Intro II - Viral Replication

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

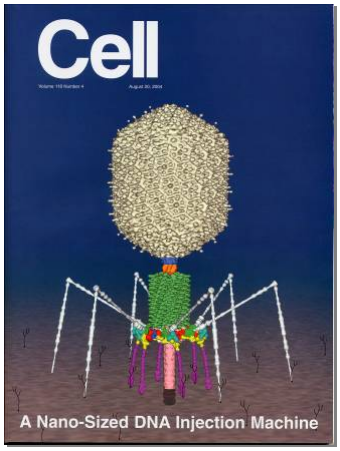
- **We carry viral genomes as part of our own genetic material**

- **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 water supply!

- 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

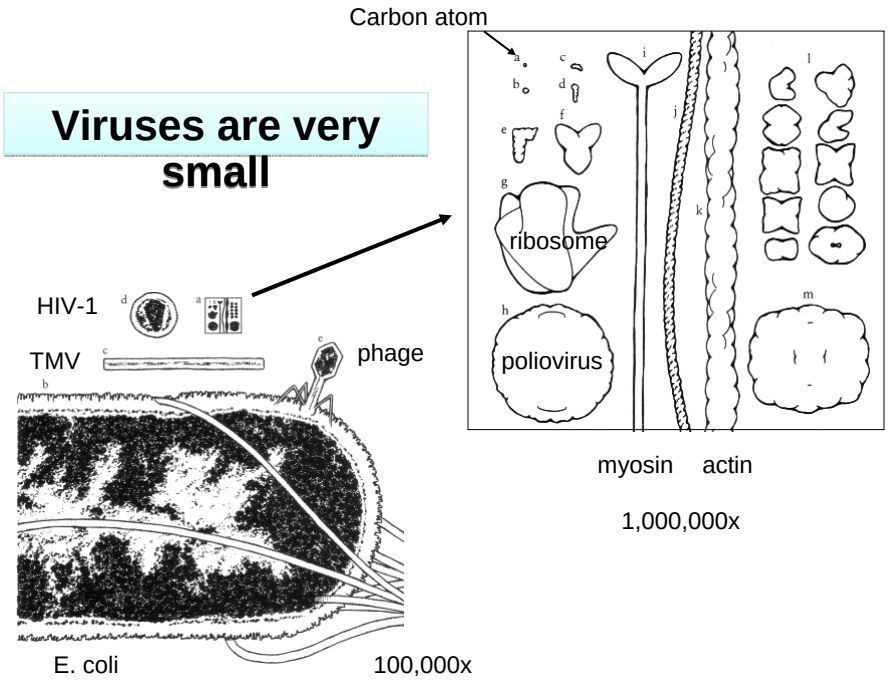
Virus particles are *not living*

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.

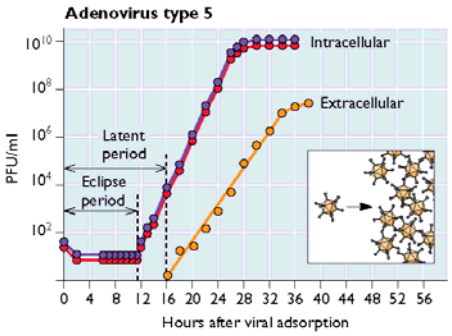
•The progeny particles are the vehicles for transmission of the viral genome to the next host cell or organism

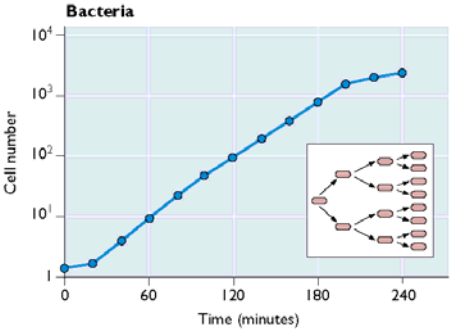
•The particles are then disassembled inside the new cell, initiating the next infectious cycle.

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:

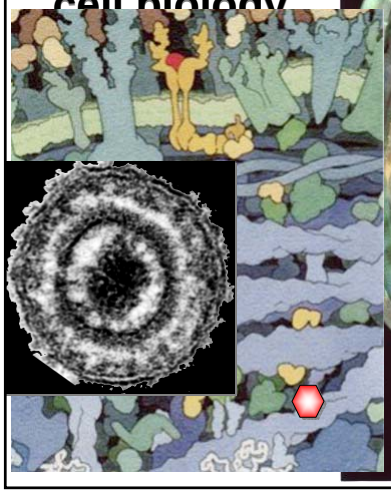
- *size, nature and topology of genomes*
- *strange particles*
- *unbelievable coding strategies*
- *amazing tissue/cell tropism*
- *degrees of pathogenesis from benign to lethal*

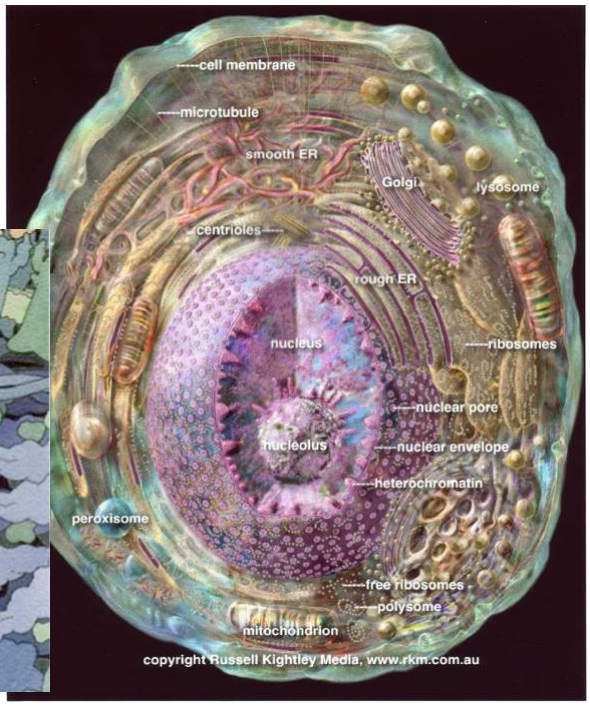
Nevertheless, there is an underlying simplicity and order to all this because of two simple facts:

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





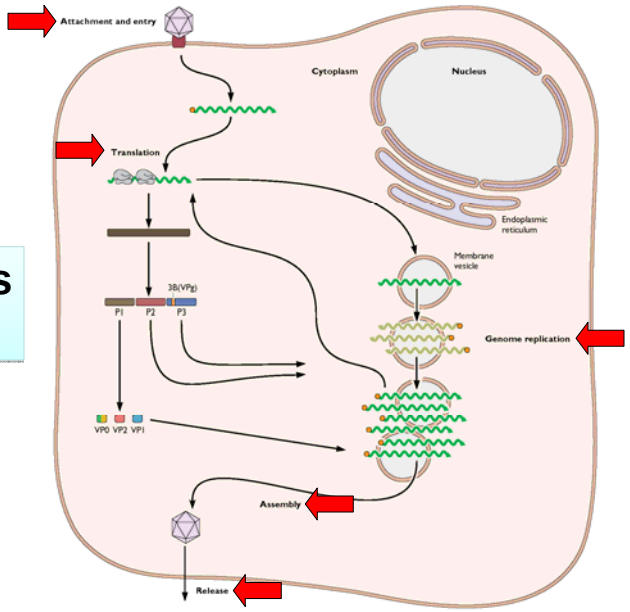
The infectious cycle is also called “virus replic

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



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.

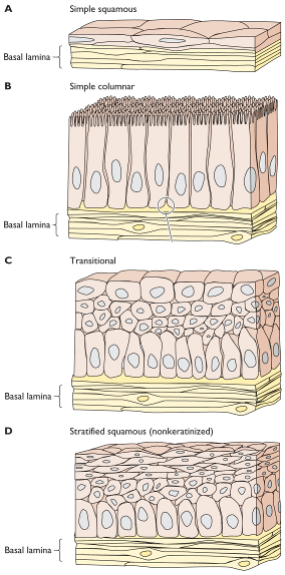
In vivo viral infections usually begin at exposed epithelial surfaces

Three topological surfaces

Apical: presented to outside ("top")

Basal: presented to inside ("bottom")

Lateral: side-to-side cell contacts



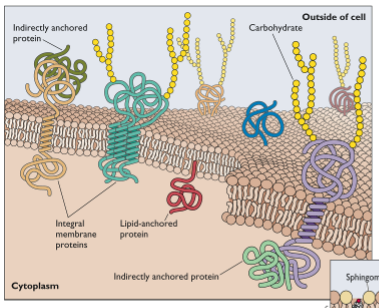
Simple Squamous: thin cells, blood vessels

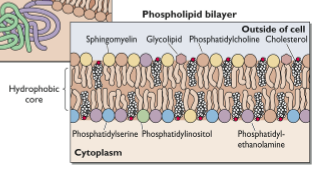
Simple columnar: thick, mucous secreting; GI tract

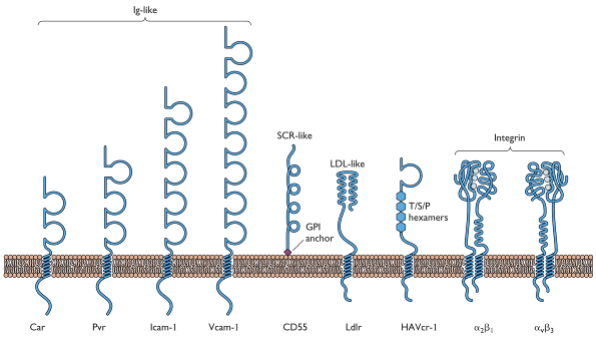
Transitional: Distinct layers: expandable

Stratified Squamous: Epidermal layer of skin, mouth, genitals

The plasma membrane

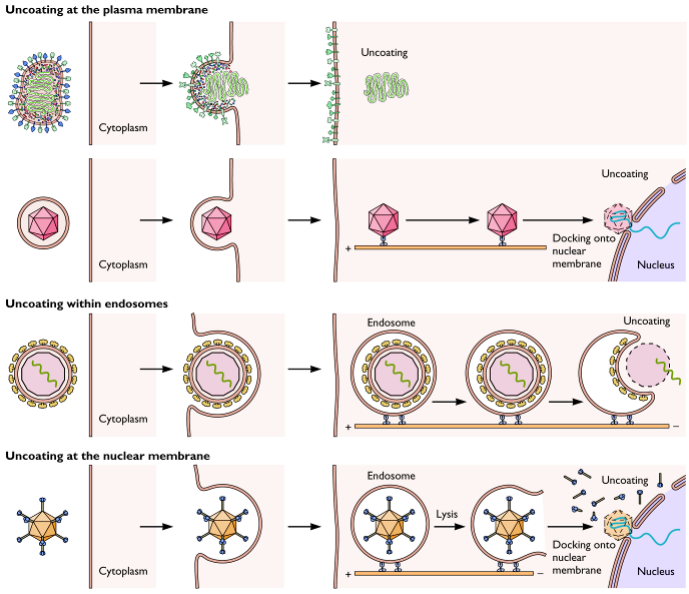






Some virus receptors

Three uncoating strategies



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

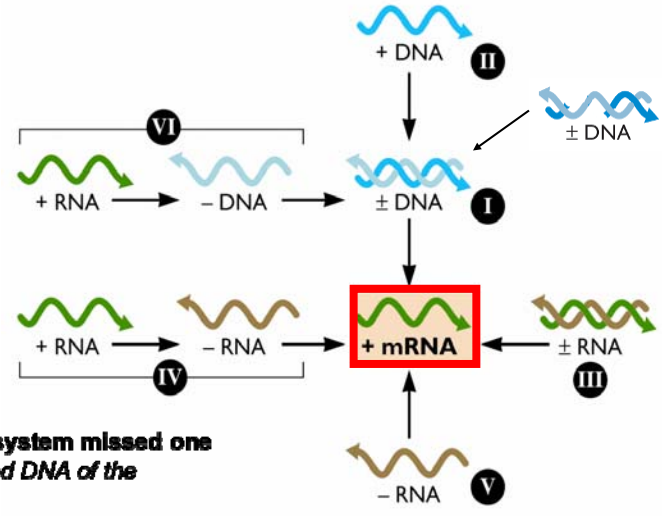
- provides a powerful rubric to organize your thinking about viruses.

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 Baltimore 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

The seven classes of viral genomes

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

Double stranded DNA (dsDNA)

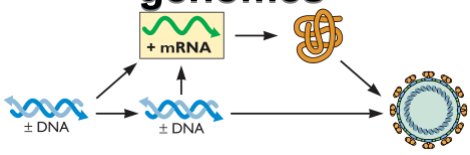
22 families of viruses have viruses with dsDNA genomes

- those that include mammalian viruses are the *Adenoviridae, Hepadnaviridae, Herpesviridae, Papillomaviridae*, *Polyomaviridae*, and *Poxviridae*.

Information extraction (making mRNA):

- mRNA is produced when host or viral DNA-dependent RNA polymerase copies the (-) strand.
- CANNOT make mRNA from ssDNA
- Can only make mRNA from dsDNA

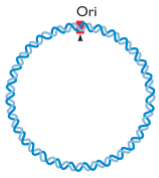
Double stranded DNA (dsDNA) genomes



Genomes use host DNA polymerase

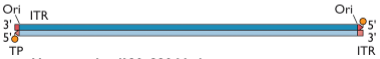
Genomes encode DNA polymerase

***Polyomaviridae* (5 kbp)**

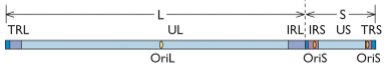


Papillomaviridae (8 kbp)

***Adenoviridae* (36–48 kbp)**



***Herpesviridae* (120–220 kbp)**

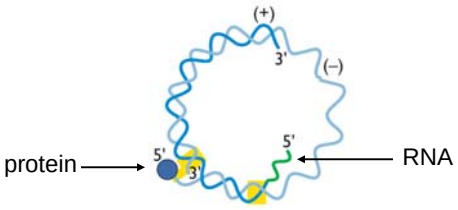


***Poxviridae* (130–375 kbp)**

Gapped DNA genomes

The strange genome of the hepadnaviruses, hepatitis B virus

- A protein is covalently attached to the 5'-end of one strand.
- A short RNA is covalently attached to the 5'-end of the other.
- One strand is complete and the other is only about half completed – hence a big gap in the DNA.



Paradox?
This genome can't make mRNA as it comes from the virion!!

Upon infection and release from the capsid, the hepadnaviral genome must be repaired and converted to dsDNA

- the protein and RNA must be removed
- the gap must be filled
- need perfectly duplex DNA to make mRNA.
- convert gapped, genome to covalently closed, ds circular DNA

This repair process must precede mRNA synthesis.

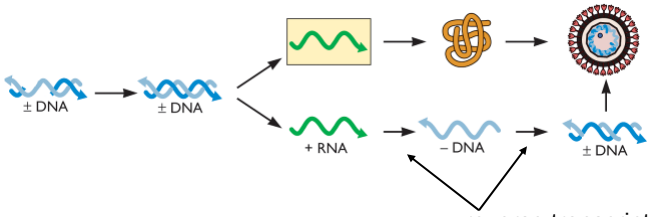
An amazing fact:

The hepadnaviral genomes encode no DNA or RNA polymerases!

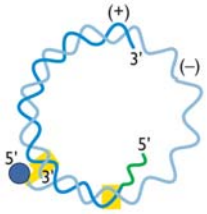
The unusual gapped DNA genome is the <u>product</u> of a curious replication process

*- produced from an RNA template by a viral encoded, **reverse transcriptase** enzyme homologous to that encoded by the retroviral genomes*

Double stranded gapped DNA genomes



reverse transcriptase



hepatitis B virus

Single stranded (ssDNA) genomes

Five viral families and one genus have viruses with ssDNA genomes

Those that include mammalian viruses**:**
- *Circoviridae* and *Parvoviridae*

A basic problem with a ssDNA genome:

RNA can only be made from a double-stranded DNA template, no matter what sense the single stranded DNA

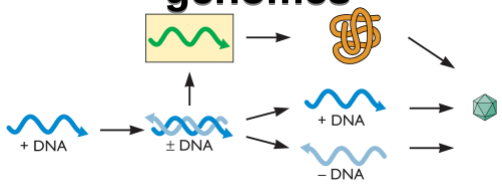
Therefore, ssDNA must be converted into dsDNA BEFORE mRNA is made

- DNA synthesis must precede mRNA production.

These tiny genomes encode NO DNA polymerase!

All replication is accomplished by **cellular DNA polymerases**.

Single stranded (ssDNA) genomes



Circoviridae (1.7–2.2 kb)

TT virus (ubiquitous human virus)

Parvoviridae (4–6 kb)

B19 parvovirus (fifth disease)

RNA genomes

The most abundant type of viral genome on the planet!

Key facts:

- Cells have no RNA-dependent RNA polymerase to replicate the genomes of RNA viruses, or to make mRNA from RNA.
- RNA virus genomes encode novel RNA dependent RNA polymerases
- Polymerases produce BOTH RNA genomes AND mRNA from RNA templates
- The mRNA produced is readable by host ribosomes

Double stranded RNA (dsRNA)

Seven viral families have viruses with dsRNA genomes

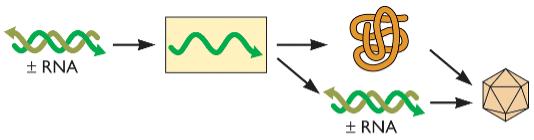
Many dsRNA genomes are segmented

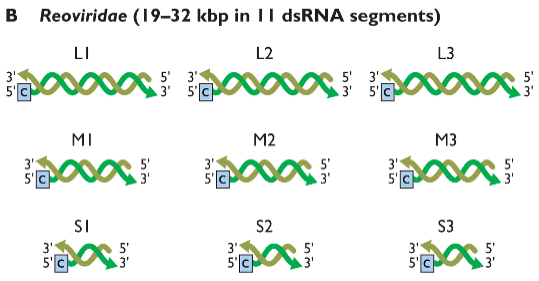
- *Reoviridae have 10-12 separate segments of dsRNA;*
  - include rotaviruses, major agents of human gastroenteritis
- *Birnaviridae have 2 segments; infect vertebrates*

dsRNA cannot be translated by ribosomes

- How does the virus produce mRNA?
- How is template strand selected? (must copy the - strand)

Double stranded RNA (dsRNA)





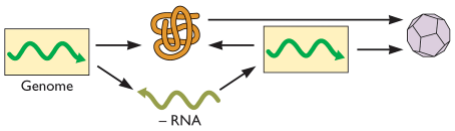
Single stranded RNA (ssRNA): (+) strand RNA

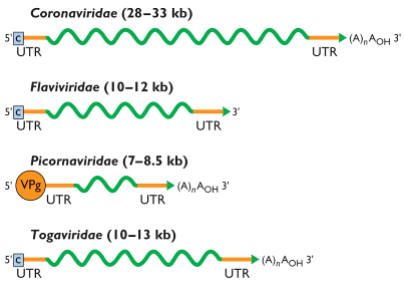
22 viral families have viruses with (+) ssRNA genomes

Eight infect mammals and are significant pathogens:

-*Picornaviridae* (poliovirus)
-*Caliciviridae* (gastroenteritis)
-*Astroviridae* (gastroenteritis)
-*Coronaviridae* (SARS)
-*Arteriviridae*
-*Flaviviridae* (Yellow Fever virus, West Nile virus, hepatitis C virus)
-*Retroviridae* (HIV)
-*Togaviridae* (Rubella virus, encephalitis)

Single stranded RNA (ssRNA): (+) strand RNA





Important fact:

The (+) strand RNA genomes are *translated directly* into protein by host ribosomes

- must be translated before any RNA replication or mRNA synthesis can occur

Single stranded (+) sense RNA with DNA intermediate

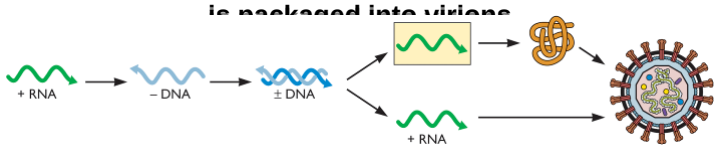
There is one viral family with viruses with (+) ssRNA genomes with DNA intermediate, *Retroviridae*

This family contains two significant human pathogens:

Human immunodeficiency virus
Human T-lymphotropic virus

The retroviral genome strategy is remarkable

RNA is copied into DNA and then back into RNA, some of which is packaged into virions



The +ssRNA in the virion is a real mRNA
- however, it is NEVER used as a message!

Upon infection, it is converted to dsDNA by a virion enzyme called **reverse transcriptase.**

This dsDNA intermediate then integrates into the host DNA and becomes a permanent part of the host genome (a “provirus”)

This “proviral” DNA serves as the template for viral mRNA and genome RNA synthesis

Cellular RNA polymerase copies the proviral DNA to make viral mRNA

- some of the mRNA is translated into viral proteins

- some of the mRNA is packaged into virions

Single strand RNA, (-) sense

Seven virus families have viruses with (-) sense RNA genomes

These families contain some very deadly viruses!

- Mammalian viruses include

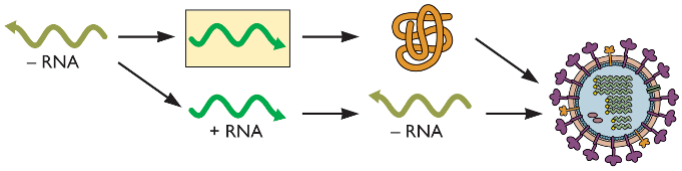
Paramyxoviridae (measles virus, mumps virus)
Rhabdoviridae (rabies virus)
Bornaviridae
Filoviridae (Ebola virus, Marburg virus)
Orthomyxoviridae (influenza virus)

Single strand RNA, (-) sense

These genomes cannot be translated directly into protein

- *must be FIRST copied to make (+) strand mRNA that can be translated*

- *always use a viral encoded, RNA-dependent, RNA polymerase that is found INSIDE the capsid*



Single strand RNA, (-) sense

There are no enzymes in the cell that can produce mRNAs from the RNA genomes of (-) strand RNA viruses

1. This unusual viral RNA dependent RNA polymerase produces functional mRNAs from the (-) strand genome

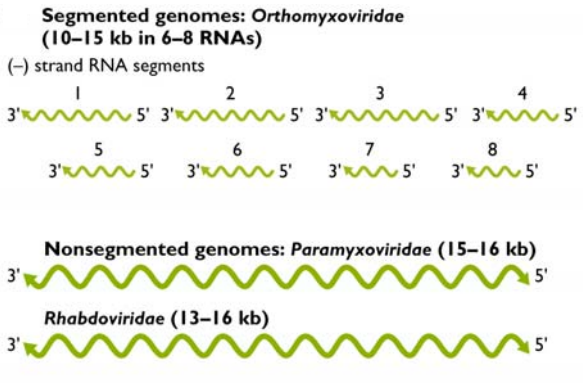
2. It also replicates the genome

- *it produces full length (+) strands that are NOT messages*

- *they are templates for making the genome*

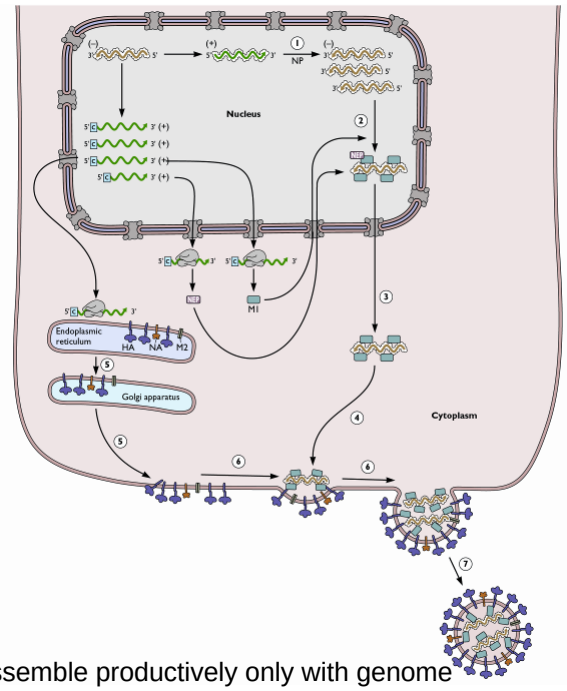
- *these templates are copied to produce (-) strand genomes*

Single strand RNA, (-) sense



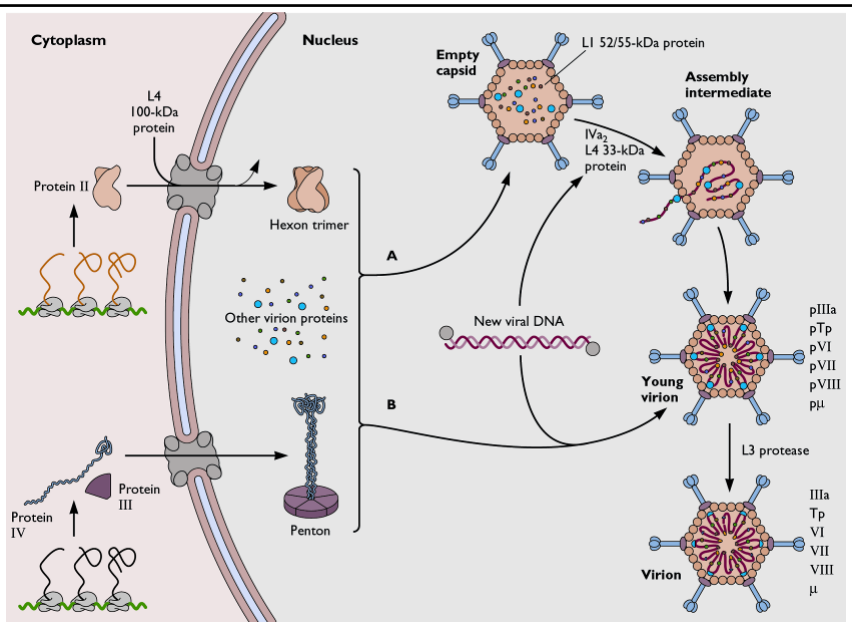
These (-)ssRNA genomes either can be single molecules (non-segmented) or segmented

Virion Assembly



Concerted assembly
Influenza virus

Structural units of shell assemble productively only with genome



Sequential Assembly
Adenovirus

Viral genome is inserted into a preformed shell

Virus release from cells

The majority of viruses leave an infected cell by one of two general mechanisms:

- release into the external environment upon budding from, or lysis of, the cell,
- move directly into a new host cell without physical release of particle (so-called “cell-cell” spread)

Extracellular and cell-to-cell spread

Extracellular spread

Cell-to-cell spread

Both extracellular and Cell-to-cell spread