The Infectious Cycle

Lecture 2
Biology 3310/4310
Virology
Spring 2018

“You know my methods, Watson”
--SIR ARTHUR CONAN DOYLE
The Infectious Cycle

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
Embryonated eggs at 10 to 12 days being inoculated by automated machinery. 1st larger needle (about 1 mm diameter) punches a hole in a shell and 2nd smaller needle injects a seed into the allantoic cavity of the egg followed by incubation for 2 to 3 days. It takes less than 10 seconds to inoculate a row of eggs. 

Courtesy: Solvay
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

Primary human foreskin fibroblasts

Mouse fibroblast cell line (3T3)

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

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>
</tr>
<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
Plaque assay

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...
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
Go to:

b.socrative.com/login/student
room number: virus

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 virions
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 clonal virus stocks
Usually done 3 times
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>
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
Particle-to-PFU ratios of some animal viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Particle/PFU ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Papillomaviridae</em></td>
<td></td>
</tr>
<tr>
<td>Papillomavirus</td>
<td>10,000</td>
</tr>
<tr>
<td><em>Picornaviridae</em></td>
<td></td>
</tr>
<tr>
<td>Poliovirus</td>
<td>30–1,000</td>
</tr>
<tr>
<td><em>Herpesviridae</em></td>
<td></td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>50–200</td>
</tr>
<tr>
<td><em>Polyomaviridae</em></td>
<td></td>
</tr>
<tr>
<td>Polyomavirus</td>
<td>38–50</td>
</tr>
<tr>
<td>Simian virus 40</td>
<td>100–200</td>
</tr>
<tr>
<td><em>Adenoviridae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20–100</td>
</tr>
<tr>
<td><em>Poxviridae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1–100</td>
</tr>
<tr>
<td><em>Orthomyxoviridae</em></td>
<td></td>
</tr>
<tr>
<td>Influenza virus</td>
<td>20–50</td>
</tr>
<tr>
<td><em>Reoviridae</em></td>
<td></td>
</tr>
<tr>
<td>Reovirus</td>
<td>10</td>
</tr>
<tr>
<td><em>Alphaviridae</em></td>
<td></td>
</tr>
<tr>
<td>Semliki Forest virus</td>
<td>1–2</td>
</tr>
</tbody>
</table>
In the ‘particle to pfu ratio’, ‘particle’ can best be described as:

A. One of the proteins which makes up the virion
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

- 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

Few cells infected

- Start/dilute
- Eclipse period
- First burst
- Second burst
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
- 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 virions
MOI

- Infection depends on the random collision of virions 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*
\[ P(k) = \frac{e^{-m}m^k}{k!} \]

\( P(k) \): 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
00.099% of the cells receive 1 particle (990)
00.0001% 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

Dilution

Sample

C  1:4  1:8  1:16  1:32  1:64  1:128  1:256  1:512  1:1,024  1:2,048  1:4,096  1:8,192
D

Virology Lectures 2018 • Prof. Vincent Racaniello • Columbia University
©Principles of Virology, ASM Press
Measurement of viral enzyme activity
<table>
<thead>
<tr>
<th>Cells split</th>
<th>LNCaP</th>
<th>DU145</th>
<th>WMPY-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Enzyme-linked immunosorbent assay (ELISA): detecting viral antigens or antibodies
Green fluorescent protein
Polymerase chain reaction (PCR)

- Research
- Industry
- Diagnosis

Exponential growth of short product
PCR product is not the same as infectious virus
Deep, high-throughput sequencing

- Metagenomics
- Identification of new viruses in environmental samples
- Identification of new pathogens
- Human genome: 10 yr/$3B vs 1 day/$1500

(this is not DHTS)
**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/

**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.

Click the arrow above to play, or right-click

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 NUWER

Virology Lectures 2018 • Prof. Vincent Racaniello • Columbia University

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0029505