Lecture 9

Viruses of Simple Eukaryotes
General properties

Most viruses of simple eukaryotes do not have an extracellular phase. Transmitted by cell-cell fusion as a consequence of the mating process.
History & Impact

• La France disease.
• Discovered in Pennsylvania, 1948.
• Pathology: gooey, yucky, slimy mushrooms.
• Infection can cause major economic impact.
• The first fungal virus discovered.
• La France isometric virus (LIV)
• Family: Partitiviridae
• dsRNA virus of Agaricus bisporus, the common mushroom.
• LIV genome contains nine dsRNA molecules
• Packaged into isometric 34 nm diameter virus particles.
• Three proteins of M(r) 120K, 115K and 90K known to be associated with LIV.
# Partitiviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host species</th>
<th>Genome size</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partitiviridae</td>
<td><em>Penicillium crysogenum</em></td>
<td>2.5, 1.4</td>
<td>Associated killer phenomenon, two segments, separate particles</td>
</tr>
<tr>
<td>PcV</td>
<td><em>Penicillium stoloniferum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PsV-F, PsV-S</td>
<td><em>Agaricus bisporus</em></td>
<td>2.2, 2.0</td>
<td>La France disease of mushrooms</td>
</tr>
<tr>
<td>AbV-4</td>
<td><em>Rhizoctonia solani</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RsV</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
History & Impact

1952-53. Culture filtrates from *Penicillium* species were found to induce resistance to viral infections in mice. Interferon induction.
Cryphonectria parasitica =
the Chestnut Blight fungus.

- Chestnut Blight caused by Cryphonectria (Endothia) parasitica
- Enters wounds, grows in and under bark
- Kills cambium in infected twigs, branches, and trunks
Before the fall: American chestnuts in the Great Smokey Mountains of North Carolina in 1910.
http://www.nature.com/news/plant-science-the-chestnut-resurrection-1.11504
# Viruses of *Cryphonectria parasitica*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host species</th>
<th>Genome size</th>
<th>Features</th>
</tr>
</thead>
</table>
| Hypoviridae<br>
* L = HAV = CHV1 | *Cryphonectria parasitica* | 12.7        | Hypovirulence-associated virus, potyvirus-like, chestnut blight |
| Reoviridae<br>
* C18, 9B21<br>Unclassified<br>NB631 RNA | *Cryphonectria parasitica* | 11 segments | B. Hillman                                             |
|           | *Cryphonectria parasitica* | 2.7         | Mitochondrial, resembles 20S and 23S RNA, hypovirulence; B. Hillman |
The CP hypovirus.

- Infection with dsRNA CP hypovirus prevents orange pigment formation in the fungus.
- Decreases pathgenicity of the fungus.
- Used to save American Chestnuts.

- EP155: Uninfected fungus
- G1-1: Infected fungus
- G2-37: Infected with viral mutant
Timeline of discovery.

1904
- First report of chestnut blight in North America (77)

1938
- First report of chestnut blight in Europe (8)

1951
- Discovery of hypovirulent C. parasitica in Italy (9)

1969
- First observation of cytoplasmic transmission of hypovirulence (45)

1977
- Identification of dsRNAs associated with hypovirulence (32)

1991
- Cloning and sequencing of first hypovirus: CHV1-713 (93)

1992
- First evidence that a hypovirus-encoded protein could alter host phenotype in the absence of virus infection (21)
- Development of an infectious cDNA clone of CHV1-713 (22)

1994
- Limited single season release of a transgenic hypovirulent C. parasitica strain (4)
- Expansion of hypovirus host range (15)
- Cloning and sequencing of CHV2-NB58 (52)

1995
- Establishment of taxonomic family Hypoviridae, genus hypovirus (51)

1999
- Development of an infectious clone for mild hypovirus CHV1-Euro7 (19)

2000
- Completion of the sequence of the third family member, CHV3-GH2 (97)
- Development of infectious chimeric hypoviruses (18)

Figure 1 Time line of important milestones in hypovirus research.
CHV1: genome and expression

12,712 bp dsRNA genome

CHV1-EP713

ORF A

ORF B

p300

AUTOCATALYTIC
# Totiviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host species</th>
<th>Genome size (kbp)</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsRNA viruses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totiviridae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-A</td>
<td><em>S. cerevisiae</em></td>
<td>4.6</td>
<td>One segment, Gag-Pol, icosahedral particles</td>
</tr>
<tr>
<td>M₁, M₂, M₃, M₄, ...</td>
<td><em>S. cerevisiae</em></td>
<td>1.6–1.8</td>
<td>Type species; Satellites of L-A; encode killer toxins</td>
</tr>
<tr>
<td>L-BC</td>
<td><em>S. cerevisiae</em></td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Hv190S</td>
<td><em>Helminthosporium victoriae</em></td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>P1-H, P4-H, P6-H</td>
<td><em>U. maydis</em></td>
<td>2.6–6.1</td>
<td>Coat protein phosphorylation; Associated killer phenomenon</td>
</tr>
<tr>
<td>AN-S, AN-F</td>
<td><em>A. foetidus</em></td>
<td>3.0, 3.1</td>
<td></td>
</tr>
<tr>
<td>YIV</td>
<td><em>Yarrowia lipolytica</em></td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>LRV</td>
<td><em>L. braziliensis</em></td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>GLV</td>
<td><em>G. lamblia</em></td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>GgV-87-1-H</td>
<td><em>Gaeumannomyces graminis</em></td>
<td>4.0, 2.9, 1.9</td>
<td>Can replicate in <em>Saccharomyces</em>; Upstream ORF(s); Extracellular Infection; transformation; Associated killer phenomenon</td>
</tr>
</tbody>
</table>

The dsRNA viruses of Yeast: L-A and its satellites The yeast Killer virus system

- **L(arge), M(iddle), S(mall), and X dsRNAs.**
- **Viral particle structure.**
  - 39nm, icosahedral with $T = 1$.
  - Asymmetric unit is a dimer of Gag (76 kDa).
  - 120 Gag units per viral particle, and 1 -2 copies of the 180 kDa Gag-pol fusion protein.
- **Small holes (10 -15Å diameter) allow exit of transcripts and influx of metabolites.**
- **dsRNAs are loosely packaged inside of the viral capsid.**
Cryoelectron micrographs of purified L-A virions.

Three-dimensional density maps of L-A capsids at 16 Å resolution. (a) Stereo views of the outer (upper panels) and inner (lower panels) surfaces of the full capsid, viewed along a fivefold axis of symmetry. (b) Transverse central sections taken from the maps of empty (left) and full (right) capsids, viewed along a twofold axis.
Structural organization of the two nonequivalent Gag monomers in the L-A capsid

b) Schematic diagram showing arrangement of Gag subunits in the L-A surface lattice, as viewed along a fivefold axis (compare with Fig. 2a). The A-subunits are green, and the B-subunits orange. (c) Diagram showing three pentons (each a Gag decamer) clustered around a threefold axis. Five- and twofold axes are also marked.
L-A Genome structure.

• The dsRNA L-A genome is in the A-form duplex.
• The 4.6 kb L-A (+) strand has 2 overlapping ORFs.
• 5' ORF is gag, encoding the Gag major coat protein.
• 3' ORF has 100 kDa of coding information.
L-A encoded Proteins

- N-terminus of *Gag* is acetylated by the host encoded Mak3p.
- His154 in the *Gag* protein steals 7MeGp caps from cellular mRNAs.
- *Gag-pol* fusion protein is made by a programmed -1 ribosomal frameshift.
- Three ssRNA binding domains have been characterized in the Pol domain.
The replication cycle.

Transcription.
- Transcription is conservative
- Newly synthesized (+) strands are extruded into cytoplasm.
- Very template specific.

Legend:
- single-stranded RNA
- double-stranded RNA
- Major Coat Protein
- 'gag-pol' fusion protein
- ribosome

Conservative (+) strand synthesis

N-acetylation of gag by MAK3

Translation:
-1 ribosomal frameshift makes gag-pol fusion protein: MGF genes

Viral Replication Cycle of L-A dsRNAs

(-) strand synthesis
Translation.

- **Programmed -1 ribosomal frameshifting.**
  - Frameshift signal is found in the 130 nt. overlap between the Gag and Pol ORFs.
  - Efficiency of the L-A promoted frameshift is 1.8 - 1.9%.
  - Provides the correct ratio of Gag to Gag-pol.

- **Bipartate signal**
  - Slippery site and RNA pseudoknot. Total of 78 nt.
  - Slippery site = G GGU UUA (gag frame is indicated).
  - RNA pseudoknot is located 8 nt 3’ of the slippery site. The RNA pseudoknot structure is required, not a specific RNA sequence.
Frameshifting efficiency is critical for virus propagation.

Normal frameshifting efficiency:
- Gag-pol
- Gag

Increased frameshifting efficiency = formation of incomplete viral particles.

Decreased frameshifting efficiency = formation of empty viral particles.
Why frameshift?

• (+) strand acts both as mRNA and genome.

• Frameshifting allows for production of >1 protein from a single, unaltered template.
Viral particle assembly and (+) strand packaging

- Viral particles self-assemble.
- A Gag-pol dimer is required for packaging.
Satellite viruses.

- Satellite viruses are viruses that rely upon another virus for their replicative machinery.
- Satellite viruses have their own genomes.
- Typically, the relationship is symbiotic.
M: A satellite virus of L-A.

The 1.4 - 1.6 kb dsRNA M genomes packaged inside of L-A viral particles.
Genome of M virus

- **Genome**: 5' 2/3 encodes the pre-protoxin.
  - Followed by variable polyA region.
  - 3' end (+) strand packaging and (-) strand replication signals like L-A.
- **M encoded proteins**. Responsible for the *Killer phenotype*.
  - The pre-protoxin is cleaved by two host-encoded proteases, Kex2 and Kex1.
  - Unprocessed form of the pre-protoxin confers immunity to the toxin on host cells.
  - Processing of the pre-protoxin by Kex1 and Kex2 led to the discovery of the human Kex homologs (Furins) that are responsible for processing many pre-prohormones.
Defective interfering particles—the S and X deletion mutants

• dsRNA’s called $S$ were isolated from cells that lost the killer phenotype.
• A dsRNA called $X$ was isolated from a cell that had also lost the killer phenotype.
• Question: why do $S$ and $X$ only exclude $M$ but not $L-A$?
Other totiviruses (dsRNA viruses of simple eukaryotes)

- **Giardia Lamblia virus**
- **Leishmania virus**
**ssRNA viruses of yeast**

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<th>Genome size</th>
<th>Features</th>
</tr>
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<tbody>
<tr>
<td>20S RNA (= W dsRNA)</td>
<td><em>S. cerevisae</em></td>
<td>2.9</td>
<td>Induced by N-starvation, high temperature</td>
</tr>
<tr>
<td>23S RNA (= T dsRNA)</td>
<td><em>S. cerevisae</em></td>
<td>2.5</td>
<td>Induced by N-starvation, high temperature</td>
</tr>
</tbody>
</table>

**20S RNA (T).**
2.5 kb, encodes a single 95 kDa protein, similarities to the RNA-dependent RNA polymerases of RNA phages and RNA viruses.
The RNA is apparently naked in the cytoplasm i.e. not encapsidated. Both single-length and double-length replication intermediates have been detected.

**23S RNA (W).**
Substantial homology with 20S RNA and likewise appears to encode an RNA-dependent RNA polymerase.
The replicative form is also known as 'T dsRNA'.
All strains that have T also have W, but not vice versa. It is not known if T depends on W.
# Retroelements

**TABLE 2. Retroelements of simple eukaryotes**

<table>
<thead>
<tr>
<th>Retrovirus</th>
<th>LTRs</th>
<th>ε</th>
<th>Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ty1, Ty2</td>
<td>S. cerevisiae</td>
<td>δ, 334–338 bp</td>
<td>5.2 kb</td>
</tr>
<tr>
<td>Ty3</td>
<td>S. cerevisiae</td>
<td>σ, 340 bp</td>
<td>4.7</td>
</tr>
<tr>
<td>Ty4</td>
<td>S. cerevisiae</td>
<td>τ, 371 bp</td>
<td>5.6</td>
</tr>
<tr>
<td>Ty5</td>
<td>S. cerevisiae</td>
<td>245 bp</td>
<td></td>
</tr>
<tr>
<td>Tf1, Tf2</td>
<td>Schizosaccharomyces pombe</td>
<td>349–358 bp</td>
<td>4.4</td>
</tr>
<tr>
<td>DIRS-1</td>
<td>Dictyostelium discoideum</td>
<td>ITRs</td>
<td>4.2</td>
</tr>
<tr>
<td>DRE</td>
<td>Dictyostelium discoideum</td>
<td>Complex</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRs</td>
<td></td>
</tr>
<tr>
<td>Tp1</td>
<td>Physarum polycephalum</td>
<td>277 bp</td>
<td>8.3</td>
</tr>
<tr>
<td>Gf1-1</td>
<td>Cladosporium fulvum</td>
<td>427 bp</td>
<td>6.1</td>
</tr>
<tr>
<td>CRE1</td>
<td>Crithidia fasciculata</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>SLACS, CZAR</td>
<td>Trypanosoma brucei</td>
<td>–</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Trypanosoma cruzi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOC1</td>
<td>Chlamydomonas reinhardtii</td>
<td>217, 237 bp</td>
<td>4.6</td>
</tr>
<tr>
<td>Tad</td>
<td>Neurospora</td>
<td>–</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*Based on amino acid sequence homology and gene order, retrotransposons may be divided into those similar to the *copia* element or the *gypsy* element of Drosophila (74). LINE-like elements are retroposons, lacking LTRs.
Retroelements

- Retroelements all use reverse transcriptase in their propagation. Retroelements include retroviruses, retrotransposons, retroposons and retrointrons.
- Retroviruses are full blown viruses, which are capable of horizontal infection, e.g. HIV.
- Retrotransposons resemble mammalian retroviruses except that they lack an env gene, and thus, they do not have an extracellular phase.
- Retroposons are one step further removed in that they lack LTRs.
- Retrointrons are introns which encode reverse transcriptase.
The Ty retrotransposable elements

- Well characterized, serve as excellent models for retroviruses. 5 different Ty elements.
- Ty1, 2, 4, 5: copia like
- Ty2: gypsy like
• Long Terminal Repeats (LTRs) of 245 - 371 bp.
• LTRs divided into conventional U3, R and U5 regions.
• 4.7 - 5.6 kb (+) strand begins at the 5' end of the 5' R, extends to the 3' end of the 3' R.
• Intervening sequence has 2 ORFs known as TYA and TYB.
• TYA encodes Gag and TYB encodes the Pol domain.
• Gag-pol fusion protein is processed into the Protease, Integrase, Reverse transcriptase and RNase H proteins like in Retroviruses.
Ty1 Replication cycle

- Resembles that of mammalian retroviruses.
- Ty transcripts are translated in the cytoplasm into Gag and Gag-pol proteins.
- After assembly, the Gag-pol protein is processed into its component fragment parts.
- The mature particle enters the nucleus and Integrase inserts the dsDNA into the host genome.