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Principles of Virology

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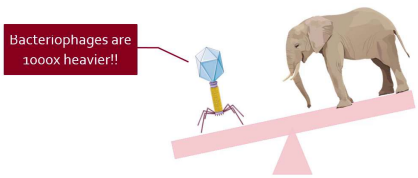
We live and prosper in a cloud of viruses

- Viruses infect all living things
- We regularly eat and breathe billions of virus particles
- We carry viral genomes as part of our own genetic material

2

The number of viruses on Earth is staggering

- There are $>10^{30}$ bacteriophage particles in the world's waters!
- A bacteriophage particle weighs about a femtogram (10^{-15} grams)
- $10^{30} \times 10^{-15}$ = the biomass on the planet of **bacterial viruses alone**



- The length of a head to tail line of 10^{30} phages is 100 million light years! (see *Phage Hunter* in the **Links** tab)

3

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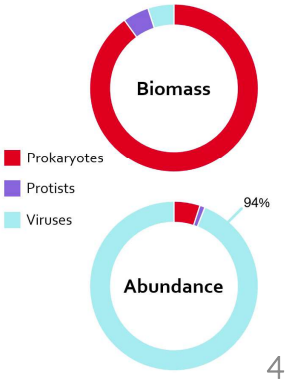
Viruses are not just purveyors of bad news

More viruses in a liter of coastal seawater than people on Earth!

Oceanic viruses are important for organic matter recycling



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There are ~10^{16} HIV genomes on the planet today

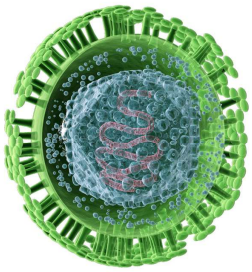
A huge number of drug-resistant genomes exist

5

How 'infected' are we?

- HSV-1
- HSV-2
- VZV
- HCMV
- EBV
- HHV-6
- HHV-7
- HHV-8

Once infected, it is for life



Herpes virus

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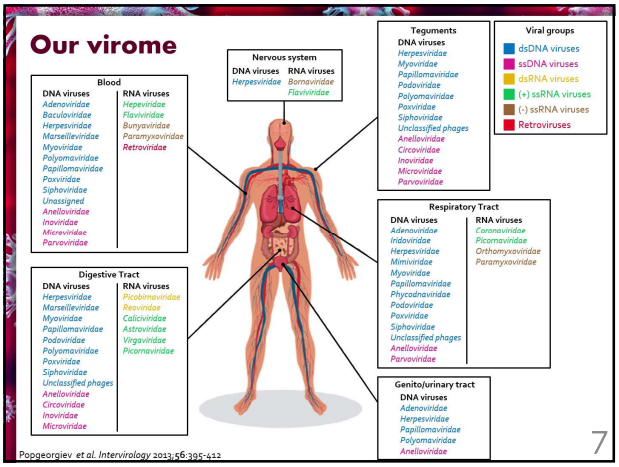
Our virome

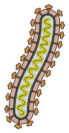


Popgeorgiev *et al. Intervirology* 2013;56:395-422

7

Most viruses just pass through us

- We ingest many non-animal viruses regularly with our foods
- ...one study found that cabbages purchased from 5 different supermarkets in the Washington D.C. area were all contaminated with baculoviruses
- To such an extent that each serving (about 100 cm^2 of leaf material) contained up to 10^8 particles of baculovirus - a virus pathogenic for the cabbage looper



Baculovirus

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Most viruses just pass through us

- Metagenomic analysis of RNA viruses in human feces revealed that most viral sequences are similar to plant viruses
- Of the 36,769 sequences obtained, 25,040 (91%) resembled plant viruses
- Most abundant human fecal virus: **pepper mild mottle virus**, 10^9 virions per gram of dry fecal matter

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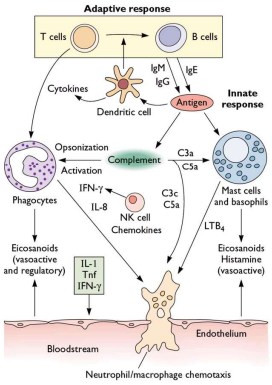
We have an amazing immune system



- Our immune system works to actively remove viruses from our bodies
- Disease can occur when the immune system is impaired and unable to do this successfully
  - Immunosuppressive drugs
  - Immunosuppressive infections *e.g.* measles or HIV-1

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What is a virus?

Can travel from host-to-host

An infectious, obligate intracellular parasite comprising genetic material (DNA or RNA) surrounded by a protein coat and/or a membrane

10

What is a virus?

Must enter a cell to replicate

An infectious, obligate intracellular parasite comprising genetic material (DNA or RNA) surrounded by a protein coat and/or a membrane

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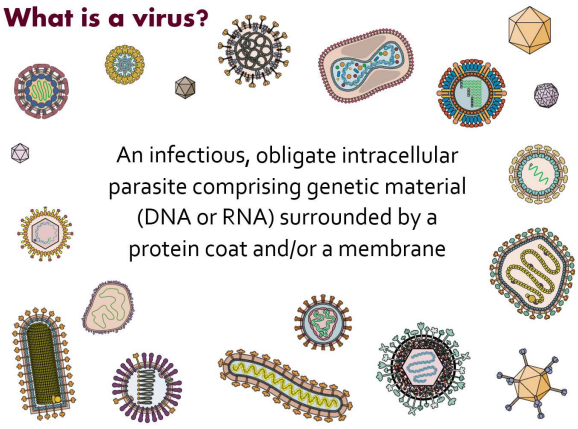
What is a virus?

Can harm the host — parasite comprising genetic material (DNA or RNA) surrounded by a protein coat and/or a membrane

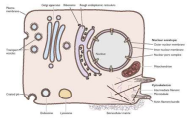
What is a virus?



An infectious, obligate intracellular parasite comprising genetic material (DNA or RNA) surrounded by a protein coat and/or a membrane

Virus hosts

As viruses are obligate molecular parasites, every solution must reveal something about the host as well as the virus



Cells

Humans & animals

Insects

Plants

Protozoans (*Cafeteria roenbergensis*)

11

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Are viruses alive?

Yes - (1917 votes)

No - (2102 votes)

They are something in between - (1959 votes)

I don't know - (295 votes)

Total Answers 6368

Total Votes 6367

www.virology.ws/are-viruses-alive - **Clickable in the 'Links' tab**

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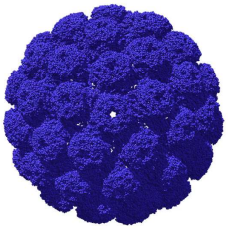
Are viruses alive?

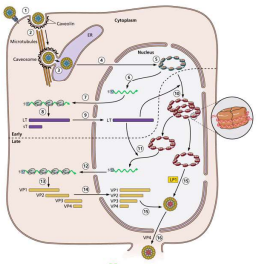
A virus is an organism with two phases

Virion
(infectious particle)



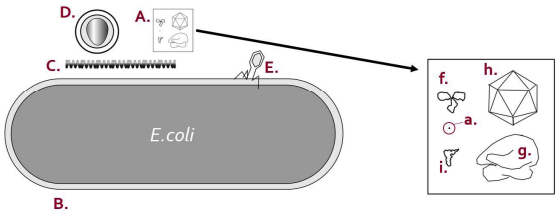
Non-living

Infected cell



Alive

Viruses are very small



100,000x

1,000,000x

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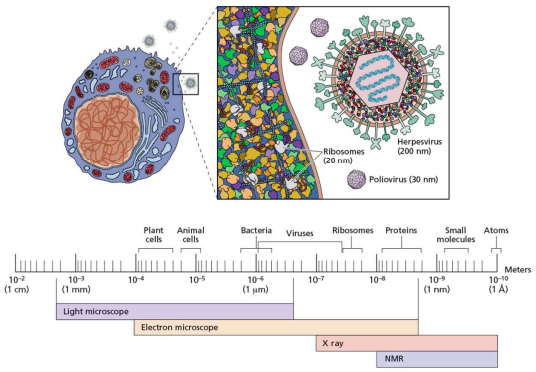
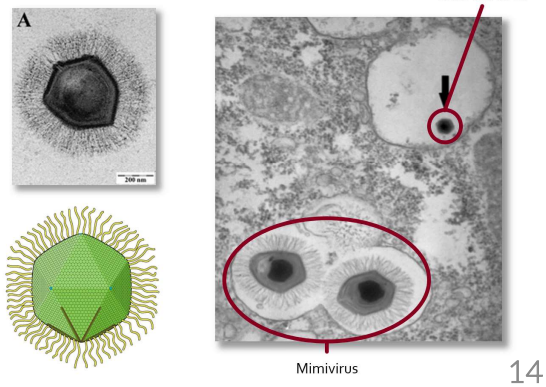
Viruses are very small



Not as small as we once thought!



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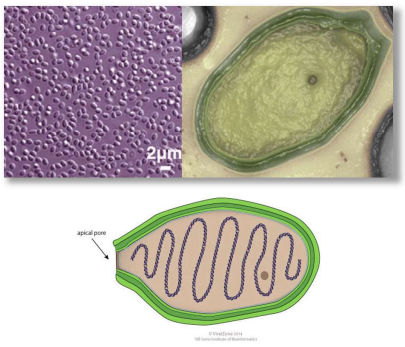
Not as small as we once thought!

Pandoravirus



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Virus replication

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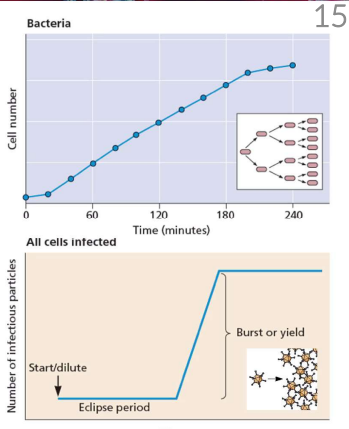
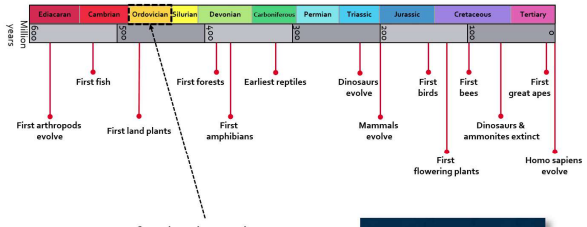
- Viruses replicate by assembly of pre-formed components into many virus particles
- Whereas cells divide by binary fission
- Viruses make the parts, then assemble the final product



How old are viruses?



- Estimates of molecular evolution suggest marine origin of some retroviruses >450 Ma, Ordovician period
- Likely originated billions of years ago - before cells?

Orthoceras, a nautiloid cephalopod (488 Ma)

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Ancient references to viral diseases

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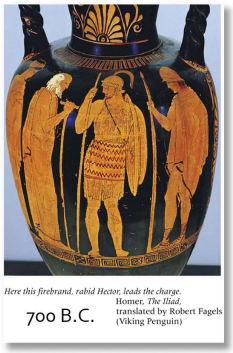
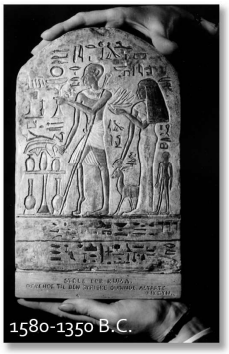


Here this firebrand, rabid Hector, leads the charge.
Homer, *The Iliad*, translated by Robert Fagels (Viking Penguin)

700 B.C.



1580-1350 B.C.

Viruses are thought to have coexisted with humans throughout their history

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Immunization

- Variolation – China (11th century), observed by Lady Montagu in the 1700s
- No knowledge of the causative agent
- Survivors of smallpox were protected against disease

- 1790s – experiments by Edward Jenner in England established vaccination

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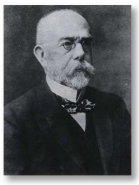
Concept of microorganisms

Leeuwenhoek
(1632 – 1723)

Pasteur
(1822 – 1895)



Koch
(1843 – 1910)

Leeuwenhoek microscope

Koch's postulates/
germ theory

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Tobacco mosaic virus

- Infected Tobacco mosaic plant leaves were ground up and passed through a filter
- 1892 – Ivanovsky
- 1898 – Beijerinck: *Contagium vivum fluidum*
- Named the agent a **virus:** slimy, liquid, poison

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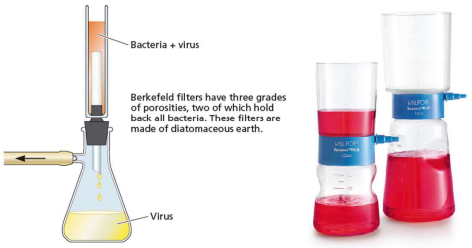
Tobacco mosaic virus



Virus discovery

- 1898 – Loeffler & Frosch discovered that the agent of foot & mouth disease is filterable
- **Key concepts:** agents are not only small, but replicate only in the host, not in a broth
- 0.2 micron filters (μm, one millionth of a meter)

Animal infected with foot and mouth disease

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Virus discovery timeline

- 1901 – First human virus, yellow fever virus
- 1903 – Rabies virus
- 1906 – Variola virus
- 1908 – Chicken leukemia virus and poliovirus
- 1911 – Rous sarcoma virus
- 1915 – Bacteriophages
- 1933 – Influenza virus

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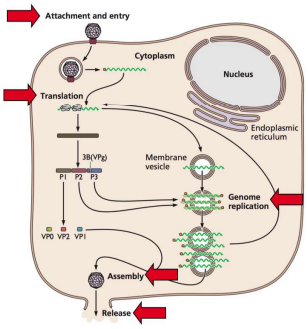
The infectious cycle

Virologists divide the infectious cycle into steps to facilitate their study, but no such artificial boundaries occur



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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

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Growing viruses

- Animal viruses at first could not be routinely propagated in cultured cells
- From the 1900s to the 1950s most viruses were grown in laboratory animals
- *E.g.*

- Since the 1950s propagation in cell culture has become the norm

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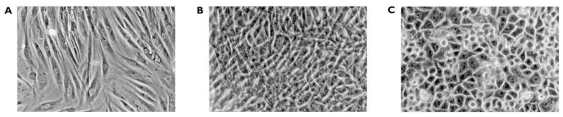
Virus cultivation



Primary human foreskin fibroblasts

Mouse fibroblast cell line (3T3)

Human epithelial cell line (HeLa)

Continuous cell lines

- Continuous cell lines are considered abnormal
- Diploid cell strains are also used (*e.g.* WI-38, human embryonic lung)
  - These are between primary and immortalised cell lines

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Cytopathic effect (CPE)

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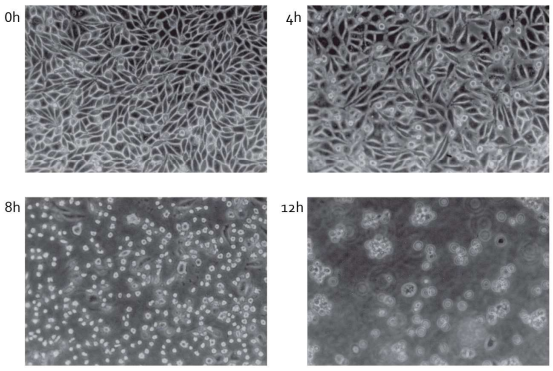

How many viruses in a sample?

Infectivity

Physical: virus particles and their components

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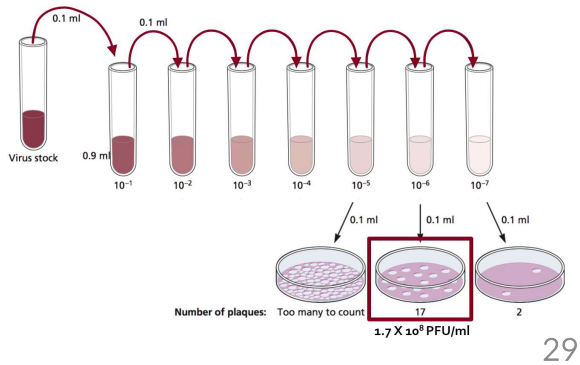
Plaque assay



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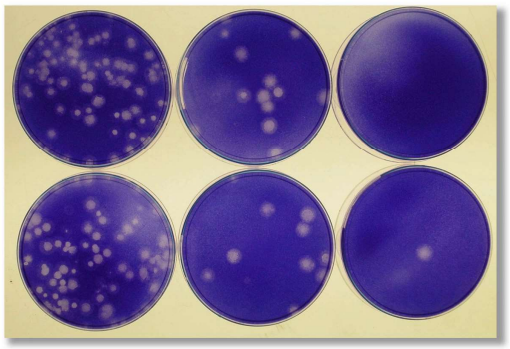
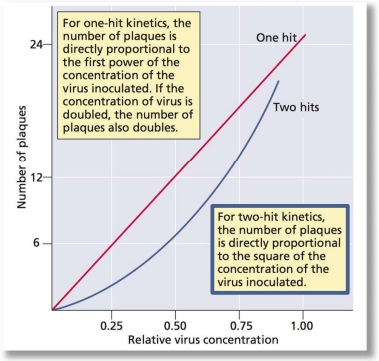
How many viruses are needed to form a plaque?



30

How many viruses are needed to form a plaque?



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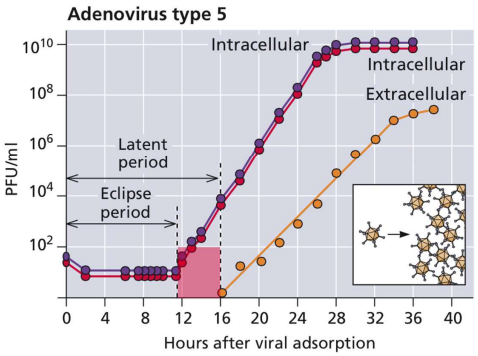
One-step growth experiment



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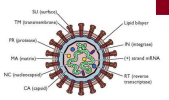
Physical measurement of virus particles

Hemagglutination

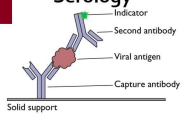
Electron microscopy

Remember
these methods don't measure infectivity!

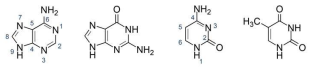
Viral enzymes



Serology



Nucleic acids:



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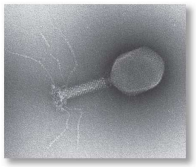
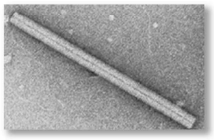
Virology breakthrough in the 1950's

- The genetic information of viruses and living things was previously unknown
- 1940s – DNA identified in bacteria
- 1950s – nucleic acid genome identified in viruses



Hershey-Chase experiment with phage T4



Fraenkel-Conrat's work with TMV

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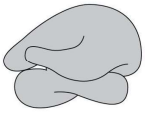
Virology breakthrough in the 1950's

- **The bigger surprise:** thousands of different virions, seemingly infinite complexity of infections
- But a finite number of viral genomes

7

Virus simplicity

- There is an underlying simplicity and order to viruses
- Viral genomes must make mRNA that can be read by host ribosomes
- All viruses on the planet follow this rule, **no known exceptions**



Ribosome

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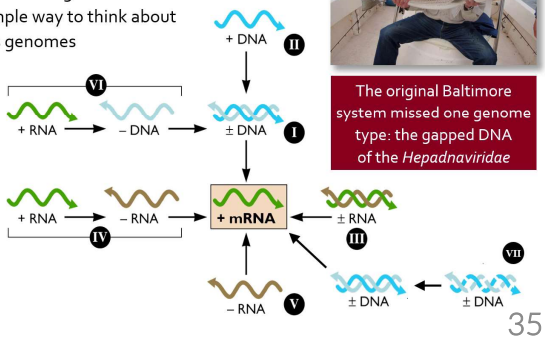
The Baltimore system

David Baltimore (Nobel laureate) used this insight to describe a simple way to think about virus genomes

David Baltimore

The original Baltimore system missed one genome type: the gapped DNA of the *Hepadnaviridae*



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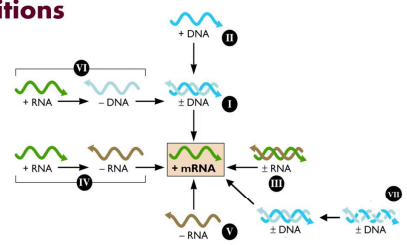
Definitions



- mRNA (ribosome ready) is always the plus (+) strand
- DNA of equivalent polarity is also the (+) strand
- RNA and DNA complements of (+) strands are negative (-) strands
- Not all (+) RNA is mRNA!

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The elegance of the Baltimore system

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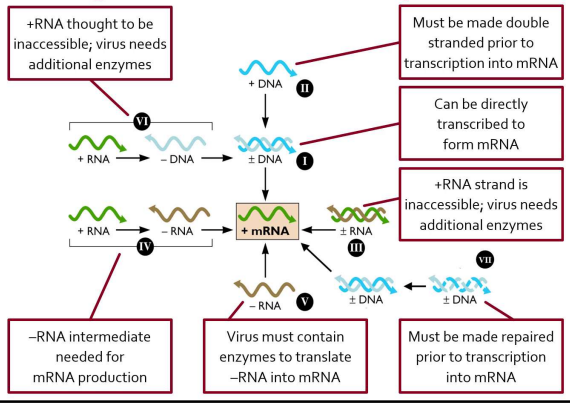
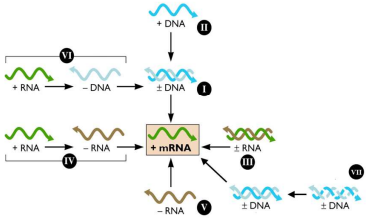


The seven classes of viral genomes

- dsDNA (I)
- gapped dsDNA (VII)
- ssDNA (II)
- dsRNA (III)
- ss (+) RNA (IV)
- ss (-) RNA (V)
- ss (+) RNA with DNA intermediate (VI)



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What is encoded in a viral genome?

Gene products and regulatory signals for:

- Replication of the viral genome
- Assembly and packaging of the genome
- Regulation and timing of the replication cycle
- Modulation of host defences
- Spread to other cells and hosts

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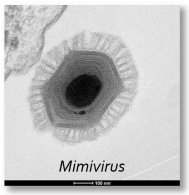
Information NOT contained in viral genomes

- No genes encoding the complete protein synthesis machinery (AARS, eIFs, tRNAs)
- No genes encoding proteins involved in energy production or membrane biosynthesis
- No classical centromeres or telomeres found in standard host chromosomes
- Probably we haven't found them yet - 90% of giant virus genes are novel



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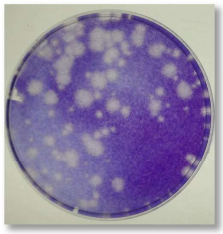
Genetic methods



- Plaque assays can be used to purify viruses
- Virus mutants could also be created and then purified
- Today - DNA copies of viral genomes are readily available

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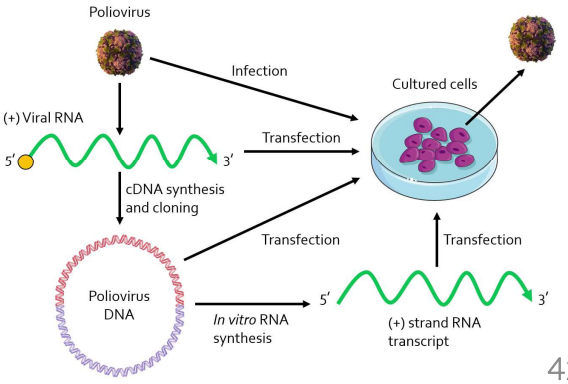
Genetic methods

- This DNA can be inserted into cells leading to the production of infectious virus particles
- Production of infectious virus after transformation of cells by viral DNA, first done with bacteriophage lambda
- **Transfection**
  - Transformation-infection
- This can be done with all viruses
- Allows easy manipulation of the viral genome
  - *E.g.* for gene therapy

Bacteriophage lambda

Infectious poliovirus DNA



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Virus particle types

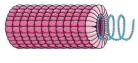
Genomes

7

Virus particles

3

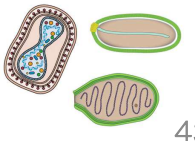
Helical



Tobacco mosaic virus

Icosahedral

Complex



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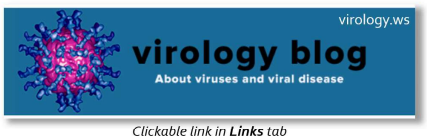
Prof. Vincent Racaniello – Columbia University, USA

Viruses are amazing!



*Clickable link in **Links** tab*

- Virology is an integrative science
- You have to understand many branches of science to understand viruses and their transmission
- Viruses need to infect cells **AND** spread within/between hosts

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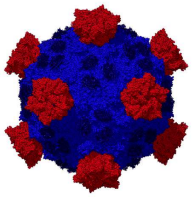
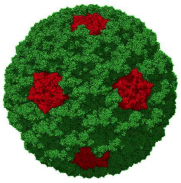
Viruses are amazing!

Virion



Inner shell



Thank you!

HSTalks

By leading world experts