The Infectious Cycle

Lecture 2
Biology W3310/4310
Virology
Spring 2014

“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
Chorioallantoic membrane inoculation

- Herpes simplex virus
- Poxvirus
- Rous sarcoma virus

Amniotic cavity

- Shell
- Albumin

Shell membrane

Air sac

Amniotic inoculation

- Influenza virus
- Mumps virus

Yolk sac inoculation

- Herpes simplex virus

Allantoic cavity

Yolk sac

Chorioallantoic membrane

Allantoic inoculation

- Influenza virus
- Mumps virus
- Newcastle disease virus
- Avian adenovirus
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

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

cytopathic effect (CPE)
Formation of syncytia
<table>
<thead>
<tr>
<th>Cytopathic effect(s)</th>
<th>Virus(es)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphological alterations</strong></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 the cytoplasm (Negri bodies)</td>
<td>Rabies virus</td>
</tr>
<tr>
<td>“Factories” in the 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 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

0.1 ml

10^{-1}

10^{-2}

10^{-3}

10^{-4}

10^{-5}

10^{-6}

10^{-7}

0.1 ml

0.1 ml

0.1 ml

Number of plaques:

Too many to count

17

1.7 \times 10^8 PFU/ml

2
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. Why?
# Endpoint dilution assay

![Endpoint dilution assay diagram](image)

TCID$_{50}$

<table>
<thead>
<tr>
<th>Virus dilution</th>
<th>Cytopathic effect</th>
</tr>
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<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>
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</table>
Particle-to-PFU ratio

- Number of virus particles in sample/number of infectious particles
- ~1 for many bacteriophages
- High for many animal viruses
- Complicates study of animal viruses
<table>
<thead>
<tr>
<th>Virus</th>
<th>Particle/ PFU ratio</th>
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</thead>
<tbody>
<tr>
<td>Adenoviridae</td>
<td>20–100</td>
</tr>
<tr>
<td>Alphaviridae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Semliki Forest virus</td>
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<tr>
<td>Herpesviridae</td>
<td></td>
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<tr>
<td></td>
<td>Herpes simplex virus</td>
</tr>
<tr>
<td>Orthomyxoviridae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Influenza virus</td>
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<tr>
<td>Papillomaviridae</td>
<td></td>
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<tr>
<td></td>
<td>Papillomavirus</td>
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<tr>
<td>Picornaviridae</td>
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<td></td>
<td>Poliovirus</td>
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<td>Polyomaviridae</td>
<td></td>
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<tr>
<td></td>
<td>Polyomavirus</td>
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<td></td>
<td>Simian virus 40</td>
</tr>
<tr>
<td>Poxviridae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1–100</td>
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<tr>
<td>Reoviridae</td>
<td></td>
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<tr>
<td></td>
<td>Reovirus</td>
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</tbody>
</table>
Particle-to-PFU ratio

• A single particle *can* initiate infection (how do we know this?)

• High particle-to-pfu ratio: not all viruses are successful. Why not?
  - Damaged particles
  - Mutations
  - Complexity of infectious cycle: failure at any one step prevents completion
One-step growth cycle

- Ellis & Delbruck, 1939, studies on *E. coli* bacteriophages
- Adsorb
- Dilute culture
- Sample
- Assay
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 $10^7$ virions to $10^6$ cells - MOI of 10 - each cell does NOT receive 10 virions
• 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
\[ P(k) = e^{-m}m^k/k! \]

\( P(k) \): 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
Physical measurements of virus particles

- Hemagglutination
- Electron microscopy
- Viral enzymes
- Serology
- Nucleic acids
Hemagglutination - measurement of virus particles

- GK Hirst, 1941
- First rapid, quantitative assay for eukaryotic viruses
Measurement of viral enzyme activity
Immunostaining

A  Direct
Indirect

B
Immunoblotting (western blot analysis)

Antigen samples

Separated proteins

Separation gel

Blotting tank
Proteins transferred to nitrocellulose sheet (blot)

Labeled antibody

Develop and fix autoradiograph

Immunostaining of blot

Antigen bands visualized

Autoradiography
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
Deep, high-throughput sequencing

- Metagenomics
- Identification of new viruses in environmental samples
- Identification of new pathogens

*(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.twiv.tv/2012/08/19/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.

Viruses and viral sequences

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

By RACHEL NUWER

January 13, 2012, 8:10 AM

Zoonotic Viruses Associated with Illegally Imported Wildlife Products

A set of Cercopithecus monkey limbs recently confiscated at O’Hare Airport in Chicago.

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 chimp. Whatever the origin, he unsuspectingly becomes host to something deadly and unseen and unwittingly carries it back home across the river or the ocean.