Unusual infectious agents

Lecture 25
Biology 4310
Virology
Spring 2020

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

*Production of potato seed stocks
Some of my favorite viroids

- Cadang-Cadang coconut viroid
  - \textit{CCCVd} causes \textit{lethal disease of coconut palms}
  - \textit{Pina colada drinkers are sad}

- Hop latent viroid
  - \textit{HLVd} - \textit{no symptoms in the hop plant}
  - \textit{Beer lovers are relieved}

- Apple scar skin viroid
  - \textit{ASSVd} - \textit{mild symptoms; apples look bad, taste good}
  - \textit{Picky consumers don’t buy these apples}
Vertical and horizontal transmission of viroids in plants via pollen
Functional regions of viroid RNA

A PSTVd - *Pospiviroidae*
- Terminal left ($T_L$)
- Pathogenesis
- Central conserved region
- Variable
- Terminal right ($T_R$)

B PLMVd - *Avsunviroidae*
- CAGACUU
- GUCUGAG
- 50
- 290
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

- 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.
Replication of satellite RNA

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
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>
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<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>
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<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
HDV and HBV

A

Genome

Self-cleavage

B

HDV virus particle

Large (L)
Medium (M)
Small (S)

Large delta antigen
Small delta antigen

HBV helper virus
Hepatitis delta-like viruses in birds and snakes

- Delta antigens divergent from human protein
- No HBV-like viruses in birds or snakes examined
- Another enveloped virus might serve as helper
- In vitro, HCV, VSV, dengue virus, WNV can be helpers for HDV
Virophages

- Derived from bacteriophage (\textit{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>Genome</th>
<th>Size (bp)</th>
<th>No. of ORFs</th>
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<tbody>
<tr>
<td>Sputnik</td>
<td>A cooling tower in Paris, France</td>
<td><em>Acanthamoeba polyphaga</em> mimivirus</td>
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<td>18,343</td>
<td>21</td>
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<td>Mavirus</td>
<td>Coastal waters of Texas</td>
<td><em>Cafeteria roenbergensis</em> virus</td>
<td></td>
<td>19,063</td>
<td>20</td>
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<td>OLV</td>
<td>Organic Lake, a hypersaline meromictic lake in Antarctica</td>
<td>Large DNA viruses</td>
<td></td>
<td>26,421</td>
<td>26</td>
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<tr>
<td>Sputnik 2</td>
<td>Contact lens fluid of a patient with keratitis, France</td>
<td>Lentiviruses</td>
<td></td>
<td>18,338</td>
<td>20</td>
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<td>Yellowstone Lake</td>
<td>Phycodnavirales or mimiviruses?</td>
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<td>ALM</td>
<td>Ace Lake in Antarctica</td>
<td>mimiviruses?</td>
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<td>17,767</td>
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</table>

![World Map](image.png)

Virology Lectures 2020 • Prof. Vincent Racaniello • Columbia University
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

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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.

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
• 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 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 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*, *(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<sub>c</sub>
- PrP<sub>c</sub> is found predominantly on the outer surface of neurons, GPI anchor
- The abnormal conformer, when introduced into the organism, causes conversion of normal PrP<sub>c</sub> into the pathogenic conformation (PrP<sub>sc</sub> for scrapie)
PrP<sub>c</sub> to PrP<sub>sc</sub>

A

Protease K digestion <i>in vitro</i>

Repeat of 8 amino acids

PrP<sub>c</sub>

H1

H2

H3

CHO

CHO

Conformational transition <i>in vivo</i>

PrP<sub>sc</sub>

H2

H3

CHO

CHO

Protease K digestion <i>in vitro</i>

PrP 27–30

H2

H3

CHO

CHO

B

PrP<sub>c</sub> protein

Conversion

PrP<sub>sc</sub>, prion conformation

Glycosyl-phosphatidylinositol
Prion hypothesis

- Mice lacking both copies of prnp are resistant to infection
- PrPsc can be introduced ('infection') or produced by rare mutations in prnp
- PrPsc 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
CJD deaths, US, 1979-2017

https://www.cdc.gov/prions/cjd/occurrence-transmission.html
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, 1993-2018

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/
Blood

Growth

PrPc
(monomers) +

PrPsc
(oligomeric seeds)

Incubation

Sonication

Repeat cycle
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
- 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
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%
Grass plants bind, retain, take up, and transport infectious prions
Chronic wasting disease

Cervid PrP<sub>Sc</sub> → No disease

Cervid PrP<sub>Sc</sub> transgenic for cervid <i>prnp</i> → Disease
Chronic wasting disease

Cervid PrP\textsubscript{Sc} → Transgenic for human \textit{prnp} → No disease
Chronic wasting 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 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?
Next time: Therapeutic viruses