The Parts List of a Virus

http://faculty.washington.edu/jais/microscopy.html
Cells and the Viruses That Infect Them

Bacteria

Note: the viral parts list is very small (10-100 pieces) with genomes roughly of 10kb. They accomplish so much with so little.

T cells

http://faculty.washington.edu/jais/microscopy.html
"Phage and the Origins of Molecular Biology"

Analogy: Put 500m of Golden Gate Bridge suspension cable in the back of a FedEx truck.

An infected bacterium: note that virus injects DNA and leaves body behind.
Views of the Lambda Phage Genome
Despite their supposedly simplicity and the existence of sequenced genomes, there is still much that we don’t know about what goes on in these genomes.

168,903 base pairs in the genome.
Life Cycle of a Bacteriophage

Rate of packing: 100bp/sec

"Some assembly required"

Rate of ejection: ≈ 100 - 10000bp/sec

Forceful ejection

Construct a physical model of these processes.

Self-assembly
Who Are the Real Predators Out There?

The density of bacteriophage in the ocean is $50 \times 10^6$/ml while the density of sharks is much less than $10^{-6}$/ml!

It is estimated that 40% of the bacterial background is infected everyday by bacteriophage - that is a predator!
Phage provide a setting within which we can ask sharp, quantitative questions and test precise models of biological phenomena.

Some universal biological themes: macromolecular assembly in a crowded environment, orchestration in space and time, polymer translocation, gene expression, evolution, nature’s nanotechnology etc.

Close to having a full census (molecular inventory) and full structure of phage.

An opinion: phage have not outlived their ability to teach!

Rossmann et al.
1955 Fraenkel-Conrat and Williams - first example of complex biological entity reconstituted outside off cell.

> 2000 protein units co-assemble with RNA molecule.

Headlines: “Life created in test tube!”
Cowpea chlorotic mottle virus - plant virus.
4 separate RNA molecules constitute its genome.
Structure known with atomic resolution.
Formation of an Infectious Nucleoprotein from Protein and Nucleic Acid Isolated from a Small Spherical Virus

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Ernest Hiebert

Department of Botany and Plant Pathology
Lilly Hall of Life Sciences
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Accepted April 4, 1967
Characteristic size scale is 30-100 nm.
Structures are known at “atomic resolution” - see Viper website.
Highly symmetric - think hard about what this implies about assembly!
- Key point: viruses hijack the host cell to make new viruses.
- Once again, many of the great themes of biology are played out in this microcosm.
- HIV a convenient and intriguing prism through which to view all of biology.
- There are many interesting physical processes that take place in this life cycle that will catch our fancy: binding, membrane fusion, transcription and its control, integration, assembly, budding!

This one picture literally provides a road map for the entire course. Almost all of our big themes are revealed right here!
Views of the HIV Genome

**Genomic Organization of HIV-1**

**LTR**
Contains transcriptional control regions and is required for transcription of the viral genome.

**vif**
Overcomes inhibition by the host factor APOBEC3G and promotes more stable reverse transcriptase complexes.

**vpu**
Promotes CD4 degradation and helps virion release.

**env**
Cleaved into two proteins: gp120 mediates viral binding to the cell, and gp41 promotes fusion of the viral and cellular membranes.

**nef**
Promotes CD4 lymphocyte activation. Blocks cell suicide, enhances infectivity, and is associated with progression of disease.

**gag**
Building block of the virus particle core.

**pol**
Encodes several enzymes, including the reverse transcriptase and protease.

**vpr**
Promotes cell-cycle arrest and facilitates infection of macrophages.

**rev**
Regulates viral RNA nuclear export.

**tat**
Enhances viral RNA transcription and promotes CD4 lymphocyte activation.

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View the original image at:
http://www.gladstone.ucsf.edu/gladstone/html/connections/fig1.html
Cryo electron microscopy provides a window on virion structure.
Mean diameter approximately 120nm.
Average volume approximately 45,000 nm$^3$
Approximate mass per virus particle is 1 fg (650 Mda)

Each picture is 160nm wide.
This is a nice and thought provoking picture, but…
Be careful, which features of this cartoon do we REALLY know?
When shown cartoons like this, you have to ask yourself what is known and how. Compare the cartoon to the actual data - what is the data? Try to make a cartoon sometime and it will reveal lots about what we don’t know.

(Turner and Summers)
Making a Virus

Yogi Berra said “You can observe a lot just by watching.” We will pursue a corrolary: you can learn a lot just by estimating.

Estimation question: how many Gag proteins does it take (roughly) to make an HIV virion? We begin by considering the immature virus.

Does the cartoon make sense?

To figure out the number of Gag proteins, we need an estimate of the area per protein.

\[
N_{Gag} \approx \frac{A_{\text{virus}}}{A_{\text{lipid}}} \approx \frac{4\pi r_{\text{virus}}^2}{\pi r_{\text{protein}}^2}
\]
"Nobody goes there anymore; it's too crowded."
"If you come to a fork in the road, take it."
"You should always go to other people's funerals; otherwise, they won't come to yours."
"It was impossible to get a conversation going; everybody was talking too much."
"You've got to be very careful if you don't know where you're going, because you might not get there."
Acquiring a Membrane: How HIV Gets Its Lipids

http://www.homepage.montana.edu/~spincus/sp_hiv.html


This scanning electron microscope picture demonstrates HIV budding (arrows) from the surface of an infected T-lymphocyte magnified 80,000X.
Fact of life: lipids come in many different shapes and chemical forms.

HIV lipid census provides a partial picture of the virion.

Brugger et al.
What Are Lipids Like?

- Typical sizes: 2.5 nm
- Typical masses: 750 Da
- Area per molecule: 1/4 nm^2
- A useful place to find stuff out is Avanti Polar Lipids.
Lipid Content of HIV Particles

- The actual molecular census of the HIV virus.

### Table 1. Phospholipid composition of MT-4 cells and HIV-1

<table>
<thead>
<tr>
<th>Lipid</th>
<th>MT-4 cells (mol % ± SD)</th>
<th>HIV-1 (mol % ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>43.0 ± 2.9</td>
<td>16.0 ± 1.0</td>
</tr>
<tr>
<td>SM + DHSM</td>
<td>10.4 ± 1.6</td>
<td>33.1 ± 1.2</td>
</tr>
<tr>
<td>PE</td>
<td>17.0 ± 1.5</td>
<td>8.2 ± 1.3</td>
</tr>
<tr>
<td>pl-PE</td>
<td>15.9 ± 0.5</td>
<td>27.0 ± 3.3</td>
</tr>
<tr>
<td>PS</td>
<td>7.4 ± 0.8</td>
<td>15.5 ± 2.2</td>
</tr>
</tbody>
</table>

Lipids were extracted and analyzed for phospholipid content as described in Materials and Methods. Values are expressed either as mol percentage of a given phospholipid to total phosphate (MT-4 cells) or as mol percentage of a given phospholipid to the total of all phospholipids quantified (HIV-1).

### Table 2. The lipid composition of HIV-1

<table>
<thead>
<tr>
<th>Lipid molecules per average HI virion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
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<tr>
<td>SM</td>
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<tr>
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<tr>
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<tr>
<td>Chol</td>
</tr>
<tr>
<td>Cer</td>
</tr>
<tr>
<td>HC</td>
</tr>
</tbody>
</table>

For details, see Supporting Text.
Capsid Structure

- Fascinating structure of the internal capsid of HIV.
- Shape conferred by geometric rules about 5-fold defects.

Sundquist et al.
Distribution of Spike Complexes

Zhu et al., Nature 2006
Distribution of Spike Proteins?

Zhu et al., Nature 2006
Viruses and the Great Themes of Biology

- How do viruses make new viruses?
- How do viruses evolve? (including to evade the host defenses)
- How do cells defend against viruses?
- How do cells make decisions and how is that decision making altered by the presence of viruses?

Korber et al.