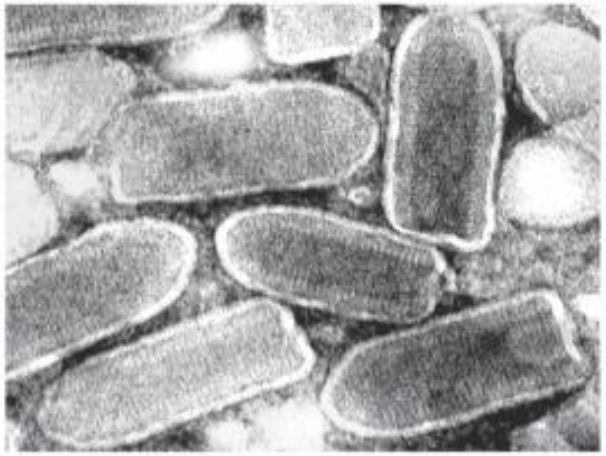
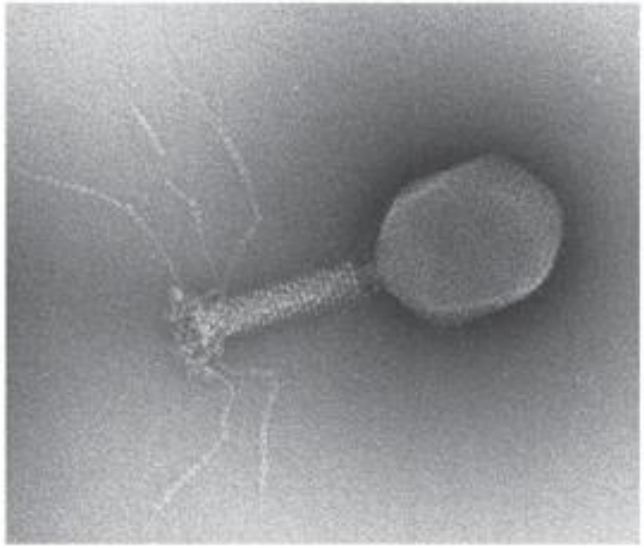
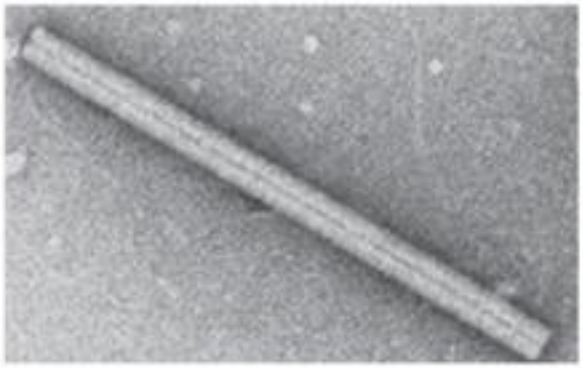


Viruses

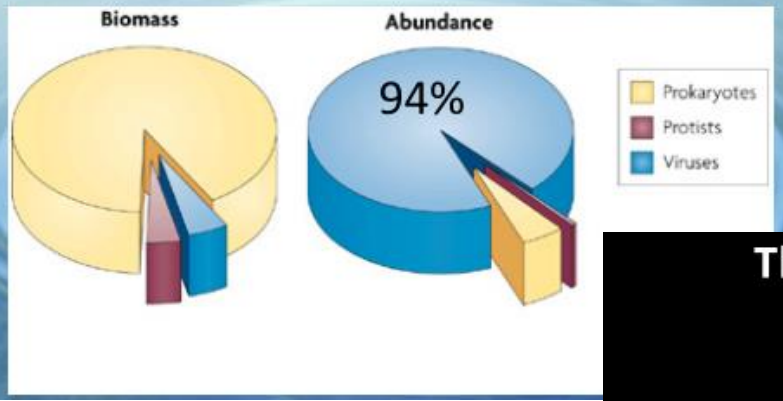




Principles of Virology, ASM Press

Viruses are not just purveyors of bad news

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



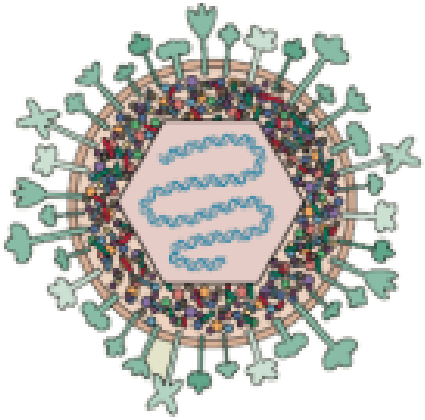
There are ~10^{16} HIV genomes on the planet today

We live in a cloud of viruses

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

How ‘infected’ are we?

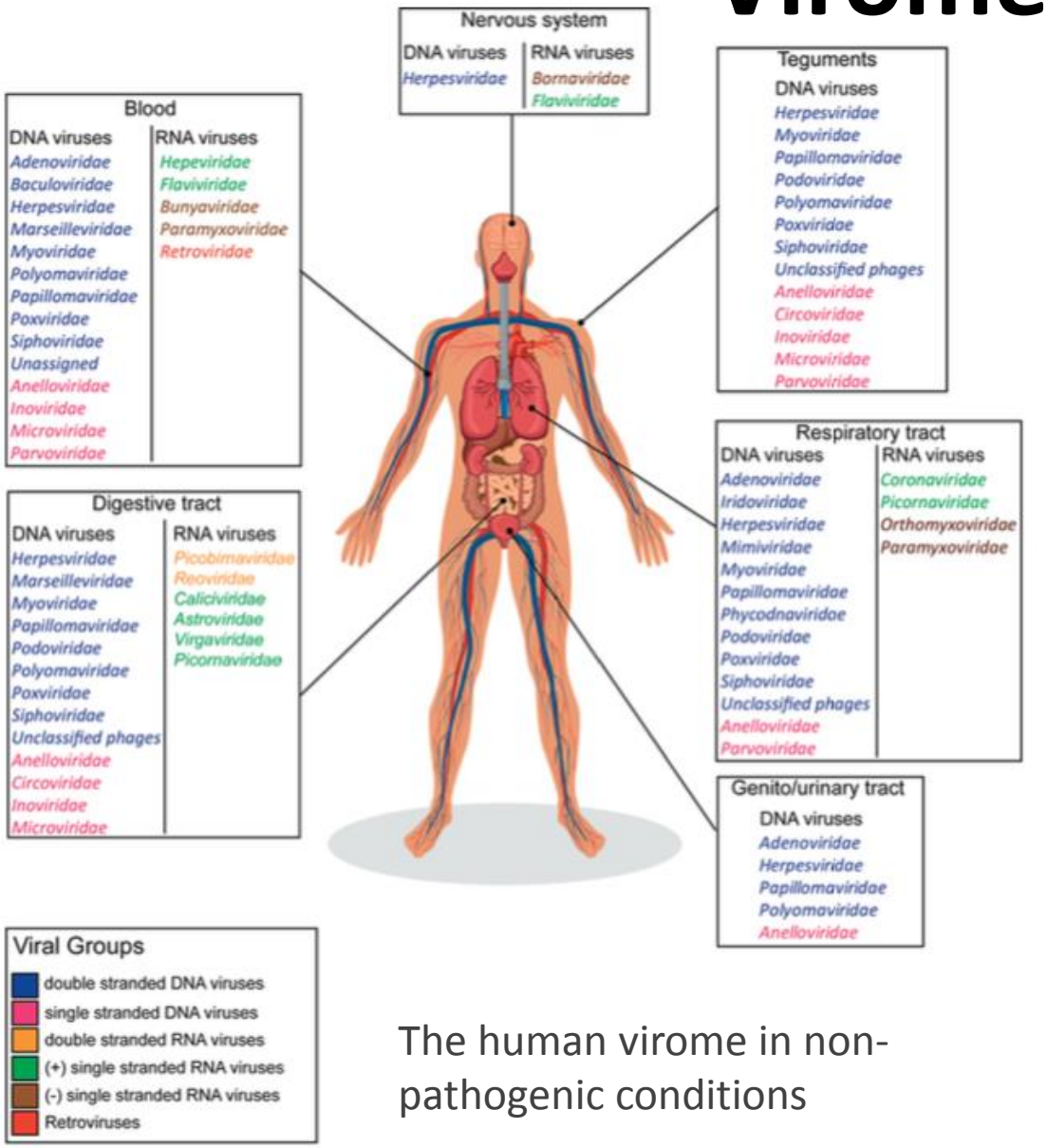
- HSV-1, HSV-2, VZV, HCMV, EBV, HHV-6, HHV-7, HHV-8
- Once infected, always infected



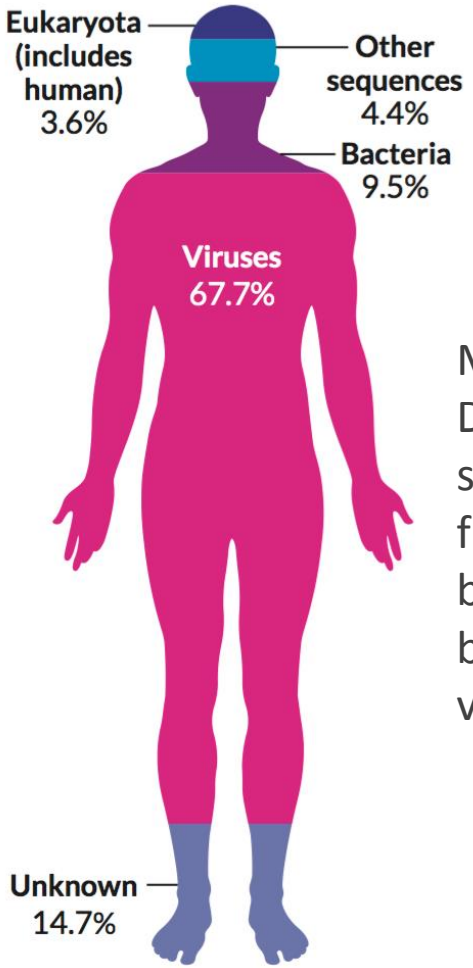
http://www.virology.ws/2013/09/11/a-retrovirus-makes-chicken-eggshells-blue/

A retrovirus makes chicken eggshells blue

Virome

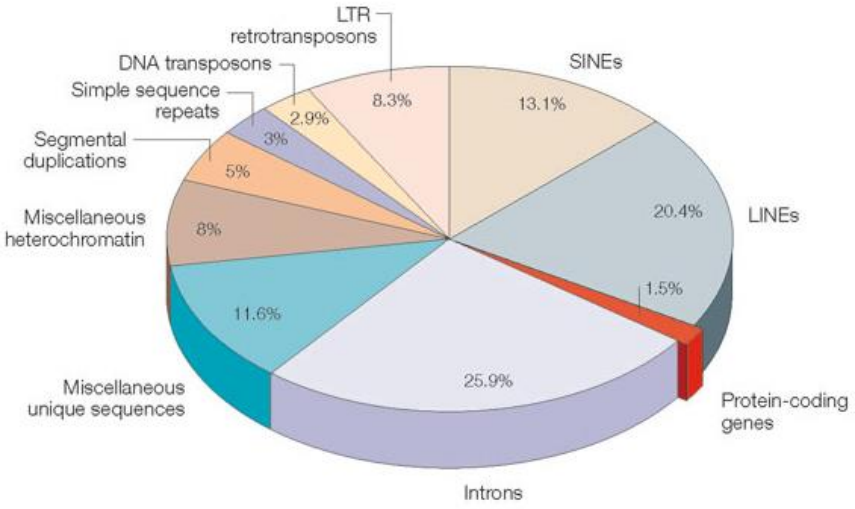


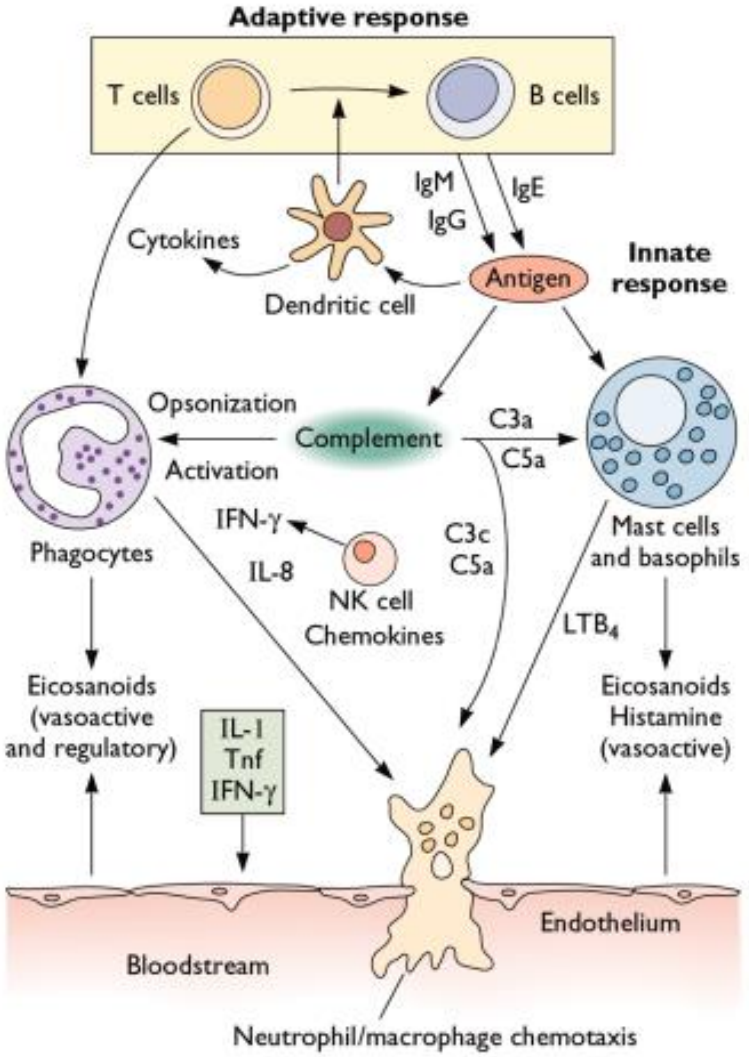
The human virome in non-pathogenic conditions



Most identified DNA sequences floating in our blood plasma belong to viruses.

Amazingly, the vast majority of the viruses that infect us have little or no impact on our health



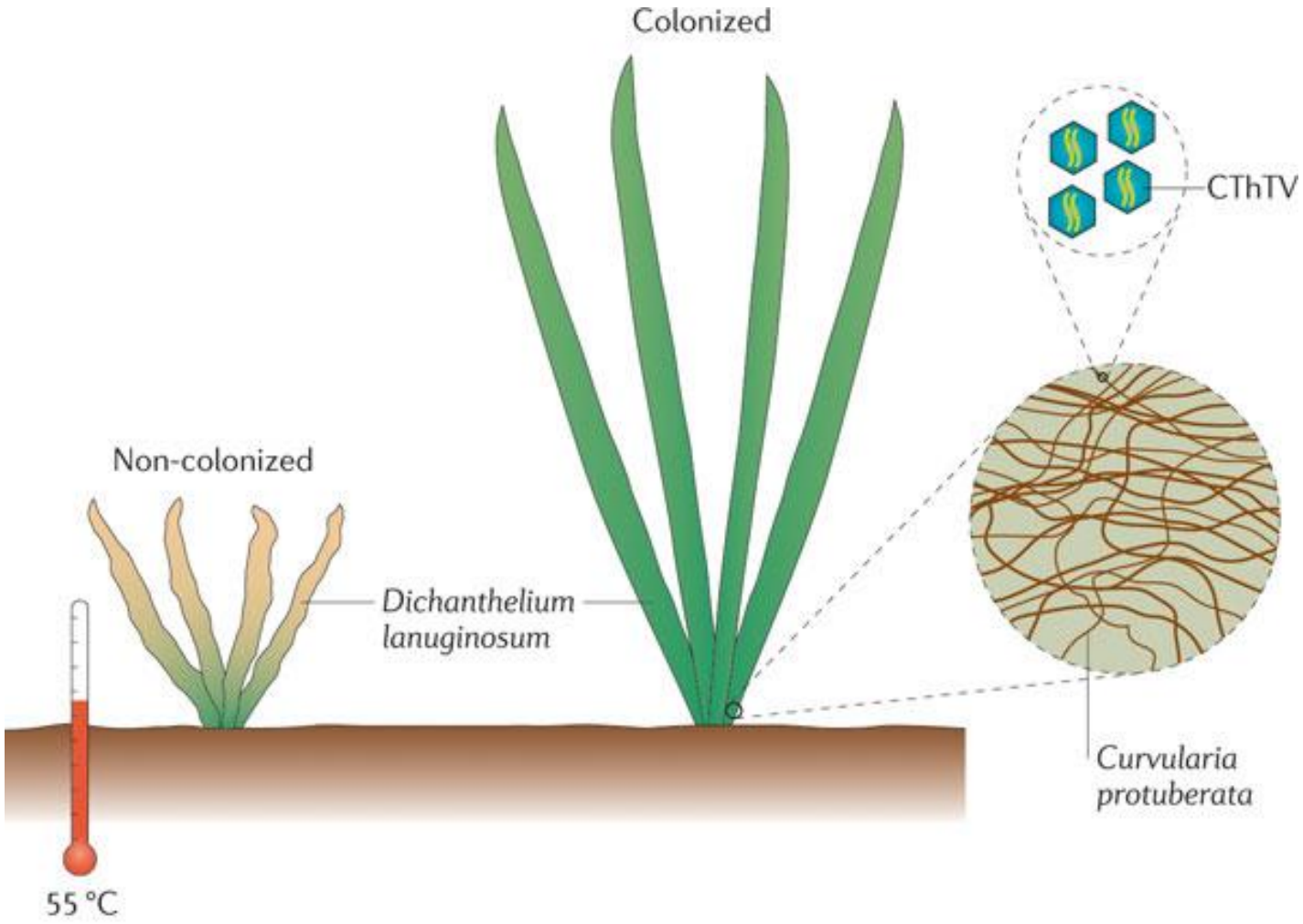


Not all viruses make you ill...

SARAH ZHANG SCIENCE 03.03.16 2:01 PM

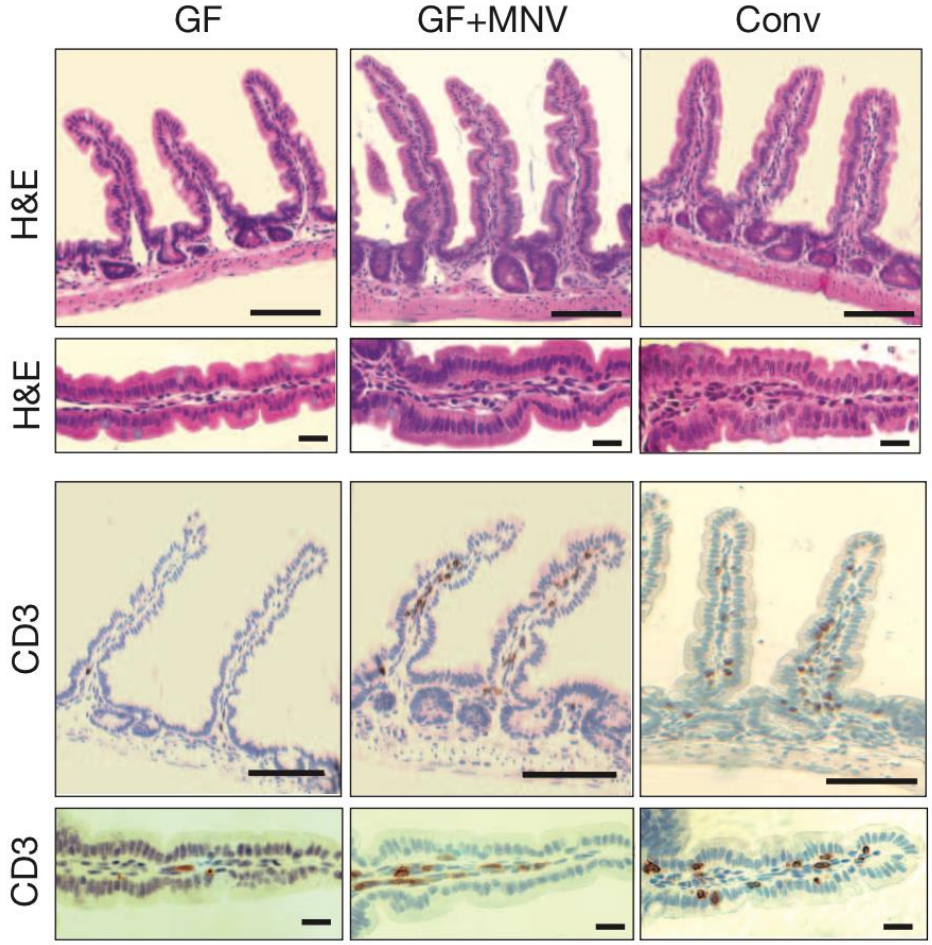
ANCIENT VIRUSES HIDDEN IN YOUR DNA FIGHT OFF NEW VIRUSES

Good viruses

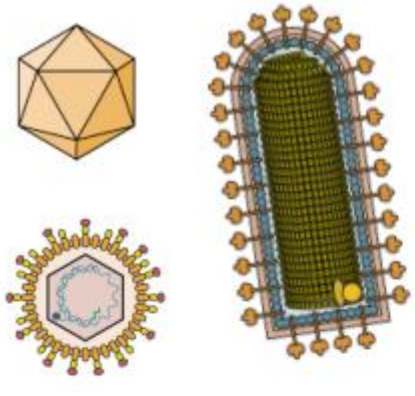
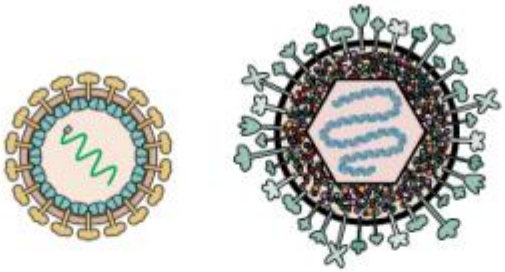
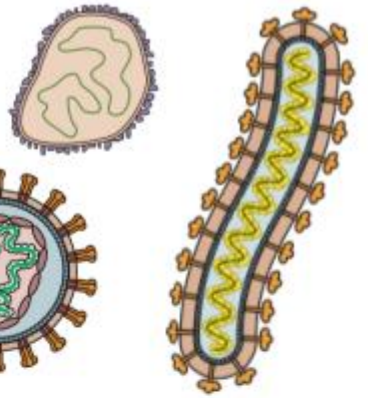


An enterovirus can replace the beneficial function of commensal bacteria

Murine norovirus (MNV) infection of germ-free or antibiotic-treated mice restored intestinal morphology and lymphocyte function without inducing overt inflammation and disease.

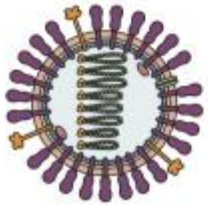
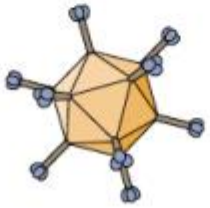


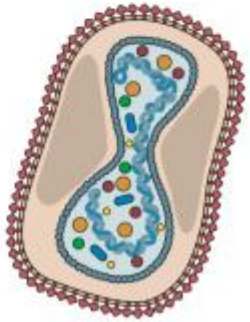
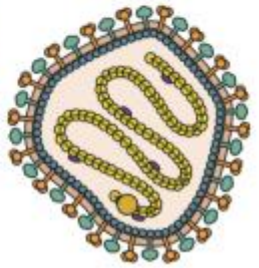
What is a virus?

An *infectious, obligate intracellular parasite comprising genetic material* (DNA or RNA) surrounded by a protein coat and/or an envelope derived from a host cell membrane

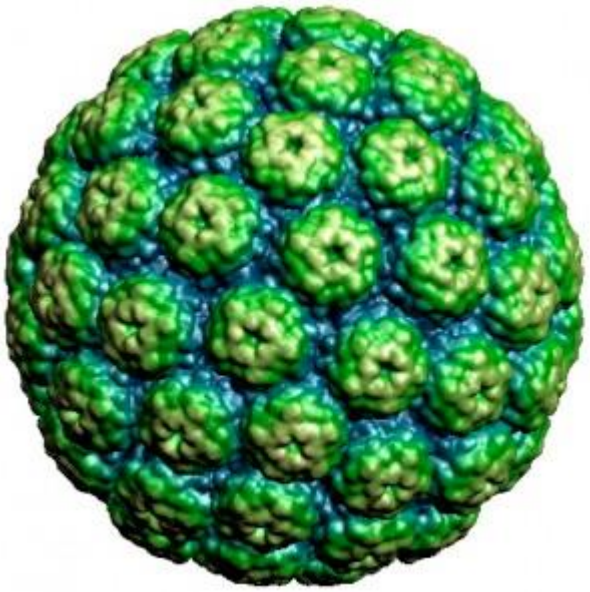


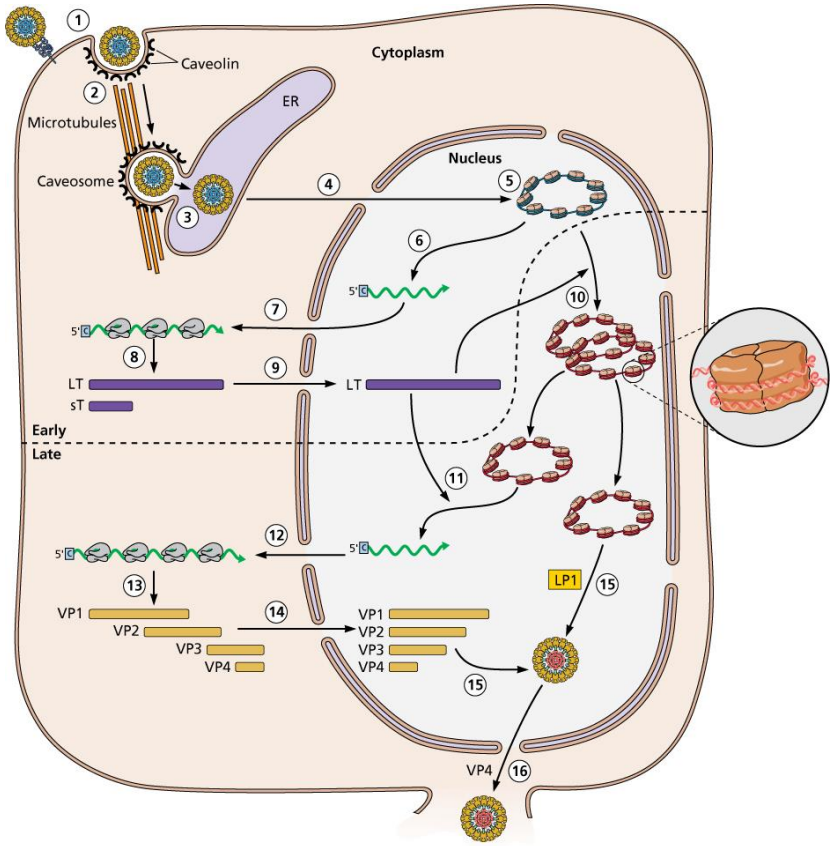



The virus and the virion

A virus is an organism with two phases



virion

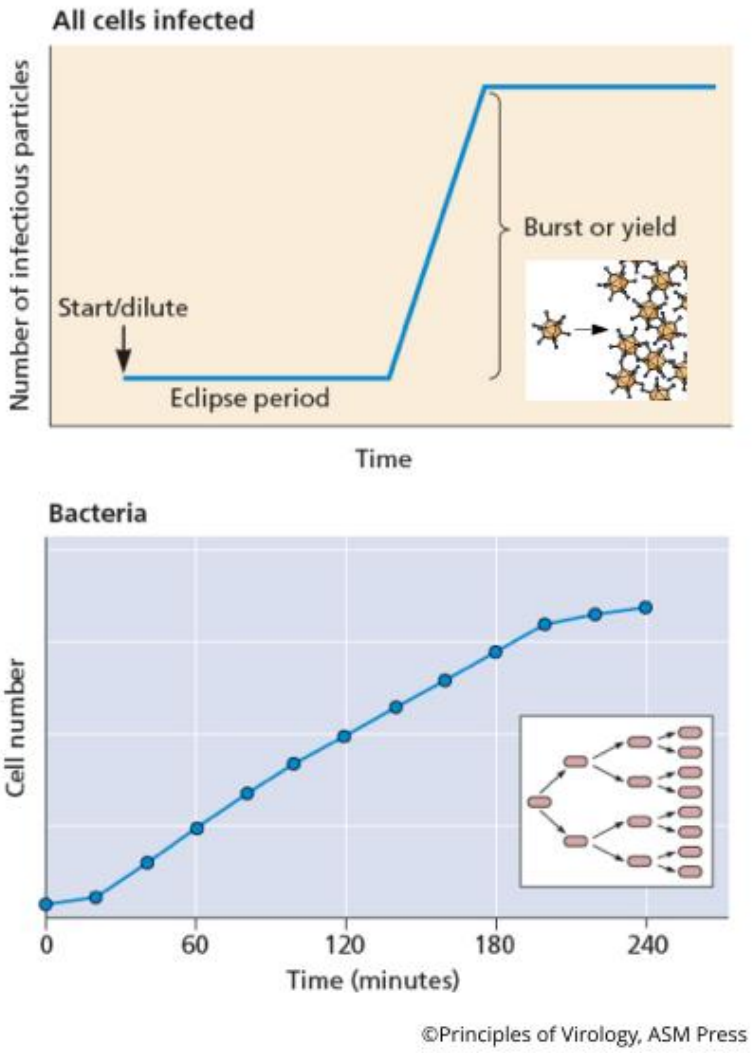


Infected cell

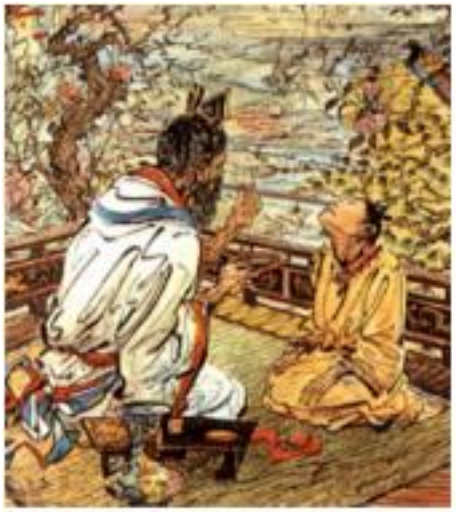
Viruses replicate by assembly of pre-formed components into many particles.

They make the parts and assemble the final product.

Not binary fission like cells.

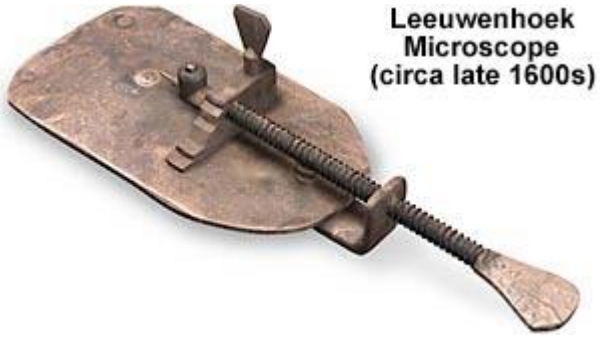


Immunization

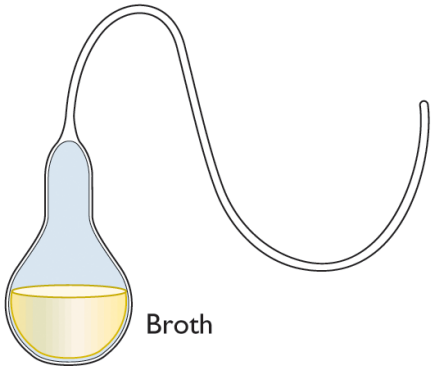


- Variolation - China (11th century), Lady Montagu (1700s)
- *No knowledge of agent*
- Survivors of smallpox protected against disease
- 1790s - experiments by Edward Jenner in England establish vaccination

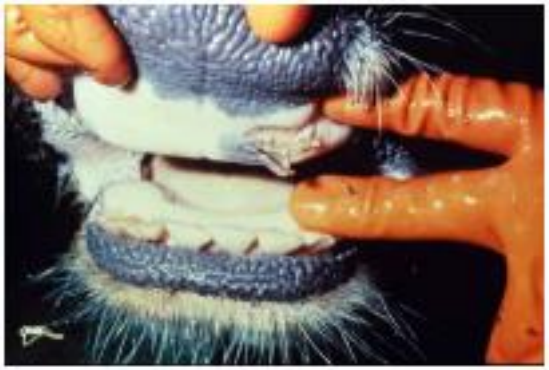
Concept of microorganisms



- Leeuwenhoek (1632 - 1723)
- Pasteur (1822 - 1895)
- Koch (1843-1910)



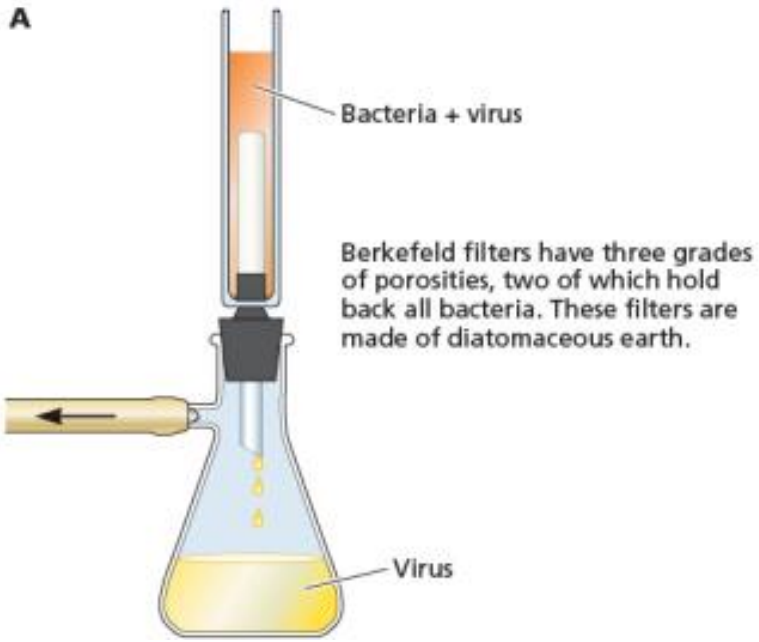
Virus discovery



- Virus discovery - filterable agents

- 1892 - Ivanovsky
- 1898 - Beijerinck: contagium vivum fluidum
- Virus: slimy liquid, poison

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



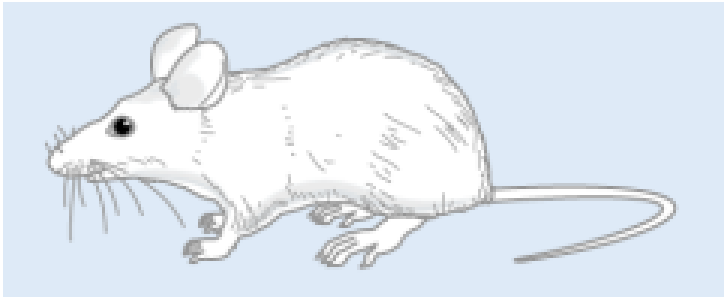
There is an underlying simplicity and order to viruses because of two simple facts

- All viral genomes are obligate molecular parasites that **can only function after they replicate in a cell**

- **All viruses must make mRNA that can be translated by host ribosomes**: they are all parasites of the host protein synthesis machinery

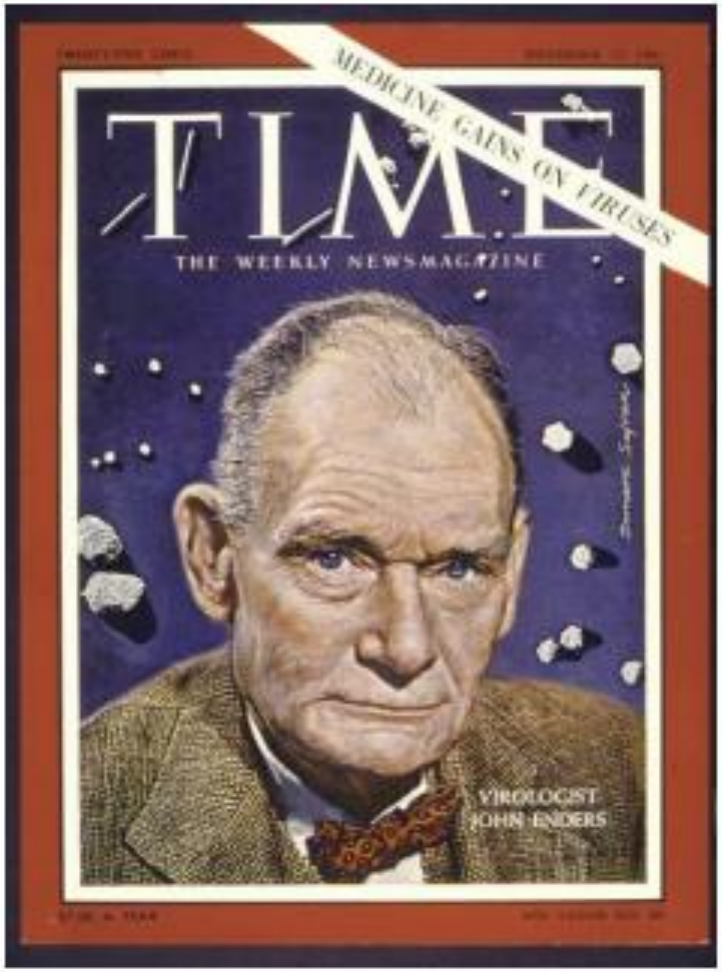
Some important definitions

- 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



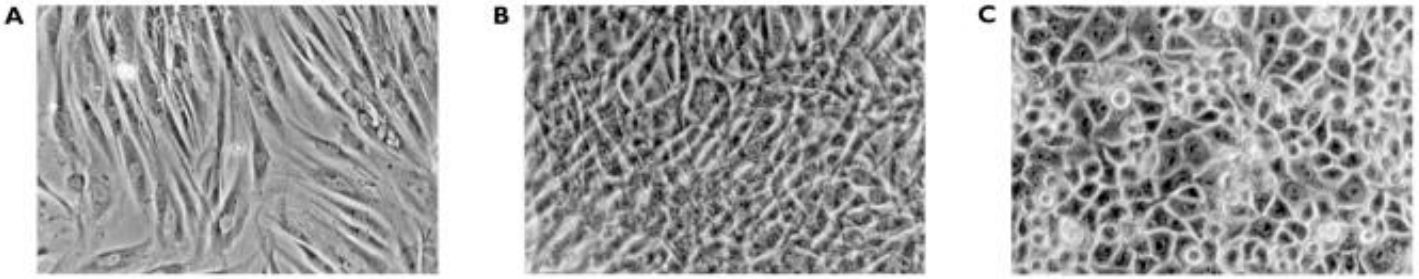
- Animal viruses at first could not be routinely propagated in cultured cells
- Most viruses were grown in laboratory animals

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



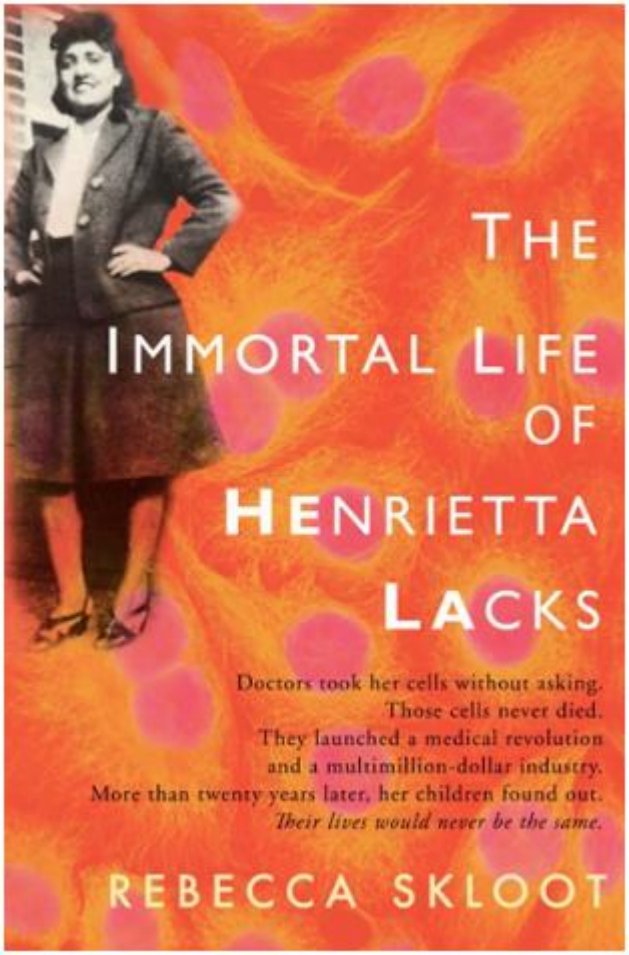
primary human foreskin fibroblasts

mouse fibroblast cell line (3T3)

human epithelial cell line (HeLa)

continuous cell lines

diploid cell strains (e.g. WI-38, human embryonic lung)



- HeLa cells *(Human cervical cells infected by HPV18)* – the first `immortal` cell line known to researchers, isolated in 1951

- Scientists have grown about 20 tons of her cells (2010)

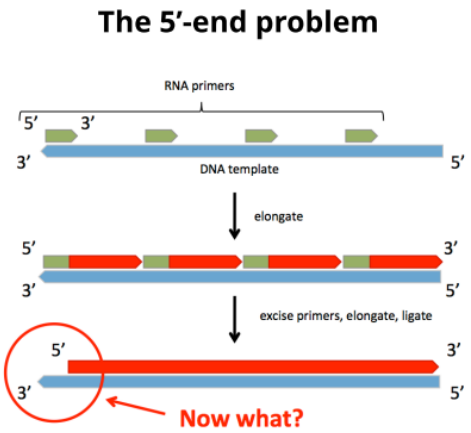
Batts DW (2010-05-10)

- Used for the study of:
- **Polio** – enabled vaccine development

- Cancer, HIV…..

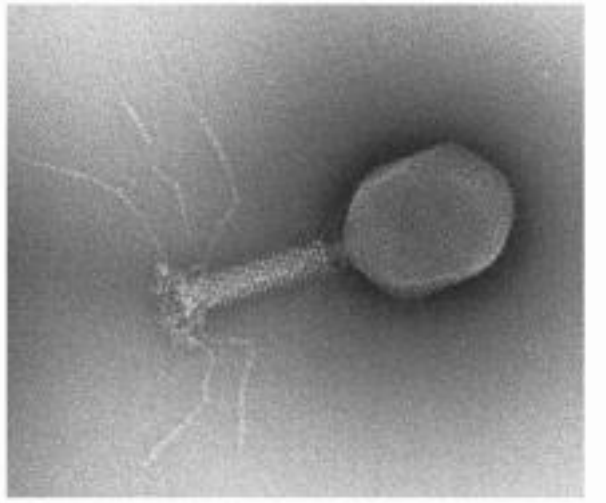
- More than 11000 patents…

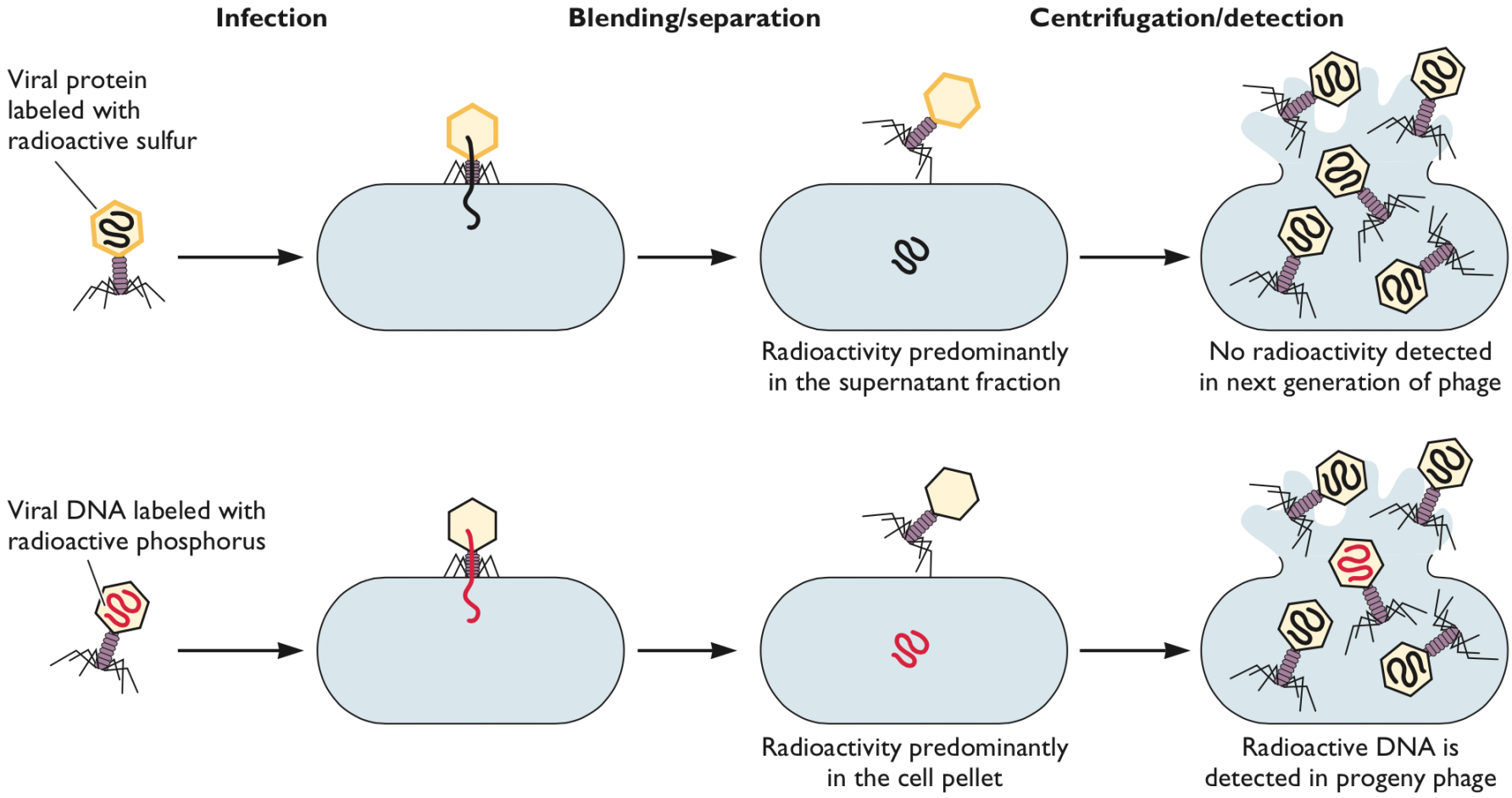
Hayflick limit



Virology breakthrough in the 1950's: The viral nucleic acid genome is the genetic code

- Hershey-Chase experiment with phage T4

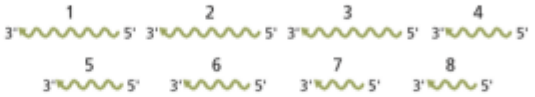


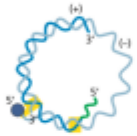


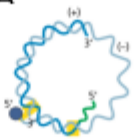
Alfred Hershey & Martha Chase, 1952

Viral DNA or RNA genomes are structurally diverse

- Linear
- Circular
- Segmented
- Gapped
- Single-stranded (+) strand
- Single-stranded (-) strand
- Single stranded, ambisense
- Double-stranded
- Covalently attached proteins
- Cross-linked ends of double-stranded DNA
- DNA with covalently attached RNA







What information **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 defenses
- Spread to other cells and hosts

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

Smallest known viral genomes

Virus	Length	Protein
Viroid	120	none
Satellite	220	none
Hepatitis delta satellite	1,700	1
Circovirus	1,759	2
Anellovirus	2,170	4
Geminivirus	2,500	4
Hepatitis B virus	3,200	7
Levivirus	3,400	4
Partitivirus	3,700	2
Barnavirus	4,000	7

Largest known viral genomes

Virus	Length	Protein
Pandoravirus salinus	2,473,870	2,541
Pandoravirus dulcis	1,908,524	1,487
Megavirus chilensis	1,259,197	1,120
Mamavirus	1,191,693	1,023
Mimivirus	1,181,549	979
Moumouvirus	1,021,348	894
Mimivirus M4	981,813	620
C. roenbergensis virus	617,453	544
Mollivirus sibericum	651,000	523
Pithovirus sibericum	610,033	467

- The biggest surprise: thousands of different virions, seemingly infinite complexity of infections
- But a small number of viral genome types

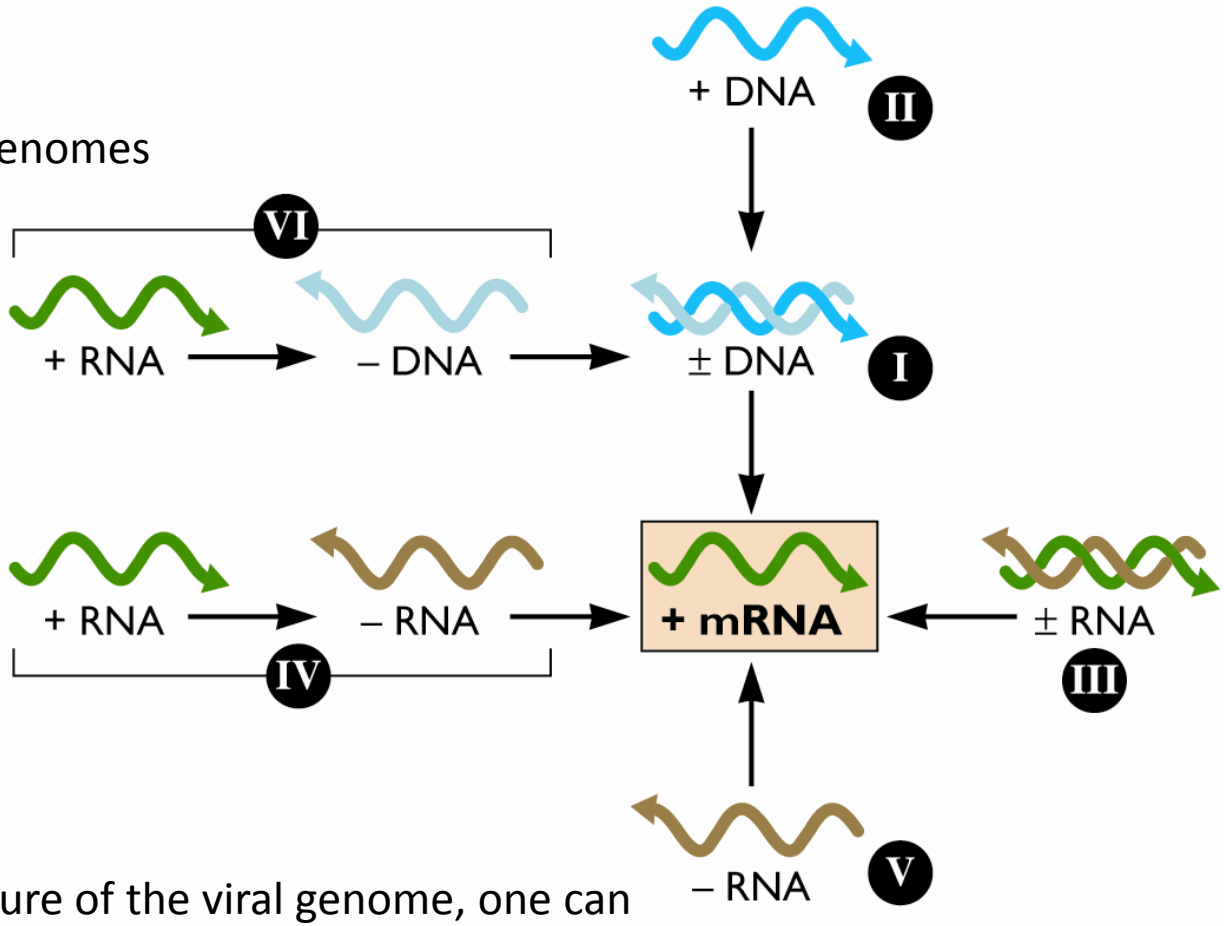
- Viral genomes must make mRNA that can be read by host ribosomes

- All viruses on the planet follow this rule, no exception to date

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

The seven classes of viral genomes

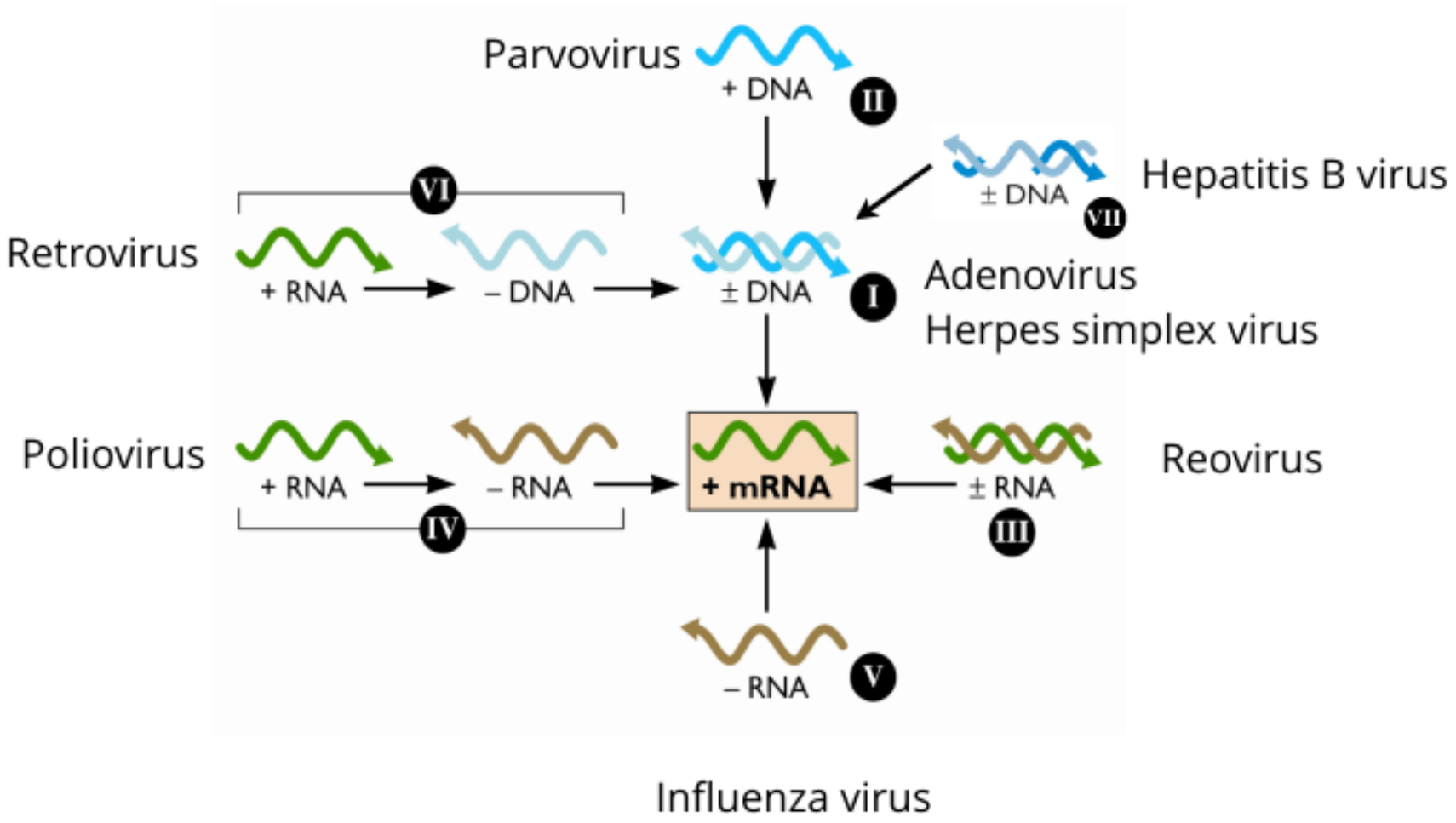
I - dsDNA
II - ssDNA
III - dsRNA
IV - ss (+) RNA
V - ss (-) RNA
VI - ss (+) RNA with DNA intermediate
VII - gapped dsDNA



Knowing only the nature of the viral genome, one can deduce the basic steps that must take place to produce mRNA

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!

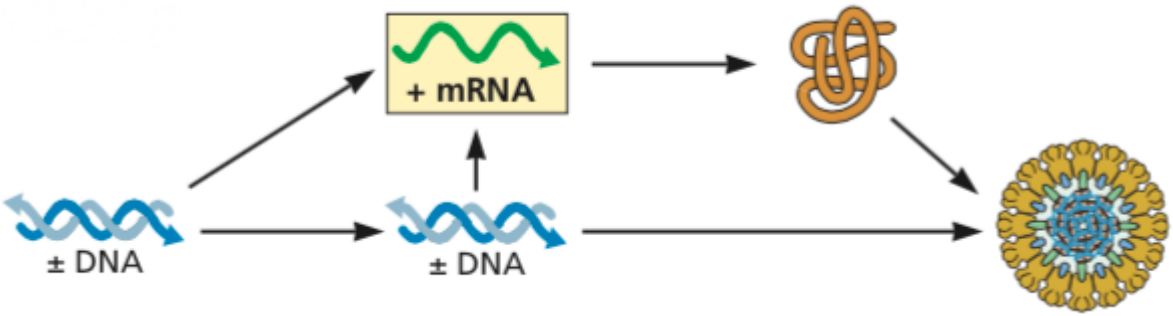

Parvovirus
+ DNA
II
± DNA
VII
Hepatitis B virus
VI
Retrovirus
+ RNA
– DNA
± DNA
I
Adenovirus
Herpes simplex virus
Poliovirus
+ RNA
– RNA
+ mRNA
± RNA
III
Reovirus
IV
– RNA
V
Influenza virus

DNA genomes

- **Transcription is the first biosynthetic reaction to occur in cells infected with dsDNA viruses**

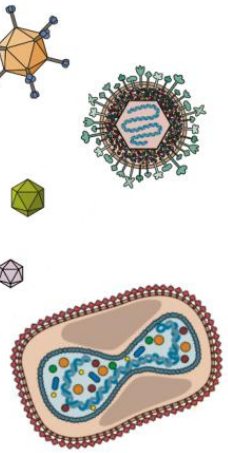
- *Viral DNA replication always requires synthesis of at least one viral protein, sometimes many - hence it is always delayed after infection*

I. dsDNA genomes



dsDNA genomes

- *Adenoviridae*
- *Herpesviridae*
- *Papillomaviridae*
- *Polyomaviridae*
- *Poxviridae*

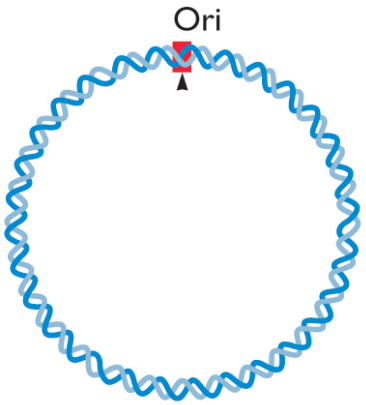


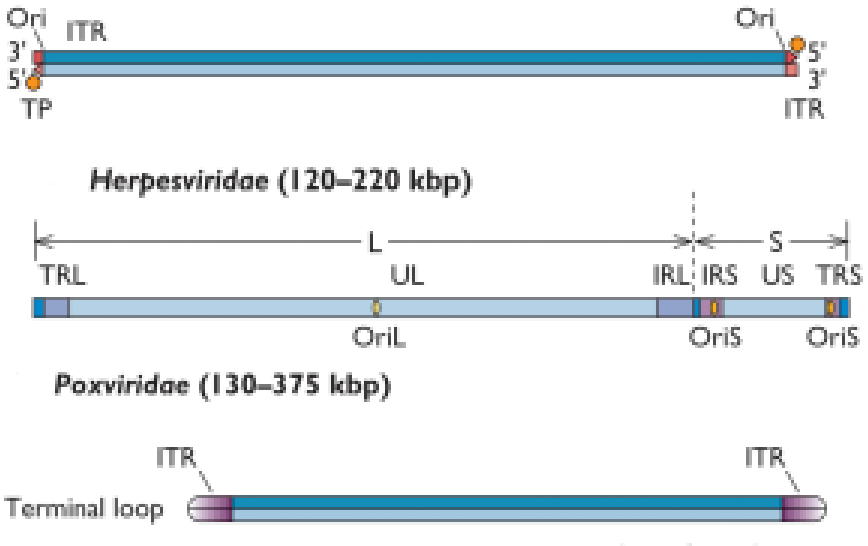
Genomes encode DNA polymerase

***Adenoviridae* (36–48 kbp)**

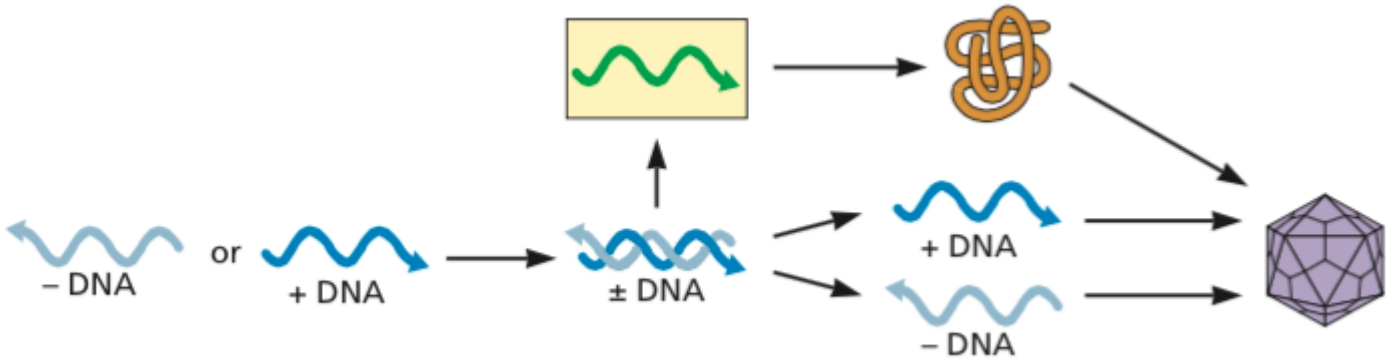
Genomes copied by host DNA polymerase

***Polyomaviridae* (5 kbp)**





II. ssDNA genomes



***Circoviridae* (1.7–2.2 kb)**

TT virus (ubiquitous human virus)

***Parvoviridae* (4–6 kb)**

B19 parvovirus (fifth disease)

RNA genomes

- **Cells have no RNA-dependent RNA polymerase** (RdRp)
- **RNA virus genomes encode RdRp**
- RdRp produce RNA genomes and mRNA from RNA templates

RNA directed RNA synthesis

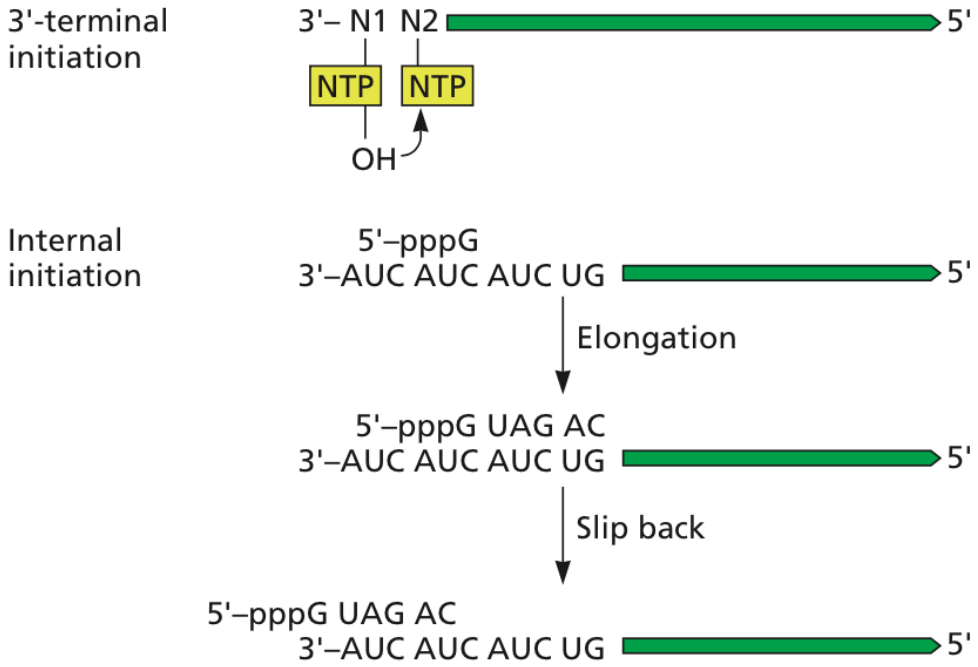
RNA in the virus particle

- (-) strand RNA genomes: coated with protein
- (+) strand RNA genomes: naked (exceptions: retrovirus, coronavirus)
- dsRNA genomes

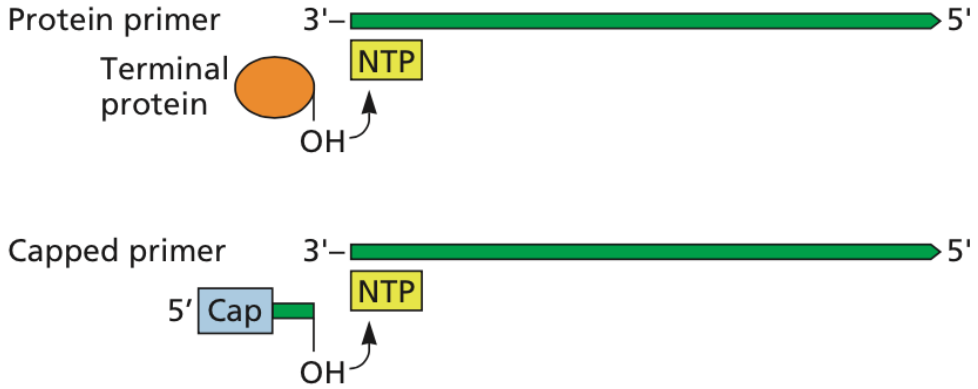
Universal rules for RNA-directed RNA synthesis

- RNA synthesis initiates and terminates at specific sites on the template
- RdRp may initiate synthesis *de novo* (like cellular DdRp) or require a primer
- Other viral and cell proteins may be required
- RNA is synthesized by template-directed stepwise incorporation of NTPs, elongated in 5'-3' direction
- Non-templated RNA synthesis

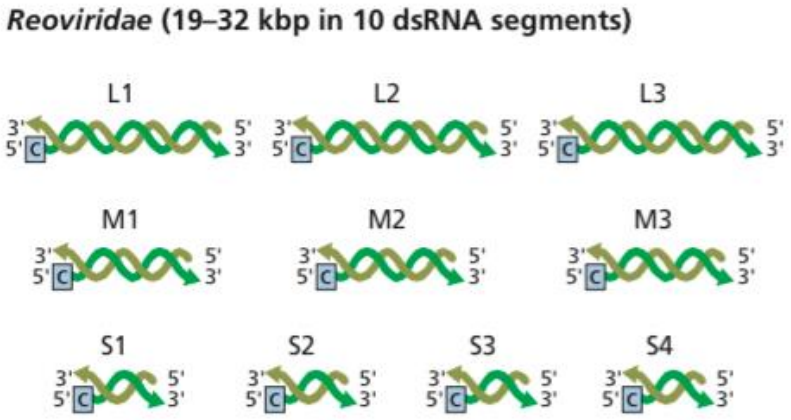
***De novo* initiation**



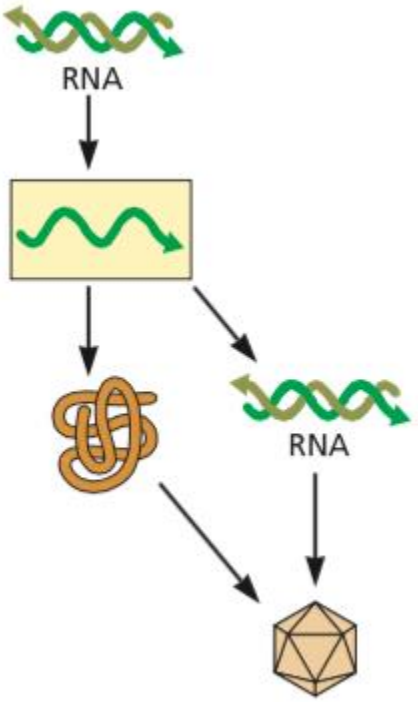
Primer-dependent initiation



III. dsRNA genome

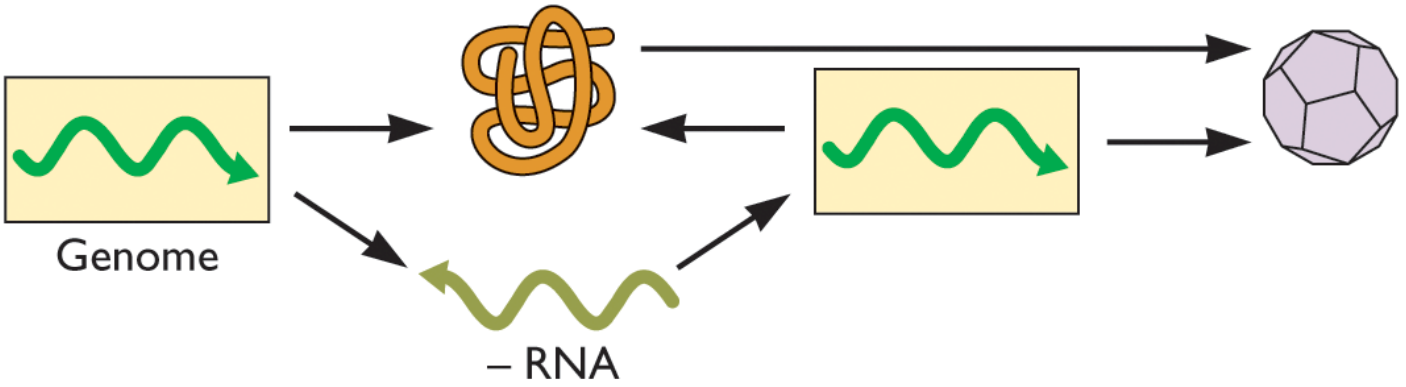


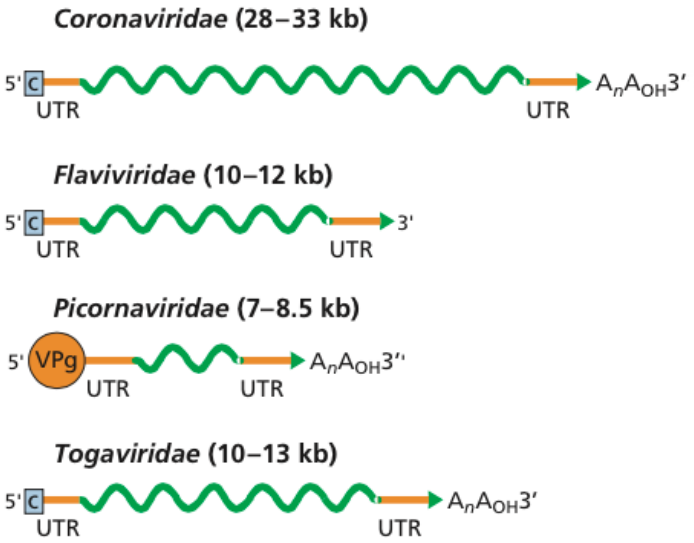
Rotavirus (human gastroenteritis)



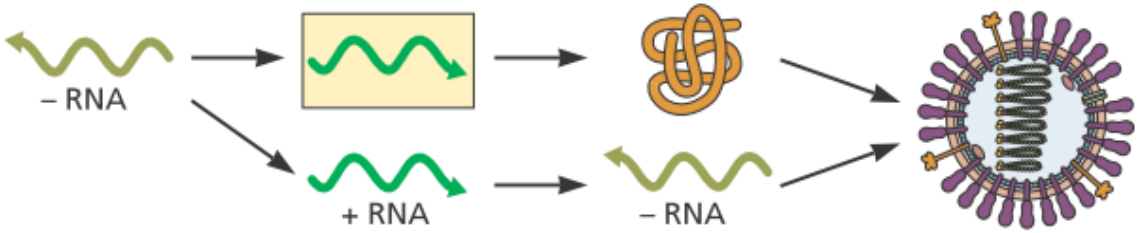
There is no incorporation into host genome

IV. ssRNA: (+) sense



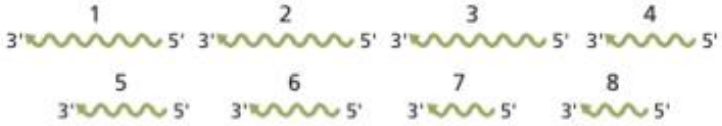


V. - ssRNA, (-) sense



Segmented genomes: *Orthomyxoviridae*
(10–15 kb in 6–8 RNAs)

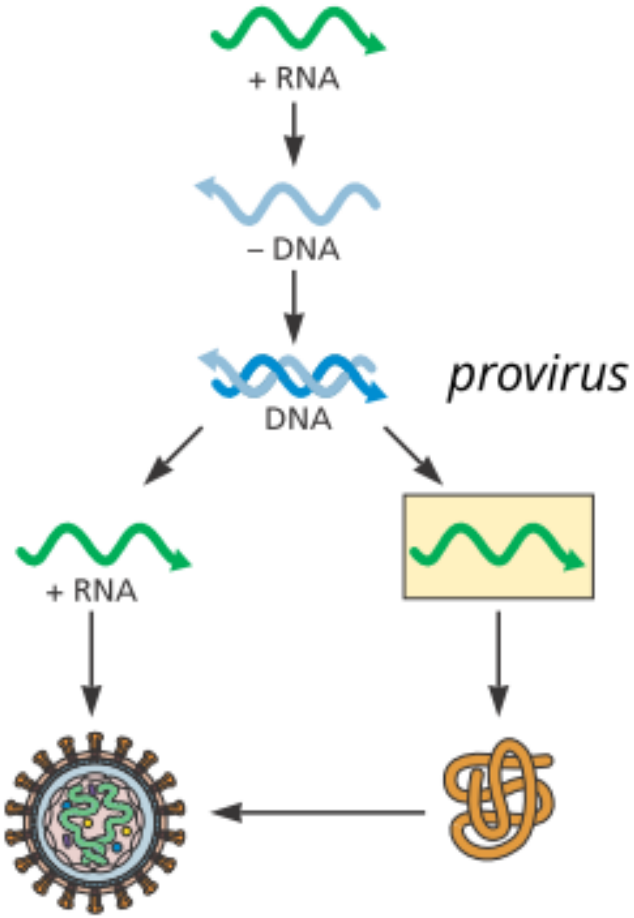
(–) strand RNA segments



Nonsegmented genomes: *Paramyxoviridae* (15–16 kb)

***Rhabdoviridae* (13–16 kb)**

VI. ssRNA(+) sense with DNA intermediate



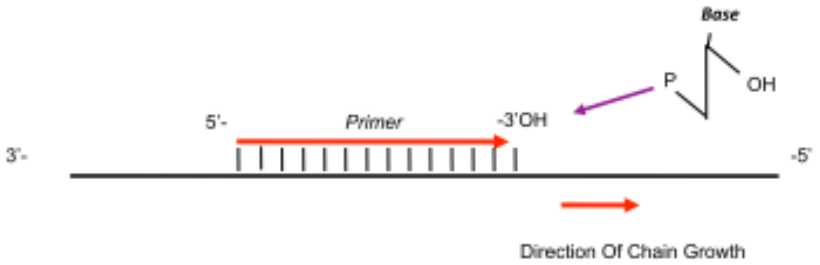
One viral family: Retroviridae
Two human pathogens:
Human immunodeficiency virus (HIV)
Human T-lymphotropic virus (HTLV)

Central dogma of molecular biology

http://hyperphysics.phy-astr.gsu.edu/hbase/organic/dogma.html

Reverse transcriptase

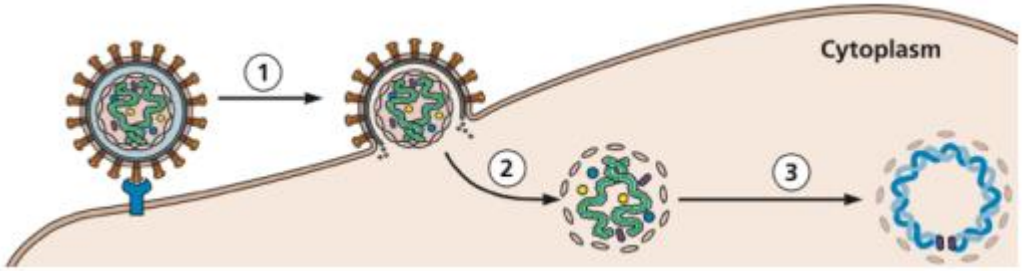
- Primer can be DNA or RNA
- Template can be RNA or DNA
- Only dNTPs, not rNTPs, are incorporated



RT

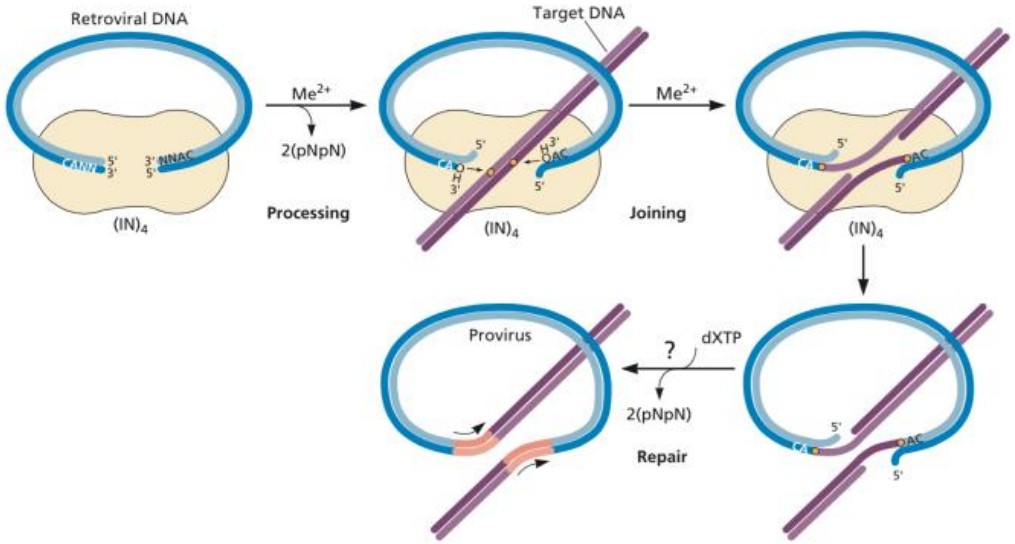
- Bacteria and Archaea have RT activity
- Therefore RT evolved before the separation of Archaea, bacteria, and eukaryotes
- RT might be the bridge between early RNA world and modern DNA world
- RT also in HBV, Caulimoviridae

DNA synthesis: cytoplasmic



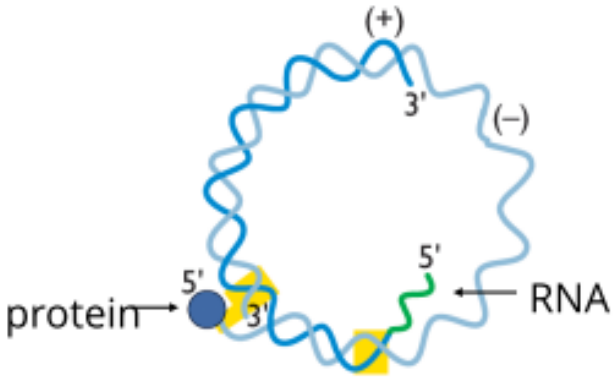
- One DNA produced from two RNAs by RT
- **Strong promoter** (the LTR) built during RT
- Proviral DNA directs the host transcription machinery to synthesize many copies of viral mRNA
- Viral mRNA is translated into viral proteins OR encapsidated into virus particles

There is no DNA replication and no RNA replication

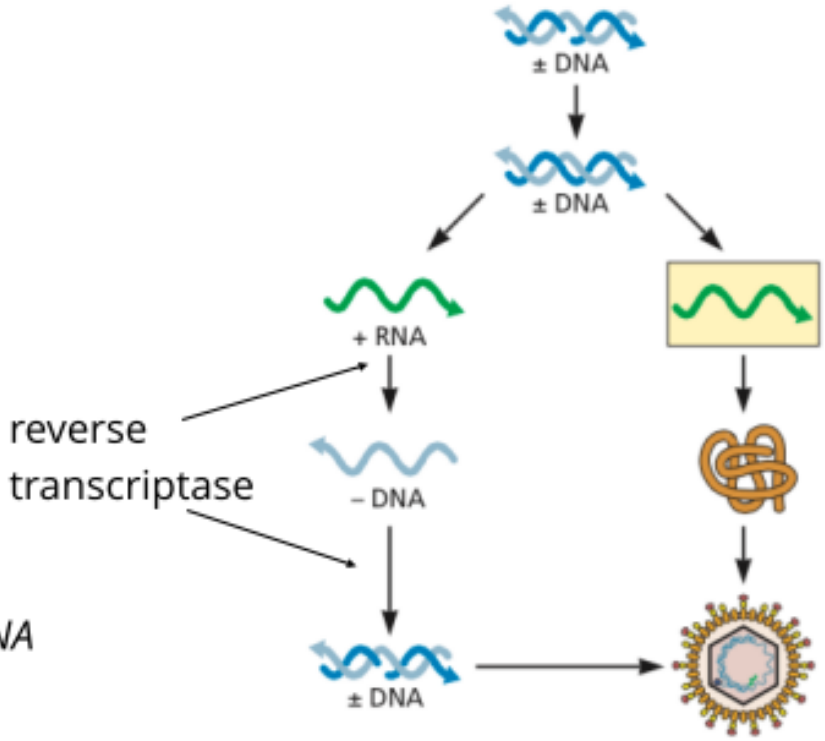


VII. Gapped dsDNA genomes

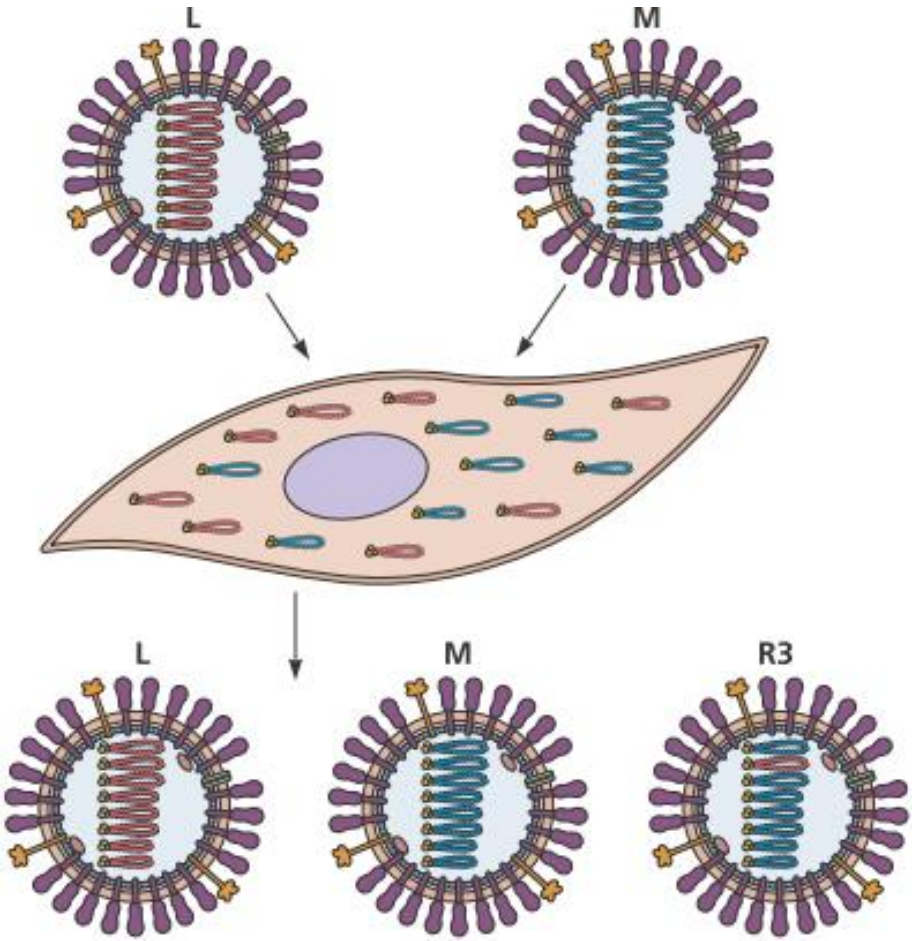
Hepadnaviridae
Hepatitis B virus



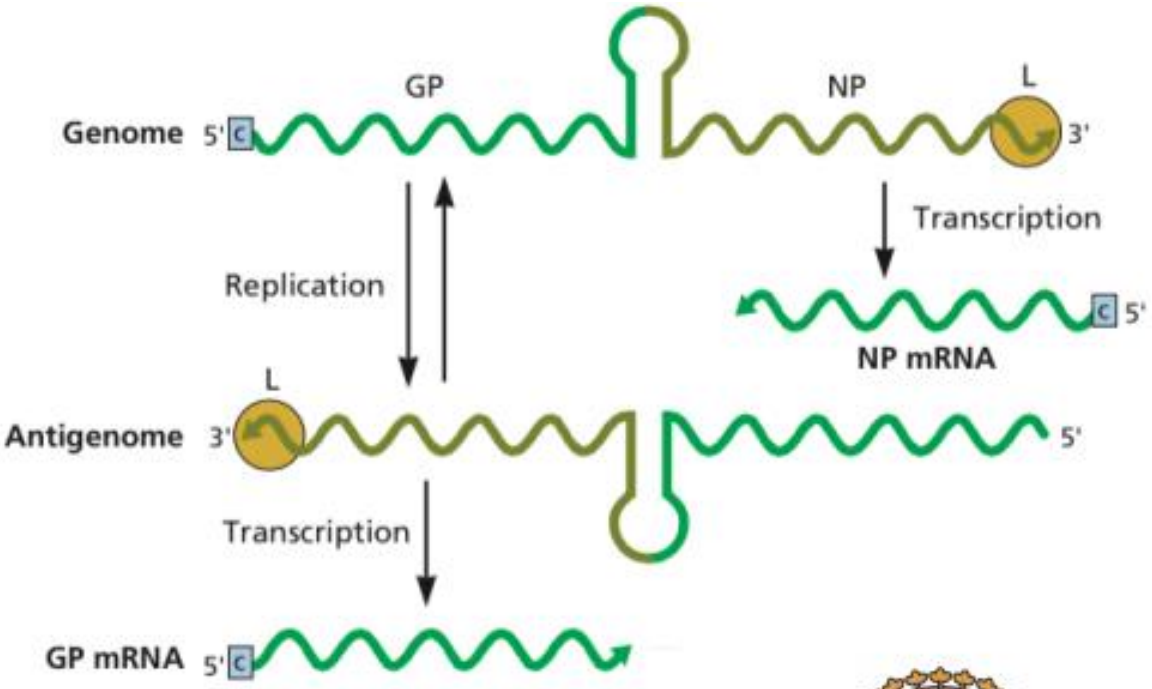
This genome cannot be copied to mRNA



Reassortment: Consequence of **segmented genome**



Ambisense RNA genomes



Arenaviridae
RNA pol in virion

Universal rules of DNA replication

- DNA is synthesized by template-directed incorporation of dNMPs into 3’-OH of DNA chain
- DNA is always synthesized 5’-3’ via semiconservative replication (two daughter strands)
- Replication initiates at specific sites on template called **origins**
- Catalyzed by DdDp + accessory proteins
- Always primer-dependent

What's the host for?

Viruses can't do it themselves

- Viral DNA replication always requires synthesis of at least one viral protein, sometimes many (hence always delayed after infection)
- Simple viruses require more host proteins - genetic economy 
- Complex viruses encode many, but not all proteins required for replication

Viral proteins

- DNA polymerase and accessory proteins
- Origin binding protein, helicases
- Exonucleases
- Enzymes of nucleic acid metabolism

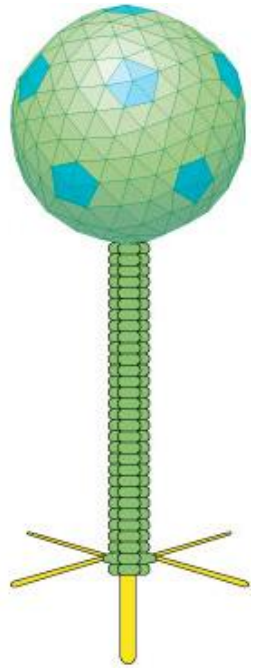
(thymidine kinase, ribonucleotide reductase, dUTPase)

Strategies of transcription of viral DNA

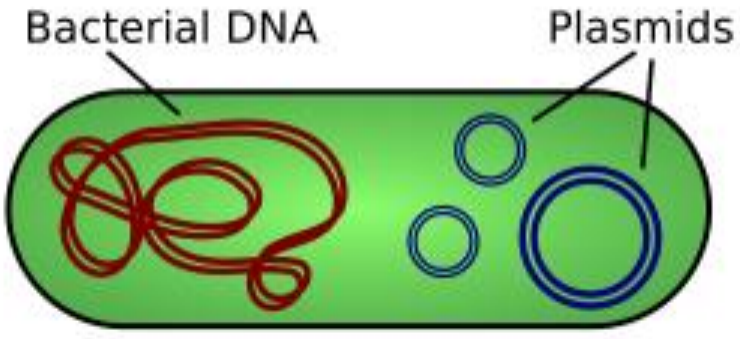
Origin of transcriptional components	Virus
Host only	Simple retroviruses
Host plus one viral protein that regulates transcription	Complex retroviruses, parvoviruses, papillomaviruses, polyomaviruses
Host plus >1 viral protein that stimulate transcription	Adenoviruses, herpesviruses
Viral	Poxviruses

There are three types of genes: immediate-early, early and late. The immediate-early genes are transcribed immediately after infection and ensure the transcription of early genes, which encode the proteins necessary for the viral replication. The late genes mostly encode structural proteins. Standing apart are genes expressed during latency.

Engineering mutations into viral genomes - the modern way



- Infectious DNA clone: transfection
- A modern validation of the Hershey-Chase experiment (1952)
- Deletion, insertion, substitution, nonsense, missense



Transfection

- - Production of infectious virus after transformation of cells by viral DNA, first done with bacteriophage lambda
- - Transformation-infection

We live and prosper in a cloud of viruses

- Most infections have no consequence
- If we do get infected, many infections are inapparent

Viral pathogenesis

- Pathogenesis: the process of producing a disease
- Two components of viral disease:

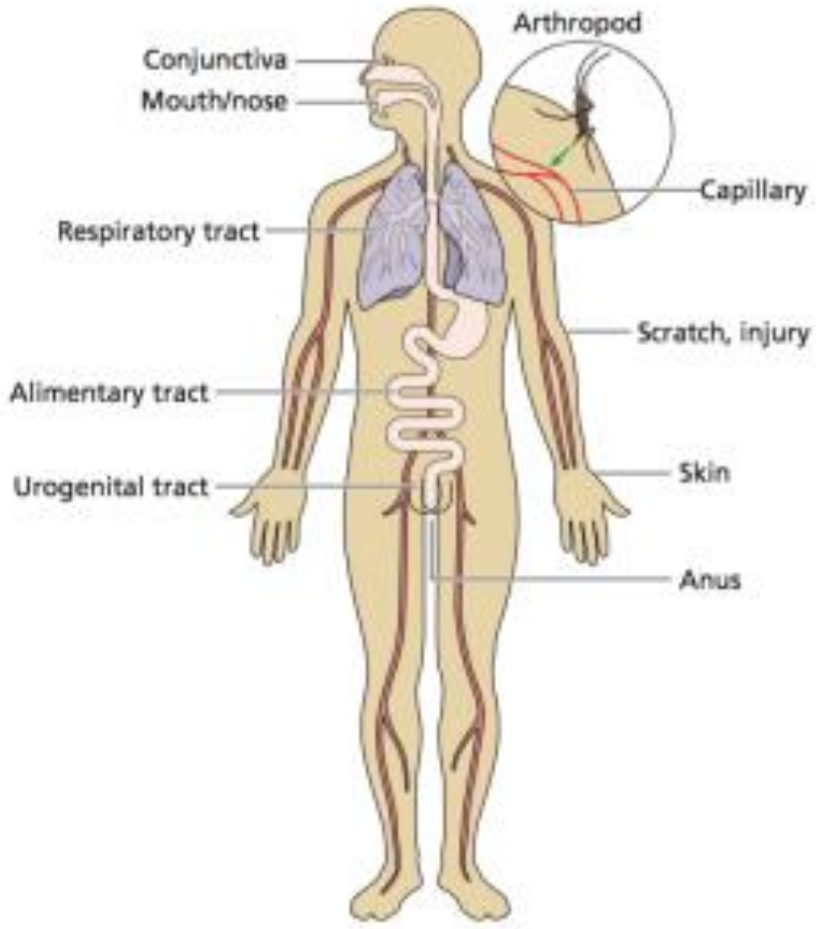
- Effects of viral replication on the host
- Effects of host response on virus and host

Three requirements for a successful infection

- Sufficient amount of virus
- Cells accessible, susceptible, permissive
- Local antiviral defense absent or overcome

Gaining access: site of entry is critical

- The human body presents only a limited spectrum of entry sites for viral infection.



Transmission of infection

- Spread of infection from one susceptible host to another; required to maintain chain of infection

Transmission terms

- Horizontal transmission - between members of same species (zoonotic - different species)
- Iatrogenic - activity of health care worker leads to infection of patient
- Nosocomial - when an individual is infected while in hospital or health care facility
- Vertical transmission - transfer of infection between parent and offspring
- Germ line transmission - agent is transmitted as part of the genome (e.g. proviral DNA)

Virulence

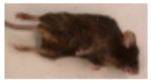
- Capacity of a virus to cause disease in a host
- Virulent vs avirulent or attenuated virus
- Virulence can be quantitated:

- Mean time to death
- Mean time to appearance of symptoms
- Measurement of fever, weight loss
- Measurement of pathological lesions (poliovirus); reduction in blood CD4+ lymphocytes (HIV-1)

Virulence depends on route of inoculation

Lymphocytic choriomeningitis virus

Dose	Route	Outcome
100,000 PFU	Intraoperitoneal	Survival
1 PFU	Intracranial	Death

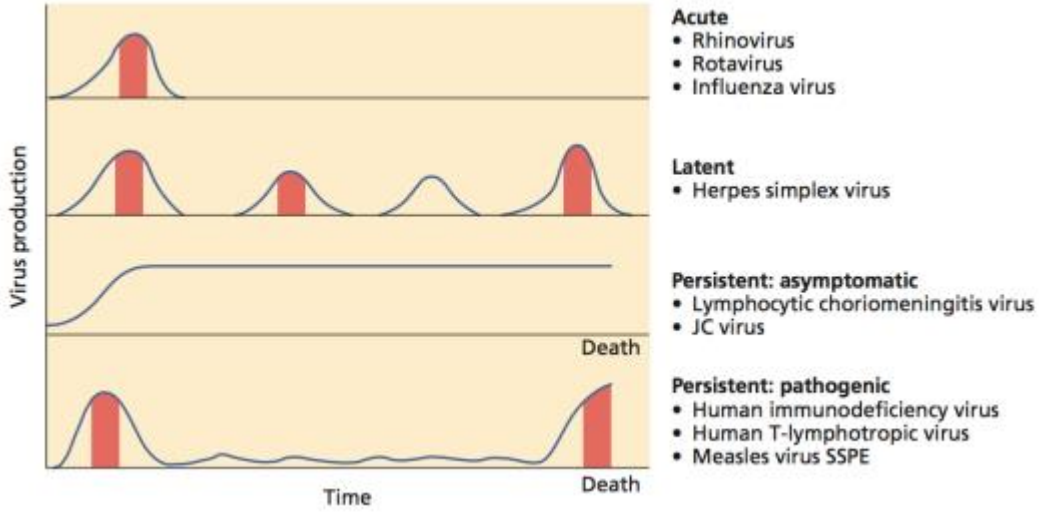


Viral virulence is a **relative property**

- Influenced by dose, route of infection, species,
- age, gender, and susceptibility of host
- Cannot compare virulence of different viruses
- For similar viruses, assays must be the same

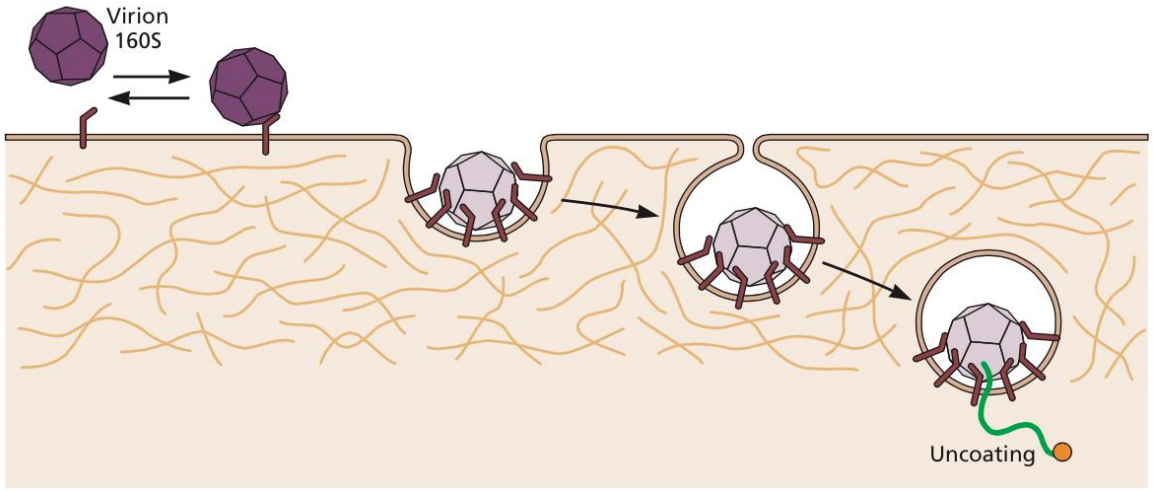
Acute vs persistent infections

- Acute infection - rapid and self-limiting
- Persistent infection - long term, for the life of host
- Stable, characteristic for each virus family
- Most persistent infections probably begin as an acute infection



Virus particles are metastable

- Must protect the genome (stable)
- Must come apart after infection (unstable)



Functions of structural proteins
Protection of the genome
- Assembly of a stable protective protein shell
- Specific recognition and packaging of the nucleic acid genome
- Interaction with host cell membranes to form the envelope

Delivery of the genome
- Bind host cell receptors
- Uncoating of the genome
- Fusion with cell membranes
- Transport of genome to the appropriate site

How is metastability achieved?

- Stable structure

- Created by symmetrical arrangement of many identical proteins to provide maximal contact

- Unstable structure

- Structure is not usually permanently bonded together

- Can be taken apart or loosened after infection to release or expose genome

Viruses are obligate intracellular parasites

Virus particles are too large to diffuse across the plasma membrane

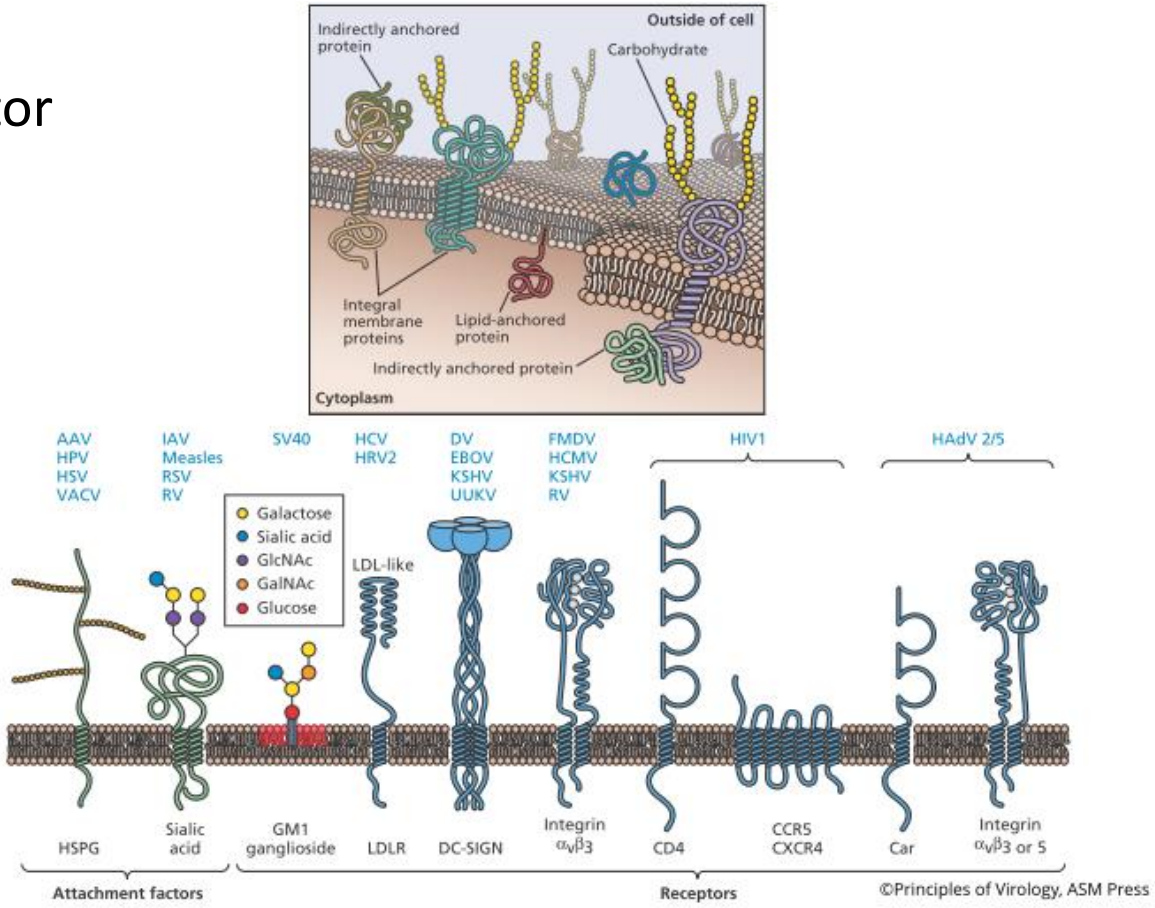
Finding the 'right' cell

- Step 1: adhere to cell surface (electrostatics)
  - No specificity
- Step 2: Attach to specific receptor molecules on cell surface
  - More than one receptor may be involved
- Step 3: Transfer genome inside the cell

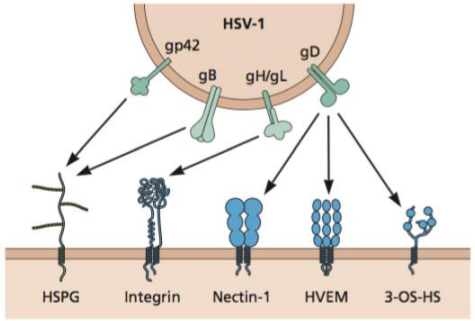
Cellular receptors for viruses

- Essential for all viruses except those of fungi (no extracellular phases) and plants (enter cells by mechanical damage)
- 1985: one receptor known, sialic acid for influenza virus

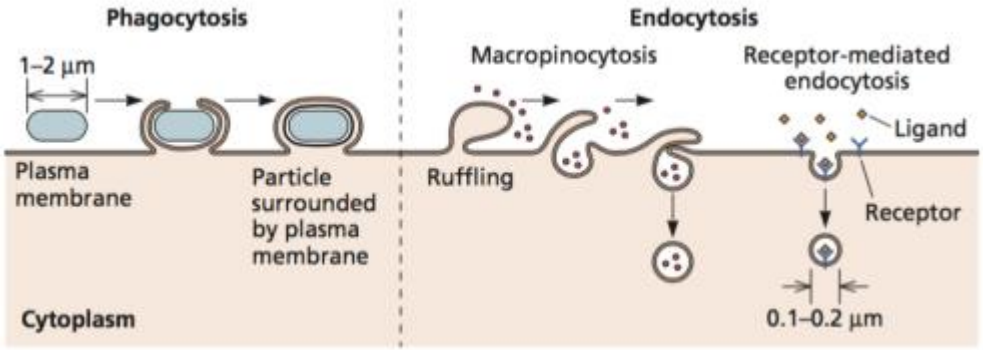
- Different viruses can bind the same receptor



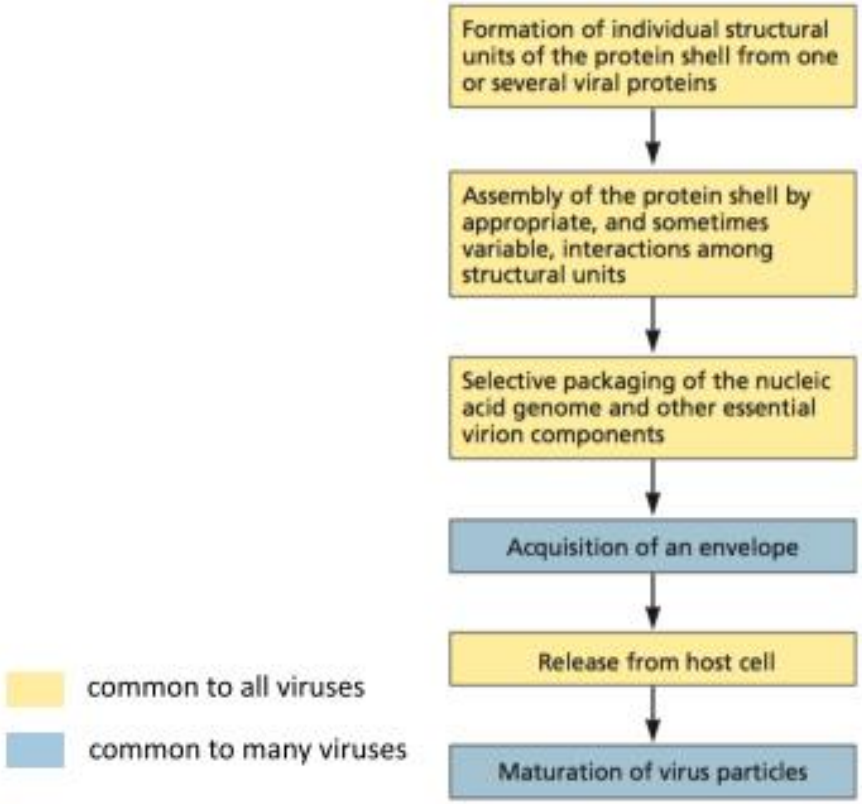
- Viruses of the same family may bind different receptors

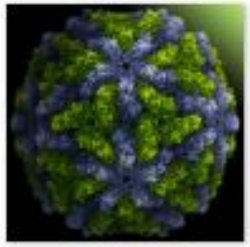


Entry into cells



All virions complete a common set of assembly reactions



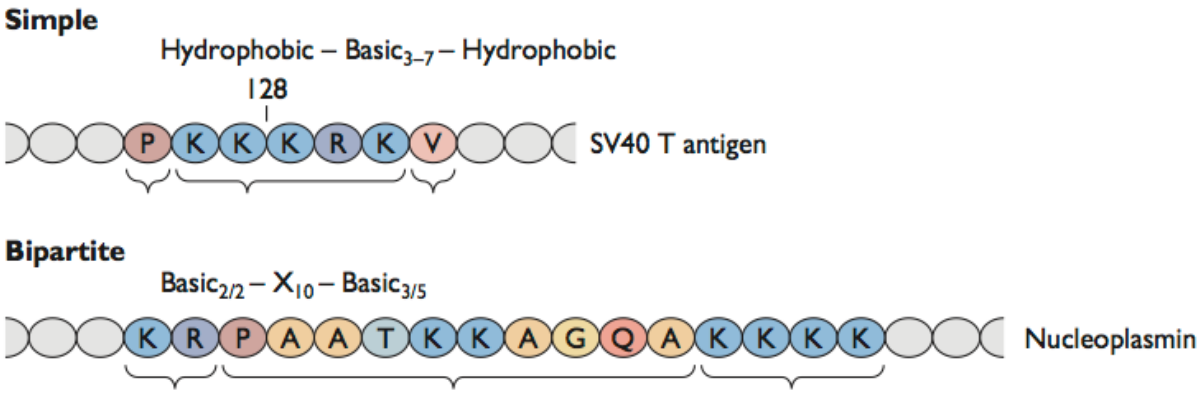


Assembly is dependent on host cell machinery

- Cellular chaperones
- Transport systems
- Secretory pathway
- Nuclear import and export machinery

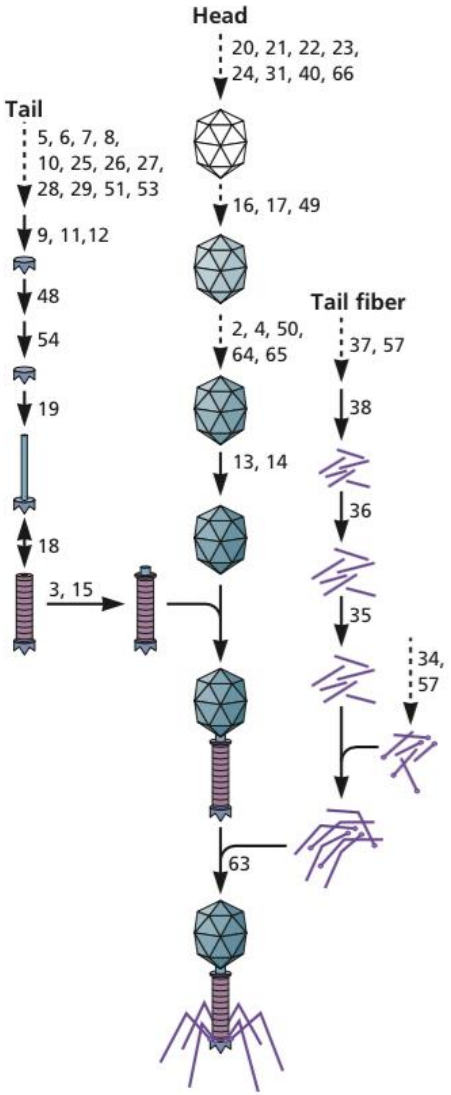
Viral proteins have ‘addresses’

- Membrane targeting: Signal sequences, fatty acid modifications
- Membrane retention signals
- Nuclear localization sequences (NLS)
- Nuclear export signals



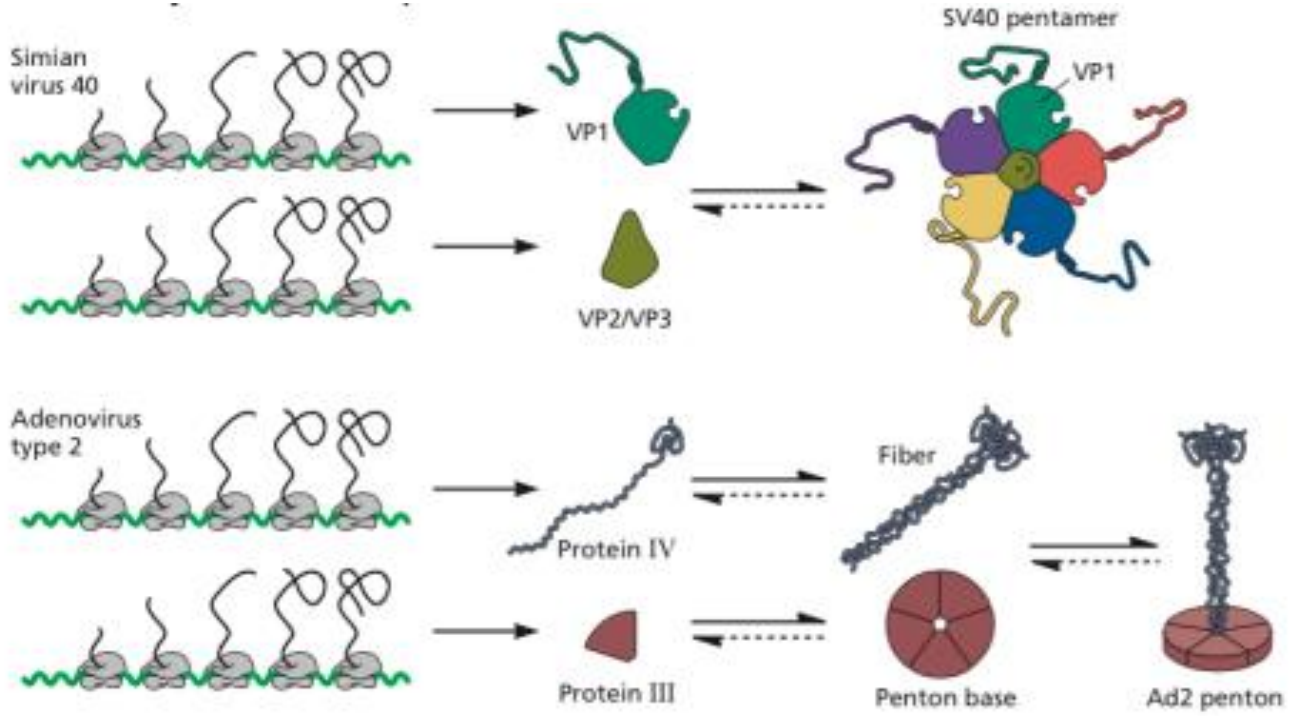
Sub-assemblies

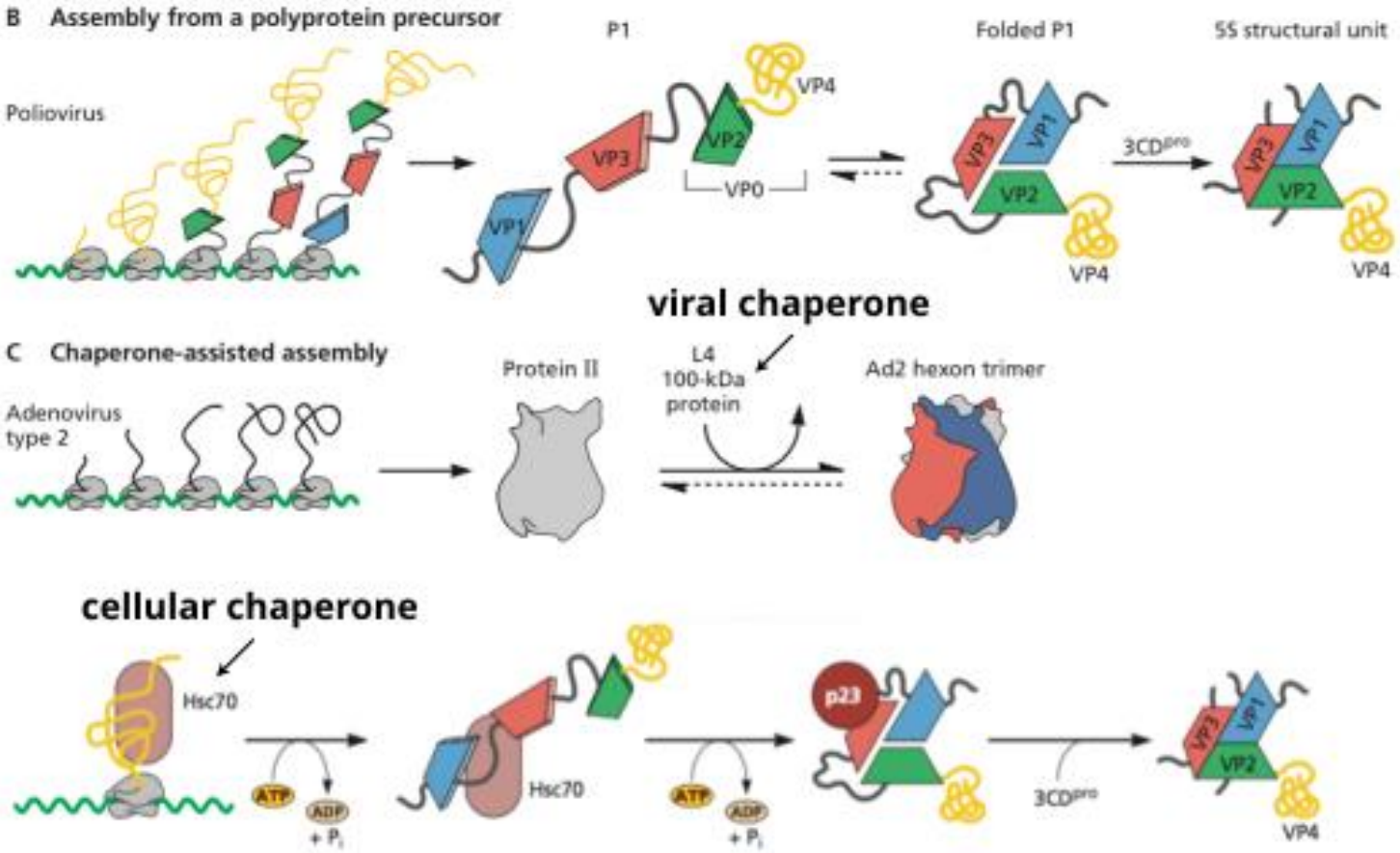
- Ensure orderly formation of viral particles and virion subunits
- Formation of discrete intermediate structures
- Can’t proceed unless previous structure is formed: quality control



Three strategies for making sub-assemblies

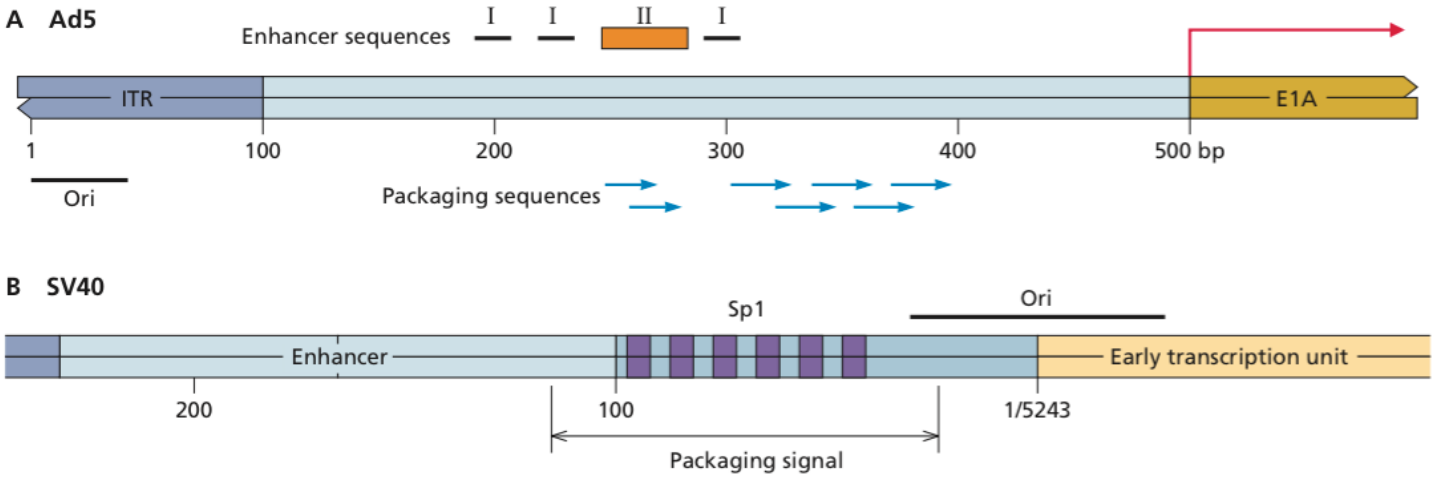
A – Assembly from individual protein molecules



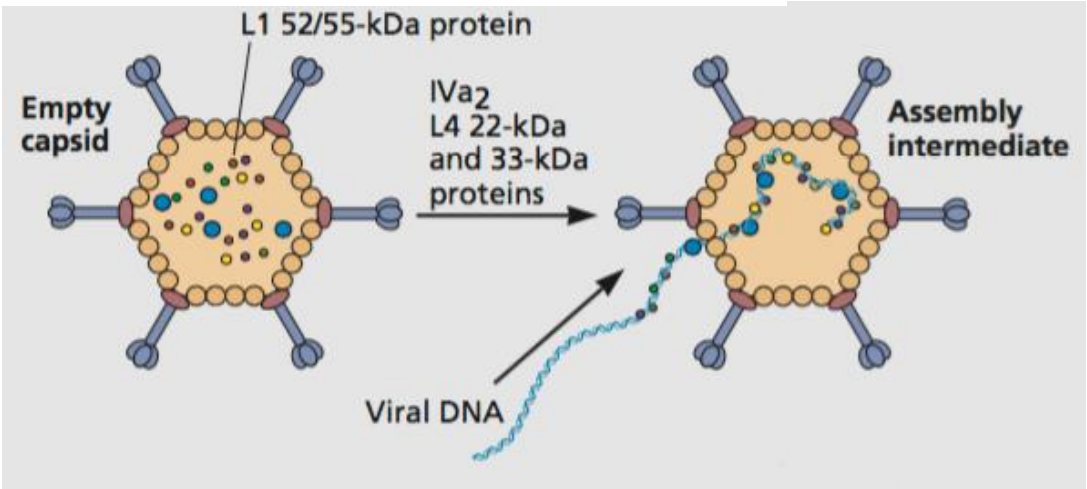

B Assembly from a polyprotein precursor
P1
Folded P1
5S structural unit
Poliovirus
VP4
VP3
VP2
VP0
VP1
3CDpro
VP4
VP4
viral chaperone
C Chaperone-assisted assembly
Protein II
L4
100-kDa
protein
Ad2 hexon trimer
Adenovirus
type 2
cellular chaperone
Hsc70
ATP
ADP
+ Pi
Hsc70
ATP
ADP
+ Pi
p23
3CDpro
VP4

Genome packaging

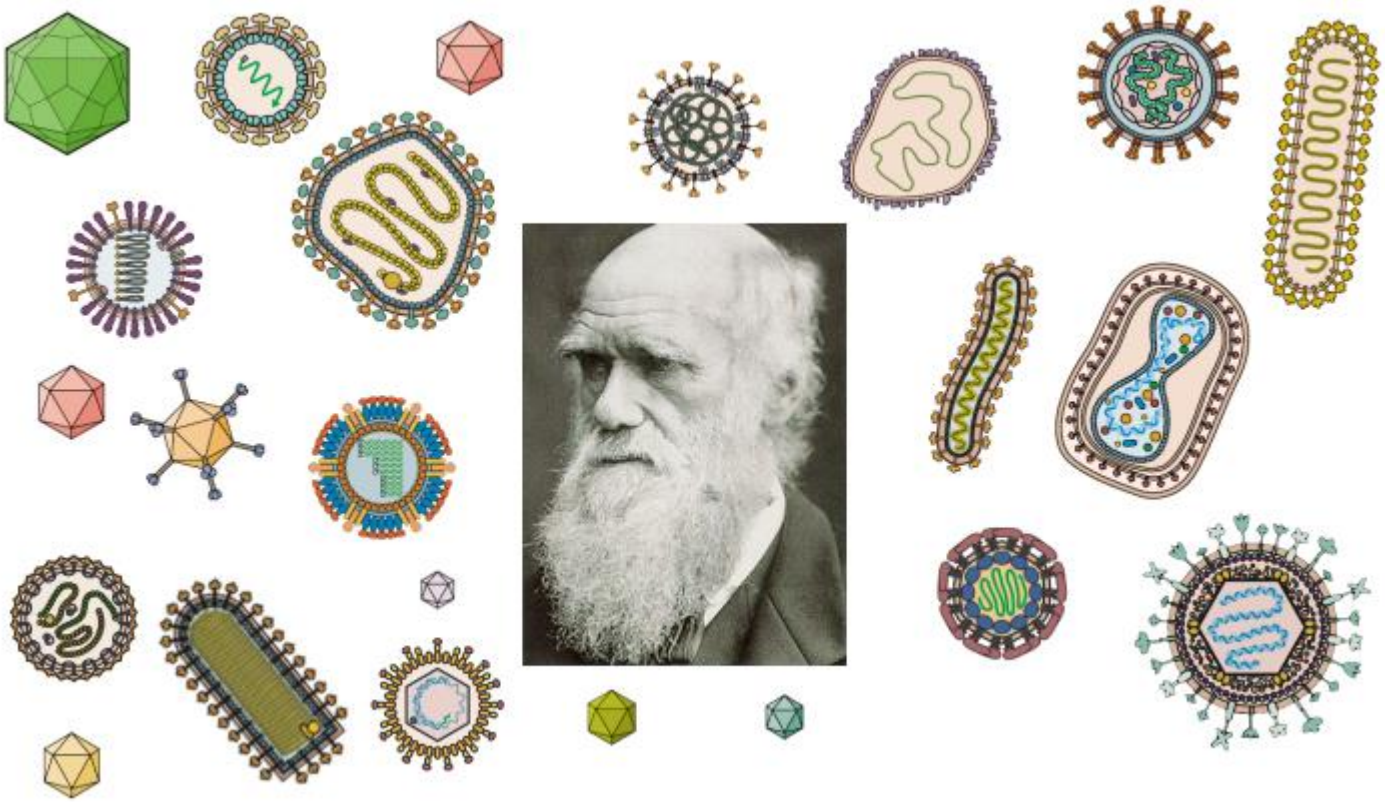
- Problem: Viral genomes must be distinguished from cellular DNA or RNA molecules where assembly takes place
- Solution: **Packaging signals** in the viral genome



Also capsid limits the amount of genetic material



Darwin would have loved viruses!



The best exemplars of evolution by natural selection, and for RNA viruses, evolution is so rapid it can be followed in real time

Modern virology has provided a window on the mechanisms of evolution

- As host populations grow and adapt, virus populations are selected that can infect them
  - New viral populations emerge every day

It also works the other way

- Viral populations can be significant selective forces in the evolution of host populations

- If a host population cannot adapt to a lethal virus infection, the population may be exterminated

The public is constantly confronted with the reality of viral evolution (even if they don't believe in evolution)

- New viral diseases: AIDS, West Nile virus in the US, HCV, Ebolavirus, Zika virus
- Regular bouts every year with influenza and common cold viruses
- Drug resistant HIV

- *Simple fact: viruses evolve faster than many can comprehend*

The interface of host defense and virus replication is fertile ground for selection and evolution

Replicating viruses produce large numbers of mutant genomes
- Evolution is possible only when mutations occur in a population
- Mutations are produced during copying of any nucleic acid molecule

Viral genomes are always mutating!

DNA viruses
- Genome replication not as error prone as RNA viruses
- Proofreading
- Most DNA viruses generate less diversity, evolve slower than RNA viruses

RNA viruses

Lack of proofreading activity in RNA dependent RNA polymerase: high error frequencies (1 misincorporation / 10^3 - 10^4 nt polymerized)
- Average error frequency: 1 in 10^4 or 10^5 nucleotides polymerized
- In a 10 kb RNA virus genome, a mutation frequency of 1 in 10^4 results in about 1 mutation per genome

The myth of consensus genome sequences

For a given RNA virus population, the genome sequences cluster around a consensus or average sequence, but virtually every genome can be different from every other

It is unlikely that a genome with the consensus sequence is actually replicating in the population

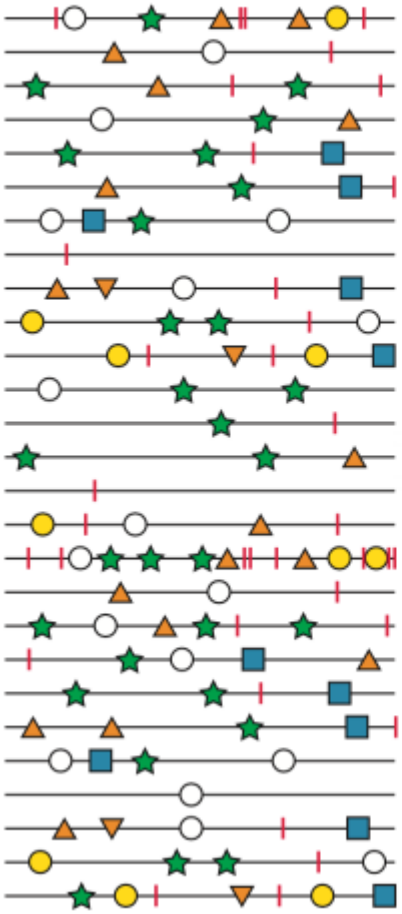
The quasispecies concept

- Analysis of an RNA bacteriophage population (Qβ):

"A Qβ phage population is in a dynamic equilibrium with viral mutants arising at a high rate on the one hand, and being strongly selected against on the other. The genome of Qβ cannot be described as a defined unique structure, but rather as a weighted average of a large number of different individual sequences."

E. Domingo, D. Sabo, T. Taniguchi, C. Weissmann. 1978. Nucleotide sequence heterogenity of an RNA phage population. Cell 13:735-744.

Viral quasispecies



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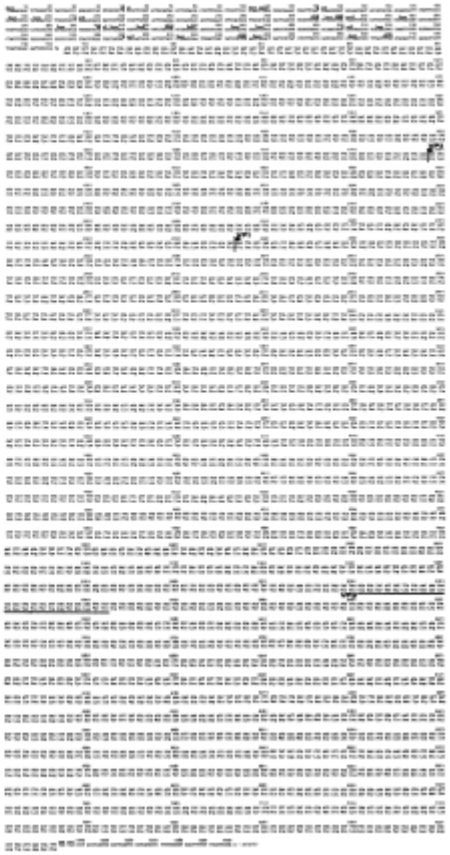
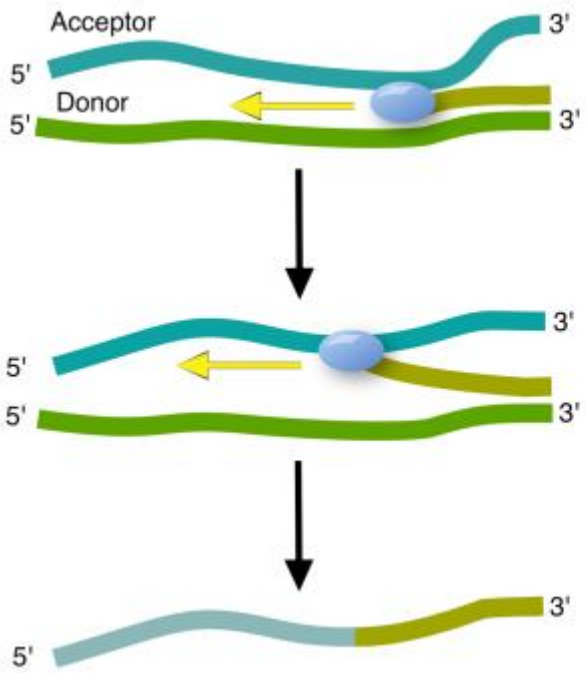
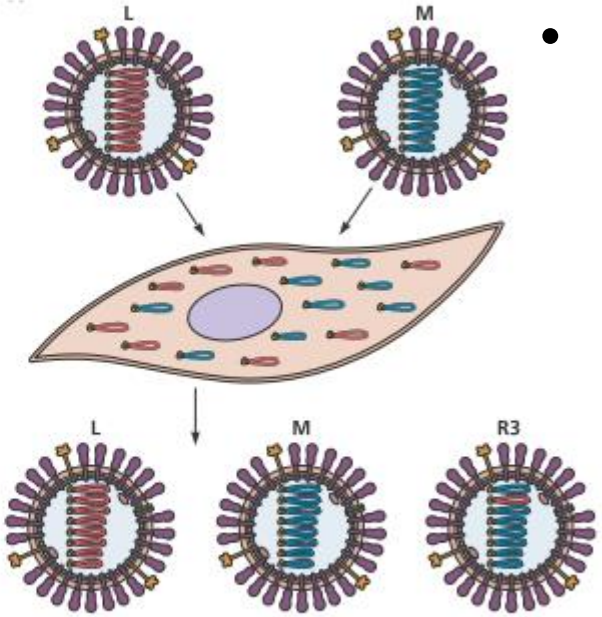


Principles of Virology, ASM Press

Quasispecies



Selection



- Variation further generated by recombination and reassortment

• **Survival of the fittest**: A rare genome with a particular mutation may survive a selection event, and this mutation will be found in all progeny genomes

• **Survival of the survivors**: However, the linked, but unselected mutations, get a free ride

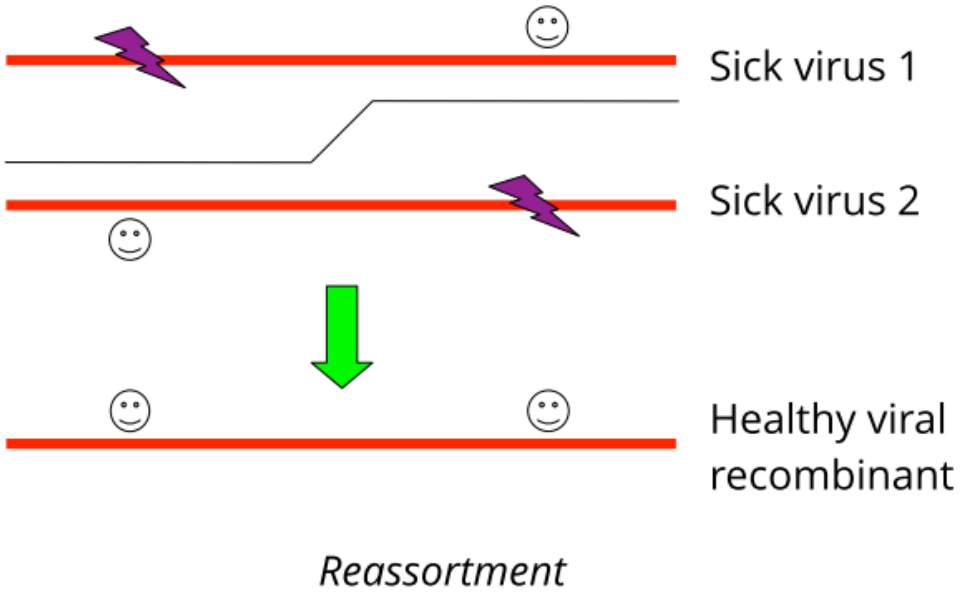
• Consequently, the product of selection after replication is a new, diverse population that shares only the selected mutations

Diversity is selected

Mutations in viral polymerases that reduce the frequency of incorporation errors:

- Do not have a selective advantage when wild type and anti-mutators are propagated together
- Lower rates are neither advantageous nor selected in nature
- Mutants are often less pathogenic

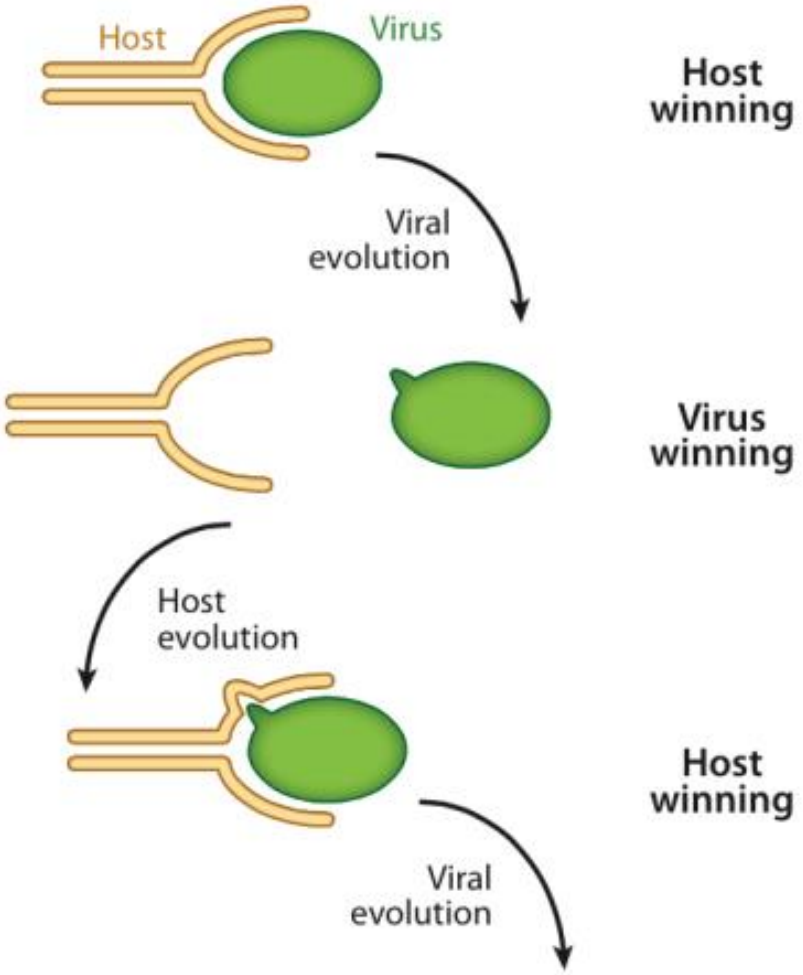
• High mutation rates are selected during virus evolution: mutation is good for viral populations



Fitness decline compared to initial virus clone after passage through a bottleneck

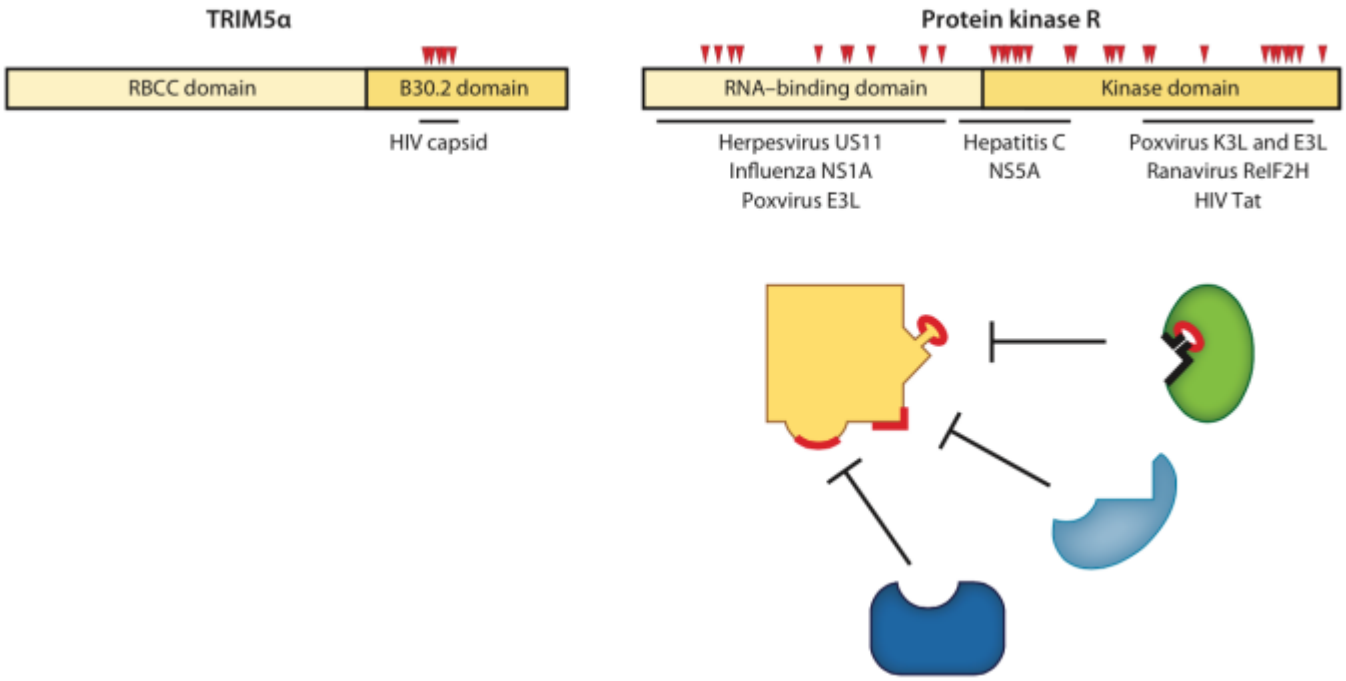
Virus	# of bottleneck passages	% Decrease in fitness
Bacteriophage ϕ6	40	22
Vesicular stomatitis virus	20	18
Foot-and-mouth disease virus	30	60
HIV	15	94
Bacteriophage MS2	20	17

Host-virus arms race



Annu. Rev. Genet. 2012. 46:677–700

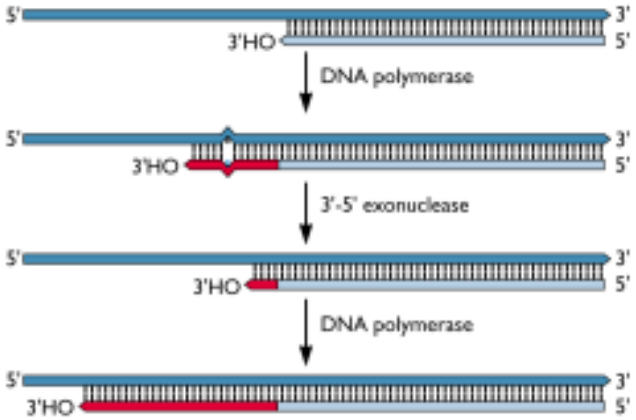
Virus-host conflicts have driven evolution of the immune system



Mechanisms of drug resistance

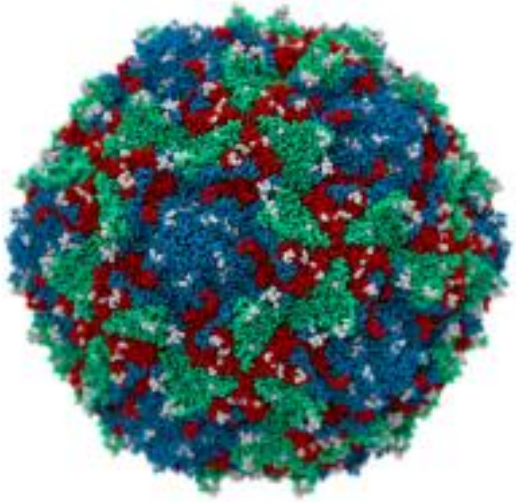
- RNA viruses: error prone RNA polymerase, no correction mechanism
- One misincorporation in 10^4 - 10^5 nucleotides polymerized (10^6 greater than host DNA genome)
- In RNA viral genome of 10 kb, this frequency leads to one mutation in 1-10 genomes

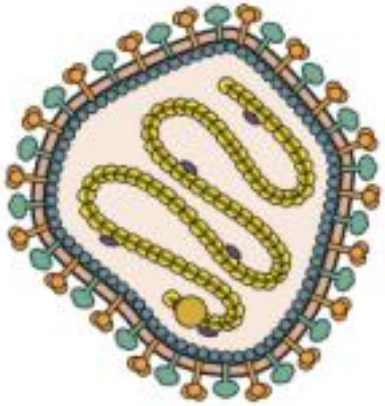
- DNA viruses: most DNA polymerases can excise and replace misincorporated nucleotides
- DNA viruses evolve more slowly than RNA viruses because they have less diversity

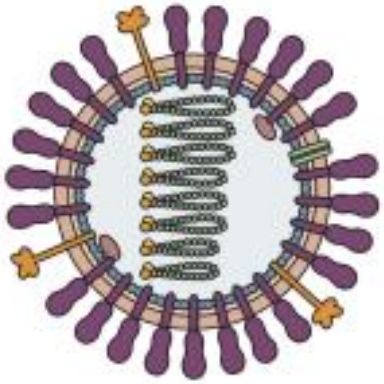


Despite this genome diversity…

- There are only 3 serotypes of poliovirus, but >150 of rhinoviruses
- One measles serotype, continuous influenza variation
- Why?







Selection: Is virulence a positive or negative trait?

- Idea: increased virulence reduces transmissibility because hosts die faster, reducing exposure to uninfected hosts
- Expectation: all viruses evolve to be maximally infectious and avirulent
- But this is not observed - there are many virulent viruses

The fundamental properties of viruses constrain and drive evolution

- Despite many rounds of replication, mutation, selection, we can recognize a herpesvirus or influenza virus genome by sequence analysis
- Viral populations often maintain master or consensus sequences, despite opportunities for extreme variation
- How is stability maintained?

Constraining viral evolution:

- Extreme alterations in viral consensus genome do not survive selection, the viral genome is one constraint

- DNA cannot become RNA, or vice versa

- Replication strategy - cannot change; consider interaction with host proteins

• Physical nature of capsid

- Icosahedral capsids: defined internal space, fixes genome size

• Selection during host infection

- A mutant too efficient in bypassing host defenses will kill host, suffer the same fate as one that does not replicate efficiently enough