Introduction to Virology I

Prof. Vincent Racaniello
Department of Microbiology
Office: HHSC 1310B

vrr1@columbia.edu

All living things survive in a sea of viruses

- **We eat and breathe billions of them regularly**

  -breathe 6 liters of air per minute, eat thousands of grams of food and its allied contaminants per day, touch heaven knows what and put our fingers in our eyes and mouths

  - every milliliter of seawater has more than a million virus particles

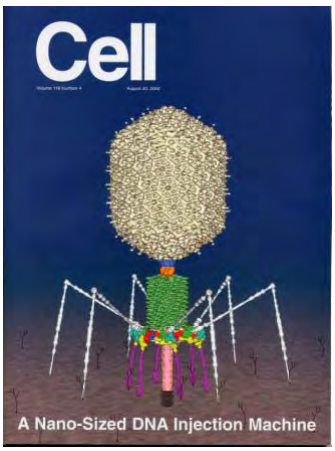
- **We carry viral genomes as part of our own genetic material**

- **Viruses infect our pets, domestic food animals, wildlife, plants, insects**

- **Viral infections can cross species barriers, and do so constantly (zoonotic infections)**

  - constant probing for new hosts
  - today's "natural host" for a virus may be a way-station in its evolution
  - viral infections influence the evolution of their hosts.

The number of viruses impinging on us is staggering



Startling facts about phage:

More than 10^{30} bacteriophage particles in the world's waters!

- A bacteriophage particle weighs about a femtogram (10^{-15} grams)

  10^{30} X 10^{-15}= the biomass on the planet of BACTERIAL VIRUSES ALONE exceeds the biomass of elephants by more than 1000-fold!

- The length of a head to tail line of 10^{30} phages is more than 200 million light years!

- **Whales are commonly infected with a tiny virus of the *Caliciviridae* family (rashes, blisters, intestinal problems, diarrhea)**

  – these whale diarrhea viruses can infect humans

- **Infected whales secrete more than 10^{13} calciviruses daily!!**

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

With this number of genomes, it is highly probable that HIV genomes exist that are resistant to every one of the antiviral drugs that we have now,
or EVER WILL HAVE!

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

We exist because we have a <u>defense system</u> that evolved to fight infections

If our immune system is down (e.g. AIDS, organ transplants), even the most common viral infection can be lethal.

A virus is a very small, infectious, obligate intracellular parasite

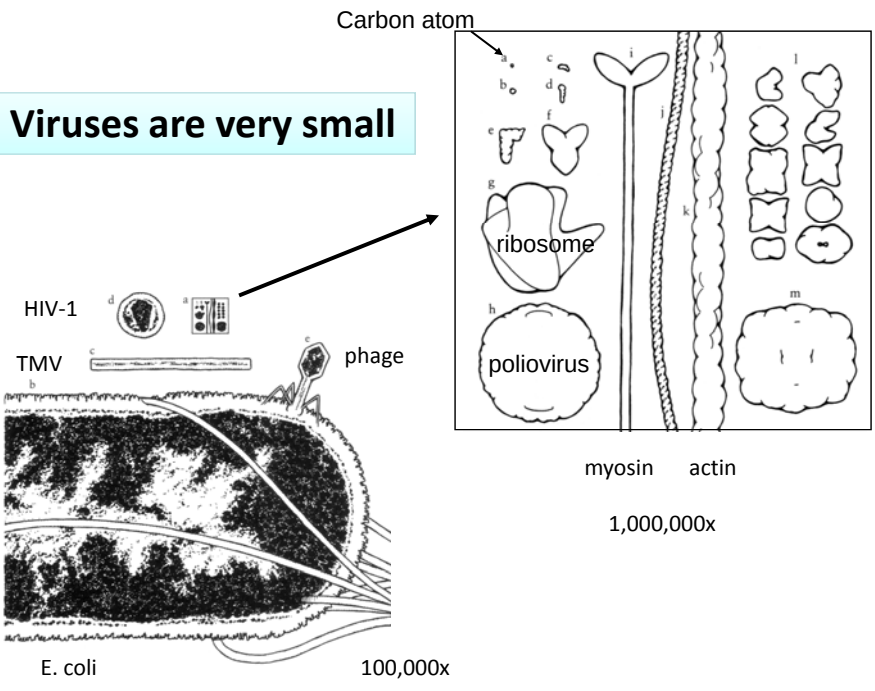
Virus particles are *not living*

They are chemicals, and by themselves cannot reproduce

A cellular host is needed for viruses to reproduce

Infected cells are the living manifestation of what is encoded in a viral genome

Viruses are very small



Defining viral attributes

•The genome is comprised of either DNA or RNA.

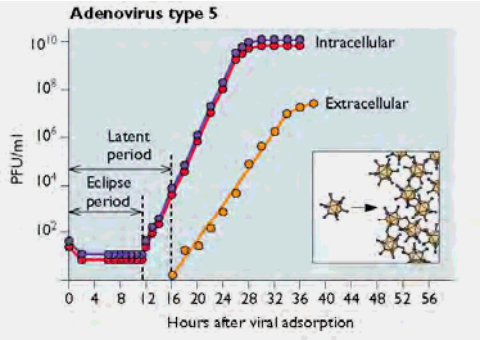
•Within an appropriate host cell, the viral genome directs the synthesis, ***by cellular systems***, of the components needed for replication of the viral genome and its transmission within virus particles.

•New virus particles are formed by *de novo* assembly from newly-synthesized components within the host cell.

•The progeny particles are the vehicles for transmission of the viral genome to the next host cell or organism

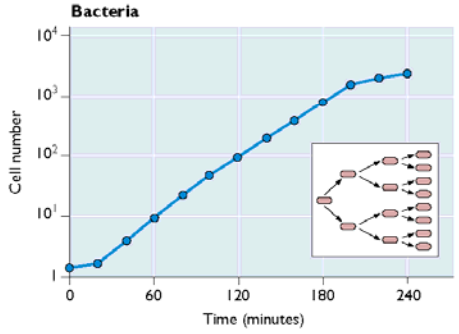
•The particles are then disassembled inside the new cell, initiating the next infectious cycle.

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



First make the parts, then assemble the final product.

Not binary fission like cells



ALL viruses follow this three-part strategy…

1. All have a nucleic acid genome packaged in a proteinaceous particle

- *This particle is the vehicle for transmission of the viral genome from host to host.*
- *The particle is a delivery device, but it is not alive*

2. The viral genome contains the information to initiate and complete an *infectious cycle* within a susceptible and permissive cell

An infectious cycle allows attachment and entry of the particle, decoding of genome information, translation of viral mRNA by host ribosomes, genome replication, assembly and release of particles containing the genome.

3. All viral genomes are able to establish themselves in a host population so that virus survival is ensured

This three-part strategy achieves one goal:
SURVIVAL

Despite this simple 3-part strategy, the tactical solutions encoded in genomes of individual virus families are incredibly diverse

There are countless virus particles out there with amazing diversity:

- *size, nature and topology of genomes*
- *strange particles*
- *unbelievable coding strategies*
- *amazing tissue/cell tropism*
- *degrees of pathogenesis from benign to lethal*

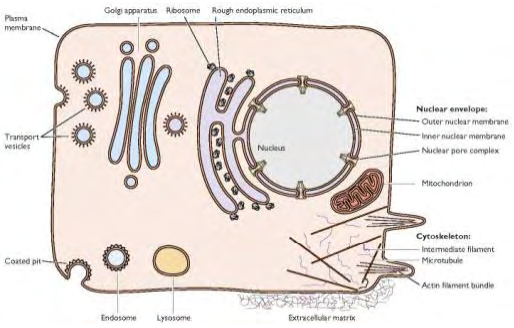
Nevertheless, there is an underlying simplicity and order to all this because of two simple facts:

1. All viral genomes are **obligate molecular parasites** that can only function after they replicate in a cell
2. All viruses must make mRNA that can be translated by host ribosomes
   - they all are parasites of the host protein synthesis machinery

A viral infection is an exercise in cell biology

Many cell functions required for viral propagation

- machinery for translation of viral mRNAs
- Energy
- enzymes for replication and assembly
- transport pathways



In the real world:

A virus particle (virion) must encounter a host

- no mean feat for nano-particles with no means of locomotion; diffusion-limited process

- environment is tough on tiny things (UV, drying, dilution; pH)

Once a host is encountered, a virus particle must evade host physical defenses

- skin (dead), low pH on skin, mucous layers, extracellular matrix surrounding cells.

Once inside the host, virions and infected cells face host defenses

- intrinsic cell defenses, innate immunity, and acquired immunity.

The infectious cycle is also called “virus replication”

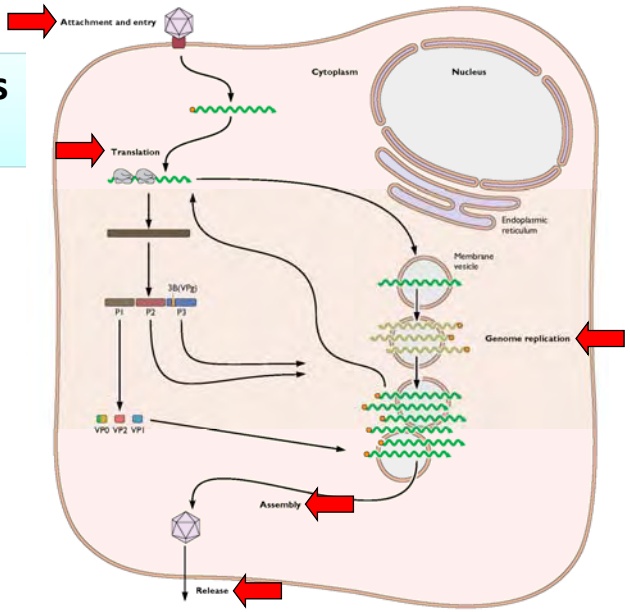
Replication is the sum total of *all the events* whereby a single particle attaches to a cell and, in a relatively short time, the cell releases many viral particles.

- Produce multiple copies of the viral genome
- Pack the genomes into particles
- One particle gives rise to hundreds or thousands of particles that can infect again.

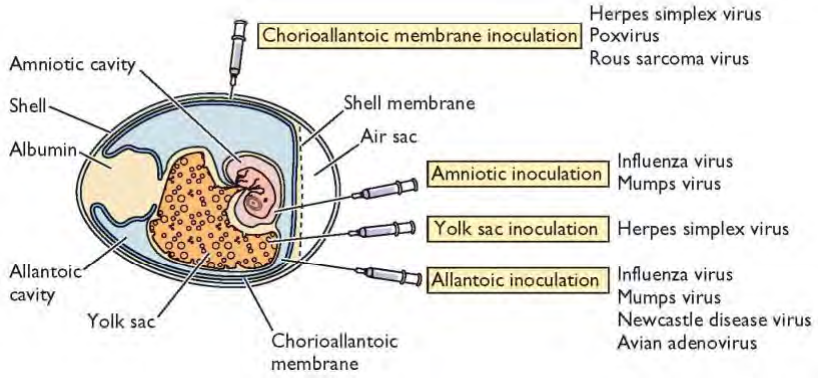
All viral infections of bacteria or elephants begin with events in a single cell

The Infectious Cycle

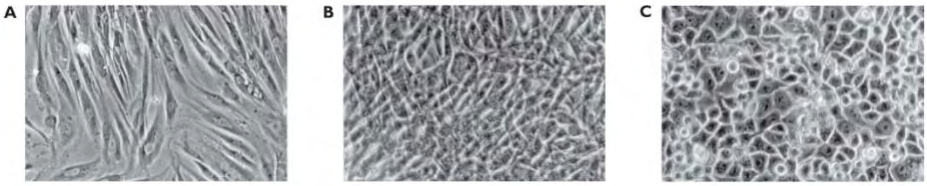
Virologists divide the infectious cycle into steps to facilitate their study, but no such artificial boundaries occur



Virus cultivation



Virus cultivation

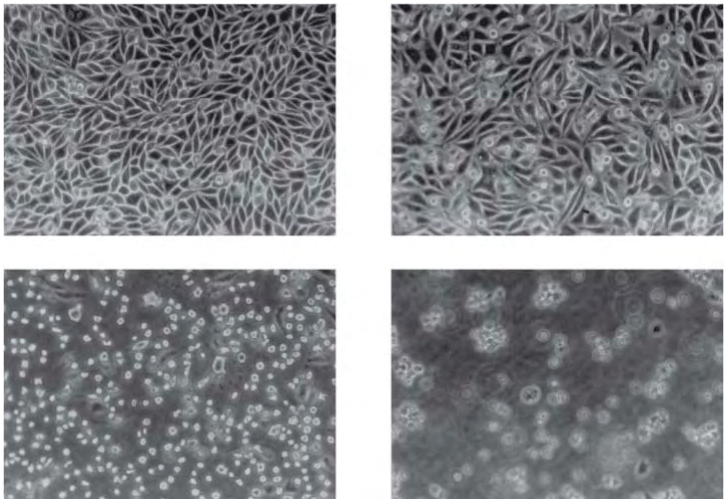


primary human foreskin fibroblasts

mouse fibroblast cell line (3T3)

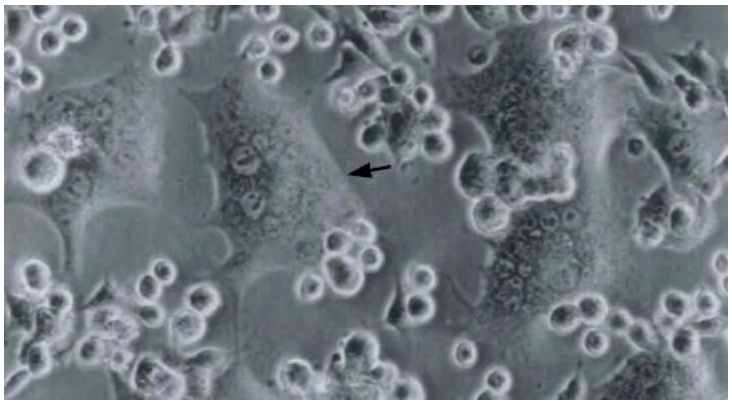
human epithelial cell line (HeLa)

Virus detection



cytopathic effect (CPE)

Virus detection



formation of syncytia

Assay of viruses

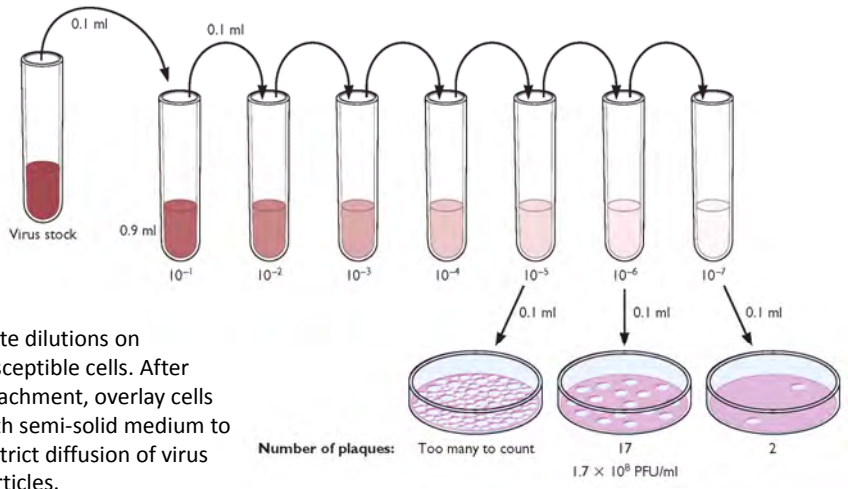
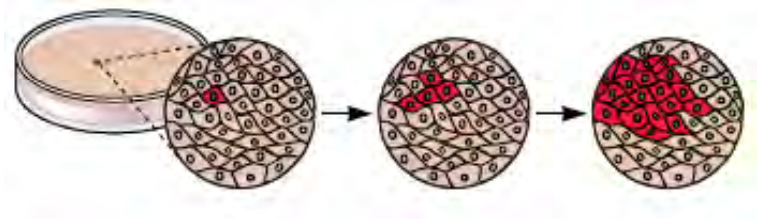
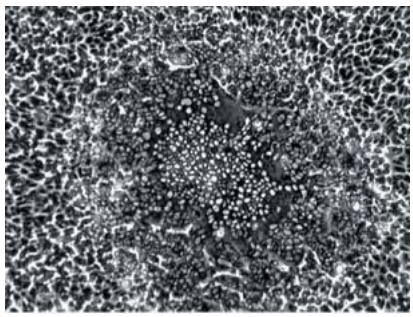


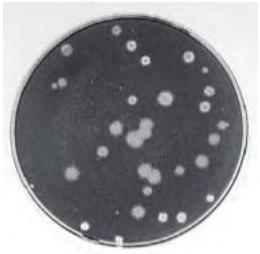
Plate dilutions on susceptible cells. After attachment, overlay cells with semi-solid medium to restrict diffusion of virus particles.

Restricted spread of virus produces localized destruction of cell monolayer visible as 'plaques'

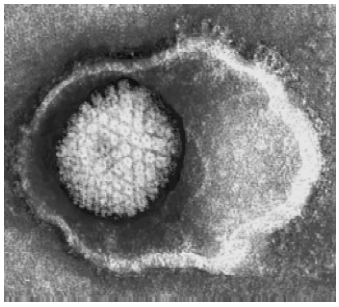
Plaque assay

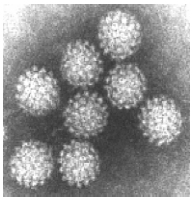


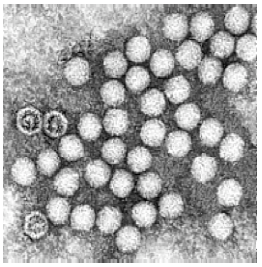


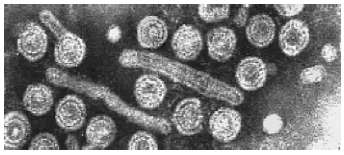


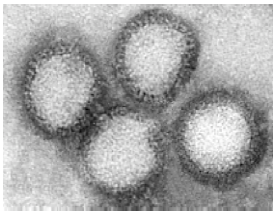
What do viruses look like?
Virus structure

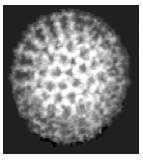


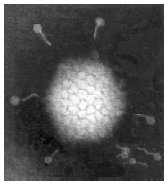


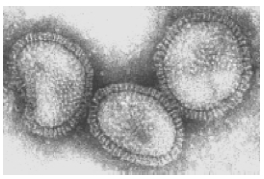


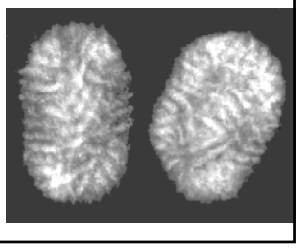












Functions of viral proteins

- Protect the viral 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
- Deliver the genome into the cell
  - Binding to host cell receptors
  - Induction of fusion with host cell membranes
  - Interaction with cell components to direct transport of genome to the appropriate site

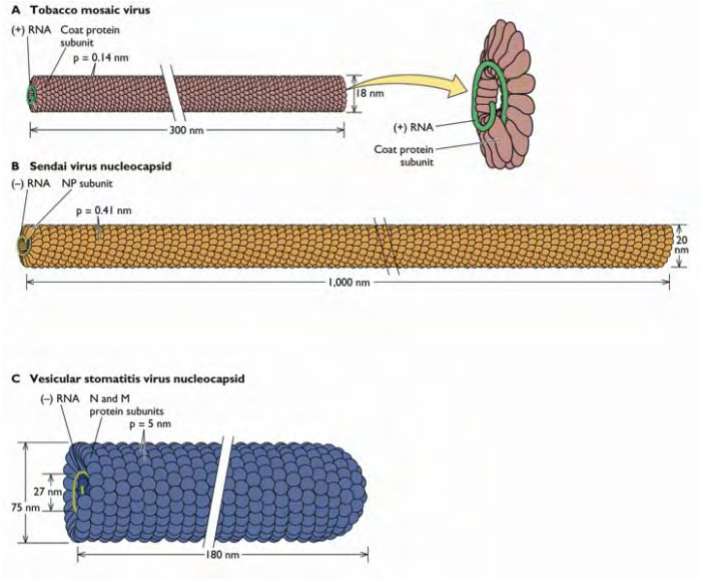
Virus particles are not inert

- They are *metastable*: have not attained minimum energy conformation
- They do so by surmounting an unfavorable energy barrier during entry into cells
- Viruses are molecular machines that play an active role in delivery of genome

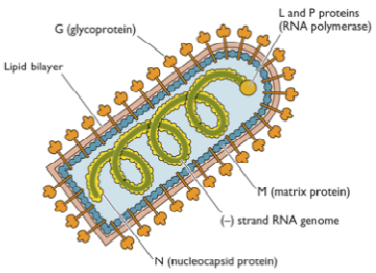
Building a protective coat

- Genetic economy dictates that a protein shell is built by using the same type of molecule over and over
- Viral proteins have structural properties that permit regular and repetitive interactions among them
- The protein coats of most viruses display helical or icosahedral symmetry

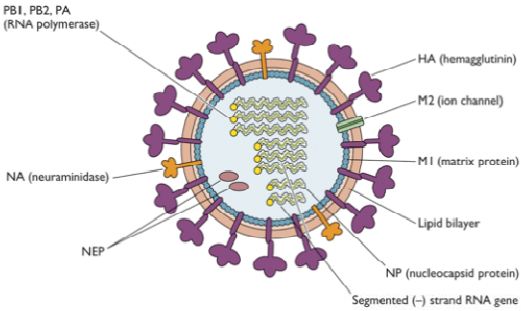
Helical symmetry



Helical symmetry



Rabies virus

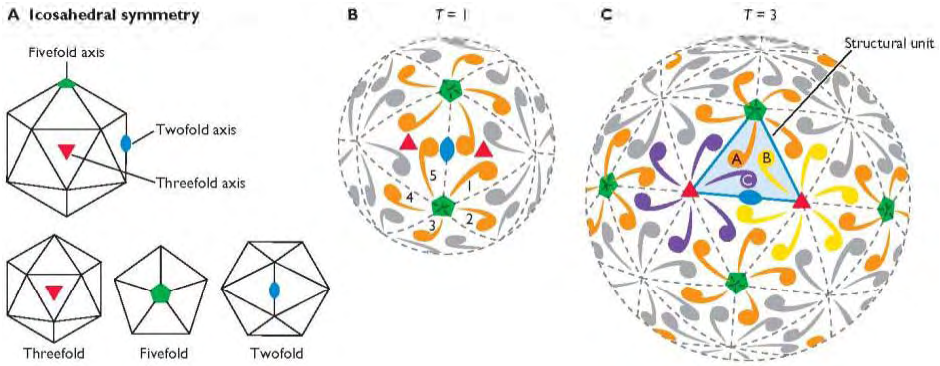


Influenza virus

Icosahedral symmetry

- Most economical way to build symmetric shell, maximal internal volume, with nonsymmetric proteins: icosahedron
- Icosahedron: solid with 20 faces, each an equilateral triangle
- 12 vertices
- Allows formation of a closed shell with the smallest number (60) of identical subunits

Icosahedral symmetry

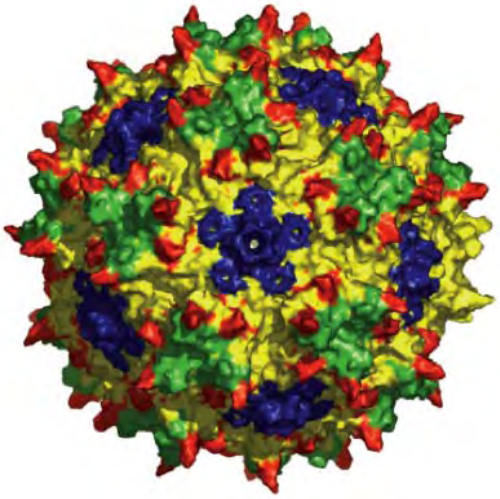


Icosahedral symmetry: Adeno-associated virus type 2

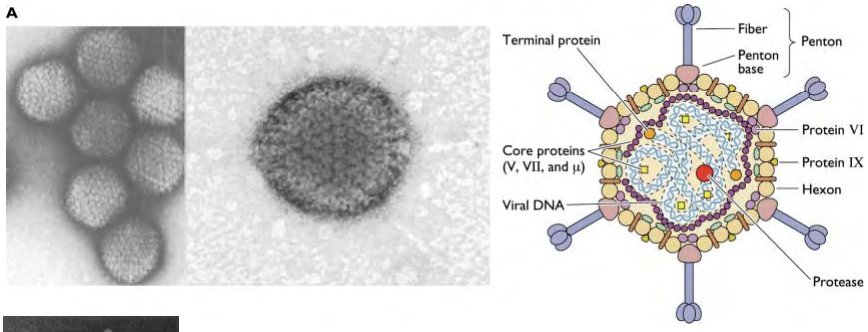
Parvovirus

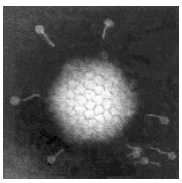
250 Å diameter

Composed of 60 copies of a single capsid protein



Icosahedral symmetry: Adenovirus



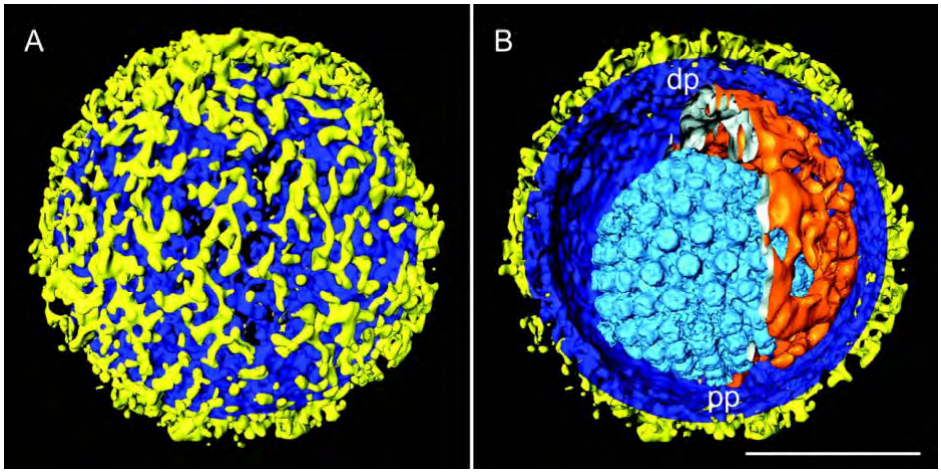


1,500 Å diameter

Composed of 240 subunits made of hexons

Fibers at 12 vertices

An icosahedral capsid may be enveloped

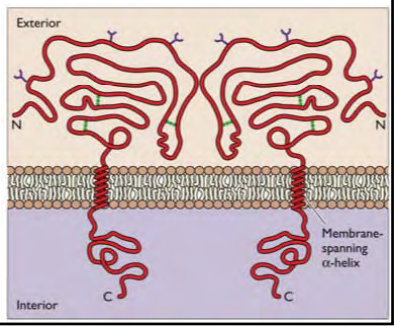


An icosahedral capsid may be enveloped

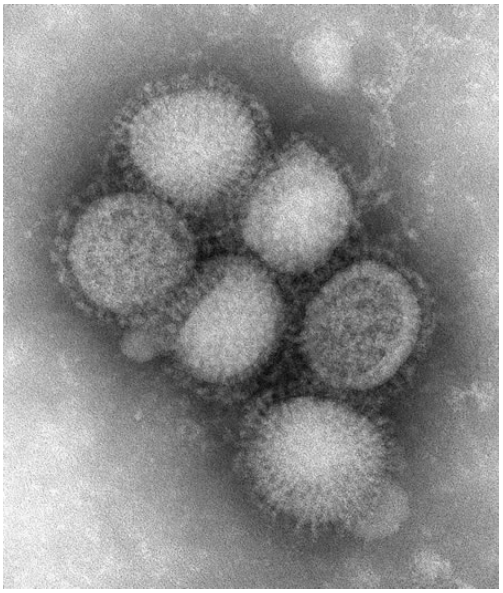
- *Herpesviridae* - over half of 80 viral genes encode proteins found in 2,000 Å virions
- Envelope proteins (13) and two distinct internal structures
  - icosahedral nucleocapsid surrounding DNA genome (4)
  - tegument (20) - role in delivery of proteins required early in infection

Viral envelope glycoproteins

- Integral membrane glycoproteins
- Ectodomain: attachment, antigenic sites, fusion
- Internal domain: assembly
- Oligomeric: spikes

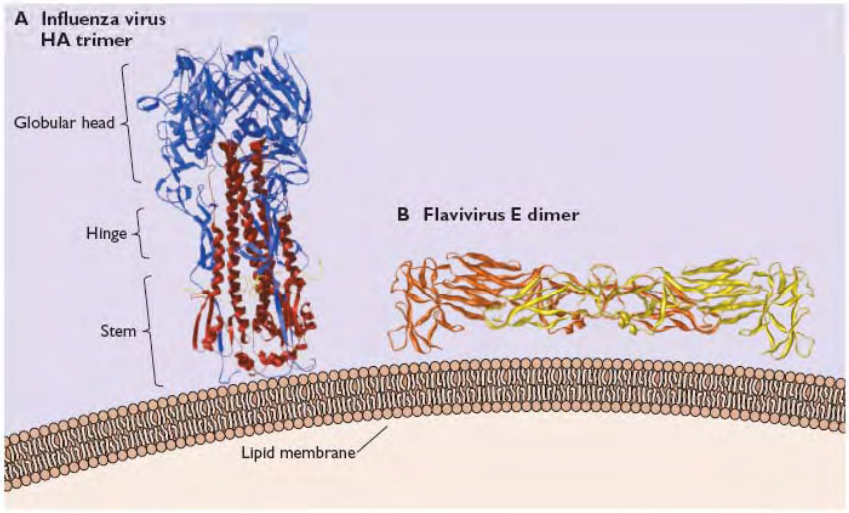


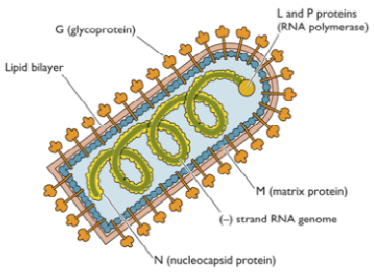
Influenza viral envelope glycoproteins



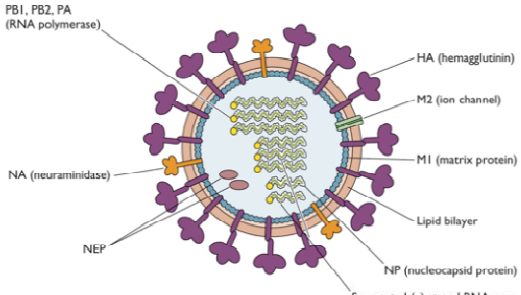
2009 pandemic H1N1 influenza virus

Viral envelope glycoproteins

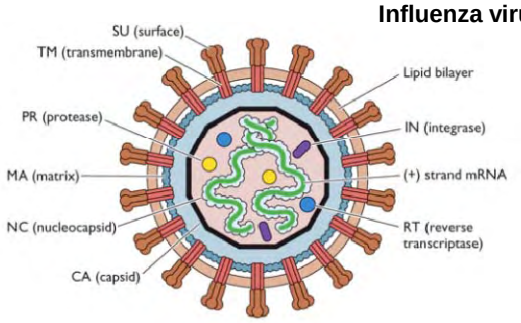




rabies virus



Influenza virus



retrovirus

Viral Genomes

BREAKTHROUGH in the 1950s:

The viral nucleic acid genome was shown to carry the information needed to replicate, build, and spread virions in the world; it IS the genetic code

- seems obvious now, but this discovery in viruses was one of the building blocks of Molecular Biology

Although there are thousands of different virions, there is only a finite number of viral genomes: There are only SEVEN genome types

Key fact makes life easier for students of virology:

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

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

~~**Viral genomes do not encode protein synthesis machinery**~~
Most viral genomes do not encode protein synthesis machinery

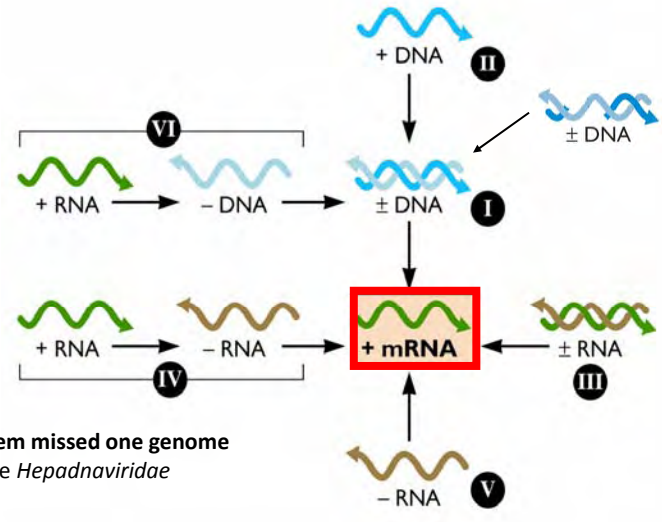
- provides a powerful rubric to organize your thinking about viruses.

All viruses are parasites of the cells mRNA translation system

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

- a major unifying principle in virology

All viral genomes must provide mechanisms for the synthesis of mRNAs that can be read by host ribosomes.



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

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

Definitions

- (+) strand: mRNA, because it can be immediately translated. A strand of DNA of the equivalent polarity is also (+) strand
- (-) strand: the complement of the (+) strand; cannot be translated

The seven classes of viral genomes

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