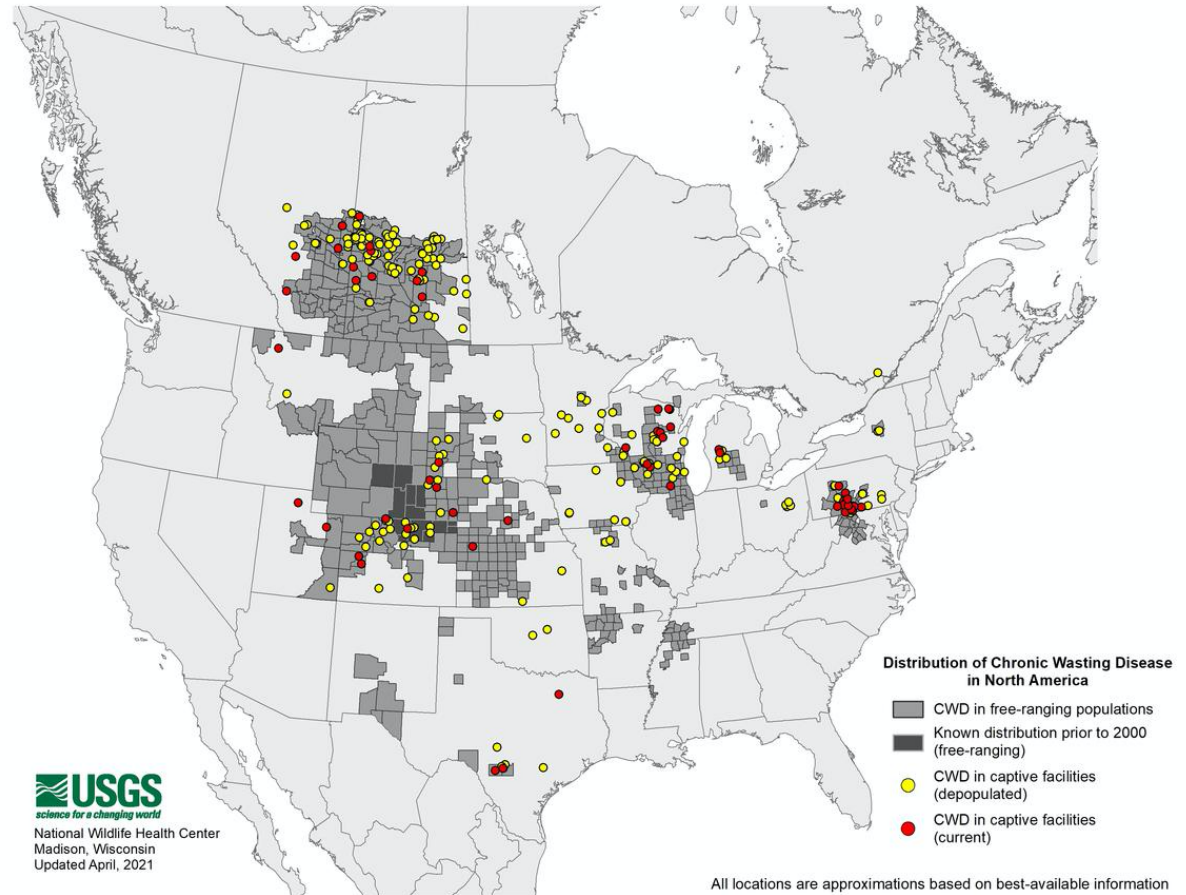
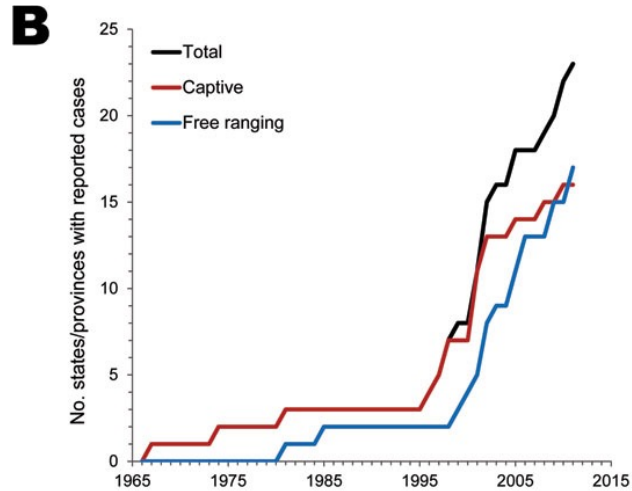
- A. Mice lacking the *prnp* gene do not develop disease
- B. Mice fed hamster PrP^{Sc} do not develop disease unless they have a hamster *prnp* transgene
- C. Humans consuming BSE beef develop disease
- D. Humans may sporadically develop prion disease
- E. Humans may transmit prion disease to other humans via corneal transplants

Chronic wasting disease - TSE of deer, elk, moose

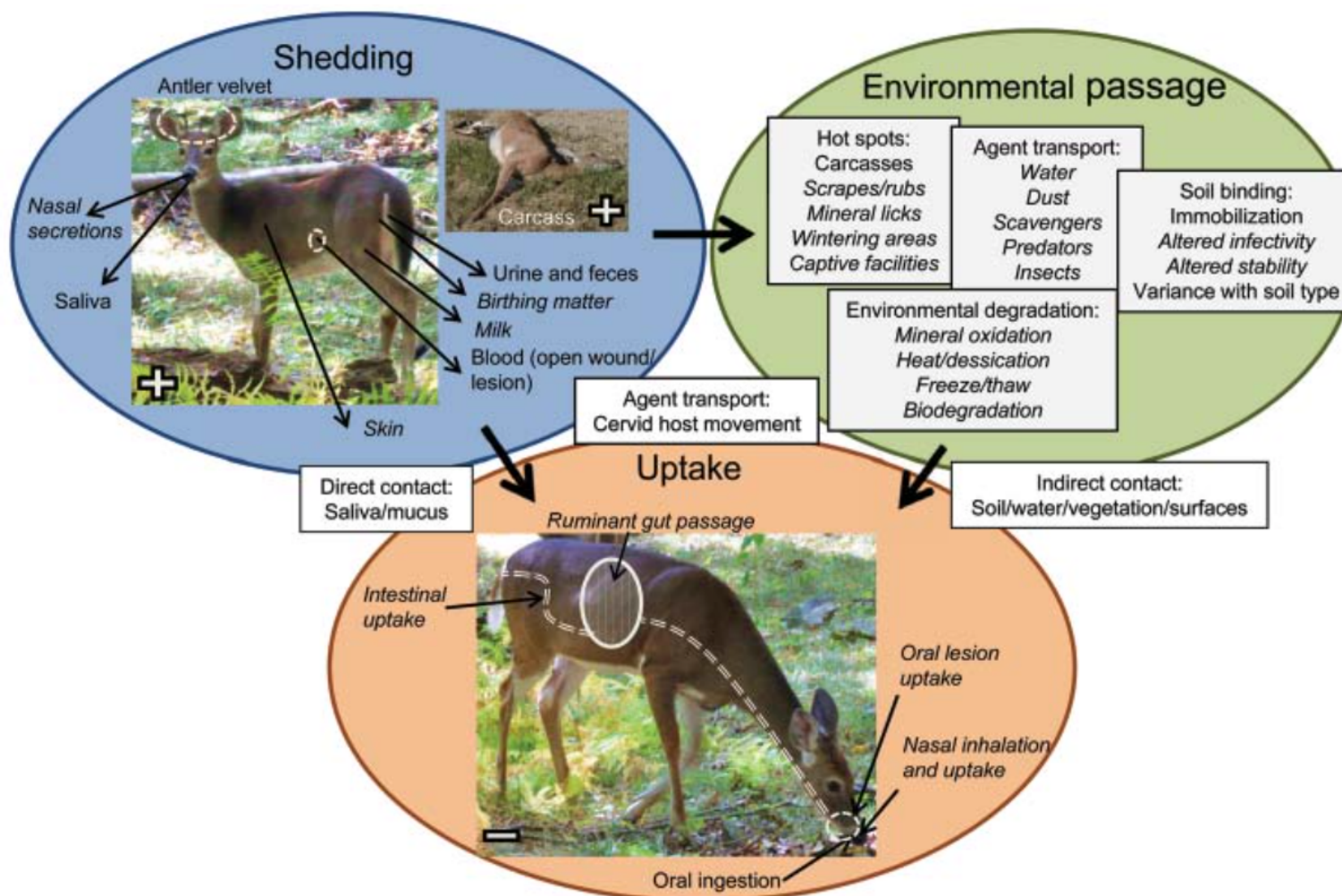


- A fatal TSE of cervids (deer, elk)
- Has been detected in 27 US states and four Canadian provinces in free-ranging cervids and/or commercial captive cervid facilities
- In standing herds up to 90% of mule deer and 60% of elk are positive
- Incidence in wild cervids as high as 15%

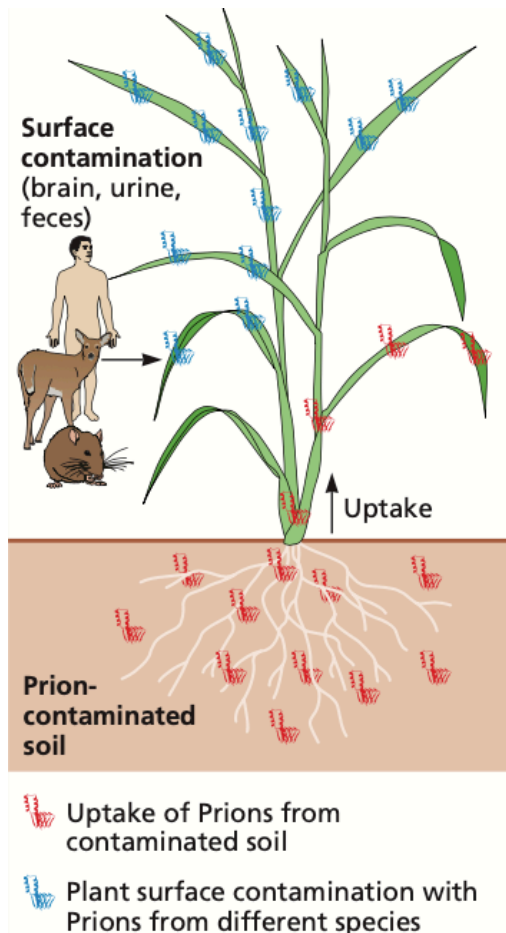
Chronic wasting disease - TSE of deer, elk, moose



Chronic wasting disease



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



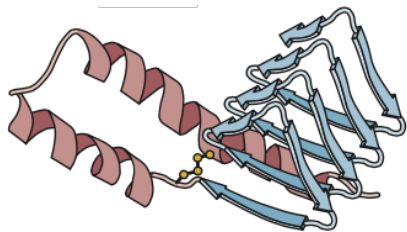
Chronic wasting disease



Cervid PrP^{Sc}



No disease



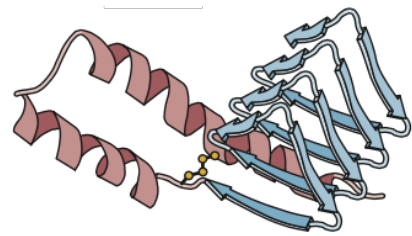
Cervid PrP^{Sc}



transgenic for
cervid *prnp*

Disease

Chronic wasting disease



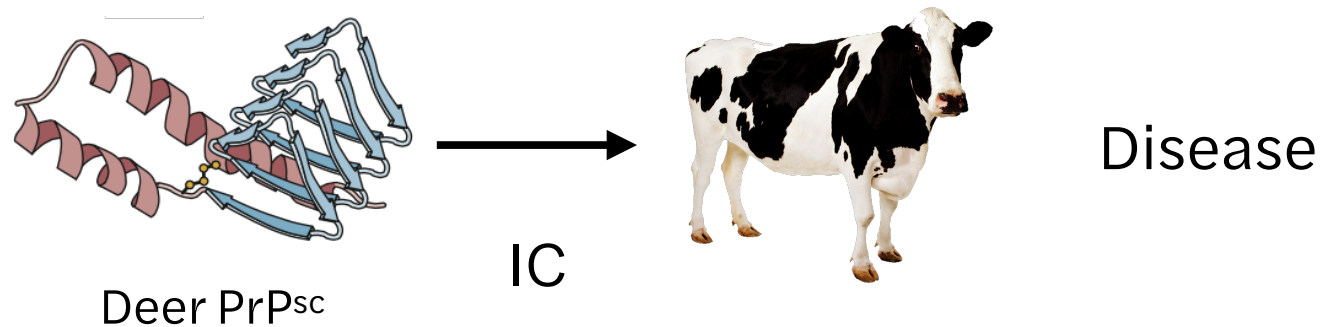
Cervid PrP^{Sc}



Transgenic for
human *prnp*

No disease

Chronic wasting disease



Could CWD prions transmit to cattle grazing in contaminated pastures?

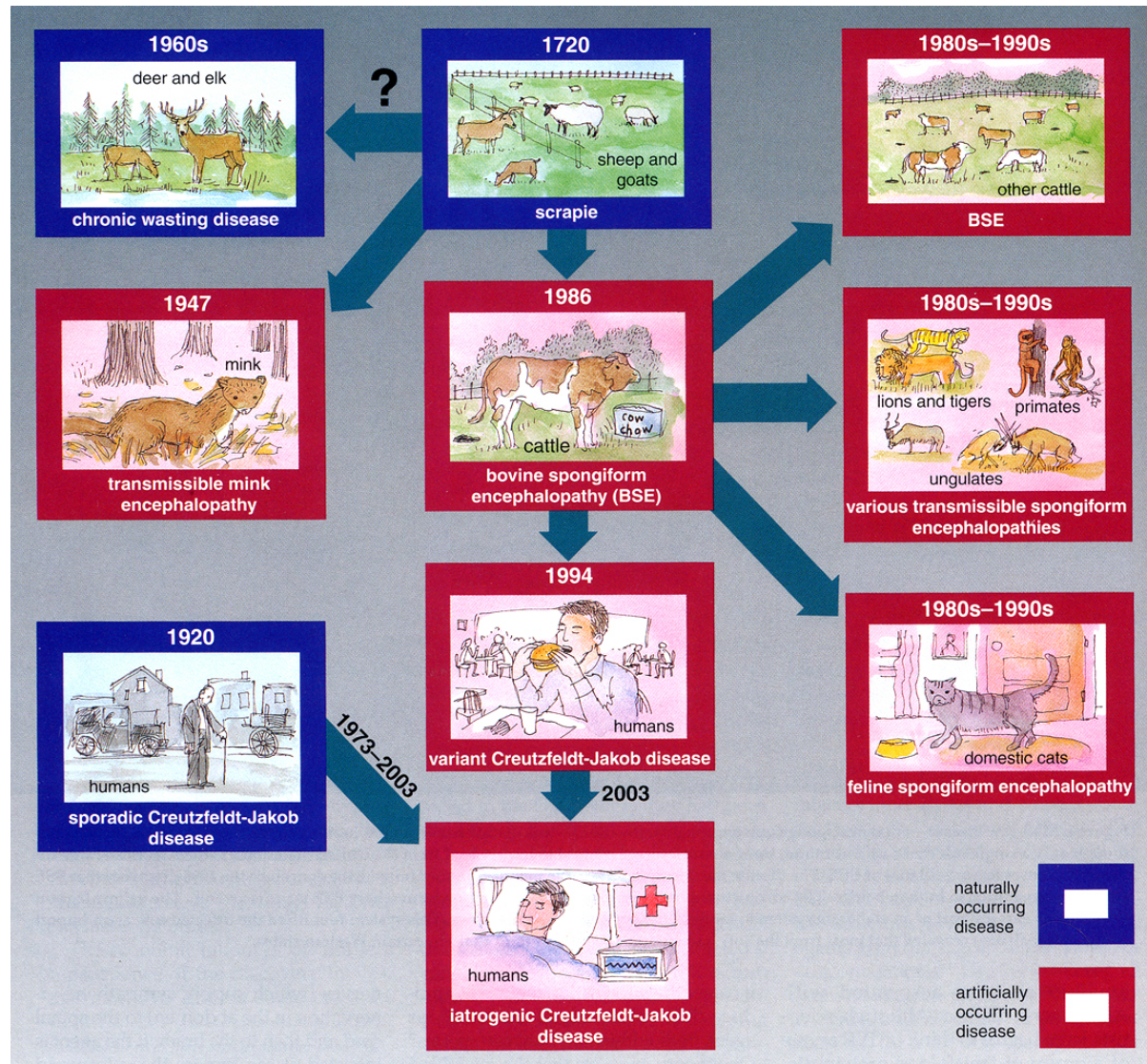
Cows so far have not been shown to be infected in pastures

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 handling 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?





VIROLOGY LIVE

WITH VINCENT RACANIELLO

Next time: Therapeutic viruses