The Infectious Cycle

Lecture 2
Biology 4310
Spring 2020

“You know my methods, Watson”
—Sir Arthur Conan Doyle
Virologists divide the infectious cycle into steps to facilitate their study, but no such artificial boundaries occur.
Some important definitions

- A **susceptible** cell has a functional receptor for a given virus - *the cell may or may not be able to support viral replication*
- A **resistant** cell has no receptor - *it may or may not be competent to support viral replication*
- A **permissive** cell has the capacity to replicate virus - *it may or may not be susceptible*
- A **susceptible** AND **permissive** cell is the only cell that can take up a virus particle and replicate it
• Animal viruses at first could not be routinely propagated in cultured cells
• Most viruses were grown in laboratory animals
Studying the infectious cycle in cells

- Not possible before 1949 (animal viruses)
- *Enders, Weller, Robbins* propagate poliovirus in human cell culture - primary cultures of embryonic tissues
- Nobel prize, 1954
**Virus cultivation**

- **A** Primary human foreskin fibroblasts
- **B** Mouse fibroblast cell line (3T3)
- **C** Human epithelial cell line (HeLa)

Continuous cell lines

Diploid cell strains (e.g. WI-38, human embryonic lung)
The Immortal Life of Henrietta Lacks

Doctors took her cells without asking.
Those cells never died.
They launched a medical revolution
and a multimillion-dollar industry.
More than twenty years later, her children found out.
Their lives would never be the same.

Rebecca Skloot

Amazing advances in cell culture

**Organoid cultures**
- Blastocyst
- Embryonic stem cell
- Endoderm
  - Gastric organoid
  - Liver organoid
  - Intestinal organoid
  - Lung organoid
- Somatic cell
- Induced pluripotent stem cell
- Endoderm
  - Optic cup organoid
  - Striated cortical organoid
  - Inner ear organoid
  - Pituitary organoid

**Air-liquid interface cultures**
- A
- B

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A ______ and ______ cell is the only cell that can take up a virus particle and replicate it (fill in the blanks)

A. Naive and resistant
B. Primary and permissive
C. Susceptible and permissive
D. Susceptible and naive
E. Continuous and immortal
cytopathic effect (CPE)
Formation of syncytia
## Examples of cytopathic effects

<table>
<thead>
<tr>
<th>Cytopathic effect(s)</th>
<th>Virus(es)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphological alterations</td>
<td></td>
</tr>
<tr>
<td>Nuclear shrinking (pyknosis), proliferation of membrane</td>
<td>Picornaviruses</td>
</tr>
<tr>
<td>Proliferation of nuclear membrane</td>
<td>Alphaviruses, herpesviruses</td>
</tr>
<tr>
<td>Vacuoles in cytoplasm</td>
<td>Polyomaviruses, papillomaviruses</td>
</tr>
<tr>
<td>Syncytium formation (cell fusion)</td>
<td>Paramyxoviruses, coronaviruses</td>
</tr>
<tr>
<td>Margination and breaking of chromosomes</td>
<td>Herpesviruses</td>
</tr>
<tr>
<td>Rounding up and detachment of cultured cells</td>
<td>Herpesviruses, rhabdoviruses, adenoviruses, picornaviruses</td>
</tr>
<tr>
<td><strong>Inclusion bodies</strong></td>
<td></td>
</tr>
<tr>
<td>Virions in nucleus</td>
<td>Adenoviruses</td>
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<tr>
<td>Virions in cytoplasm (Negri bodies)</td>
<td>Rabies virus</td>
</tr>
<tr>
<td>“Factories” in cytoplasm (Guarnieri bodies)</td>
<td>Poxviruses</td>
</tr>
<tr>
<td>Clumps of ribosomes in virions</td>
<td>Arenaviruses</td>
</tr>
<tr>
<td>Clumps of chromatin in nucleus</td>
<td>Herpesviruses</td>
</tr>
</tbody>
</table>
How many viruses in a sample?

- Infectivity
- Physical: virus particles and their components
Plaque assay

1930s: used to study multiplication of bacteriophages
1952, Renato Dulbecco developed *plaque* assay for animal viruses

Nobel Prize, 1975

*PRODUCTION OF PLAQUES IN MONOLAYER TISSUE CULTURES BY SINGLE PARTICLES OF AN ANIMAL VIRUS*

BY RENATO DULBECCO

CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Read before the Academy, April 29, 1952

Research on the growth characteristics and genetic properties of animal viruses has stood greatly in need of improved quantitative techniques, such as those used in the related field of bacteriophage studies.

The requirements for a quantitative virus technique are as follows: (1) The use of a uniform type of host cell; (2) an accurate assay technique; (3) the isolation of the progeny of a single virus particle; and (4) the separate isolation of each of the virus particles produced by a single infected cell.
**Plaque assay**

- **Virus stock**
  - 0.1 ml
  - 0.9 ml
  - 10^{-1}
  - 10^{-2}
  - 10^{-3}
  - 10^{-4}
  - 10^{-5}
  - 10^{-6}
  - 10^{-7}

- **Number of plaques**:
  - Too many to count
  - 17
  - $1.7 \times 10^8$ PFU/ml
  - 2
When doing a plaque assay, what is the purpose of adding a semi-solid agar overlay on the monolayer of infected cells?

A. To stabilize progeny virus particles
B. To ensure that cells remain susceptible and permissive
C. To act as a pH indicator
D. To keep cells adherent to the plate during incubation
E. To restrict viral diffusion after lysis of infected cells
How many viruses are needed to form a plaque?
For one-hit kinetics, the number of plaques is directly proportional to the first power of the concentration of the virus inoculated. If the concentration of virus is doubled, the number of plaques also doubles.

For two-hit kinetics, the number of plaques is directly proportional to the square of the concentration of the virus inoculated.
Plaque purification

A method for producing virus stocks
Usually done 3 times
For viruses that do not form plaques: Endpoint dilution assay

<table>
<thead>
<tr>
<th>Virus dilution</th>
<th>Cytopathic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-2}$</td>
<td>+</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>+</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>–</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>–</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>–</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>–</td>
</tr>
</tbody>
</table>
Not all virus particles are infectious!

<table>
<thead>
<tr>
<th>Virus</th>
<th>Particle/PFU ratio</th>
<th># of physical particles</th>
<th># of infectious particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillomaviridae</td>
<td></td>
<td></td>
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<tr>
<td>Papillomavirus</td>
<td>10,000</td>
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<tr>
<td>Picornaviridae</td>
<td></td>
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<tr>
<td>Poliovirus</td>
<td>30–1,000</td>
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<tr>
<td>Herpesviridae</td>
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<tr>
<td>Herpes simplex virus</td>
<td>50–200</td>
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<tr>
<td>Polyomaviridae</td>
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<td></td>
<td></td>
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<tr>
<td>Polyomavirus</td>
<td>38–50</td>
<td></td>
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<tr>
<td>Simian virus 40</td>
<td>100–200</td>
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<td></td>
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<tr>
<td>Adenoviridae</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>20–100</td>
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<tr>
<td>Poxviridae</td>
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<tr>
<td></td>
<td>1–100</td>
<td></td>
<td></td>
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<tr>
<td>Orthomyxoviridae</td>
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<tr>
<td>Influenza virus</td>
<td>20–50</td>
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<tr>
<td>Reoviridae</td>
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<tr>
<td>Reovirus</td>
<td>10</td>
<td></td>
<td></td>
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<tr>
<td>Alphaviridae</td>
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<tr>
<td>Semliki Forest virus</td>
<td>1–2</td>
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</table>
Particle-to-PFU ratio

- # of *physical* particles ÷ # of *infectious* particles
- A single particle *can* initiate infection
- Not all viruses are successful
  - Damaged particles
  - Mutations
  - Complexity of infectious cycle
- Complicates study

Virology Lectures 2020 • Prof. Vincent Racaniello • Columbia University
Go to:

b.socrative.com/login/student

room number: virus

In the ‘particle to pfu ratio’, ‘particle’ can best be described as:

A. One of the proteins which makes up the virus
B. A virus which may or may not be infectious
C. A virus which is infectious
D. A virus which is not infectious
E. Elementary or composite
One-step growth cycle:
A method to study virus reproduction in cells

- Ellis & Delbruck, 1939, studies on *E. coli* bacteriophages
- Adsorb
- Dilute culture
- Sample
- Assay
Single and multi-step growth cycles

**All cells infected**
- Start/dilute
- Eclipse period
- Burst or yield
- Number of infectious particles vs. Time

**Few cells infected**
- Start/dilute
- Eclipse period
- First burst
- Second burst
- Number of infectious particles vs. Time

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Synchronous infection - key to one-step growth cycle

To achieve this, we need to infect all the cells - but how do we know?
Multiplicity of infection (MOI)

- Number of infectious particles ADDED per cell
- Amount of virus (PFU) ÷ # of cells
- Not the number of infectious particles each cell receives
- Add $10^7$ virus particles to $10^6$ cells - MOI of 10 - each cell does NOT receive 10 virus particles
MOI

• Infection depends on the random collision of virus particles and cells

• When susceptible cells are mixed with virus, some cells are uninfected, some receive one, two, three or more particles

• The distribution of virus particles per cell is best described by the Poisson distribution
\[ P(k) = e^{-m} \frac{m^k}{k!} \]

\( P(k) \): fraction of cells infected by \( k \) virus particles

\( m \): multiplicity of infection (moi)

uninfected cells: \( P(0) = e^{-m} \)

cells receiving 1 particle: \( P(1) = me^{-m} \)

cells multiply infected: \( P(>1) = 1-e^{-m}(m+1) \)

[obtained by subtracting from 1 {the sum of all probabilities for any value of \( k \)}
the probabilities \( P(0) \) and \( P(1) \)]
Examples:
If $10^6$ cells are infected at moi of 10:
45 cells are uninfected
450 cells receive 1 particle
the rest receive $>1$ particle

If $10^6$ cells are infected at moi of 1:
37% of the cells are uninfected
37% of the cells receive 1 particle
26% receive $>1$ particle

If $10^6$ cells are infected at moi of .001:
99.9% of the cells are uninfected
0.099% of the cells receive 1 particle (990)
0.0001% receive $>1$ particle
If cells are infected at an MOI=10 in a one-step growth cycle experiment, in the growth curve you will likely see...

A. Multiple bursts of virus release  
B. Multiple eclipse periods  
C. A single burst of virus release  
D. No burst of virus release  
E. Asynchronous infection
Physical measurements of virus particles

- Hemagglutination
- Electron microscopy
- Viral enzymes
- Serology
- Nucleic acids
Hemagglutination

Sample

<table>
<thead>
<tr>
<th>Dilution</th>
<th>C</th>
<th>D</th>
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<tbody>
<tr>
<td>1:4</td>
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<td>1:8</td>
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<td>1:16</td>
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<td>1:32</td>
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<td>1:64</td>
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<td>1:128</td>
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<td>1:256</td>
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<td>1:512</td>
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<td>1:1,024</td>
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<td>1:2,048</td>
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<tr>
<td>1:4,096</td>
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<tr>
<td>1:8,192</td>
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</tbody>
</table>
Measurement of viral enzyme activity
Enzyme-linked immunosorbent assay (ELISA): detecting viral antigens or antibodies
Green fluorescent protein
Polymerase chain reaction (PCR)

- Research
- Industry
- Diagnosis
Deep, high-throughput sequencing

- Metagenomics
- Identification of new viruses in a sample
- Identification of new pathogens
- Human genome: 10 yr/$3B vs 1 day/$1000
Phylogenetic trees

Degree of genetic change
Scale = # changes/length of sequence

Measure of support:
Sequences to right of node cluster together better than other sequences

Root = presumed ancestor
Isolation of 2019-nCoV

China CDC Weekly

A Novel Coronavirus Genome Identified in a Cluster of Pneumonia Cases — Wuhan, China 2019–2020

Wenjie Tan1,*, Xiang Zhao1; Xuejun Ma1; Wenling Wang1; Peihua Niu1; Wenbo Xu1;
George F. Gao1; Guizhen Wu1,2,*

Emerging and re-emerging pathogens are great challenges to the public health (1). A cluster of pneumonia cases with an unknown cause occurred in Wuhan starting on December 21, 2019. As of January 20, 2020, a total of 201 cases of pneumonia in China have been confirmed. A team of professionals from the National Health Commission and China CDC conducted epidemiological and etiological investigations. On January 3, 2020, the first complete genome of the novel β genus coronaviruses (2019-nCoVs) was identified in samples of bronchoalveolar lavage fluid (BALF) from a patient from Wuhan by scientists of the National Institute of Viral Disease Control and Prevention (IVDC) through a combination of Sanger sequencing, Illumina sequencing, and nanopore sequencing. Three distinct strains have been identified, the virus has been designated as 2019-nCoV, and the disease has been subsequently named novel coronavirus-infected pneumonia (NCIP).

Phylogenetic analysis was conducted to determine the relationship between 2019-nCoVs and other
Preliminary maximum likelihood phylogenetic analysis of novel Wuhan, China human CoV in red, GenBank (accession MH889947). Novel CoV seq data from: http://virological.org/t/initimate-release-of-novel-coronavirus-2019. The Shanghai Public Health Clinical Center & School of Public Health, in collaboration with the Central Hospital of Wuhan, Huazhong University of Science and Technology, the Wuhan Center for Disease Control and Prevention, the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control, and the University of Sydney, Sydney, Australia.

PhyML tree based on partial RdRp gene sequence (410bp), aligned with representative human and animal CoV sequences from Genbank compiled by Alice Latinne; tree by Kevin Ollivol. Analysis by EcoHealth Alliance - 11 Jan 2020 (12:30pm EST)
**TWiV 196: An arena for snakes**

AUGUST 19, 2012

Hosts: Vincent Racaniello, Alan Dove, Rich Condit, Dickson Despommier, Kathy Spindler, Mark Stenglein, and Joseph DeRisi

The TWIVites meet with Mark Stenglein and Joseph DeRisi to discuss their discovery of a novel arenavirus in snakes with inclusion body disease.

http://www.microbe.tv/twiv/twiv-196-an-arena-for-snakes/

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**TWiV 199: Of mice, ticks, and pigs**

SEPTEMBER 16, 2012


Vincent, Alan, Rich, and Kathy discuss recent outbreaks of hantavirus pulmonary syndrome in Yosemite National Park and novel swine-origin influenza in the US midwest, and isolation of the Heartland virus from two patients in Missouri with severe febrile illness.

PCR product is not the same as infectious virus

Frequent Zika Virus Sexual Transmission and Prolonged Viral RNA Shedding in an Immunodeficient Mouse Model

https://doi.org/10.1016/j.celrep.2017.01.056
Viruses and viral sequences

Zoonotic Viruses Associated with Illegally Imported Wildlife Products

From the Jungle to J.F.K., Viruses Cross Borders in Monkey Meat
By RACHEL NUIWER

It's a familiar story: deep within the jungle, an intrepid explorer or hunter awakens something he shouldn't have. Maybe he bagged the wrong bat or came into contact with an ailing chimpanzee. Whatever the origin, he unsuspectingly becomes host to something deadly and unseen and unwittingly carries it back home across the river or the ocean.

And then the pandemic begins.

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0029505
Next time: Genomes and Genetics