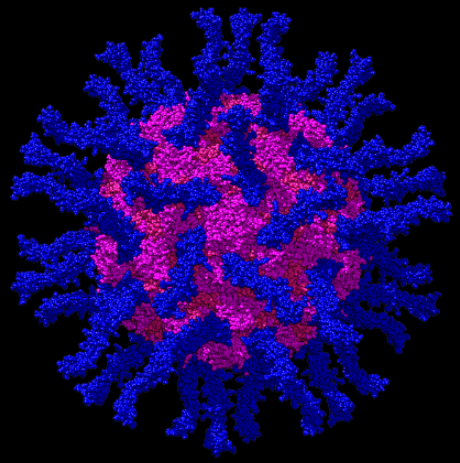


VIROLOGY LIVE

WITH VINCENT RACANIELLO

Genomes and Genetics

Session 3

Virology Live

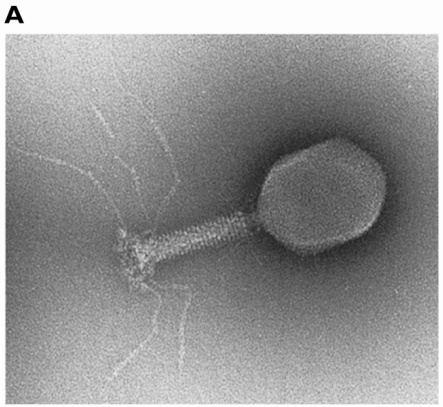
Fall 2021

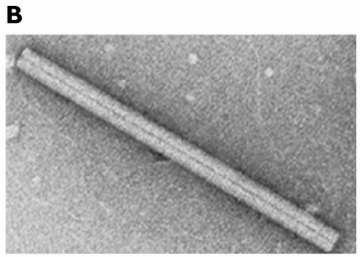
"...everywhere an interplay between nucleic acids and proteins; a spinning wheel in which the thread makes the spindle and the spindle the thread"
--Erwin Chargaff

Virology breakthrough in the 1950's:

The viral nucleic acid genome is the genetic code

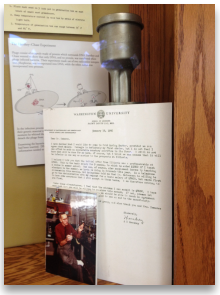
Hershey-Chase experiment with phage T2



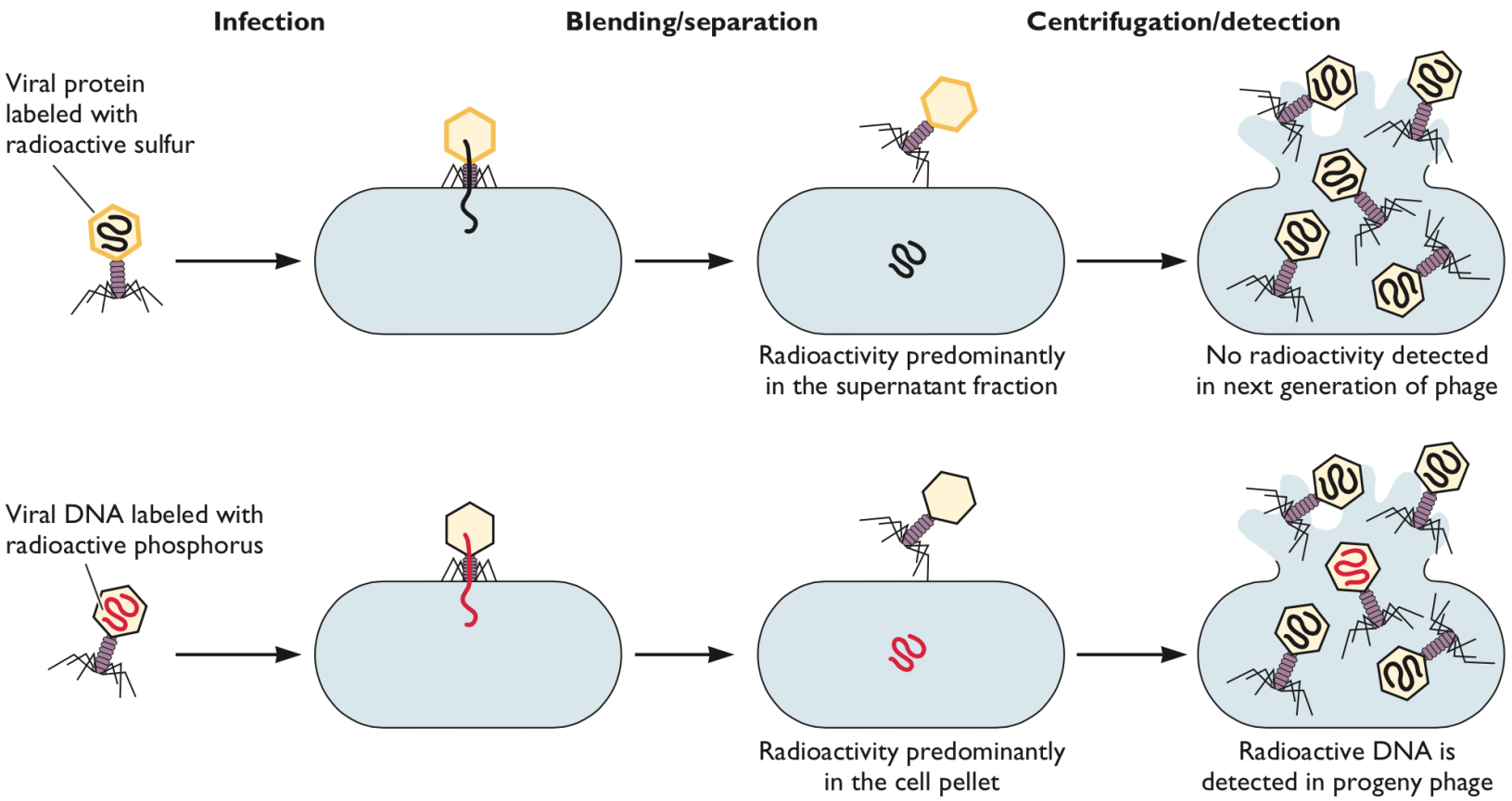


Fraenkel-Conrat's work with TMV

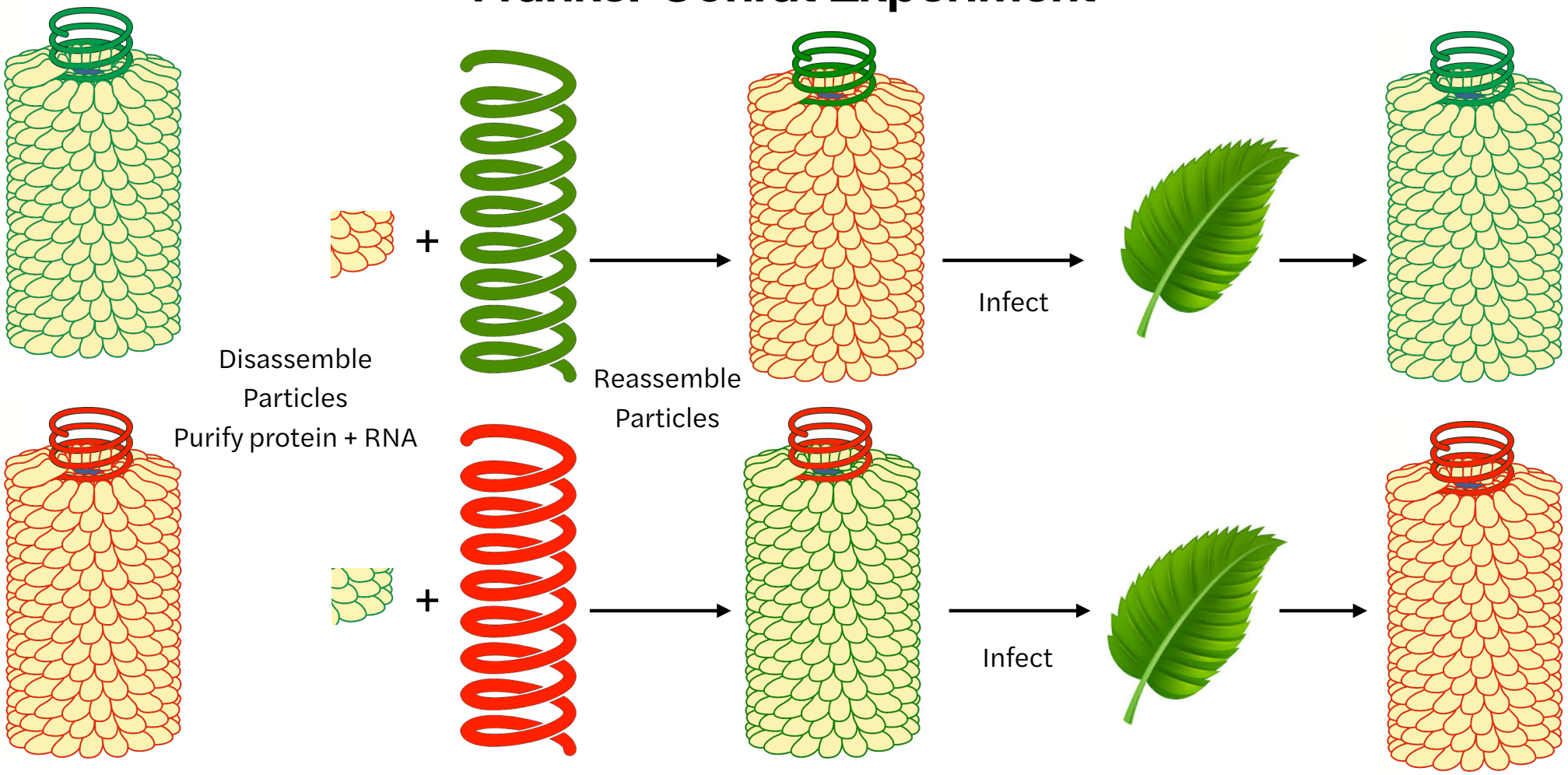
Hershey-Chase Experiment



Alfred Hershey &
Martha Chase, 1952



Frankel-Conrat Experiment

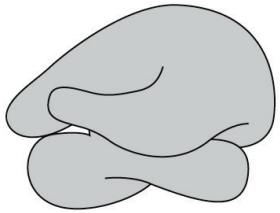


The bigger surprise: thousands of different virions, seemingly infinite complexity of infections

But a finite number of viral genomes

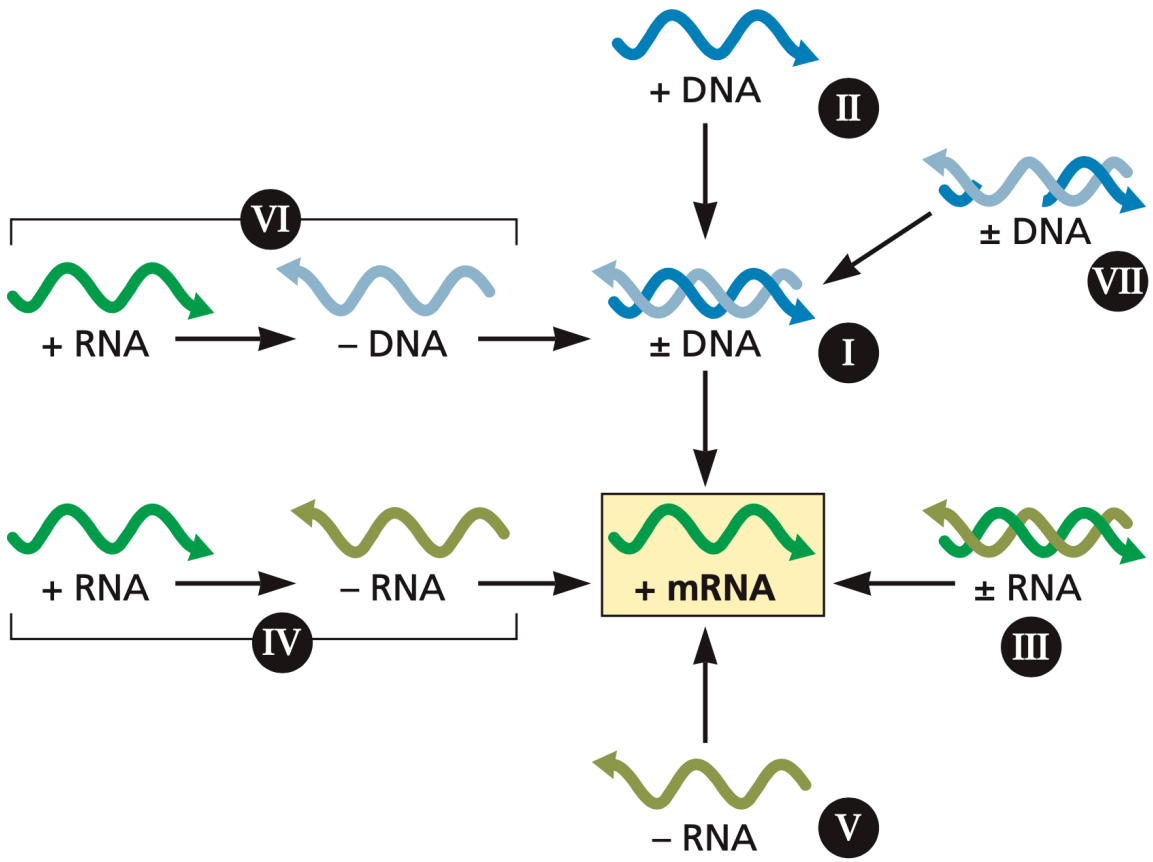
Key fact makes your life easier:

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



All viruses on the planet follow this rule, no known exception

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

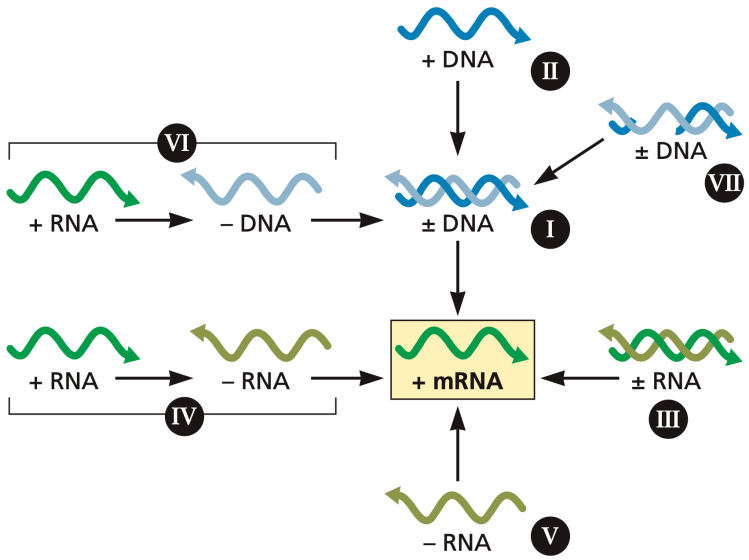


The original Baltimore system missed one genome type:

gapped DNA of *Hepadnaviridae*

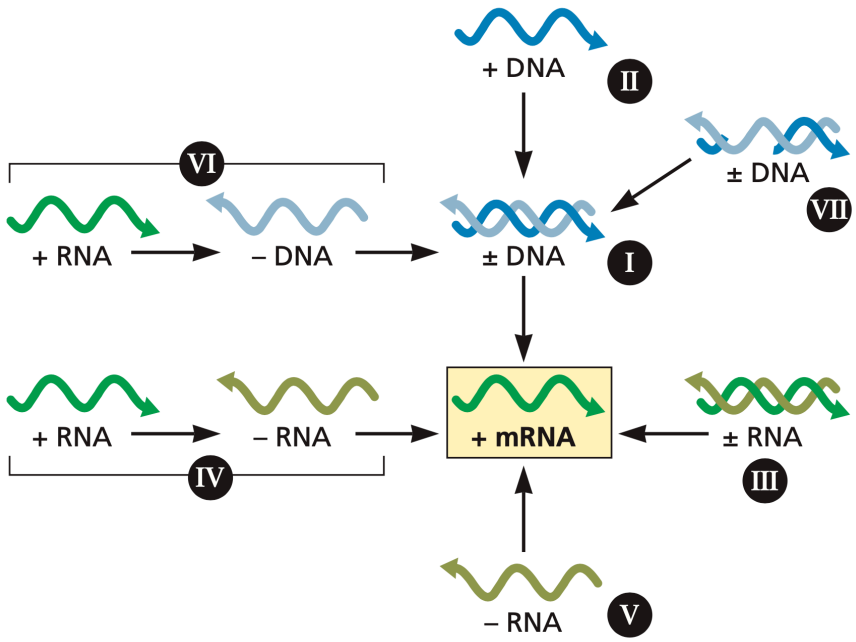
Definitions

- 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!



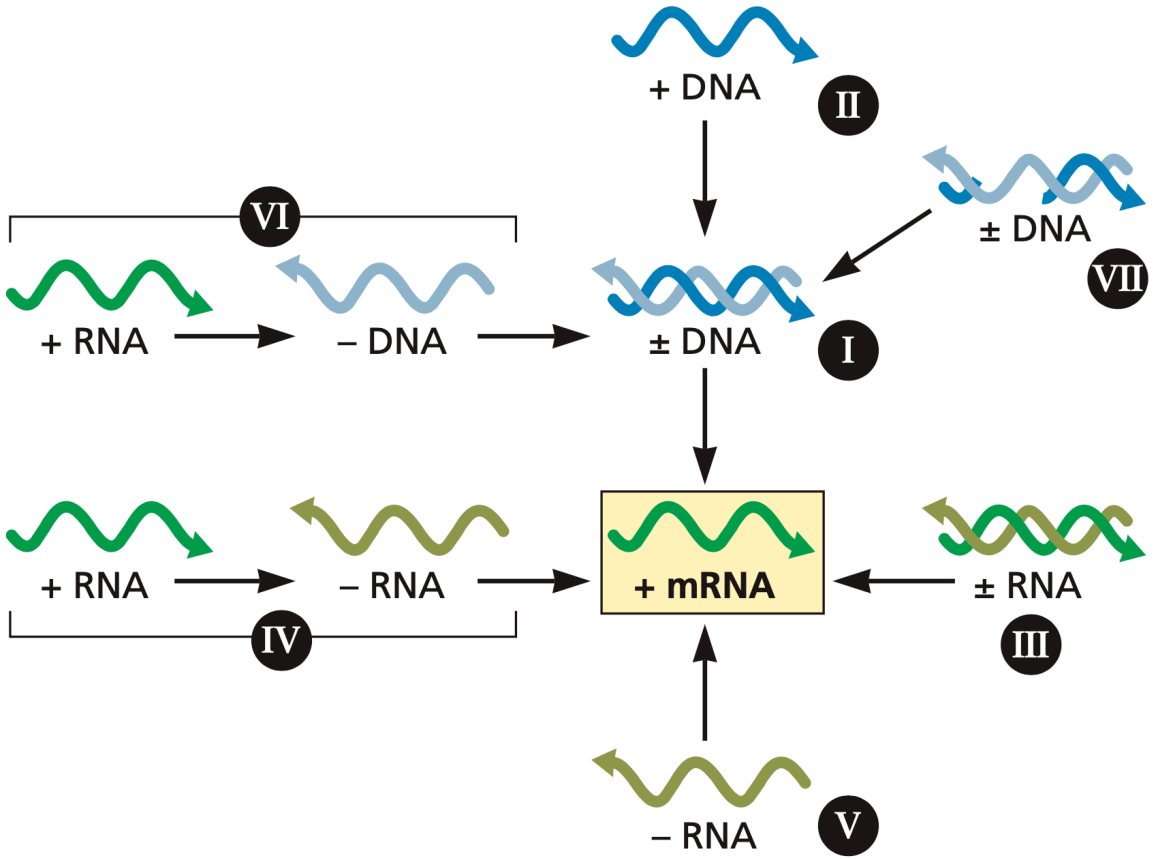
The elegance of the Baltimore system

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



The seven classes of viral genomes

- dsDNA
- gapped dsDNA
- ssDNA
- dsRNA
- ss (+) RNA
- ss (-) RNA
- ss (+) RNA with DNA intermediate



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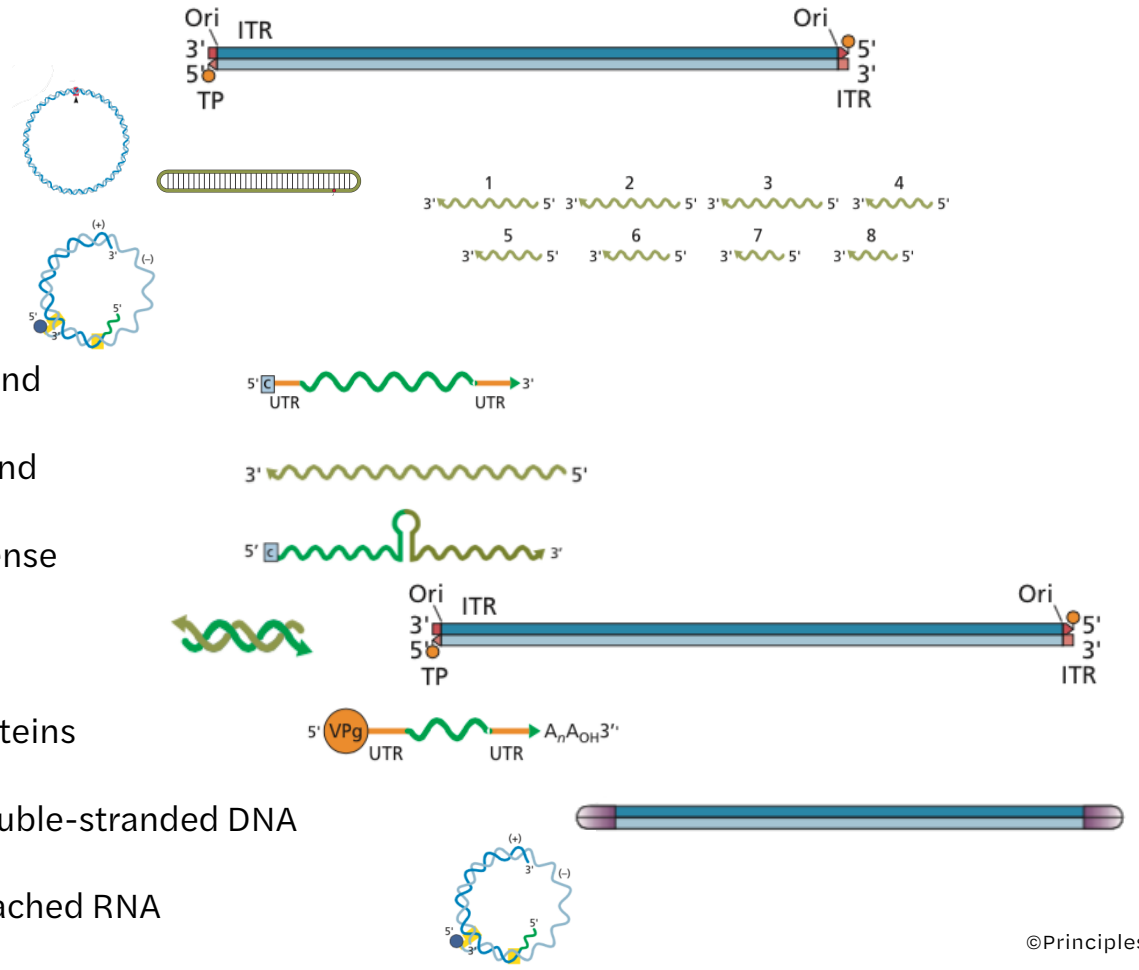
room number: virus

Why is mRNA placed at the center of the Baltimore scheme?

A. Because all virus particles contain mRNA
B. There is no specific reason
C. Because all viral genomes are mRNAs
D. Because mRNA must be made from all viral genomes
E. Because Baltimore studied mRNA

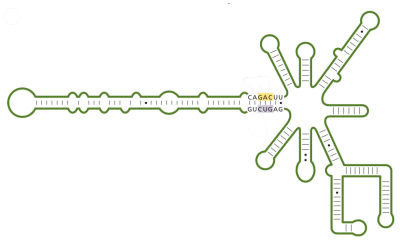
Viral DNA or RNA genomes are structurally diverse

- 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

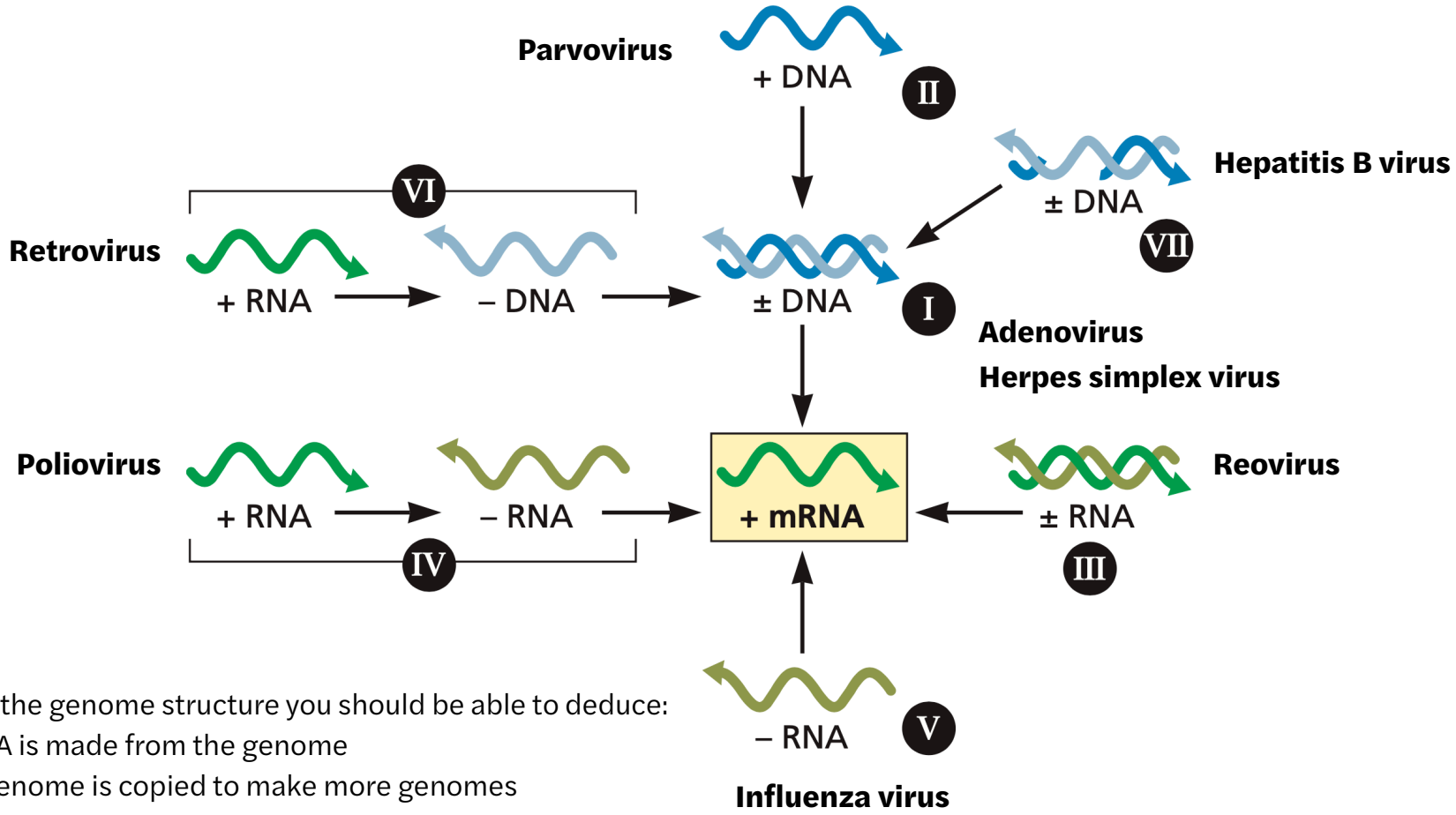


What is the function of genome diversity?

- DNA *and* RNA based
  - RNA genomes appeared first in evolution (RNA World)
  - Switch to DNA genomes - bigger!
  - Only RNA genomes on planet today are viral
  - Viroids: Relics of RNA world?
- Linear, circular, segmented, ds, ss, (+), (-)



Memorize 7 genome types and key virus families



If you know the genome structure you should be able to deduce:
- How mRNA is made from the genome
- How the genome is copied to make more genomes

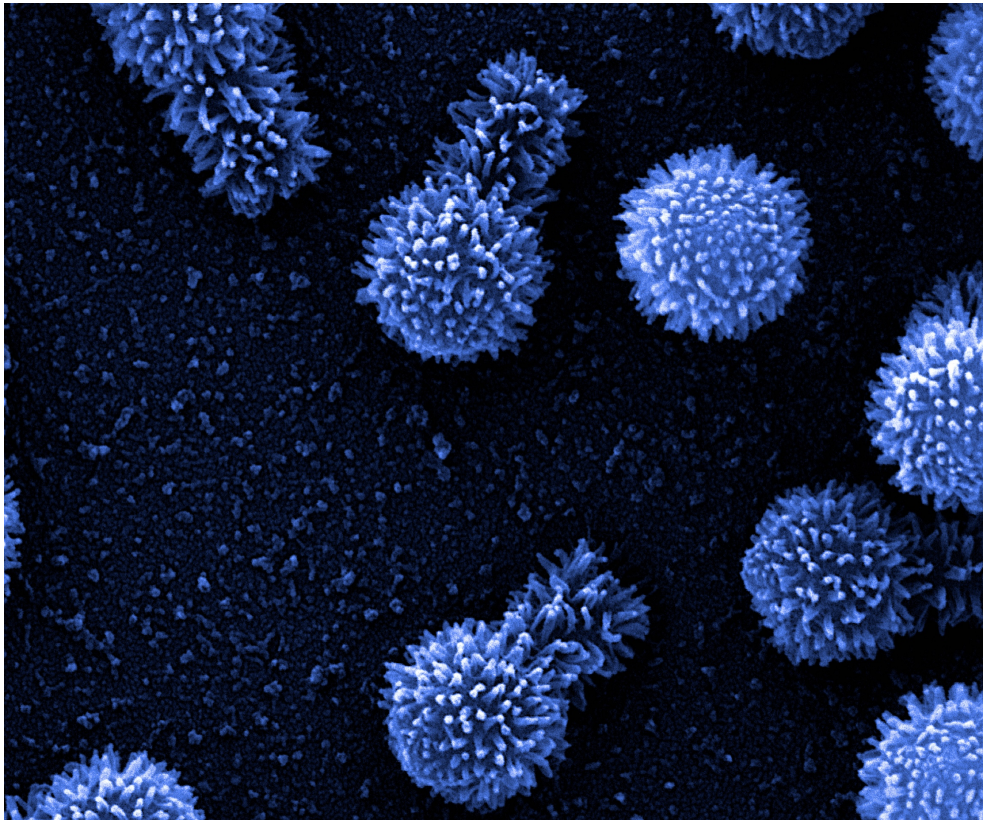
What information is encoded in a viral genome?

Gene products and regulatory signals for:

- Protein synthesis (mainly giant viruses)
- 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

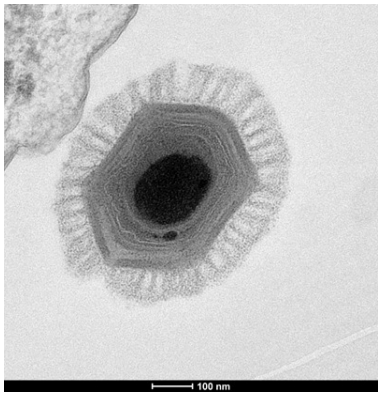
Tupanvirus - Only the ribosome is lacking

- 20 aminoacyl tRNA syntheses
- 70 tRNAs
- Multiple translation initiation and elongation proteins
- Multiple translation related genes e.g. for tRNA, mRNA maturation
- Most complete translational apparatus of the virosphere



Information NOT contained in viral genomes

- No genes encoding the *complete* protein synthesis machinery
- No genes encoding proteins involved in 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



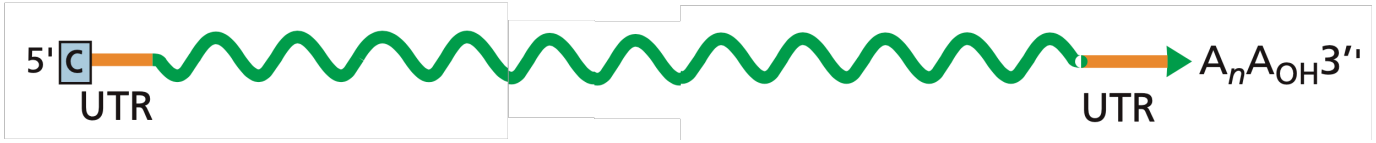
Largest known viral genomes

Virus	Length	Protein
Pandoravirus salinus	2,473,870	2,541
Pandoravirus dulcis	1,908,524	1,487
Tupanvirus	1,516,267	1,425
Bodo saltans virus	1,385,869	1,227
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

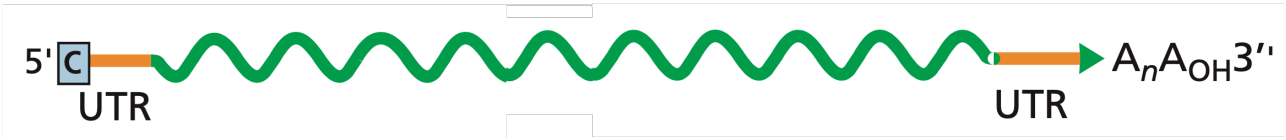
<= *Hemophilus* 1.8 Mb

<= *Nasuia 112,000 bp*

Largest RNA virus genomes



41.1 kb
Planarian secretory cell nidovirus



35.9 kb
Aplysia california nidovirus

33.5 kb
Ball python nidovirus

Smallest known viral genomes

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

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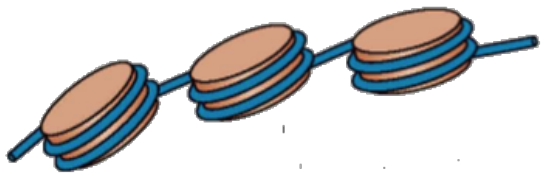
What information may be encoded in a viral genome?

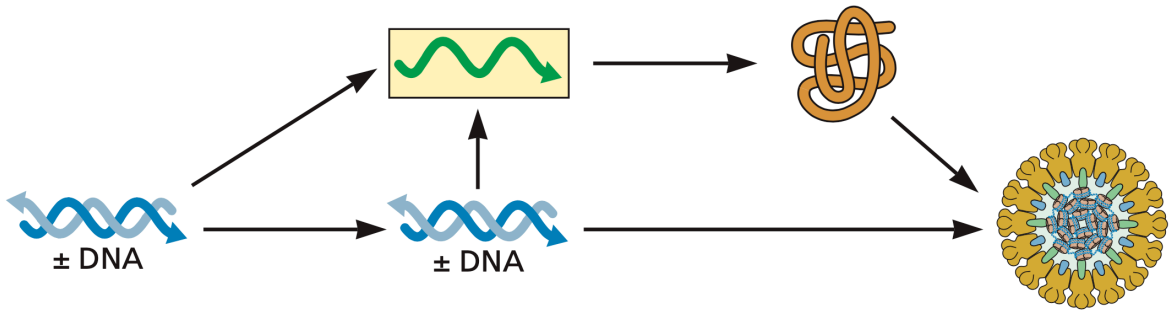
A. Gene products that catalyze membrane biosynthesis
B. Complete protein synthesis systems
C. Centromeres or telomeres
D. Enzymes to replicate the viral genome

Viral DNA genomes

- dsDNA viruses dominate the bacterial virosphere
- Many DNA viruses emulate the host, based on DNA
- However, almost all viral DNA genomes are NOT like cell chromosomes
- Unexpected tricks have evolved

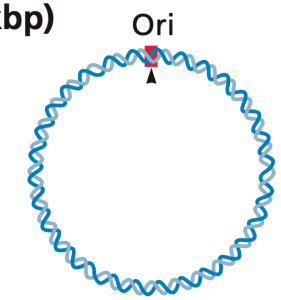


dsDNA genomes

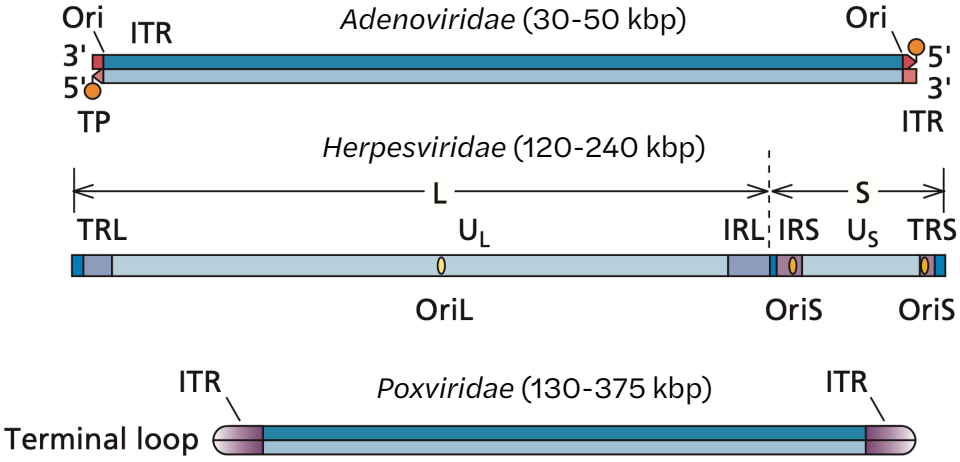


Genomes copied by host DNA polymerase

***Polyomaviridae* (5 kbp)**

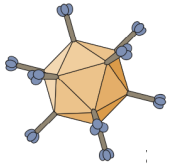


Genomes encode DNA polymerase

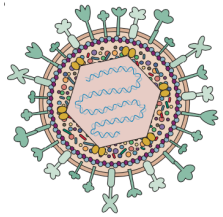


dsDNA genomes

Adenoviridae



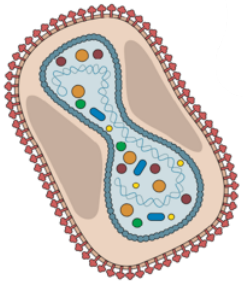
Herpesviridae

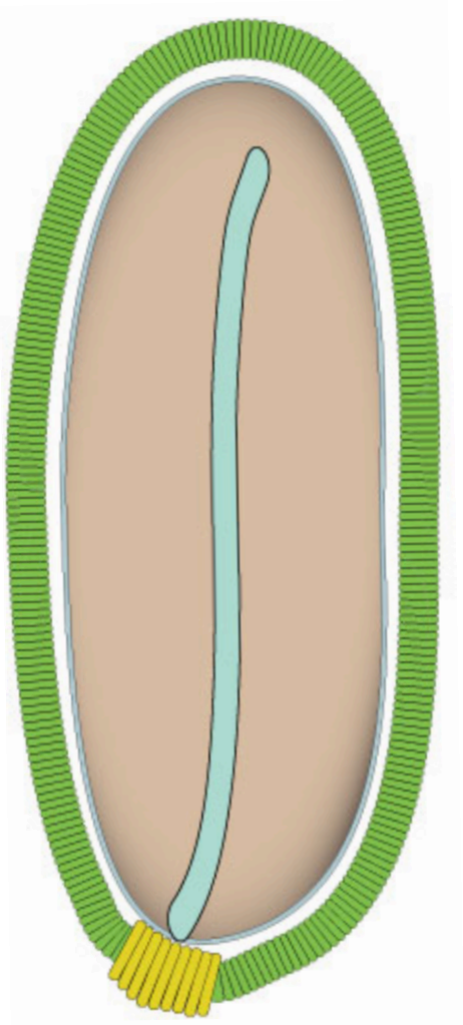


Papillomaviridae

Polyomaviridae

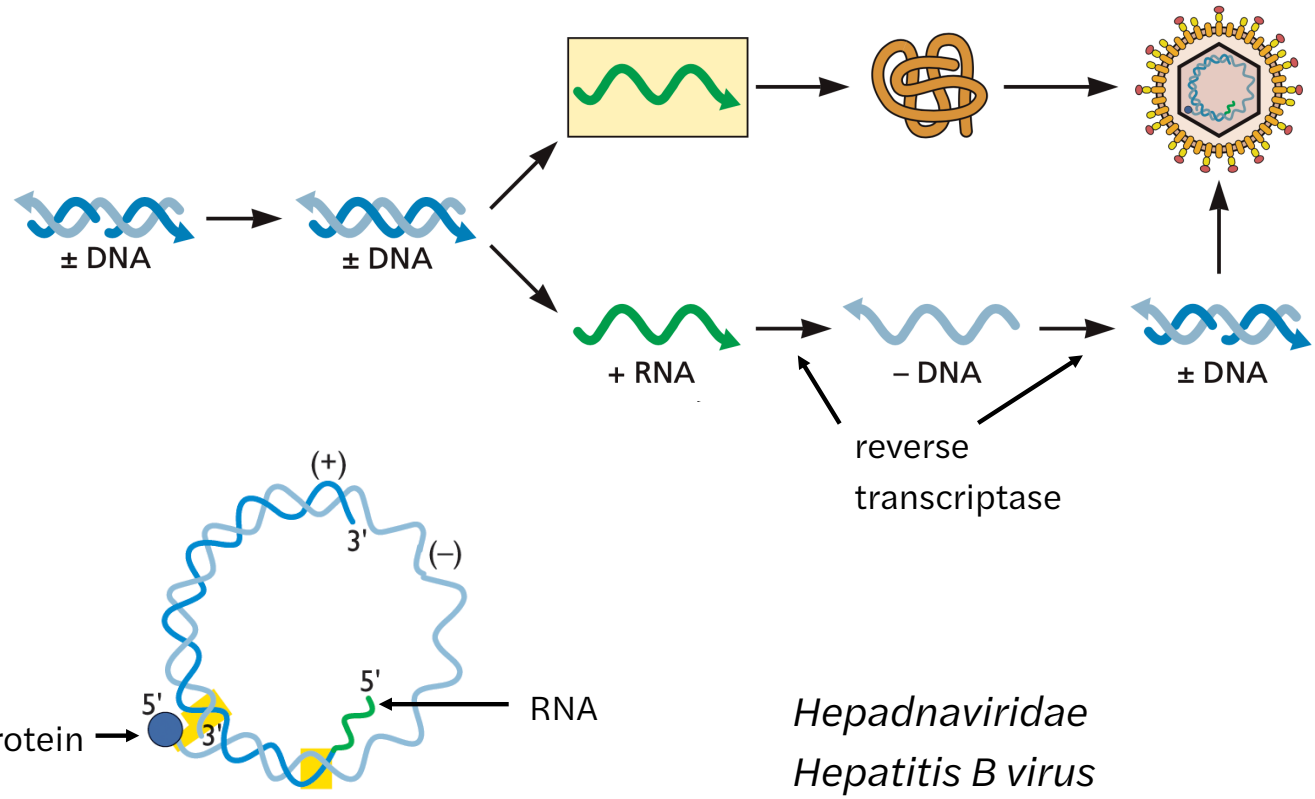
Poxviridae





Pithovirus

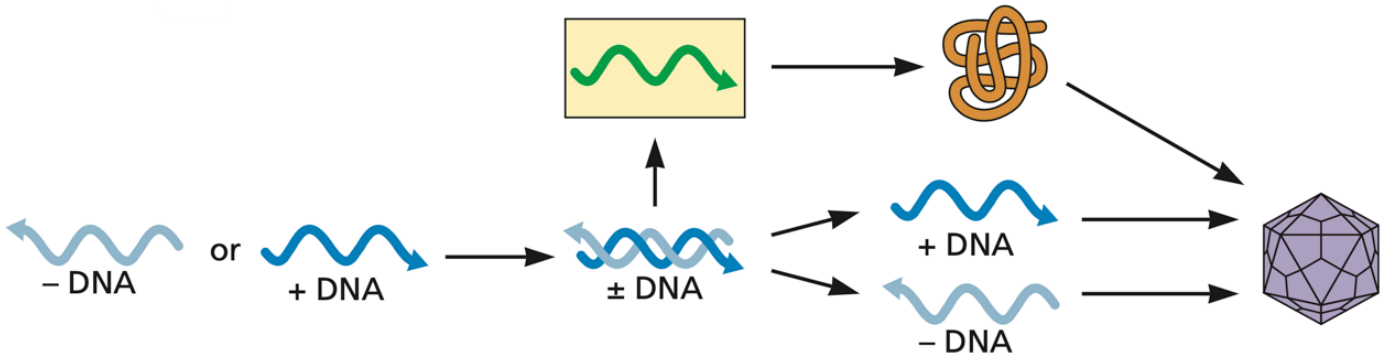
Gapped dsDNA genomes



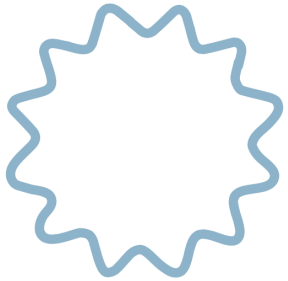
Hepadnaviridae
Hepatitis B virus

This genome cannot be copied to mRNA

ssDNA genomes

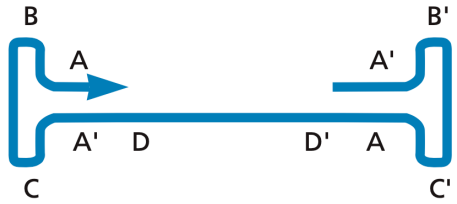


***Circoviridae* (1.8–2.3 kb)**



TT virus (ubiquitous human virus)

C *Parvoviridae* (4–6 kb)



B19 parvovirus (fifth disease)

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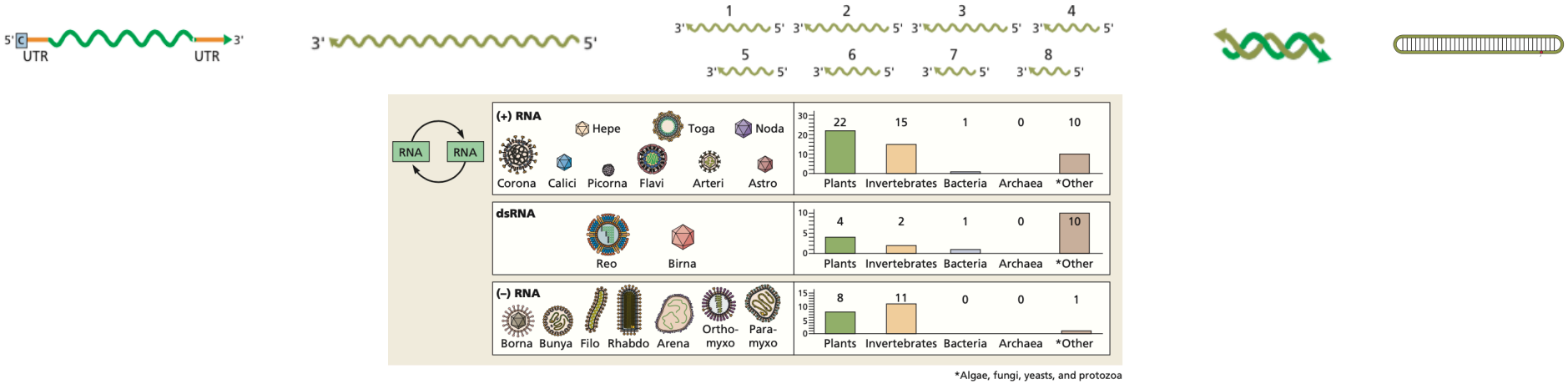
Which DNA genome, on entry into the cell, can be immediately copied into mRNA?

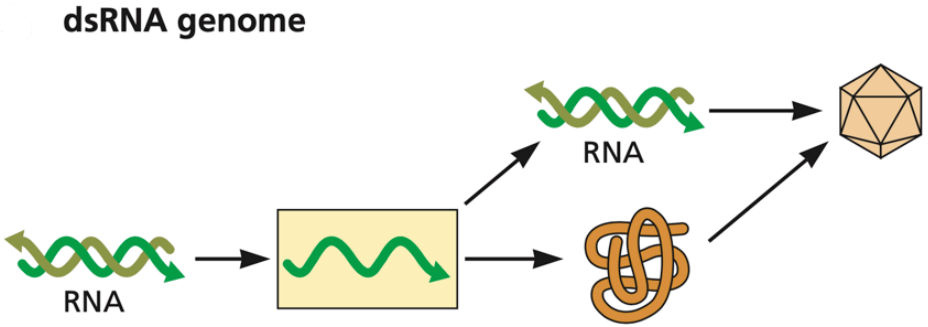
A. dsDNA
B. gapped dsDNA
C. circular ssDNA
D. linear ssDNA
E. All of the above

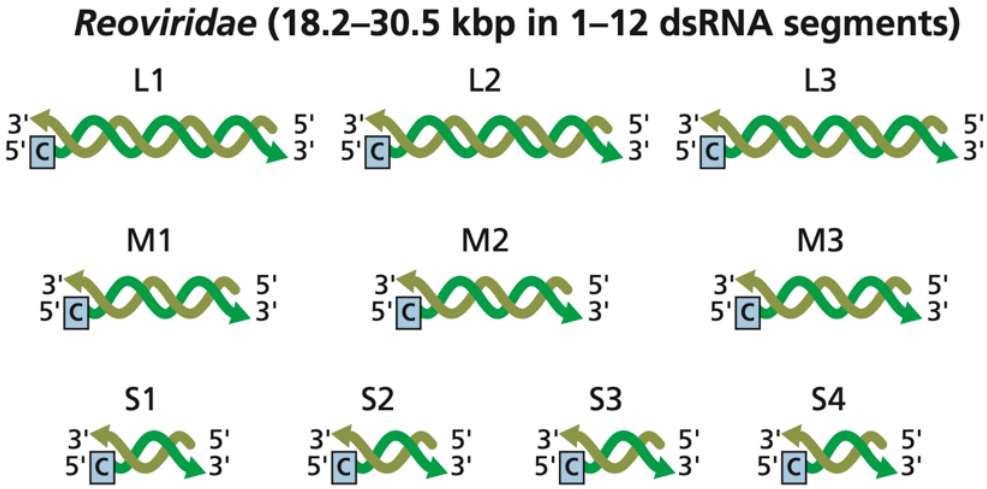
RNA genomes

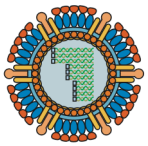


- RNA viruses dominate the eukaryotic virosphere, rare in bacteria
- Cells have no RNA-dependent RNA polymerase (RdRp)
- RNA virus genomes encode RdRp
- RdRp produce RNA genomes and mRNA from RNA templates

dsRNA genome

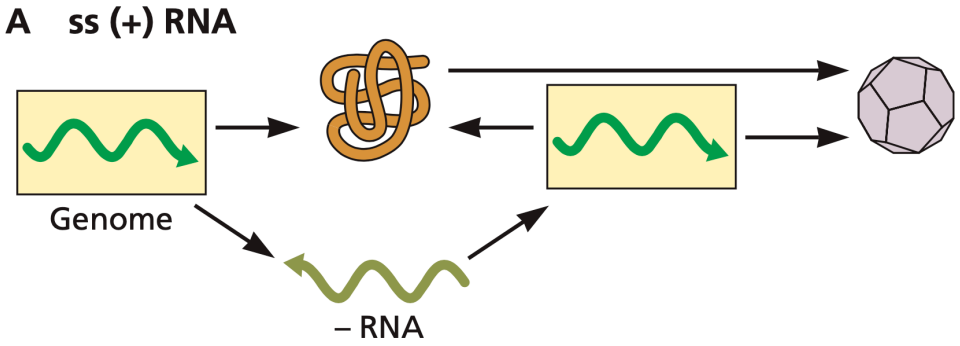


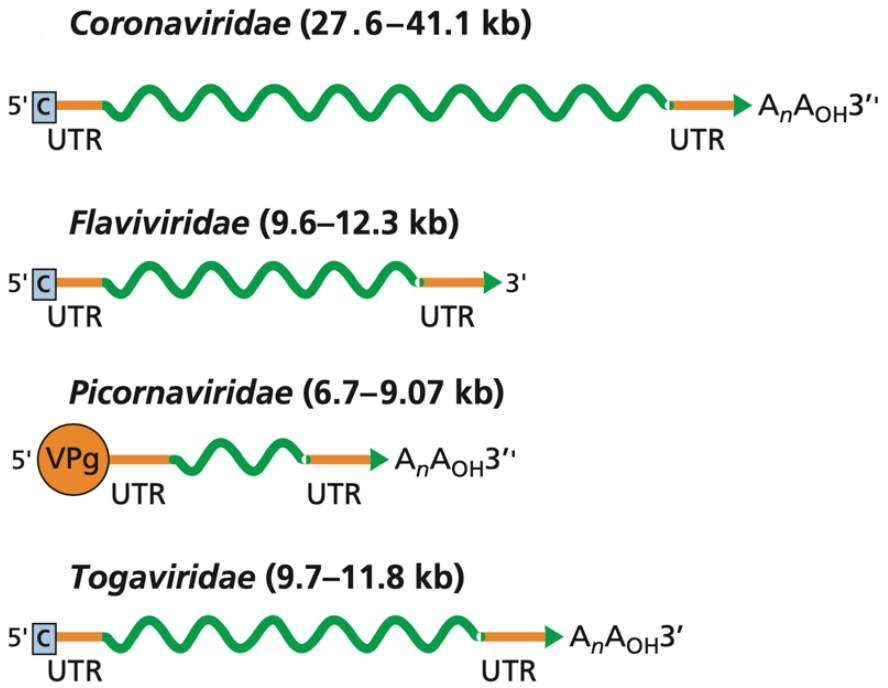




Rotavirus (human gastroenteritis)

ssRNA: (+) sense



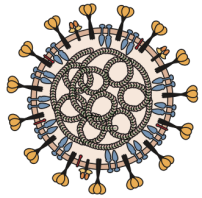


ssRNA: (+) sense

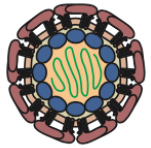
Picornaviridae (Poliovirus, Rhinovirus)

Caliciviridae (gastroenteritis)

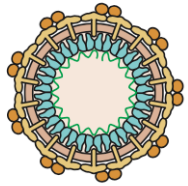
Coronaviridae (SARS-CoV, MERS-CoV, SARS-CoV-2)



Flaviviridae (Yellow fever virus, West Nile virus, Hepatitis C virus, Zika virus)

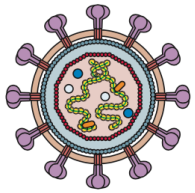


Togaviridae (Rubella virus, Equine encephalitis virus)



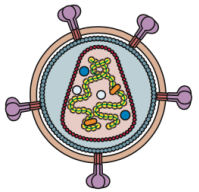
ssRNA(+) sense with DNA intermediate

One viral family: *Retroviridae*

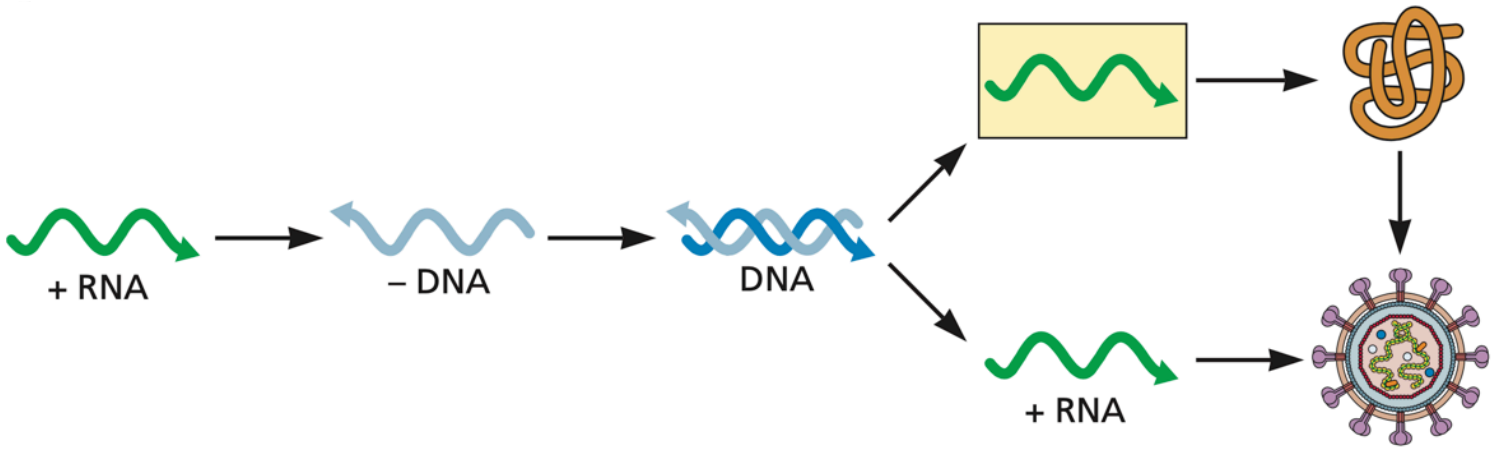


Two human pathogens:

Human immunodeficiency virus (HIV)
Human T-lymphotropic virus (HTLV)

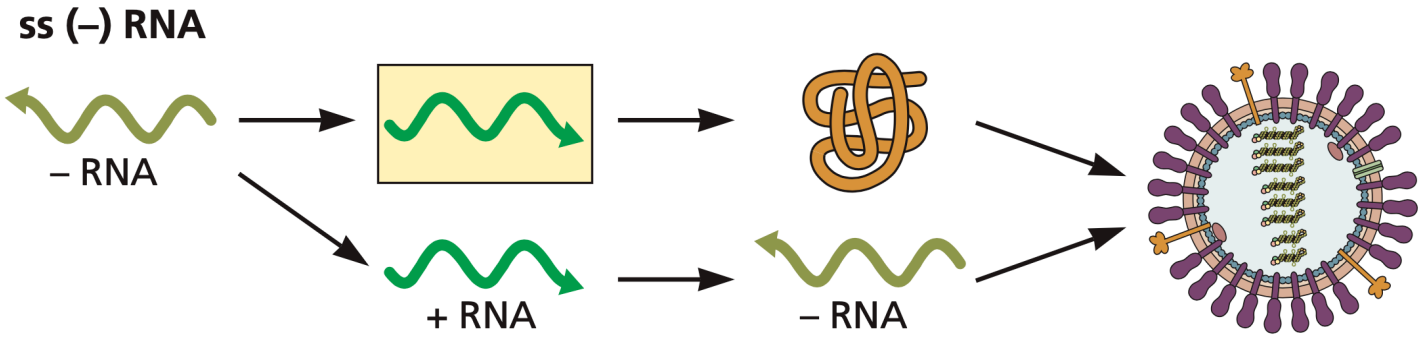


The remarkable retroviral genome strategy



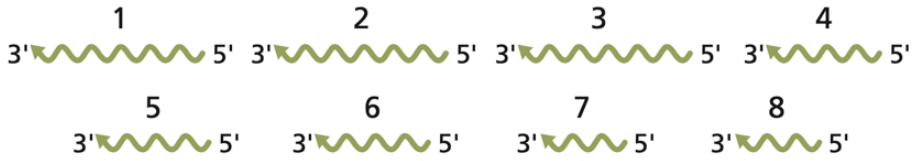
***Retroviridae* (7–11 kb)**

ssRNA, (-) sense



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

(–) strand RNA segments

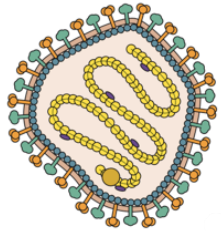


Nonsegmented genomes: *Paramyxoviridae* (15.1–18.2 kb)

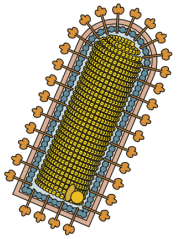
***Rhabdoviridae* (11–15 kb)**

ssRNA, (-) sense

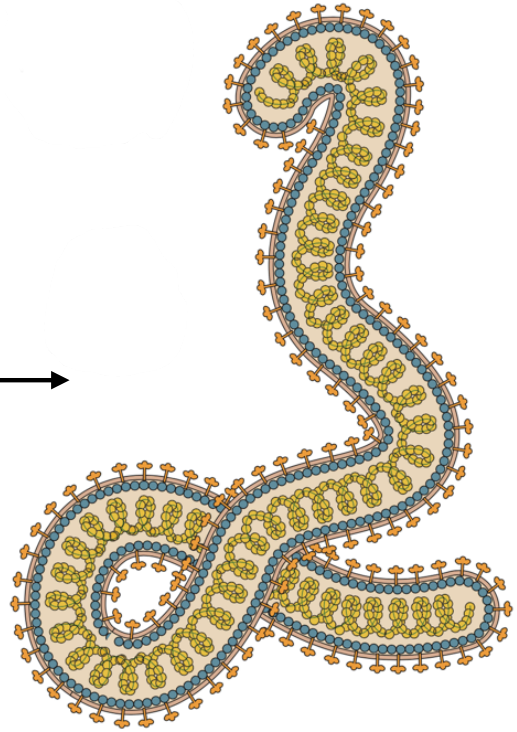
Paramyxoviridae (Measles virus, Mumps virus)



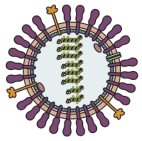
Rhabdoviridae (Rabies virus)



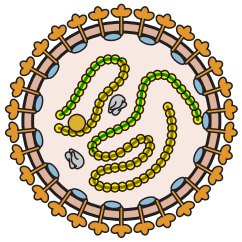
Filoviridae (Ebolavirus, Marburg virus)

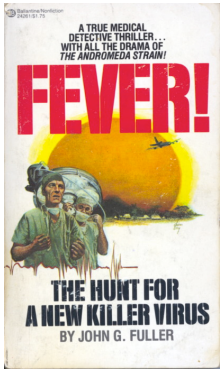


Orthomyxoviridae (Influenza virus)

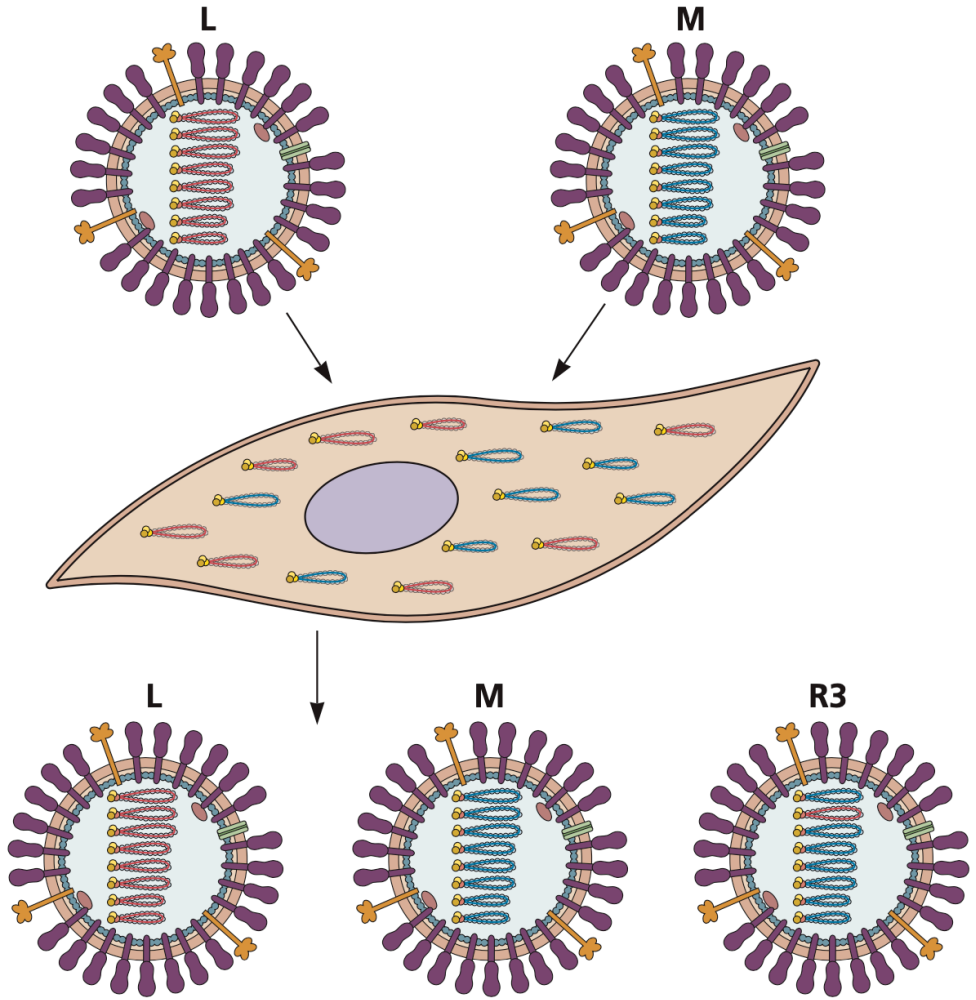


Arenaviridae (Lassa virus)





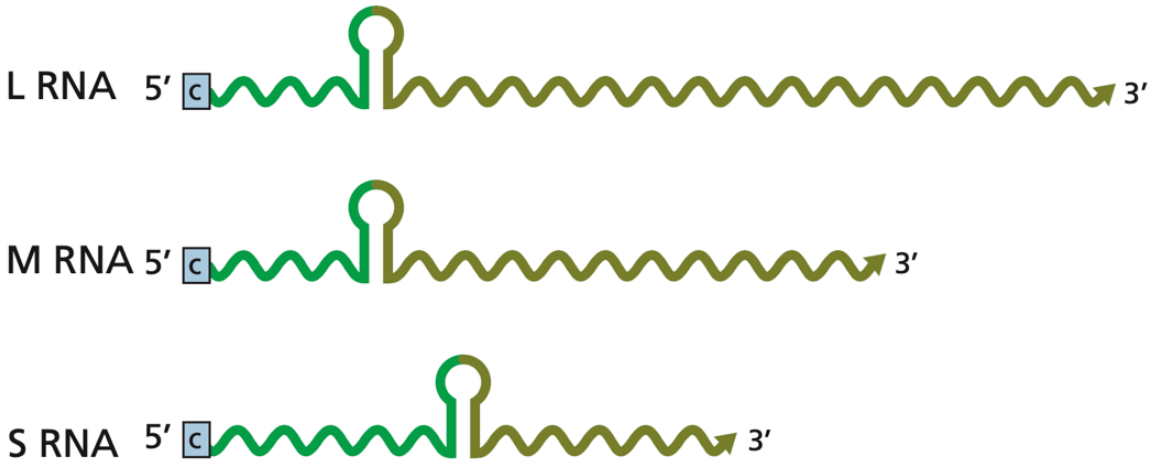
Reassortment: Consequence of segmented genome

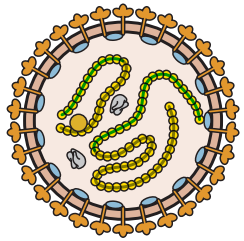


Ambisense RNA genomes

Ambisense (–) strand RNA

***Arenaviridae* (11 kb in 2 RNAs)**
***Peribunyaviridae* (12.4–16.6 kb in 3 RNAs)**





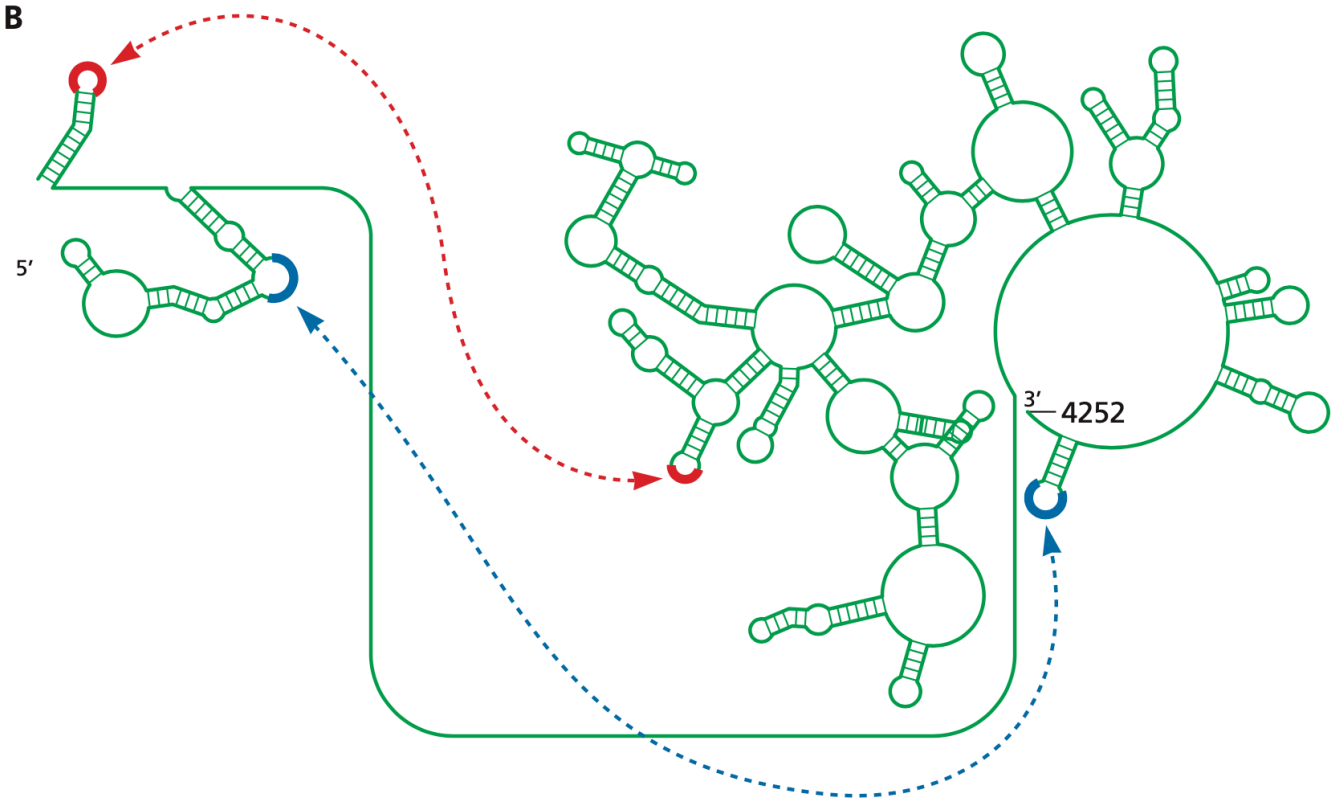
Arenaviridae
RNA pol in virus particle

A

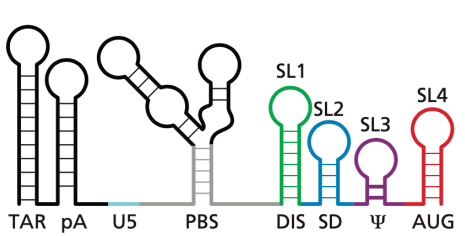
Linear (+) strand RNA genome of a picornavirus

RNA secondary structure elements in HIV-1 5′ leader

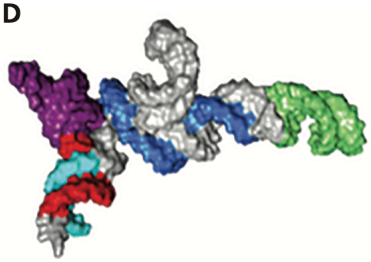
B



C



D



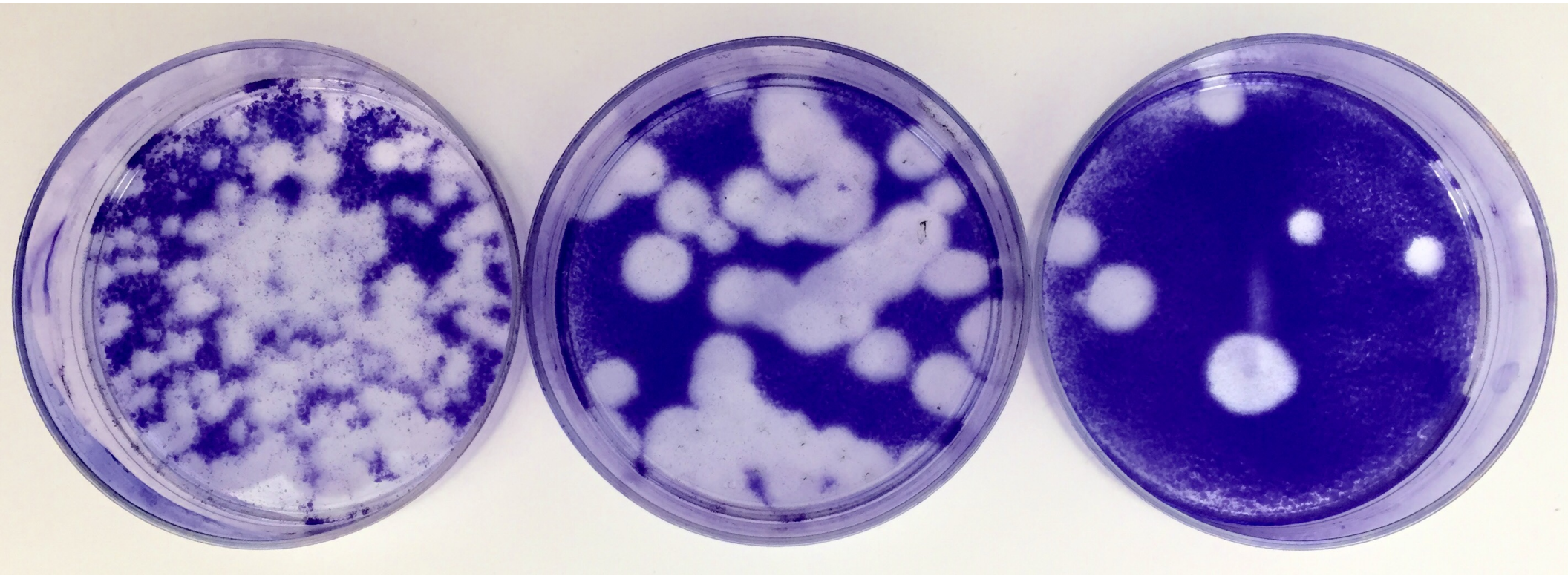
Go to:

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

Which statement about viral RNA genomes is correct?

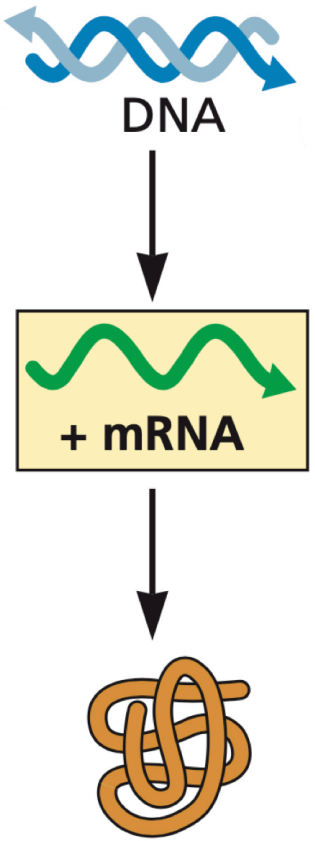
A. (+)ssRNA genomes may be translated to make viral protein
B. dsRNA genomes can be directly translated to make viral protein
C. (+)ssRNA virus replication cycles do not require a (-) strand intermediate
D. RNA genomes can be copied by host cell RNA-dependent RNA polymerases
E. All of the above

This method allowed the application of genetic methods to animal viruses



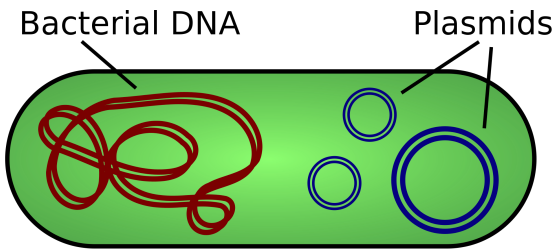
Changes in nucleic acids and proteins

- A *mutation* is a change in DNA or RNA (nucleotide addition, deletion, rearrangement)
- Mutations may lead to amino acid substitutions, additions, or truncations in proteins
- In the old days, mutations were introduced by mutagenesis (UV, chemicals)

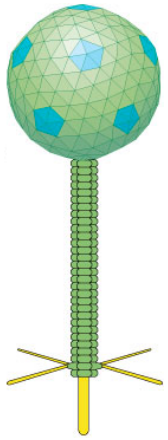


Engineering mutations into viral genomes - the modern way

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

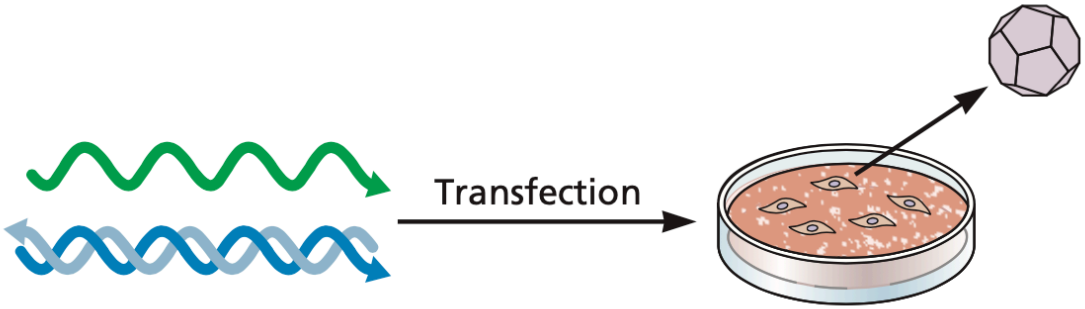


Genetic methods

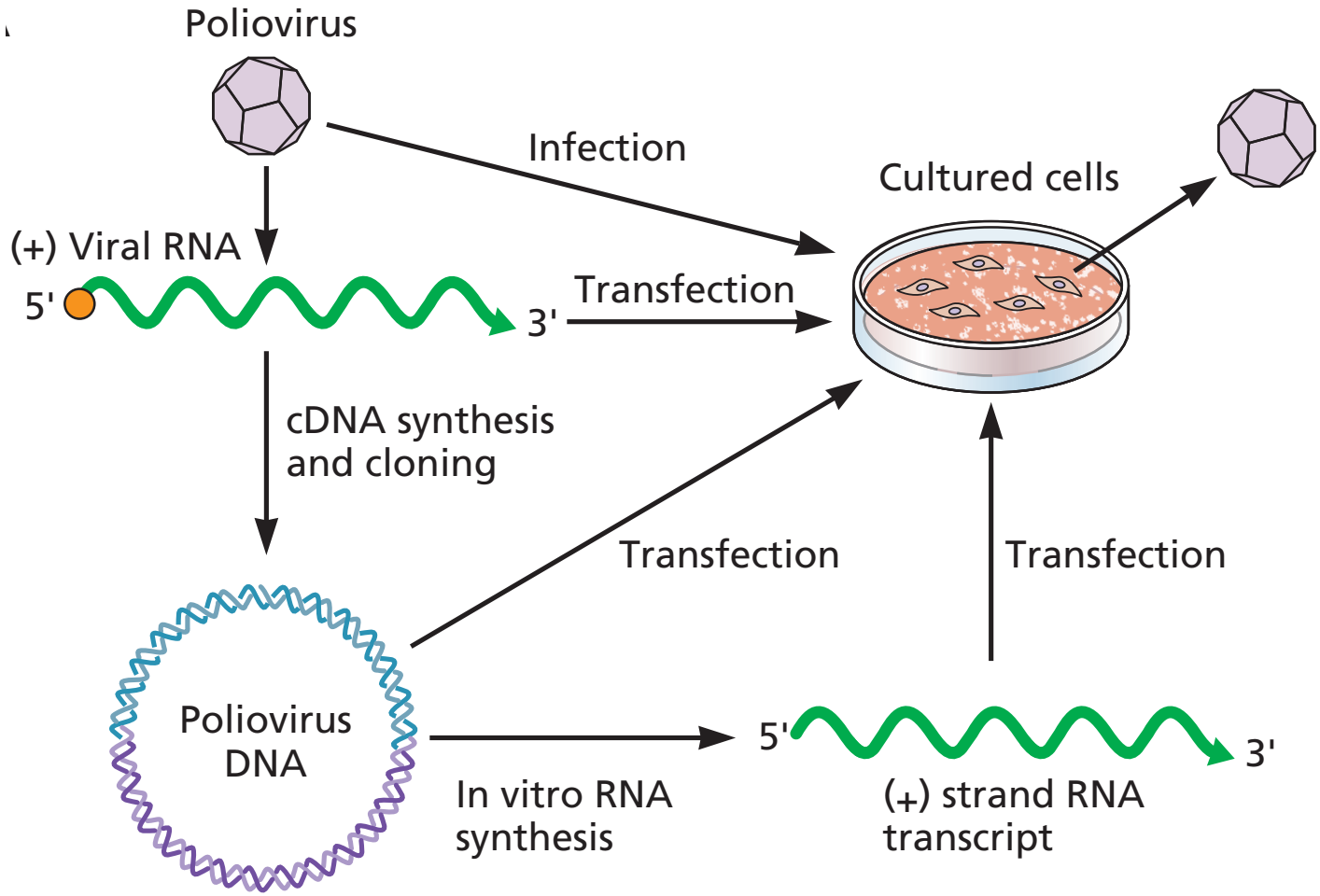


Transfection

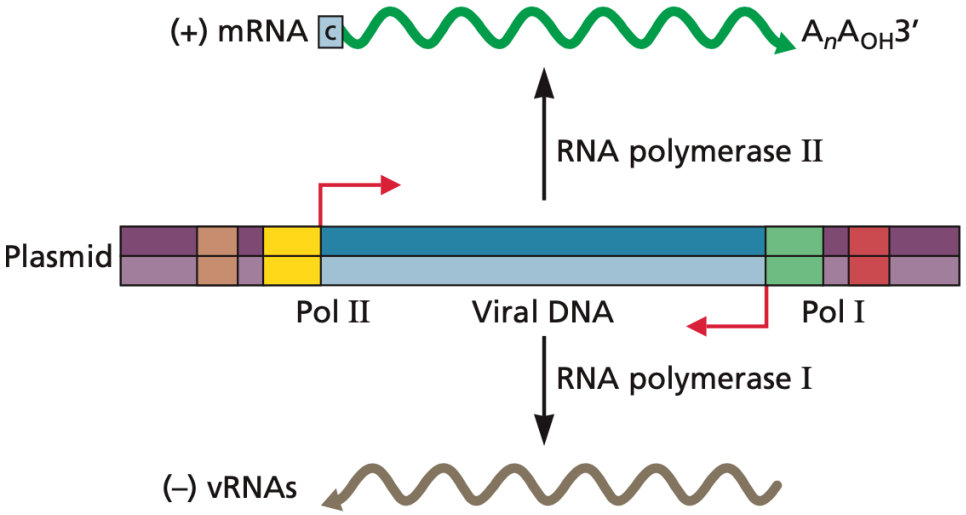
- Production of infectious virus after transformation of cells by viral DNA, first done with bacteriophage lambda
- Transformation-infection

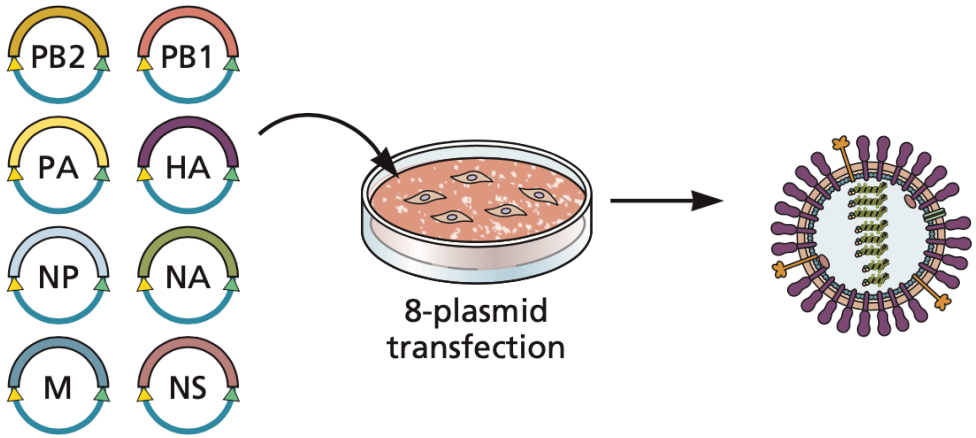


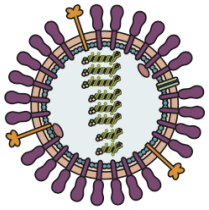
Infectious poliovirus DNA



Infectious influenza virus DNA

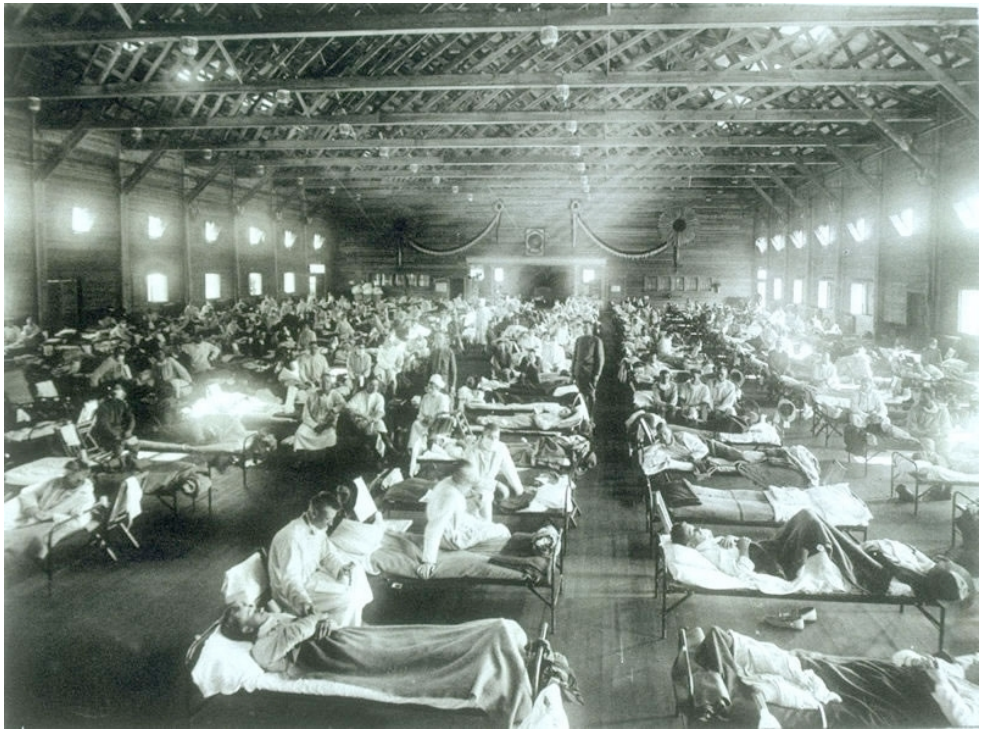




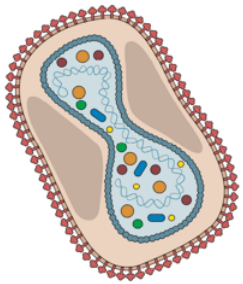


Recovering the 1918 influenza virus

- 1918 influenza pandemic killed millions
- Influenza virus not isolated until 1933
- In 2005, influenza RNA isolated from formalin-fixed, paraffin-embedded lung tissue sample from autopsy of victim of 1918 influenza
- Influenza RNA also isolated from frozen sample obtained by in situ biopsy of the lung of a victim buried in permafrost since 1918
- Complete nucleotide sequence of all 8 RNA segments determined
- Virus recovered by transfection of cells with 8 plasmids containing genome sequences

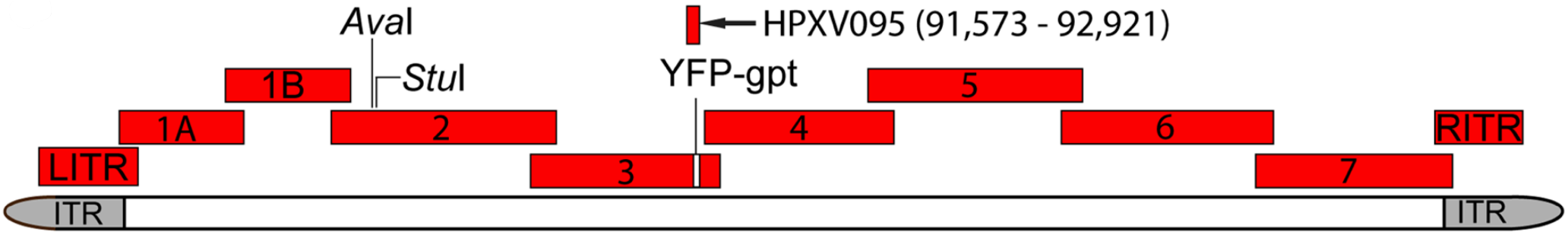


http://www.sciencemag.org/content/310/5745/77.long



Cloning viral DNA isn't always necessary!

- Horsepox virus is extinct, but have 212,633 bp genome sequence
- Chemically synthesized 10 overlapping DNAs ($150,000)
- Transfected into cells => infectious virus!



https://doi.org/10.1371/journal.pone.0188453

Synthetic Virology and Biosecurity

Infectious viral DNA enables experiments not previously possible

https://phys.org/news/2017-07-scientists-extinct-horsepox-virus-lab.html

Scientists bring back extinct horsepox virus in lab, raising important biosecurity questions

July 11, 2017, Johns Hopkins University

In a laboratory in Alberta, Canada, a team of scientists recently pieced together overlapping segments of mail order DNA to form a synthetic version of an extinct virus.

Their ominous milestone - successfully synthesizing horsepox, a relative of the deadly smallpox virus, which was declared eradicated in 1980 - has raised a conundrum in the scientific community: What are the implications of conducting research that has the potential to grow biological knowledge, but also harm public health and safety?

NSABB, National Science Advisory Board for Biosecurity: Federal advisory committee to provide advice, guidance, and leadership regarding biosecurity oversight of *dual use research of concern*

http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb

Gain of function research

- Giving a new phenotype to a virus
- Can be done the old ways (chemical mutagenesis, passing virus in a different host)
- Or can be done by modification of infectious cloned viral DNA
- Some examples follow

Gain of function research

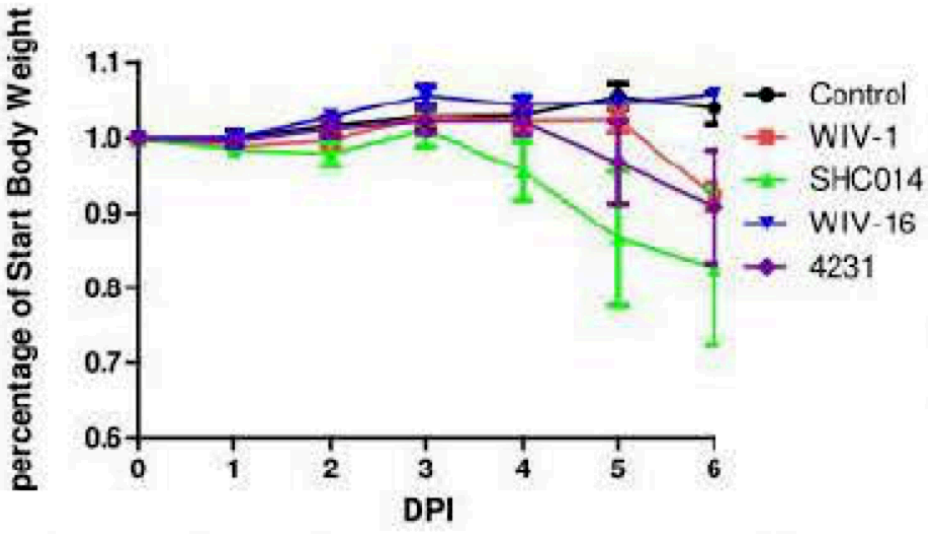
SUCCESSFUL TRANSFER OF THE LANSING STRAIN OF POLIOMYELITIS VIRUS FROM THE COTTON RAT TO THE WHITE MOUSE[1]

By Charles Armstrong, *Senior Surgeon, United States Public Health Service*

In an earlier paper (*1*) the successful transmission of a strain of poliomyelitis to the eastern cotton rat, *Sigmodon hispidus hispidus*, was recorded. This strain has now been carried through 26 serial transfers in this species to which it has become progressively better adapted. The incubation period has shown a tendency to stabilize at from 3 to 5 days when the inoculating dose is maintained at 0.06 cc. of a 5 percent saline suspension of virus-infected fresh cord and brain, administered intracerebrally. Attempts to transmit the infection by the intranasal route have so far been without success. Cotton rats are apparently quite uniformly susceptible to intracerebral

[1] From the Division of Infectious Diseases, National Institute of Health.

Recombinant CoVs - from EcoHealth Alliance NIH grant via FOI



Moving beyond metagenomics to find the next pandemic virus

Vincent Racaniello[a,1]

The critics of gain-of-function experiments frequently cite apocalyptic scenarios involving the release of altered viruses and subsequent catastrophic effects on humans (8). Such statements represent personal opinions that are simply meant to scare the public and push us toward unneeded regulation. Virologists have been manipulating viruses for years—this author was the first to produce, 35 y ago, an infectious DNA clone of an animal virus (9)—and no altered virus has gone on to cause an epidemic in humans. Although there have been recent lapses in high-containment biological facilities, none have resulted in harm, and work has gone on for years in many other facilities without incident (10). I understand that none of these arguments tell us what will happen in the future, but these are the data that we have to calculate risk, and it appears to be very low. As shown by Menacherry et al. (2) in PNAS, the benefits are considerable.

A major goal of life science research is to improve human health, and prohibiting experiments because they may have some risk is contrary to this goal. Being overly cautious is not without its own risks, as we may not develop the advances needed to not only identify future pandemic viruses and develop methods to prevent and control disease, but to develop a basic understanding of pathogenesis that guides prevention. These are just some of the beneficial outcomes that we can predict. There are many examples of how science has progressed in areas that were never anticipated, the so-called serendipity of science. Examples abound, including the discovery of restriction enzymes that helped fuel the biotechnology revolution, and the development of the powerful CRISPR/Cas9 gene-editing technology from its obscure origins as a bacterial defense system.

Banning certain types of potentially risky experiments is short sighted and impedes the potential of science to improve human health. Rather than banning experiments, such as those described by Menachery et al. (2), measures should be put in place to allow their safe conduct. In this way science's full benefits for society can be realized, unfettered by artificial boundaries.

doi: 10.1073/pnas.1601512113

Next time: Structure of viruses