Unusual infectious agents

Lecture 23
Biology W3310/4310
Virology
Spring 2015

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 1742 viroid sequences
  - [http://subviral.med.uottawa.ca/cgi-bin/home.cgi](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

A PSTVd - Pospiviroidae

B 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
• **Satellite viruses**: Encode structural proteins that encapsidate the genome (form distinct particles)
• **Satellite RNAs**: Packaged by helper virus genome
• Lack genes required for replication
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

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

Copies per average liver cell
300,000
50,000

Polyadenylation signal

ORF for $\delta$Ag-S

$\delta$Ag-S (195 aa)
$\delta$Ag-L (214 aa)
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

<table>
<thead>
<tr>
<th>Virophage</th>
<th>Location</th>
<th>Host</th>
<th>Eukaryote</th>
<th>Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Virus</td>
<td>Eukaryote</td>
<td>Size (bp)</td>
</tr>
<tr>
<td>Sputnik</td>
<td>A cooling tower in Paris, France</td>
<td>Acanthamoeba polyphaga mimivirus</td>
<td>A. polyphaga</td>
<td>18,343</td>
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<tr>
<td>Mavirus</td>
<td>Coastal waters of Texas</td>
<td>Cafeteria roenbergensis virus</td>
<td>Marine phagotrophic flagellate (C. roenbergensis)</td>
<td>19,063</td>
</tr>
<tr>
<td>OLV</td>
<td>Organic Lake, a hypersaline meromictic lake in Antarctica</td>
<td>Large DNA viruses</td>
<td>Prasinophytes (phototrophic algae)</td>
<td>26,421</td>
</tr>
<tr>
<td>Sputnik 2</td>
<td>Contact lens fluid of a patient with keratitis, France</td>
<td>Lentille virus</td>
<td>A. polyphaga</td>
<td>18,338</td>
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<tr>
<td>YSLV1</td>
<td>Yellowstone Lake</td>
<td>Phycodna- or mimiviruses?</td>
<td>Microalgae?</td>
<td>27,849</td>
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<td>YSLV2</td>
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<td>Phycodna- or mimiviruses?</td>
<td>Microalgae?</td>
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<td>Phycodna- or mimiviruses?</td>
<td>Microalgae?</td>
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<td>ALM</td>
<td>Ace Lake in Antarctica</td>
<td>mimiviruses?</td>
<td>Phagotrophic protozoan?</td>
<td>17,767</td>
</tr>
</tbody>
</table>
Virophages

• Mavirus - virophage of giant virus of *Cafeteria roenbergensis*, a marine phagotrophic 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
• 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)

• 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 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
proteinaceous infectious particles

- 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, \( \text{PrP}^c \)
- \( \text{PrP}^c \) is found predominantly on the outer surface of neurons, GPI anchor
- The abnormal conformer, when introduced into the organism, causes conversion of normal \( \text{PrP}^c \) into the pathogenic conformation (\( \text{PrP}^{sc} \) for scrapie)
PrP\textsuperscript{c} to PrP\textsuperscript{sc}

A

Protease K digestion \textit{in vitro}

Repeat of 8 amino acids

PrP\textsuperscript{c}

H1

H2

H3

CHO

CHO

Glycosyl-phosphatidylinositol

PrP\textsuperscript{sc}

Conformational transition \textit{in vivo}

H2

H3

CHO

CHO

Glycosyl-phosphatidylinositol

Protease K digestion \textit{in vitro}

PrP 27–30

CHO

CHO

Glycosyl-phosphatidylinositol

B

PrP\textsuperscript{c} protein

Conversion

PrP\textsuperscript{sc}, prion conformation

Principles of Virology, ASM Press
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
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.

http://www.cdc.gov/ncidod/dvrd/cjd/
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

**Chart:**
- **Orange bars:** Bovine spongiform encephalopathy (thousands)
- **Blue bars:** Variant Creutzfeldt-Jakob disease

**Key Events:**
- Ban on meat-and-bone meal
- Ban on mechanically recovered meat

**Years of Onset:**
- 1987 to 2003
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
Prion species barrier

• Inoculation of diseased brain material into same species reproduces disease
• Inoculation into different species is inefficient
• Sequences of PrP\textsuperscript{Sc} in inoculum and PrP\textsuperscript{C} in host should be isologous
• Transgenic mice synthesizing bovine PrP\textsuperscript{C} can be efficiently infected with BSE prions
• Barrier to interspecies transmission is in the sequence of PrP protein
Prion species barrier

Hamster PrP\textsuperscript{sc} → no disease

Hamster PrP\textsuperscript{sc} → transgenic for hamster prnp → disease
Prion species barrier

- BSE PrP\textsuperscript{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

cervid PrP\textsuperscript{sc} → no disease

cervid PrP\textsuperscript{sc} transgenic for cervid \textit{prnp} → disease
Chronic wasting disease

cervid PrP$^\text{sc}$ → transgenic for human *prnp* → no disease
Could CWD prions transmit to cattle grazing in contaminated pastures?
Hunters beware!

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.

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