

# Unusual infectious agents

Lecture 23

Biology W3310/4310

Virology

Spring 2016

*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?

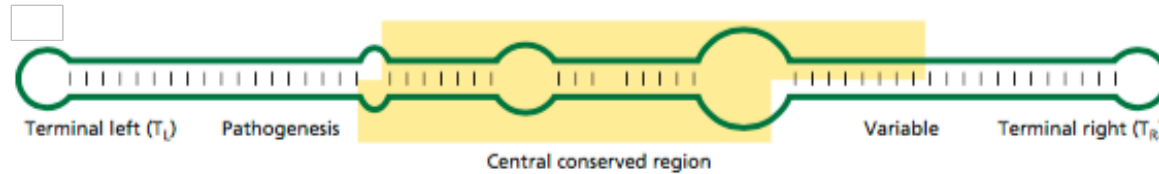
*Could an infectious agent exist without ANY genome?*

**Viroids, satellites, and prions provide answers**

# **Viroids: pure RNA, no protein**

- No protective coat, yet migrate from host to host (no receptors required)
- Subviral RNA database lists 1949 viroid sequences
  - *<http://subviral.med.uottawa.ca/cgi-bin/home.cgi>*

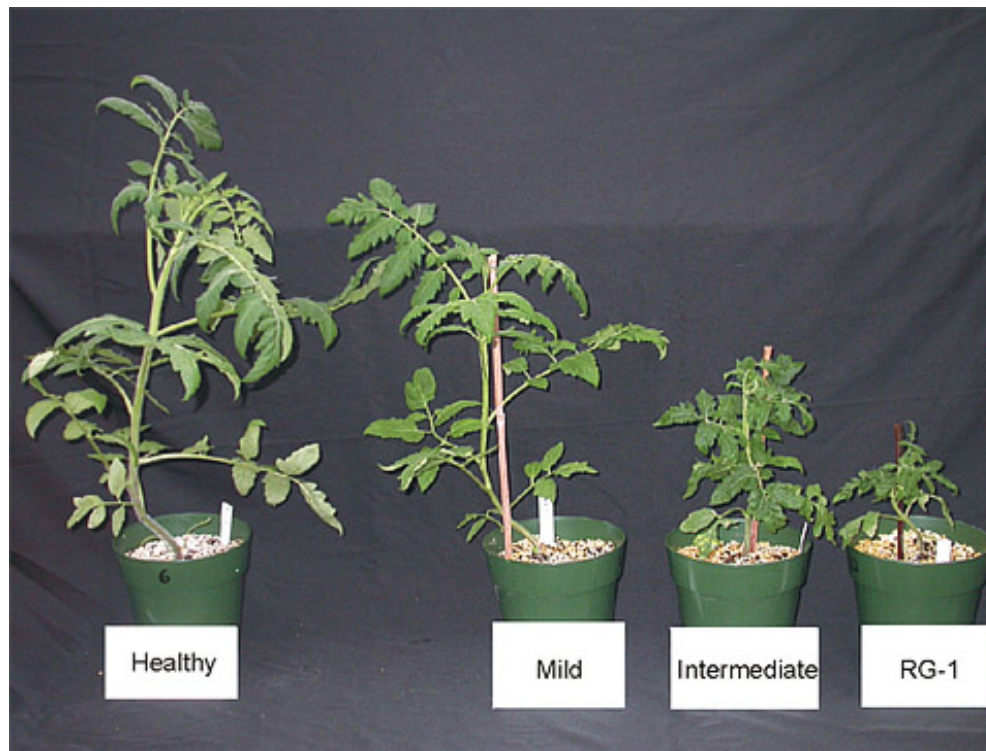
# Viroids



- Small, circular ssRNA
- No protein coding regions
- Replicate when introduced into plants
- Families *Pospiviroidae* (replicate in nucleus) and *Avsunviroidae* (replicate in chloroplasts)



- 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

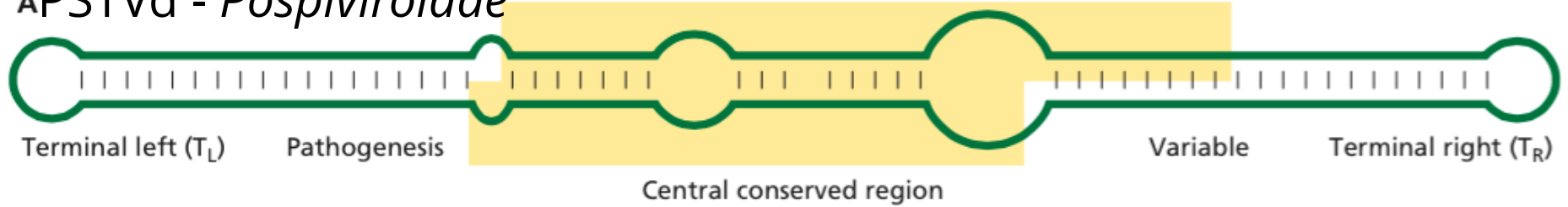


# **Viroids do not encode proteins or mRNA**

- 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*

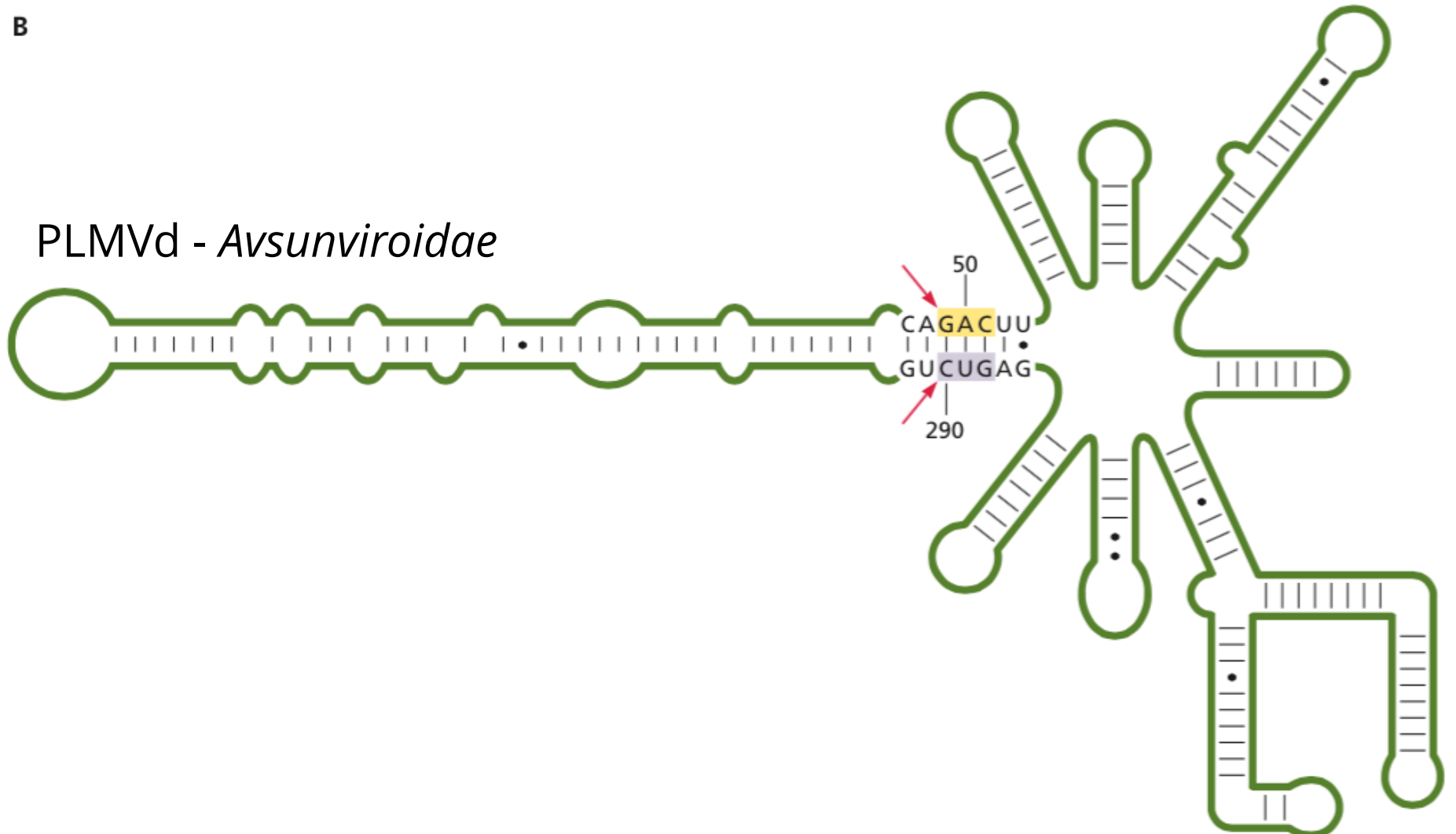
# Functional regions of viroid RNA

APSTVd - *Pospiviroidae*



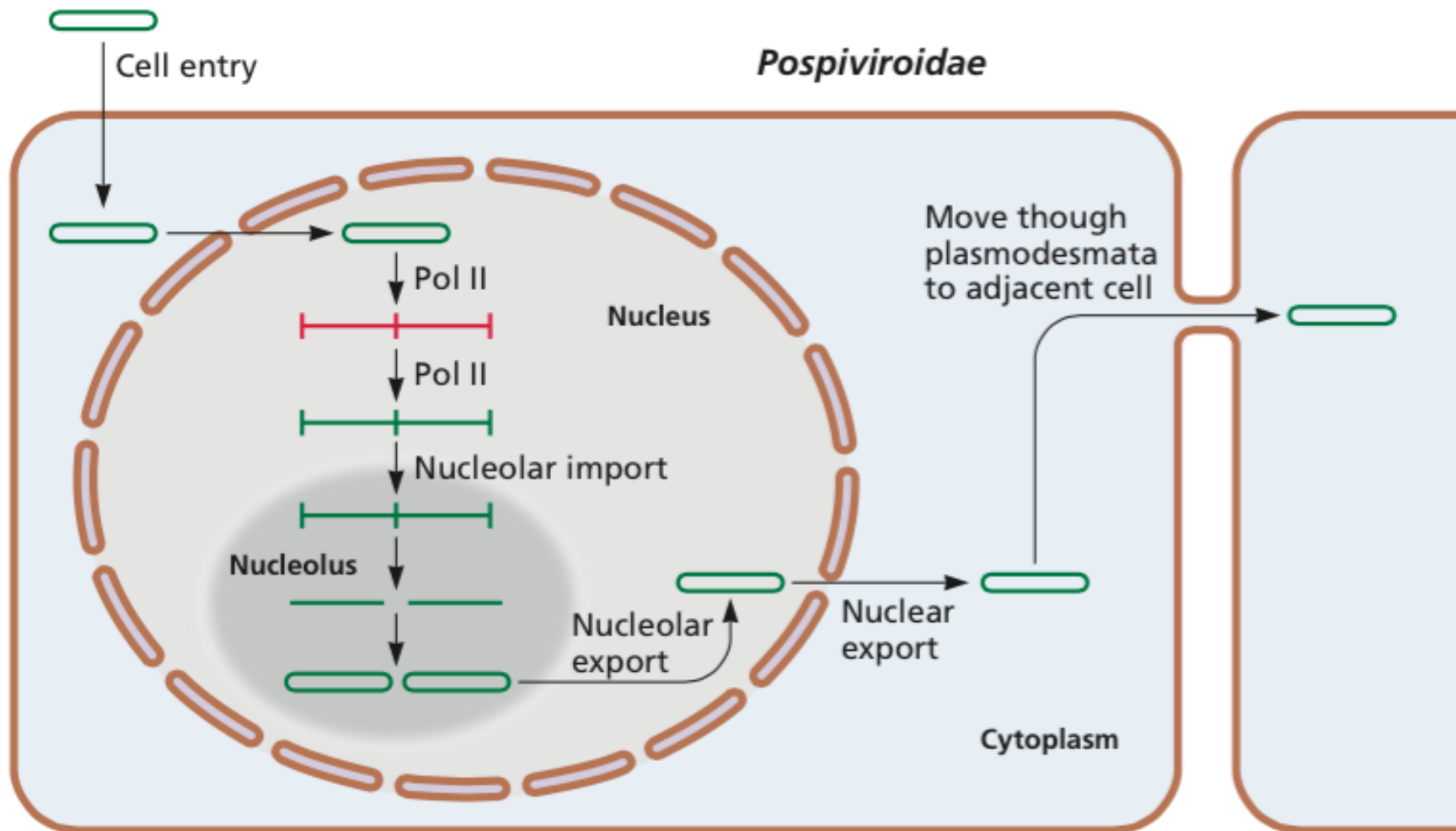
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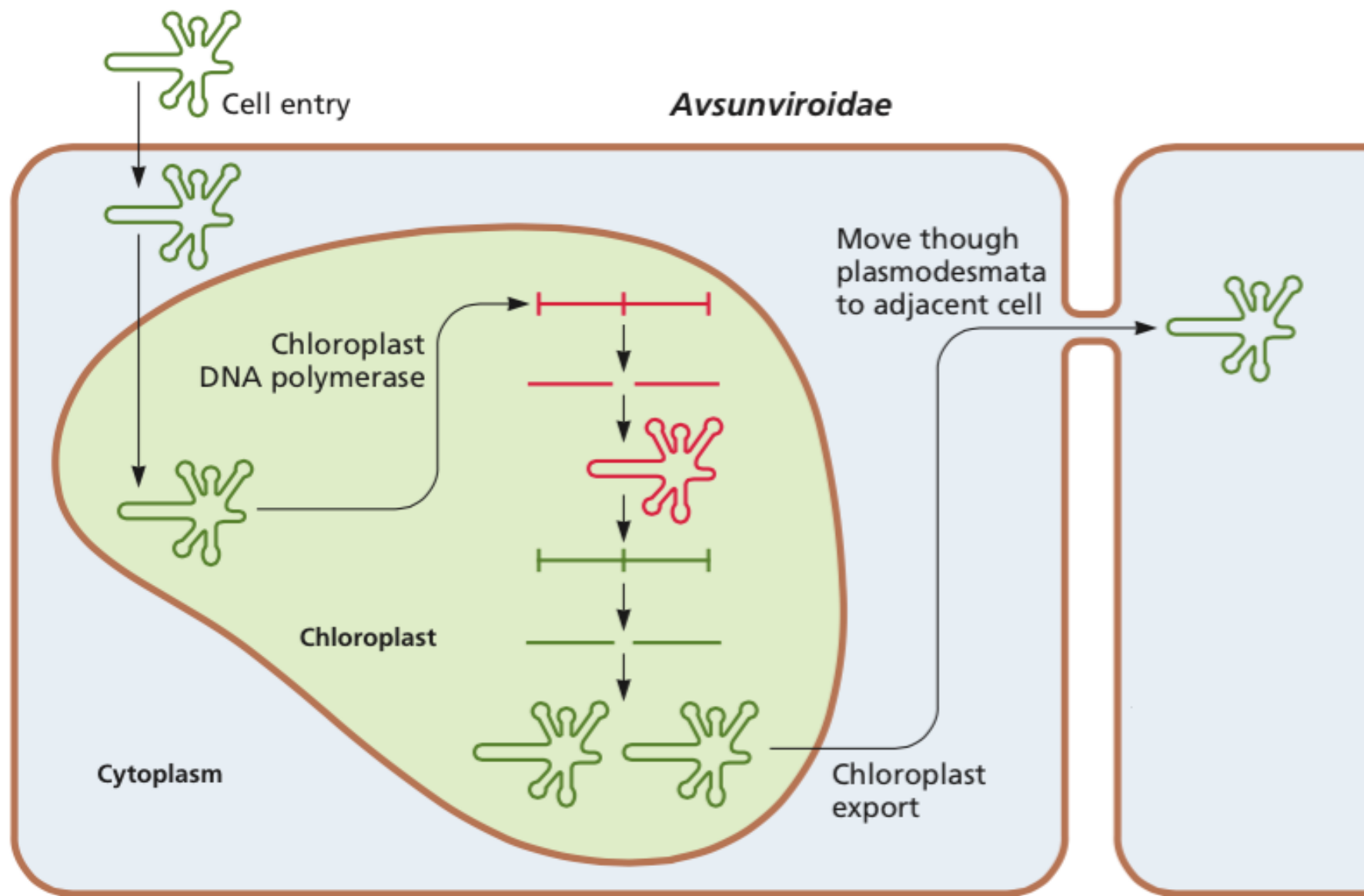
PLMVd - *Avsunviroidae*



# How do viroids replicate?

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





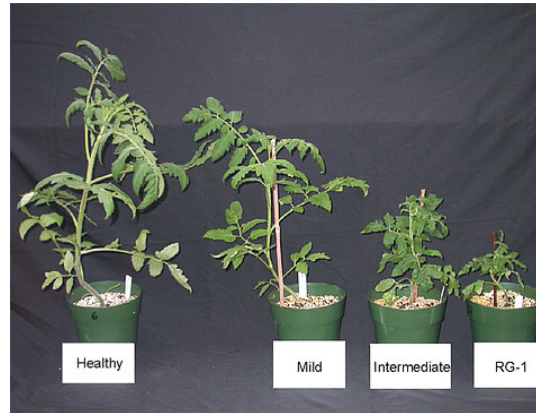
Hammerhead ribozyme

# Origin of viroids

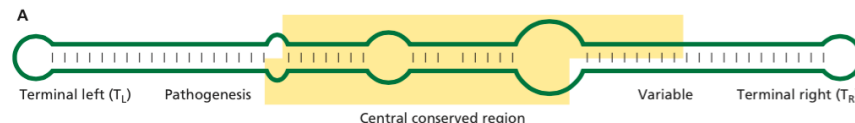
- Originated in the 20th century by chance transfer from wild plants used in breeding modern crops
- Worldwide use of genetically identical plant breeding lines (monoculture)
- Mechanical transmission by contaminated farm machinery, equipment, hands, plant to plant



# How do viroids cause disease?

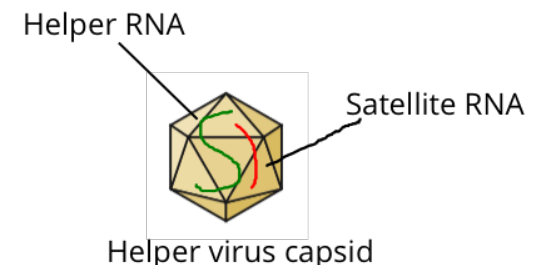


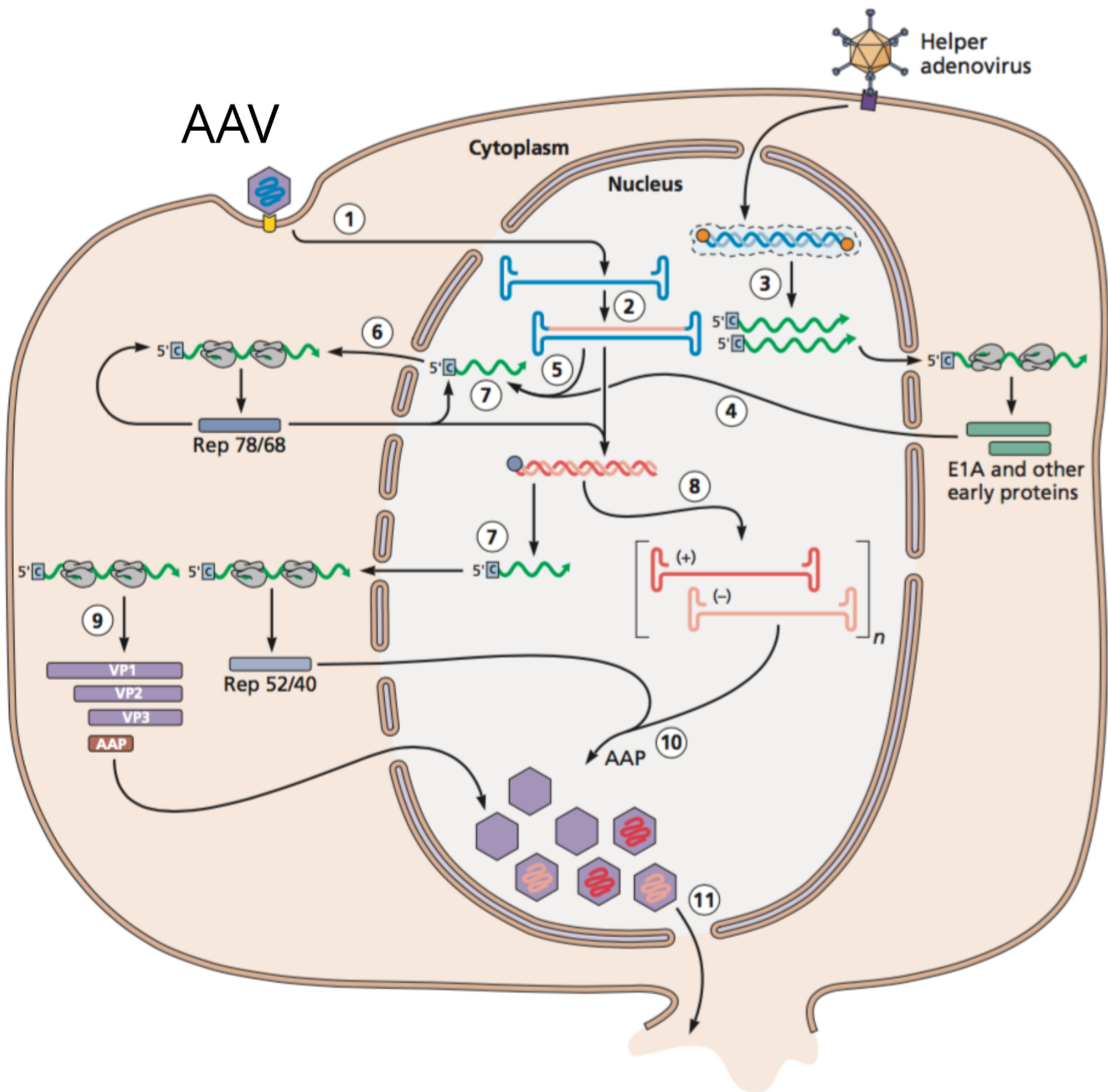
- Small 21-24 nt RNAs (siRNAs) derived from viroid RNAs in plants may guide RNA silencing of host genes and induce disease
- Symptom development correlates with production of small RNAs
- Many siRNAs map to pathogenicity-modulating domain of viroid



# 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.





# 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

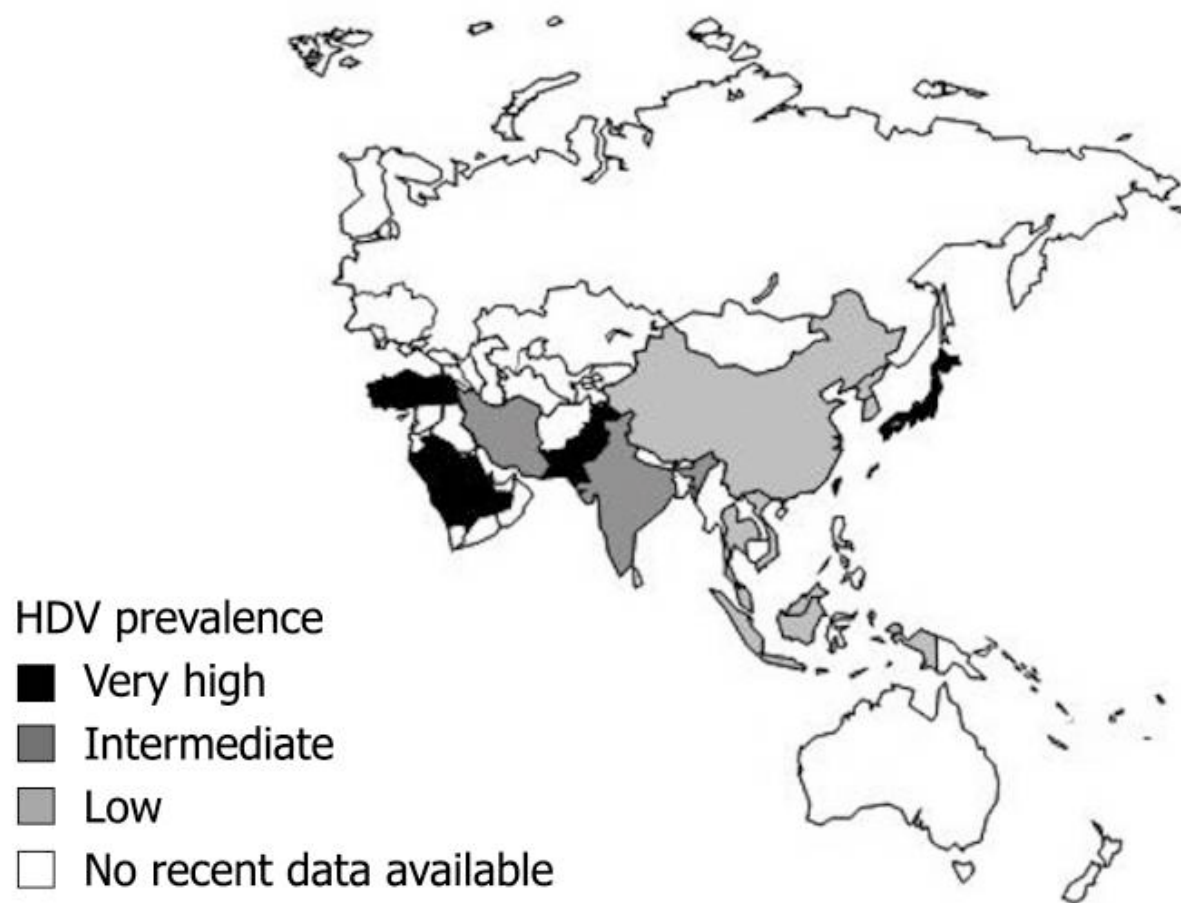
# Hepatitis delta virus

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

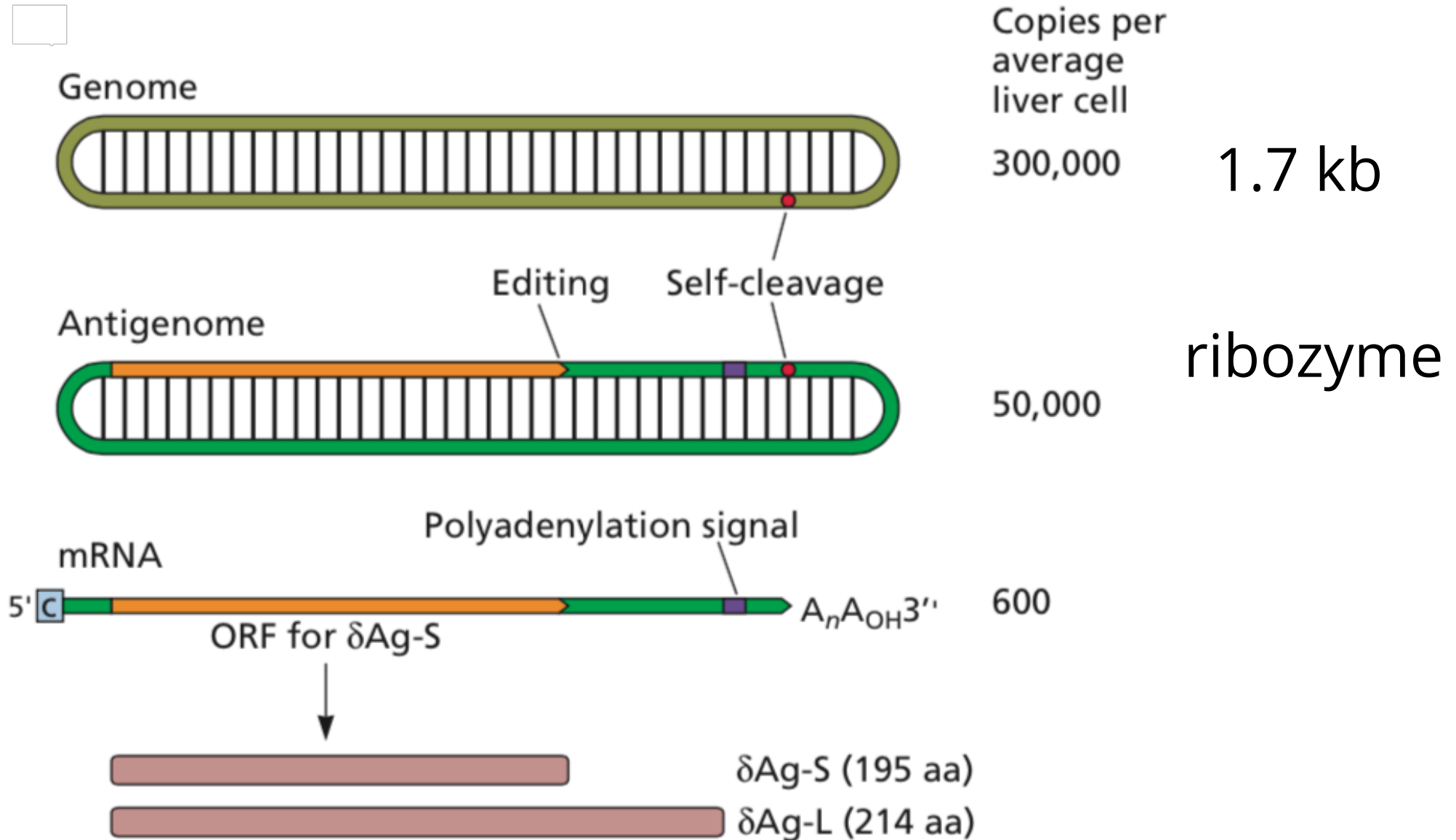
# HDV global distribution

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

Hepatitis Delta-Scenario in the Asia-Pacific region



# HDV genome

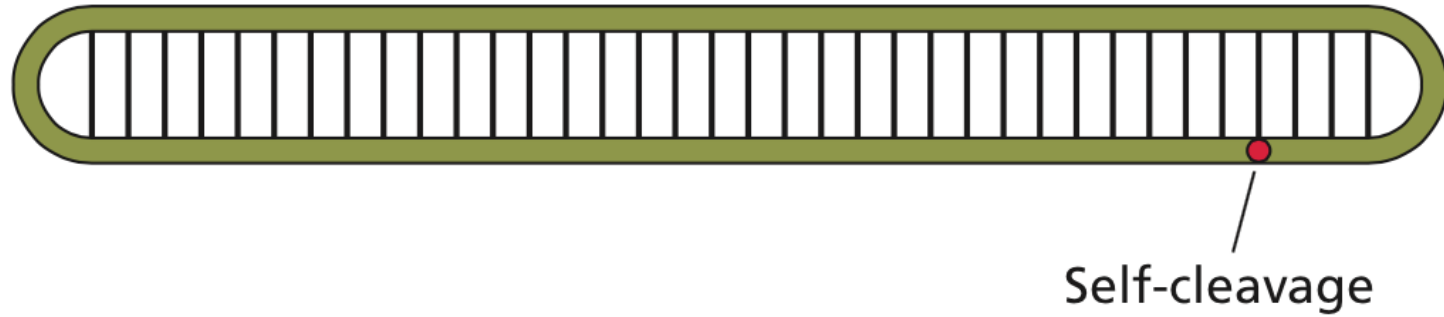




# HDV and HBV

**A**

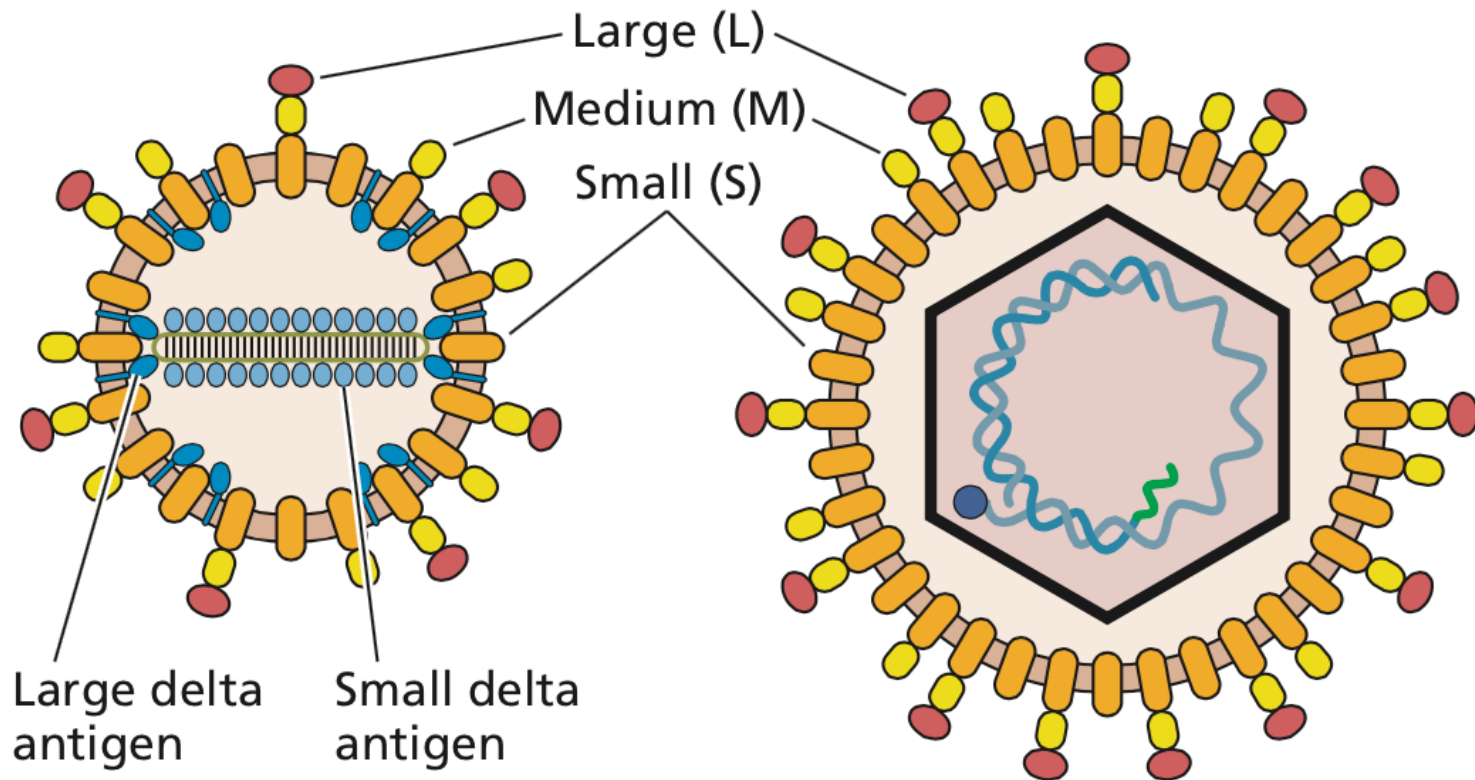
Genome

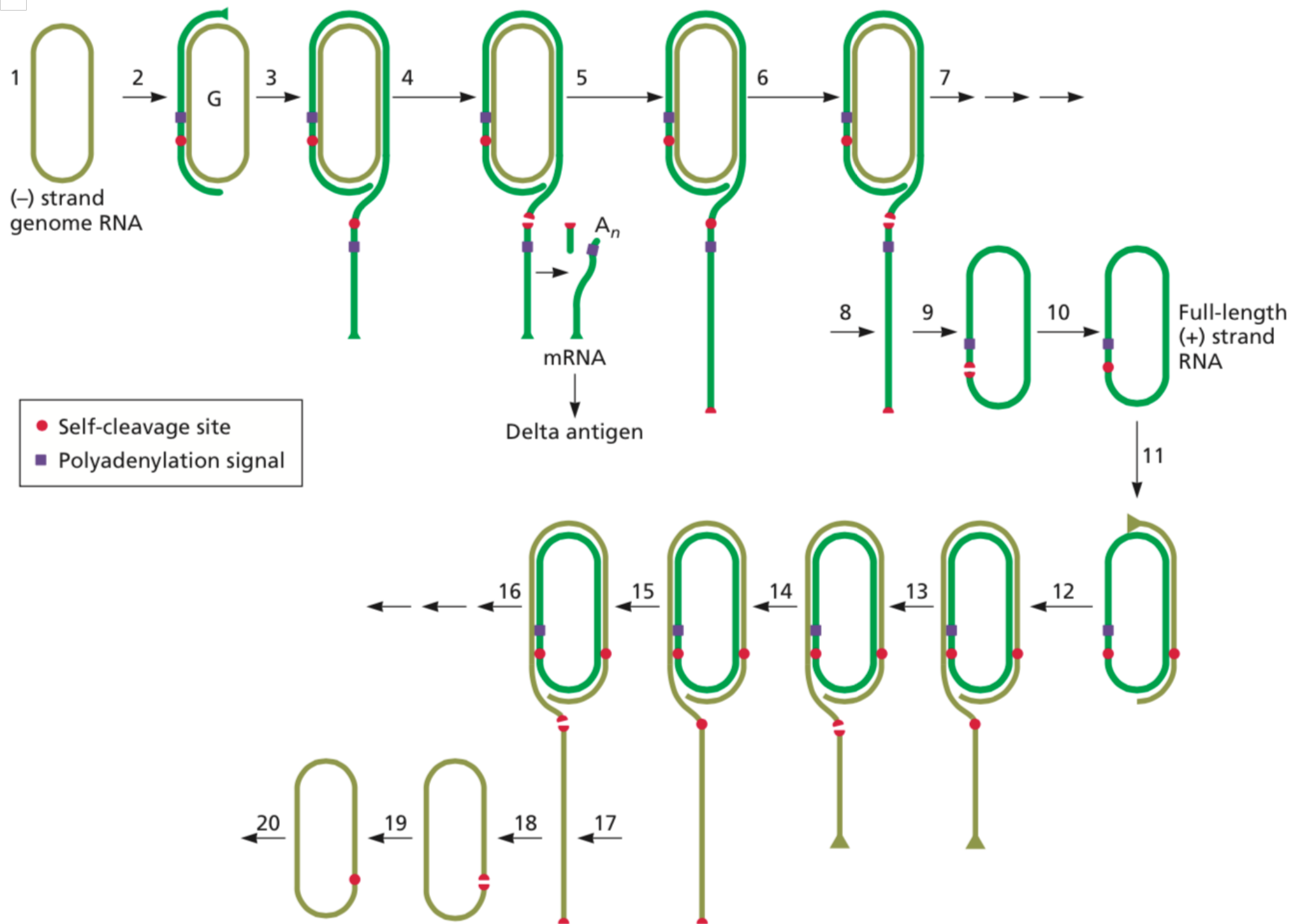


**B**

HDV virus particle

HBV helper virus



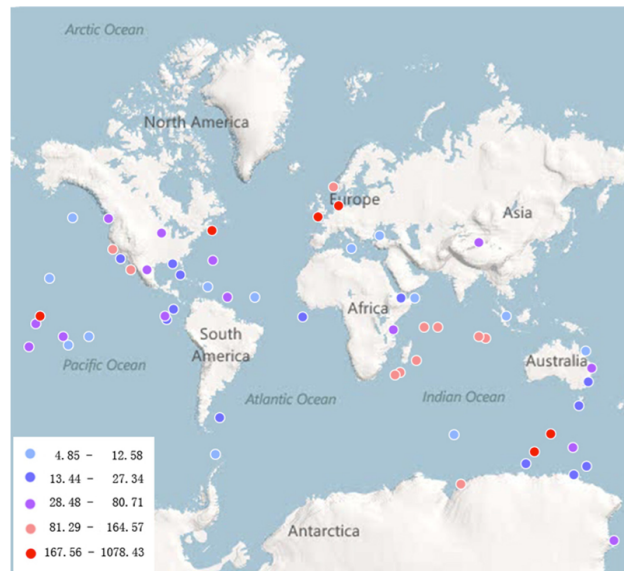


# Virophages

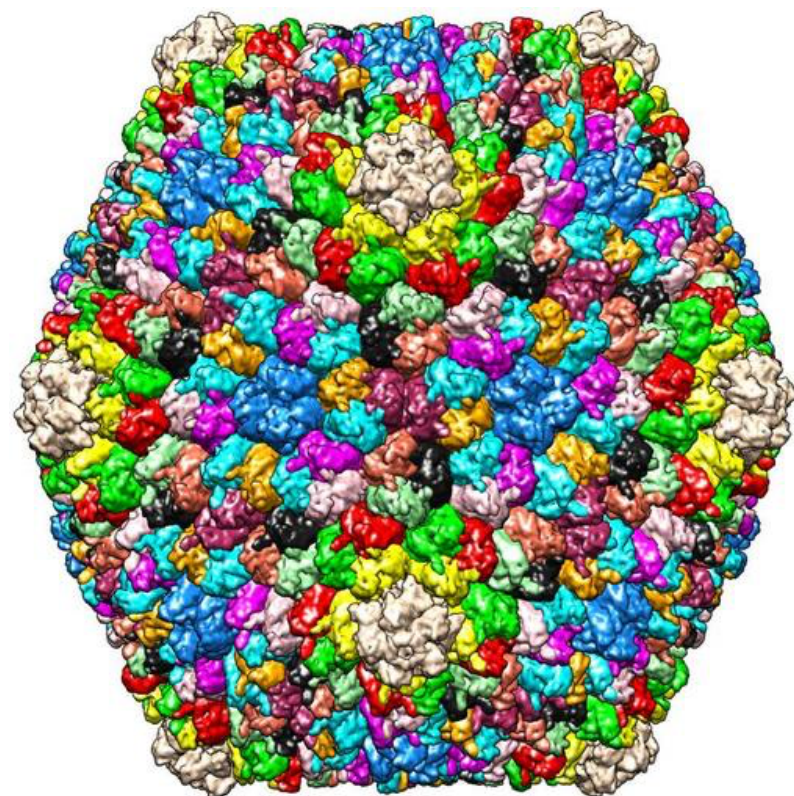
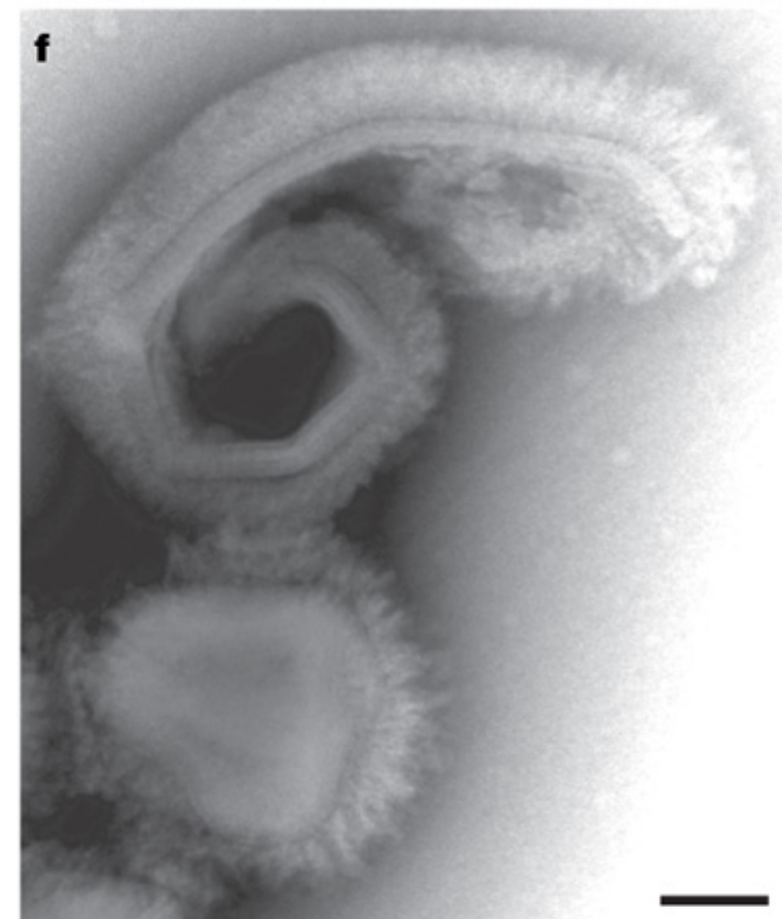
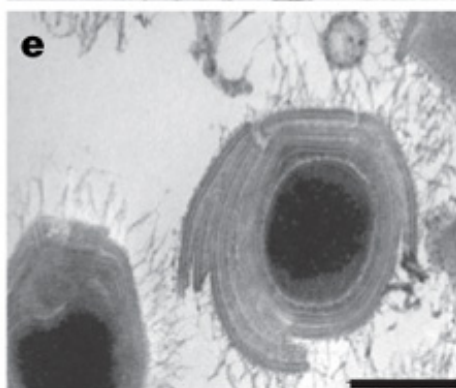
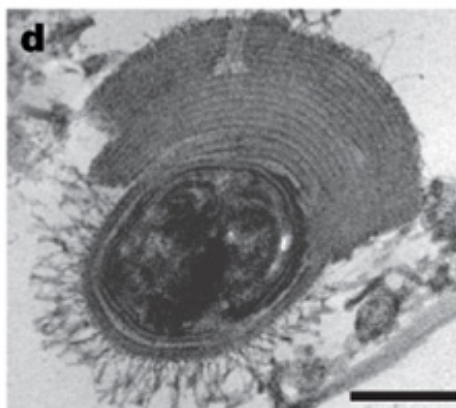
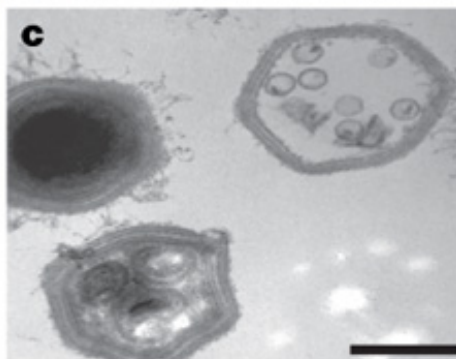
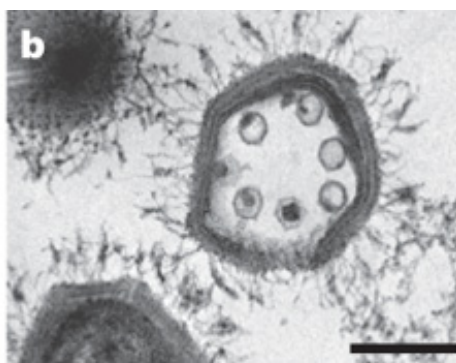
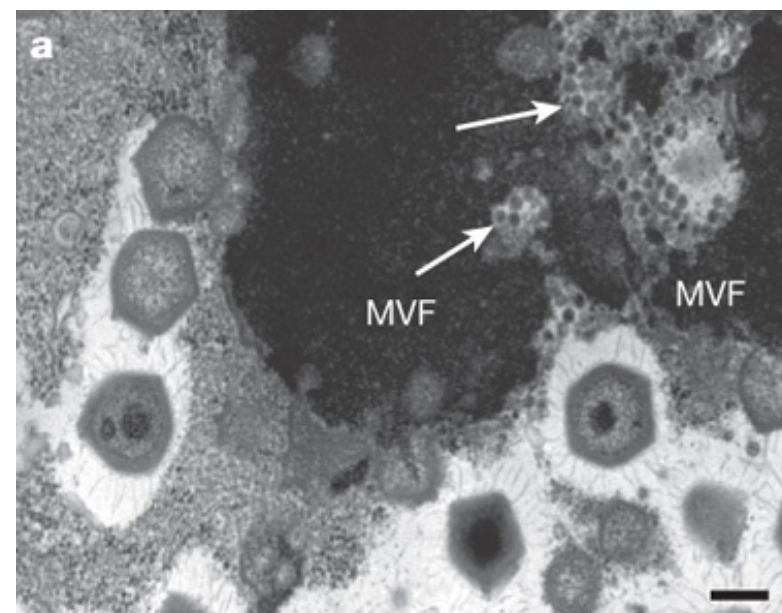
- Derived from bacteriophage (*phagein* = Greek, to eat), means 'virus eater'
- Circular dsDNA viruses, icosahedral
- Replicate only in cells infected with a giant virus
- Interfere with helper virus replication

# Virophages

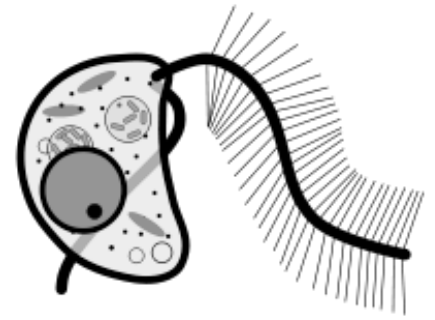
Virophage	Location	Host		Genome	
		Virus	Eukaryote	Size (bp)	No. of ORFs
Sputnik	A cooling tower in Paris, France	<i>Acanthamoeba polyphaga</i> mimivirus	<i>A. polyphaga</i>	18,343	21
Mavirus	Coastal waters of Texas	<i>Cafeteria roenbergensis</i> virus	Marine phagotrophic flagellate ( <i>C. roenbergensis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





# **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)



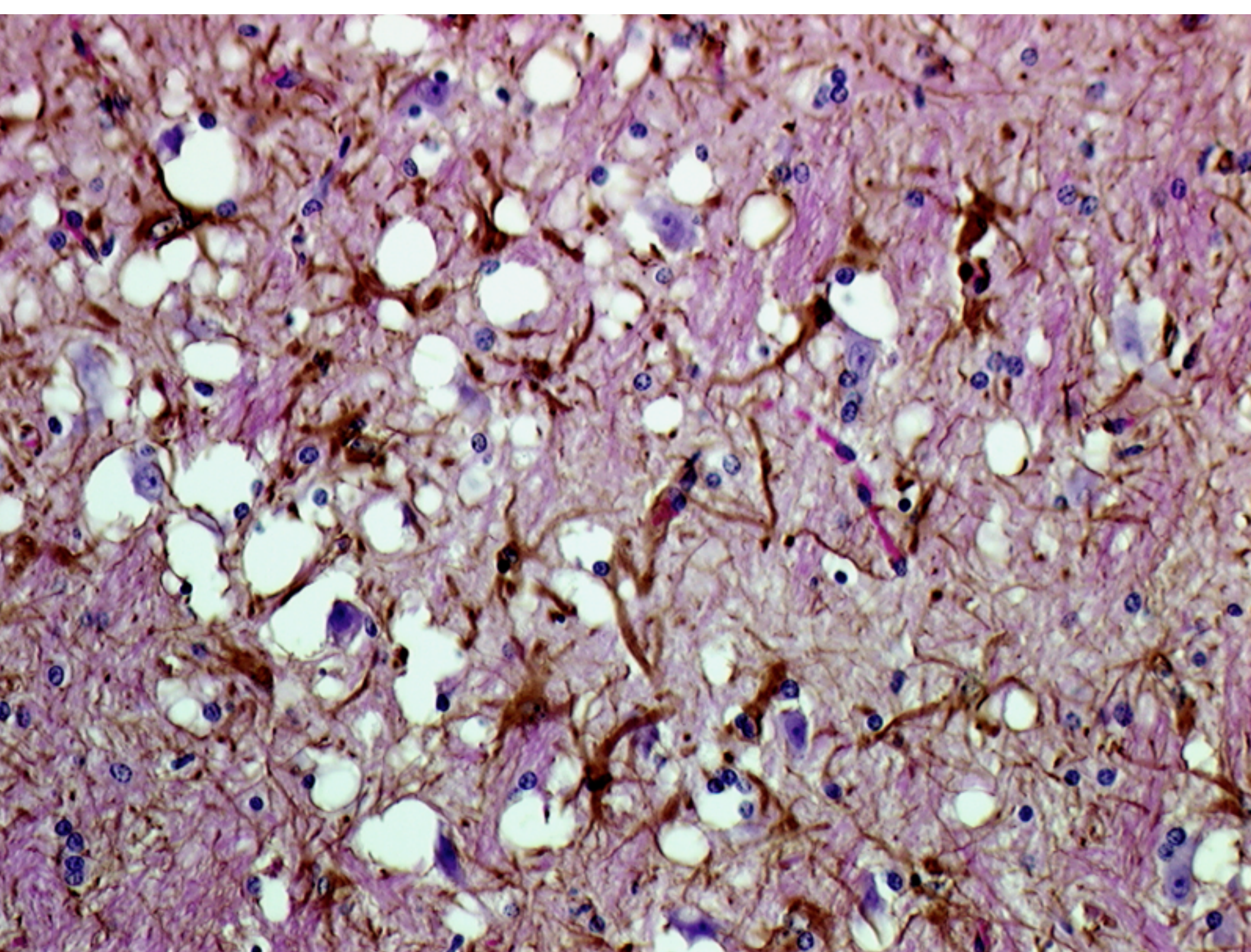
# Transmissible spongiform encephalopathies

- 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







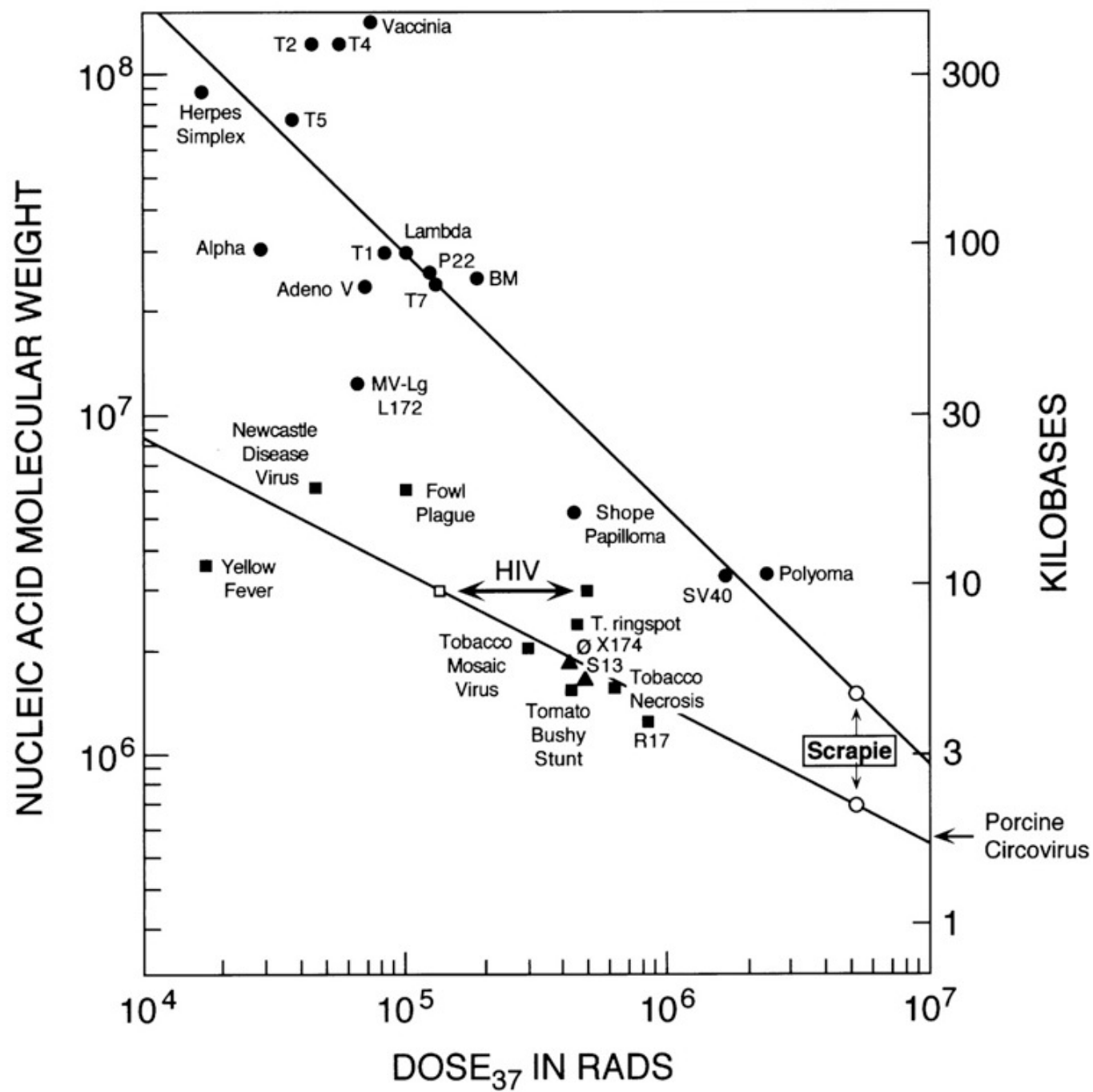
# Scrapie



- 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 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 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

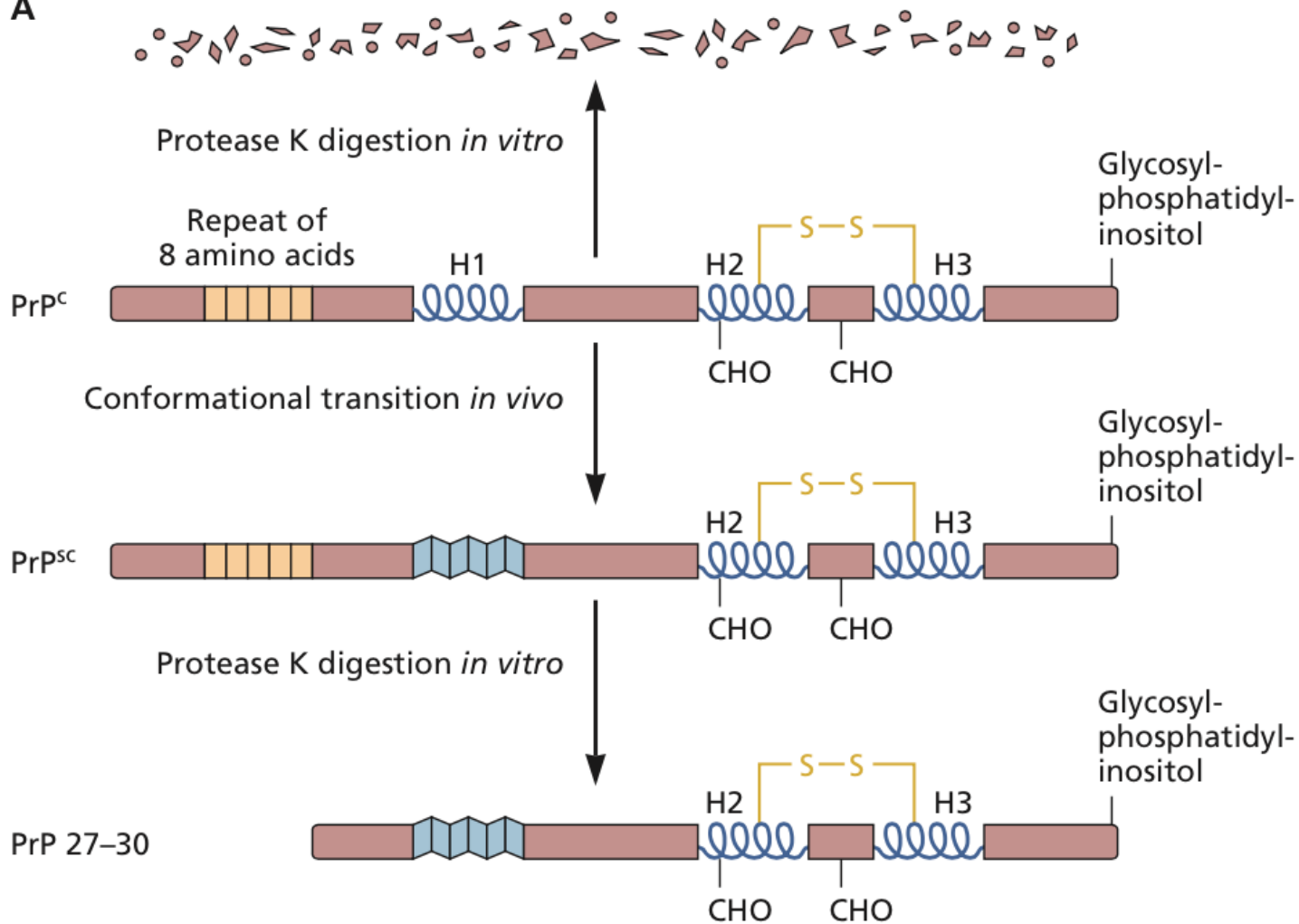
- 1967 Griffith suggested that TSE agents were protein
- 1981 Prusiner identified infectious protein complexes in scrapie brain, purified protein, transmitted to animals
- Called the agent a *prion*, (*proteinaceous and infectious particle*)
- Encoded by *prnp* gene, essential for pathogenesis of TSEs

# Current view of prions

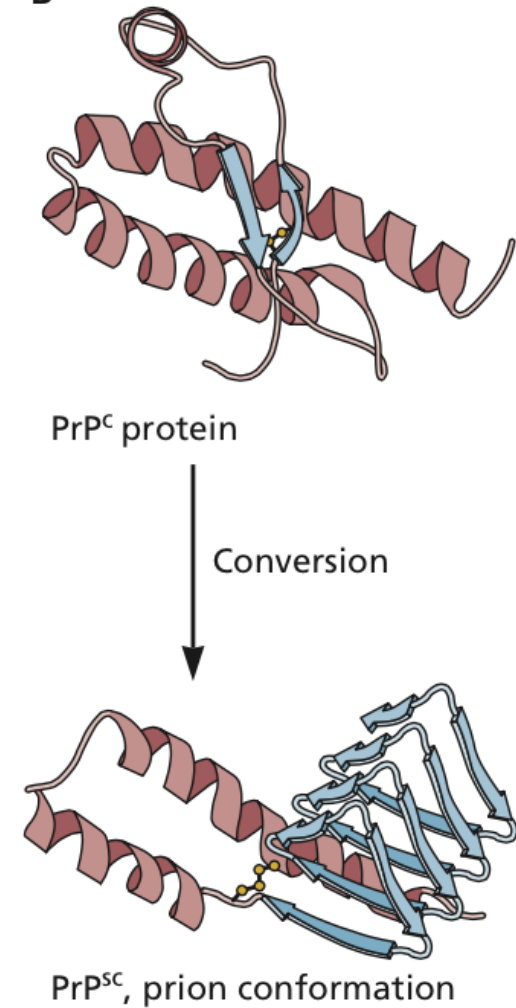
- Pathogenic prion is a conformational isoform of a normal host protein, PrP<sup>c</sup>
- PrP<sup>c</sup> is found predominantly on the outer surface of neurons, GPI anchor
- The abnormal conformer, when introduced into the organism, causes conversion of normal PrP<sup>c</sup> into the pathogenic conformation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

# PrP<sup>c</sup> to PrP<sup>sc</sup>

A



B



# Prion hypothesis

- Mice lacking both copies of *prnp* are resistant to infection
- PrP<sup>sc</sup> can be introduced ('infection') or produced by rare mutations in *prnp*
- PrP<sup>sc</sup> 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

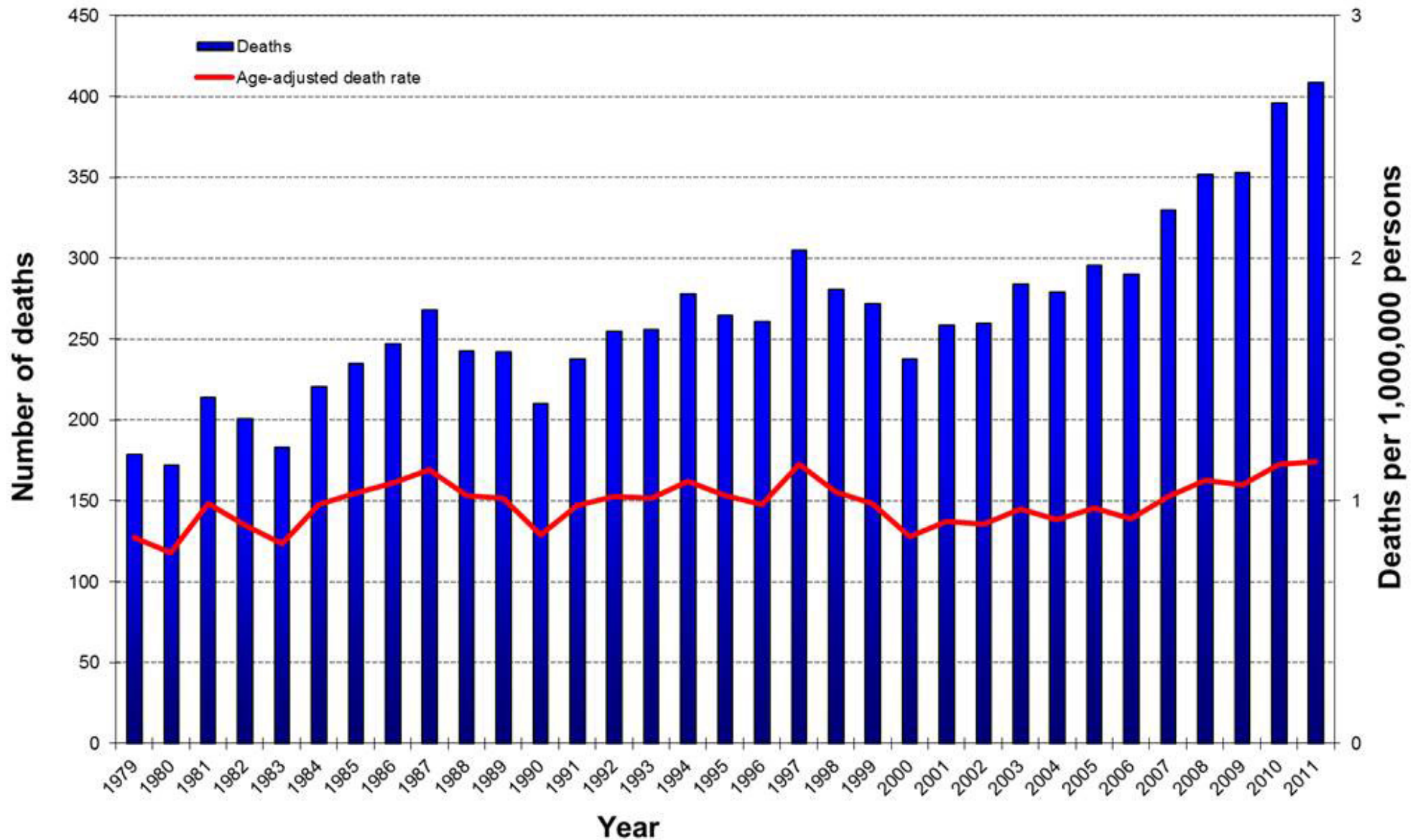
# Sporadic CJD

- 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

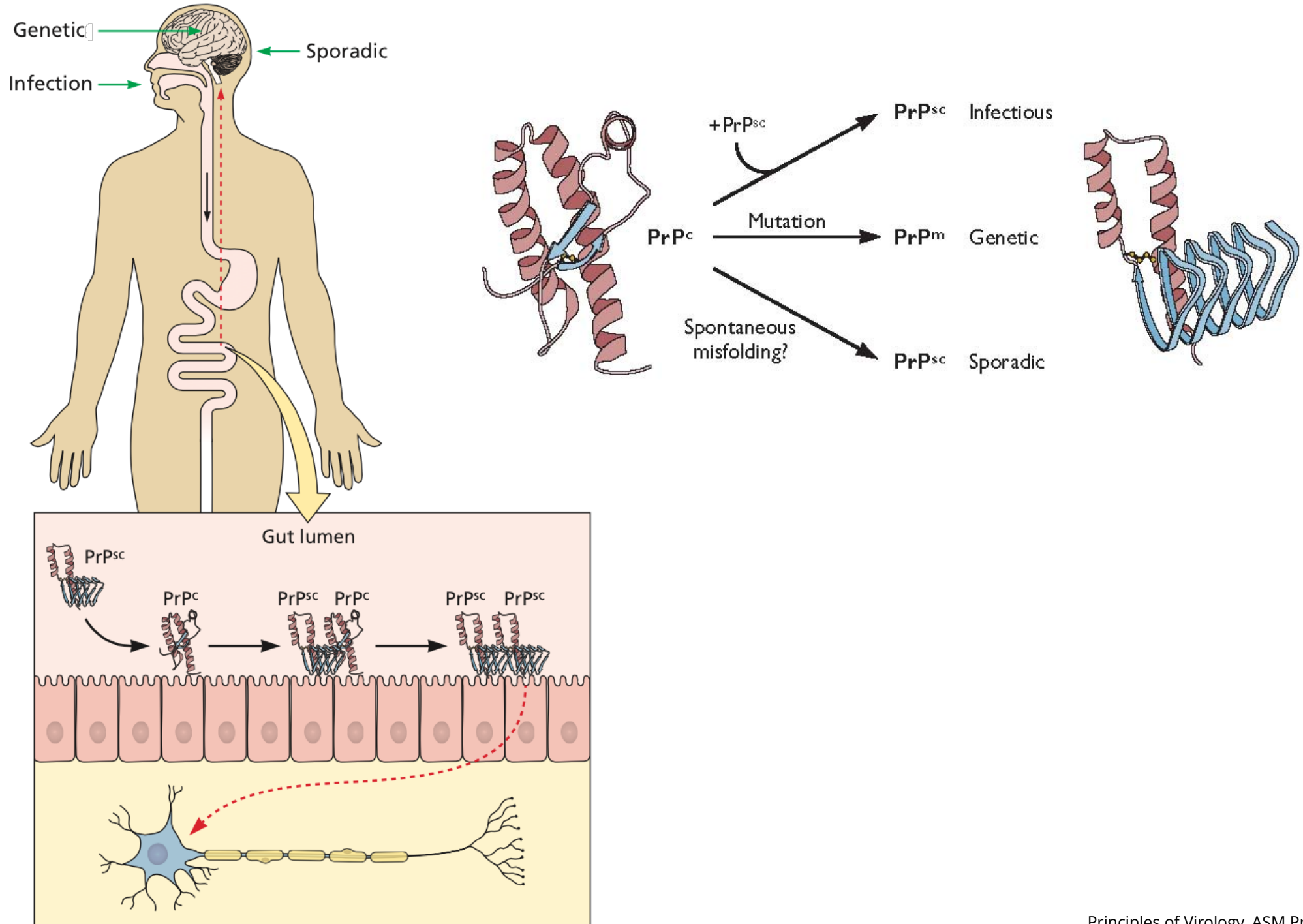
- An inherited disease
- Autosomal dominant mutation in *prnp* gene
- Organs, corneas, blood products from people afflicted with sporadic CJD can be infectious, transmit CJD to others

# Creutzfeldt-Jakob disease deaths and age-adjusted death rate, United States, 1979-2011\*



\* Deaths obtained from the multiple cause-of-death data for 1979-1998 are based on ICD-9 codes, and those beginning in 1999 are based on ICD-10 codes with available computerized literal death certificate data. Death information was also obtained from other surveillance mechanisms; includes familial prion disease. Rates are adjusted to the US standard 2000 projected population.

# 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

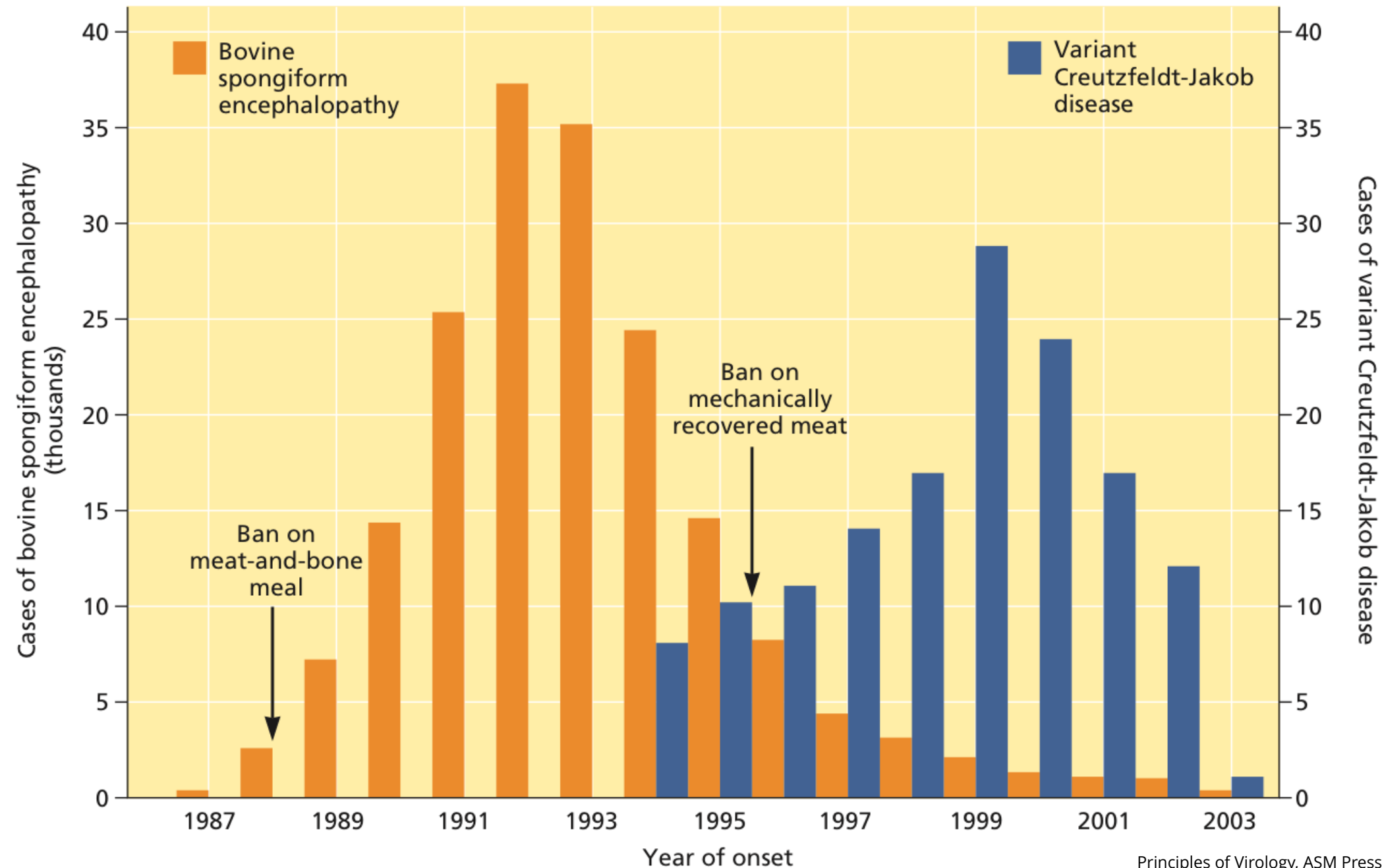


# Forced cannibalism spreads BSE

- 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

# 1-2 million cattle were infected with prions

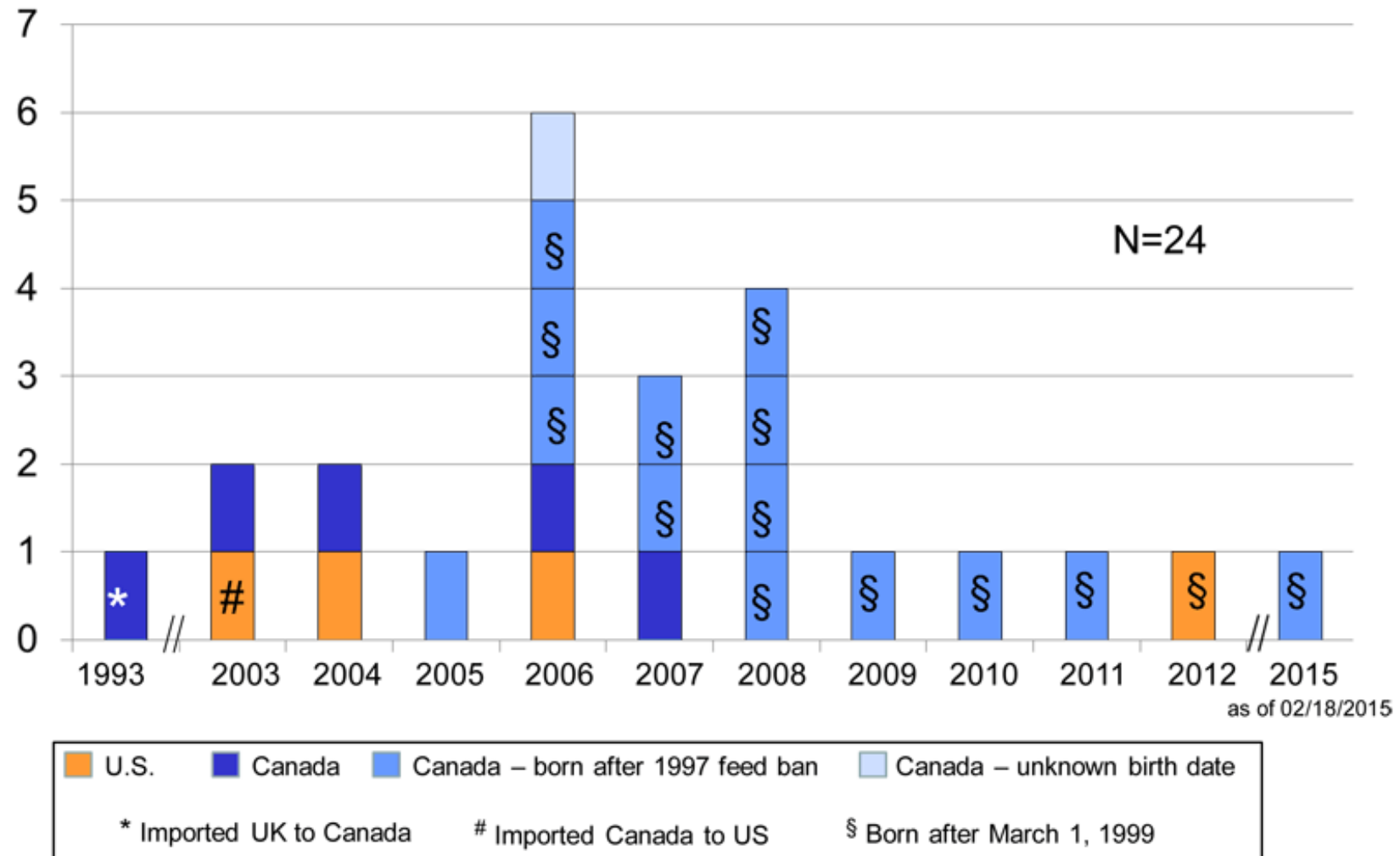
Incubation time 5 yr, slaughtered 2-3 yr



# 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 of Death, 1993 – Feb 2015

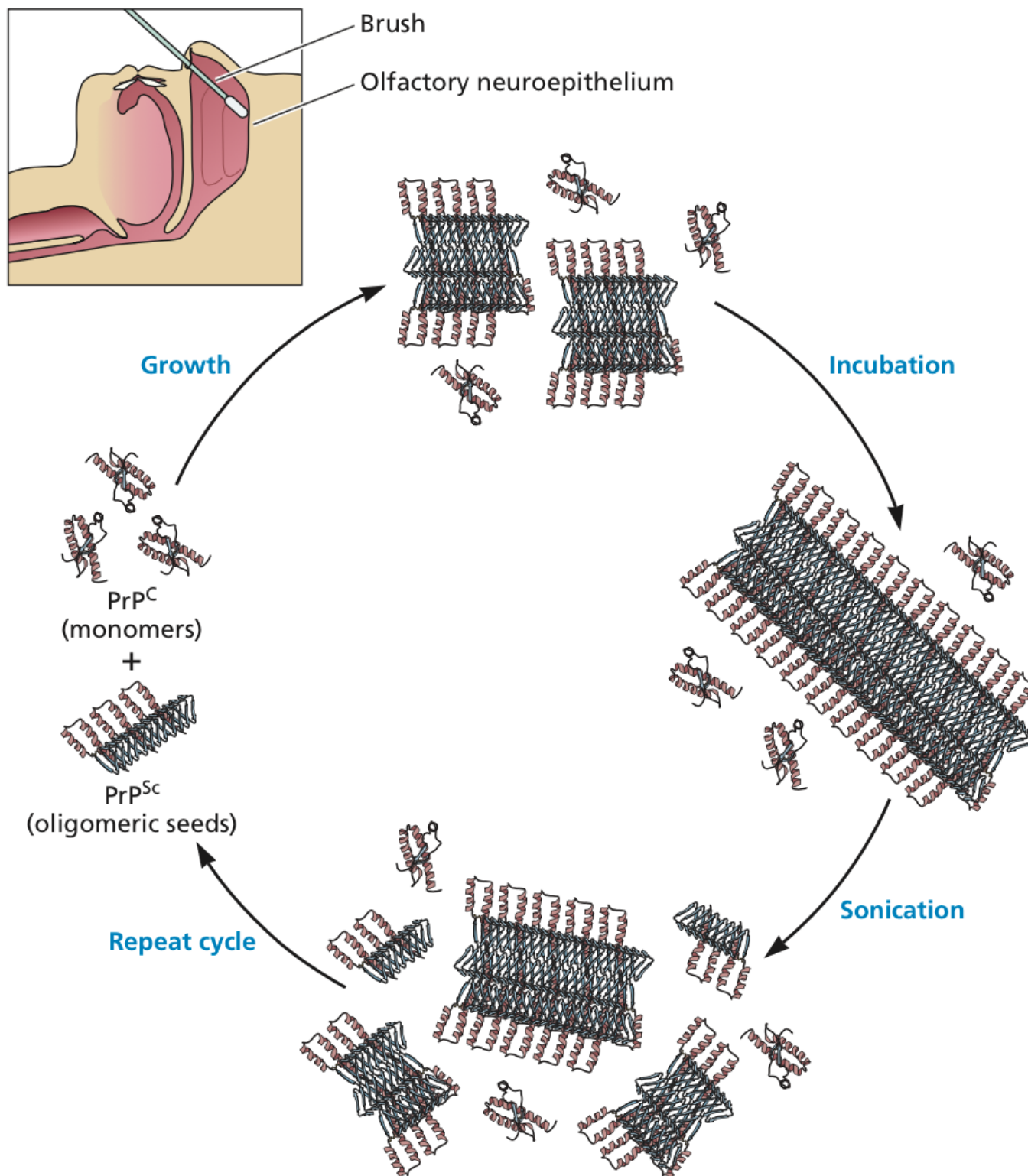


# **Case of prion disease from contaminated beef**

<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/>





# Prion species barrier

- Inoculation of diseased brain material into same species reproduces disease
- Inoculation into different species is inefficient
- Sequences of PrP<sup>Sc</sup> in inoculum and PrP<sup>C</sup> in host should be isologous
- Transgenic mice synthesizing bovine PrP<sup>C</sup> can be efficiently infected with BSE prions
- Barrier to interspecies transmission is in the sequence of PrP protein

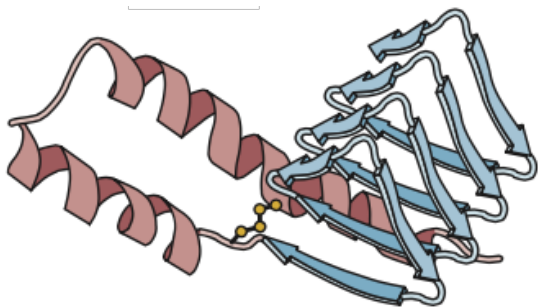
# Prion species barrier



Hamster PrP<sup>Sc</sup>



No disease



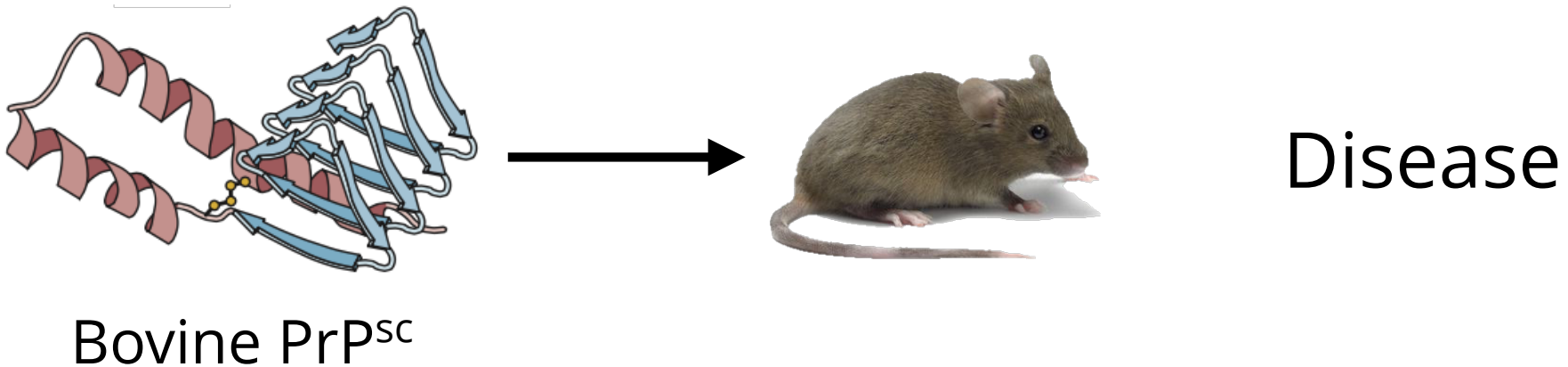
Hamster PrP<sup>Sc</sup>



Disease

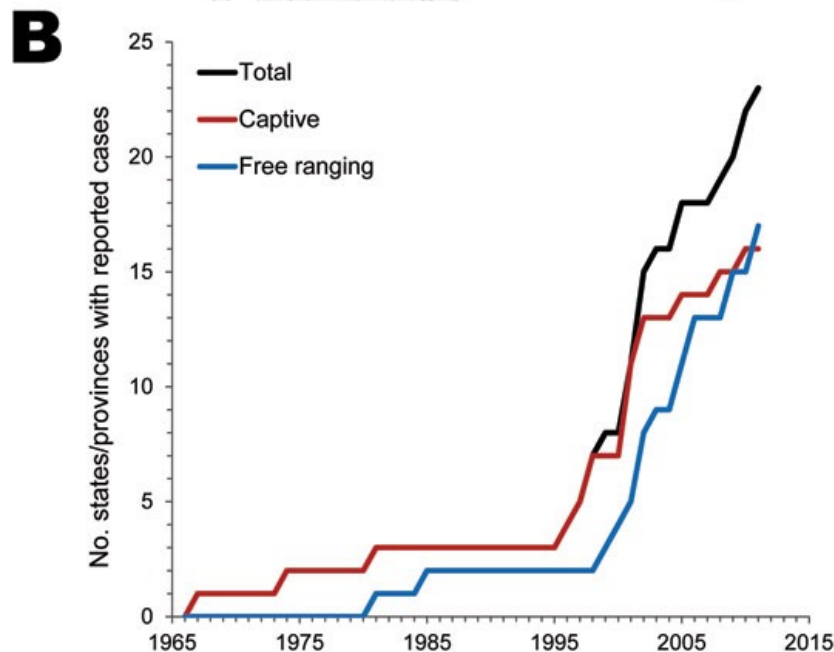
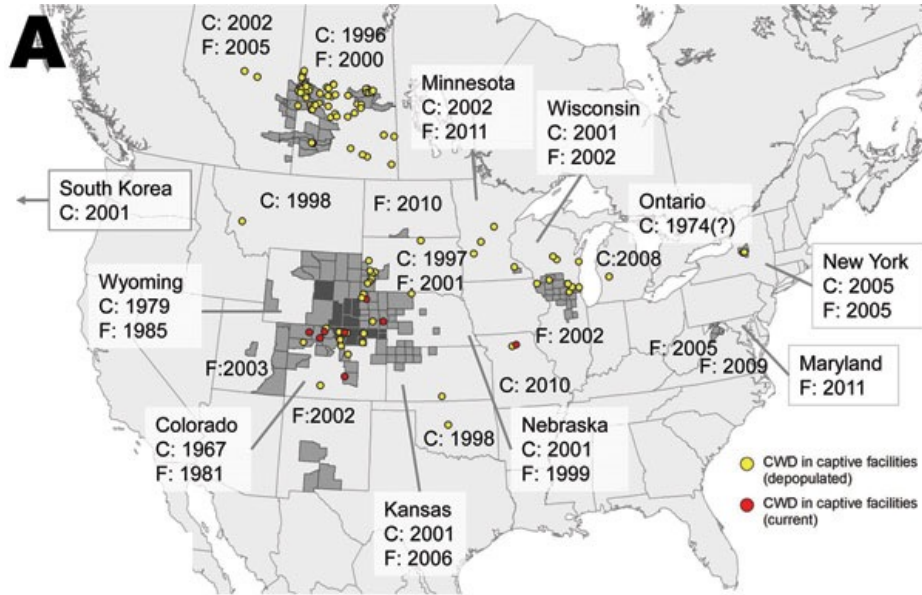
transgenic for  
hamster *prnp*

# Prion species barrier



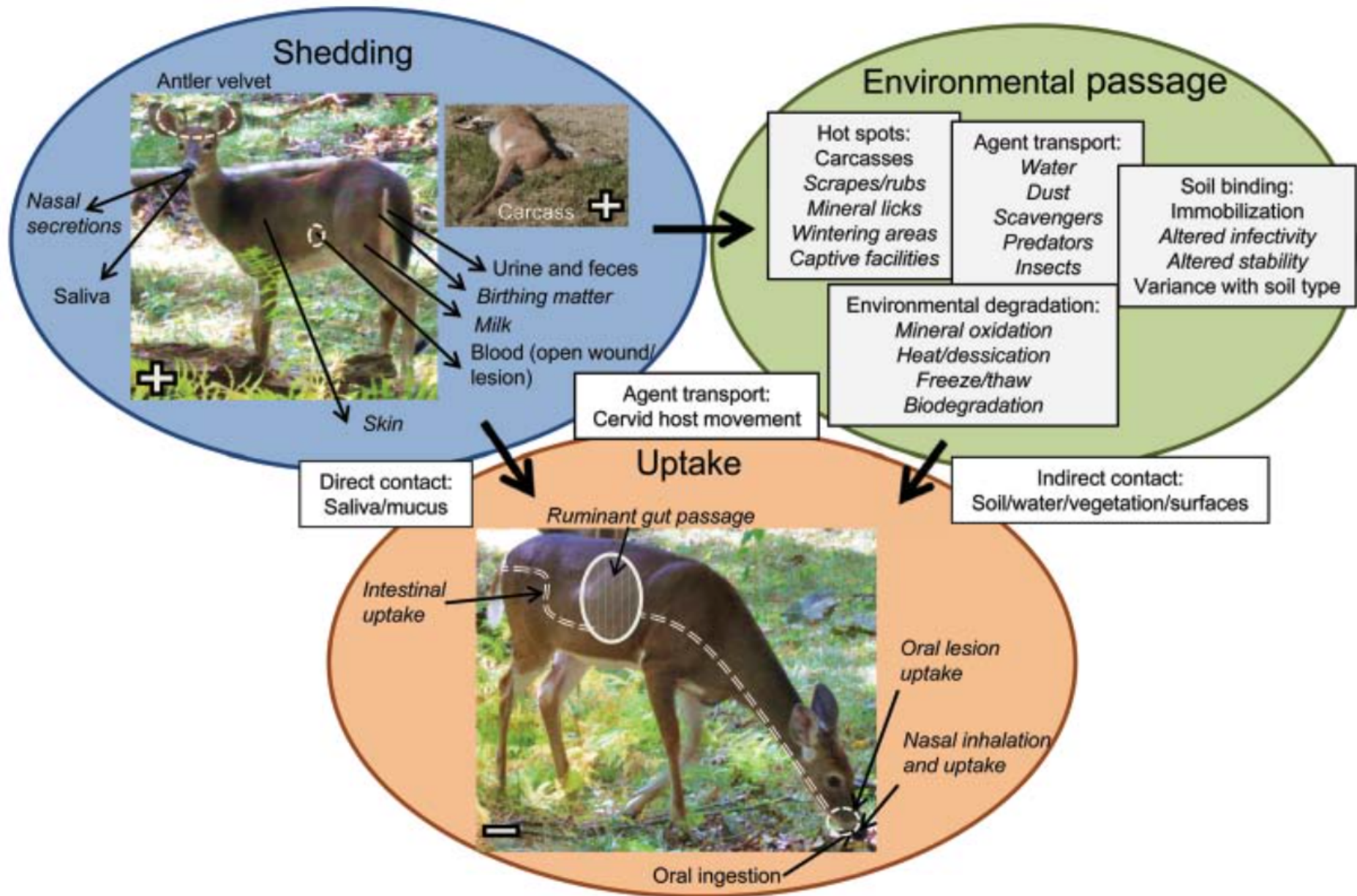
- BSE PrP<sup>Sc</sup> has broad host range, infects many meat eating mammals including humans
- Clearly some prions overcome the influence of primary sequence on host range
- This is why BSE is a concern

# Chronic wasting disease - TSE of deer, elk, moose



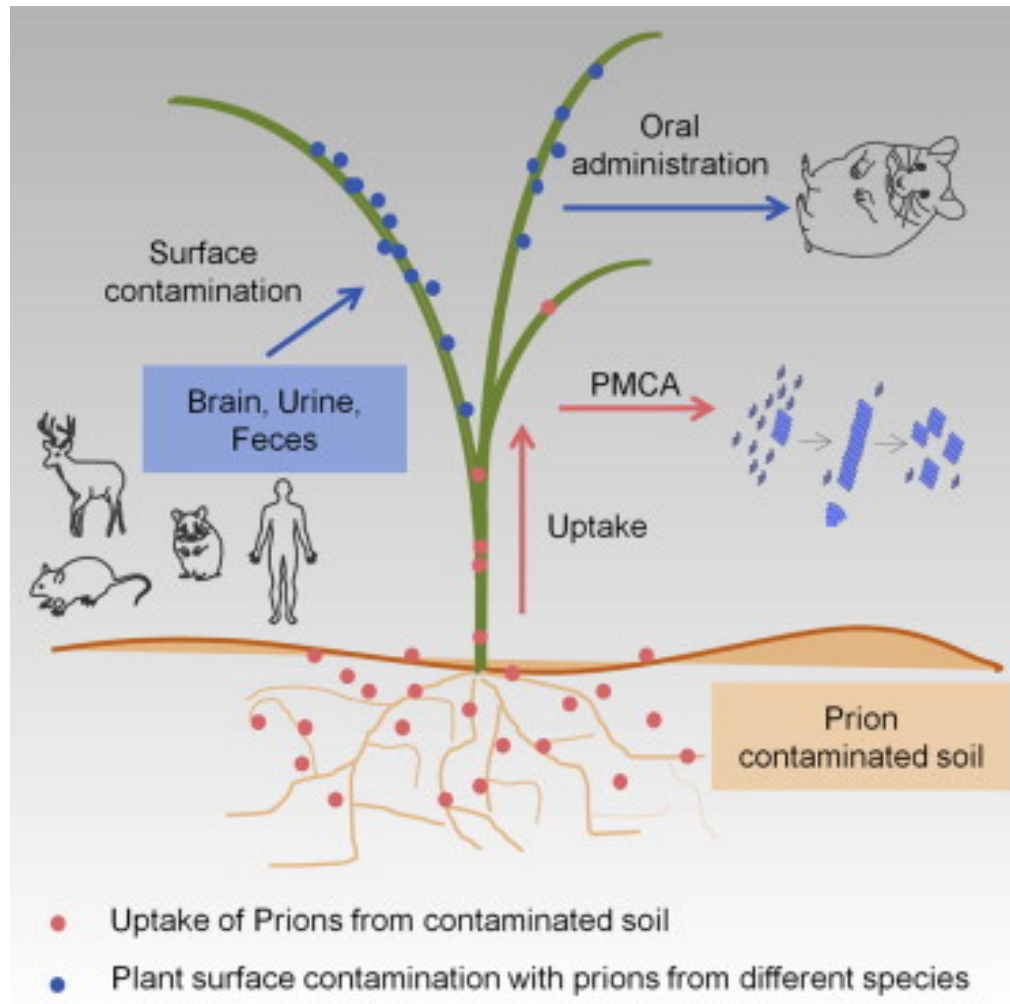
- In standing herds up to 90% of mule deer and 60% of elk are positive
- Incidence in wild cervids as high as 15%

# Chronic wasting disease

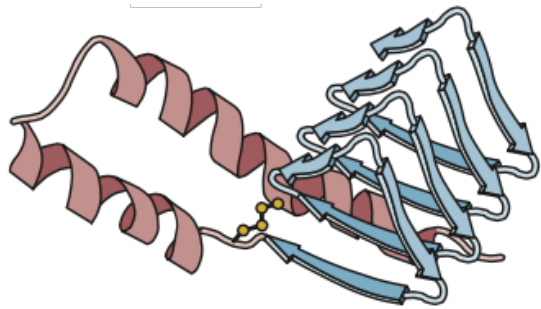




# Grass plants bind, retain, uptake, and transport infectious prions



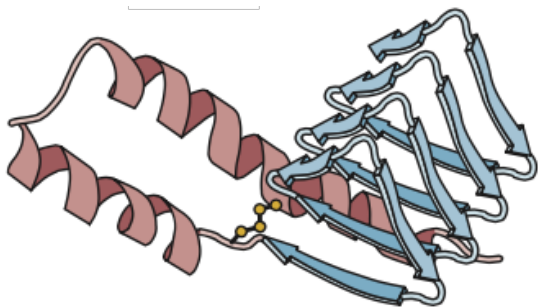
# Chronic wasting disease



Cervid PrP<sup>Sc</sup>



No disease



Cervid PrP<sup>Sc</sup>



Disease

transgenic for  
cervid *prnp*



# Chronic wasting disease



Cervid PrP<sup>Sc</sup>



Transgenic for  
human *prnp*

No disease

# Chronic wasting disease



Deer PrP<sup>Sc</sup>



Disease

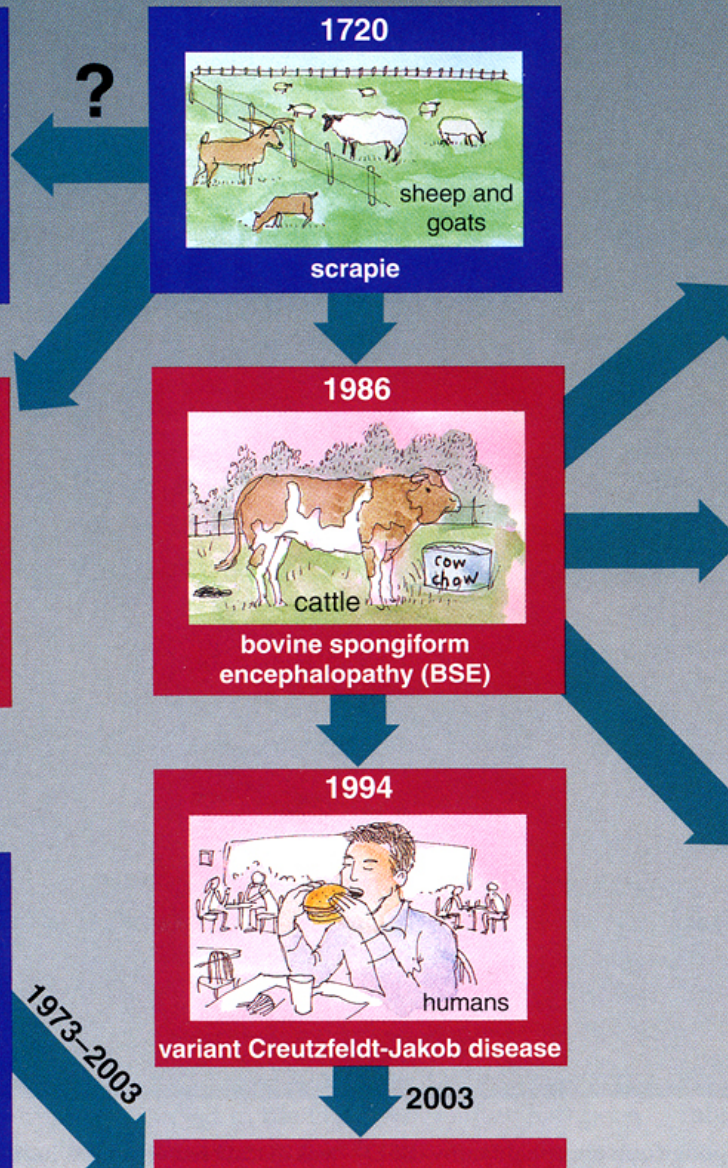
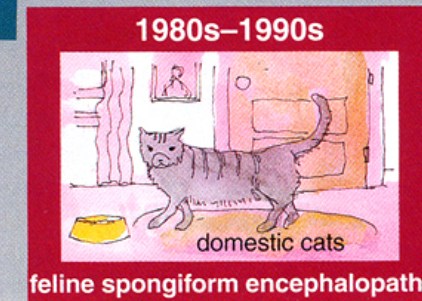
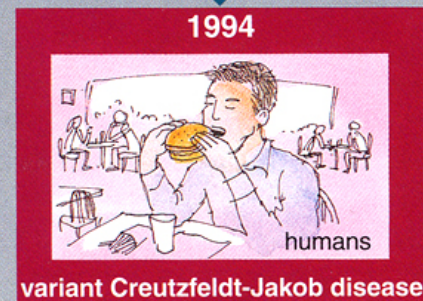
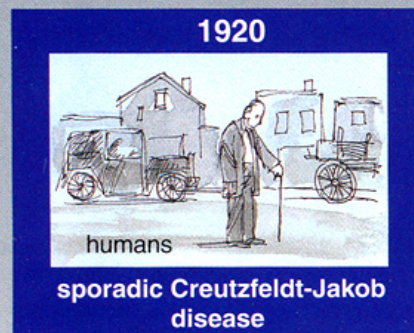
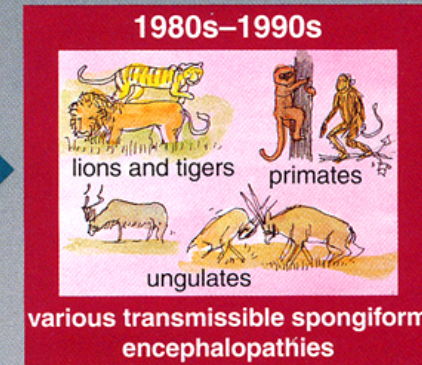
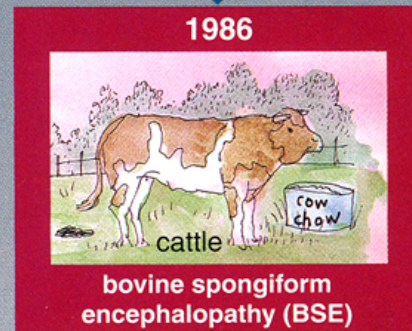
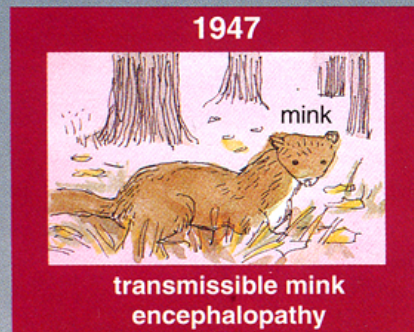
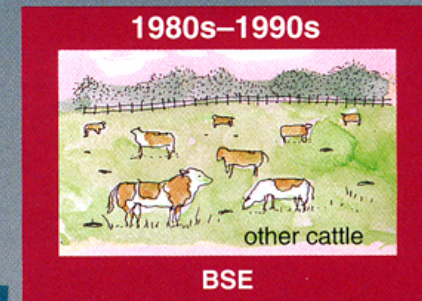
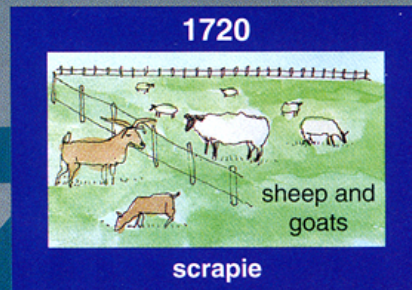
*Could CWD prions transmit to cattle grazing in contaminated pastures?*

# Hunters beware!

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

Do not shoot, handle or consume an elk or deer that is acting abnormally or appears to be sick. When field-dressing game, wear rubber gloves and minimize the use of a bone saw to cut through the brain or spinal cord (backbone). Bone out the meat. Minimize contact with and do not consume brain or spinal cord tissues, eyes, spleen, or lymph nodes. Always wash hands thoroughly after dressing and processing game meat.





naturally  
occurring  
disease



artificially  
occurring  
disease

