Introduction to Virology II

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Double stranded DNA (dsDNA)

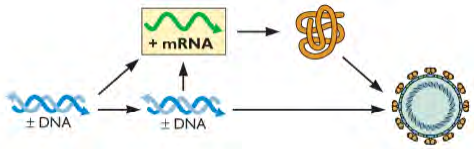
22 families of viruses have viruses with dsDNA genomes

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

Information extraction (making mRNA):

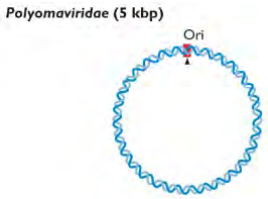
- mRNA is produced when host or viral DNA-dependent RNA polymerase copies the (-) strand.
- CANNOT make mRNA from ssDNA
- Can only make mRNA from dsDNA

Double stranded DNA (dsDNA) genomes

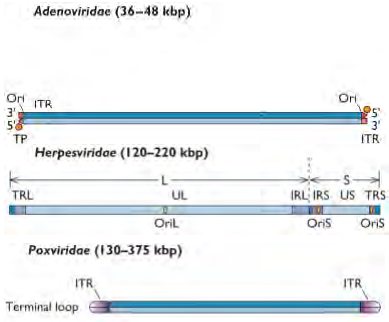


Genomes use host DNA polymerase

Genomes encode DNA polymerase



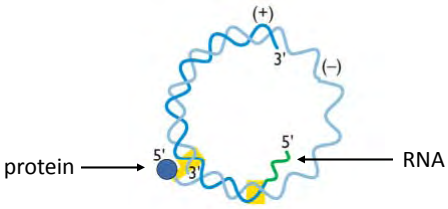
Papillomaviridae (8 kbp)



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

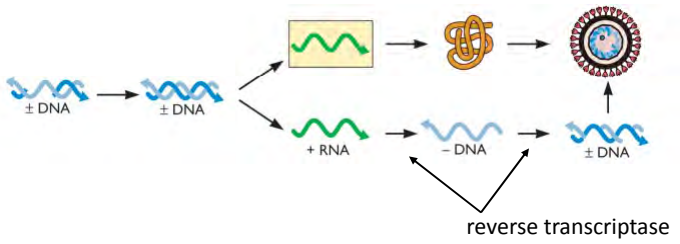
This repair process must precede mRNA synthesis.

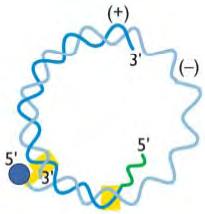
An amazing fact:

The 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





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

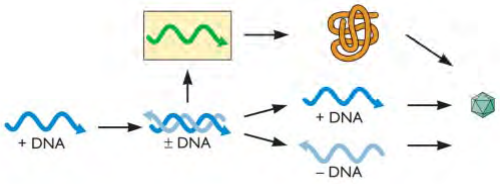
Therefore, ssDNA must be converted into dsDNA BEFORE mRNA is made

- 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

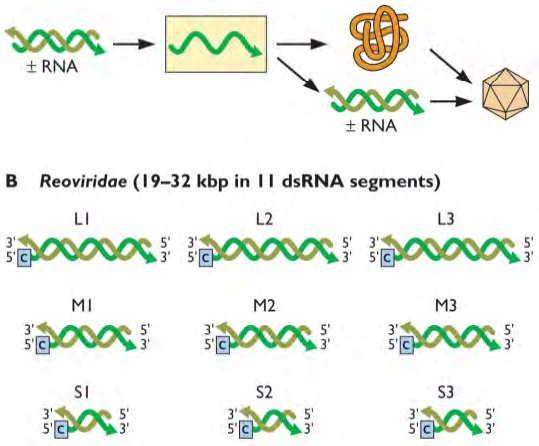
Many dsRNA genomes are segmented

- *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)



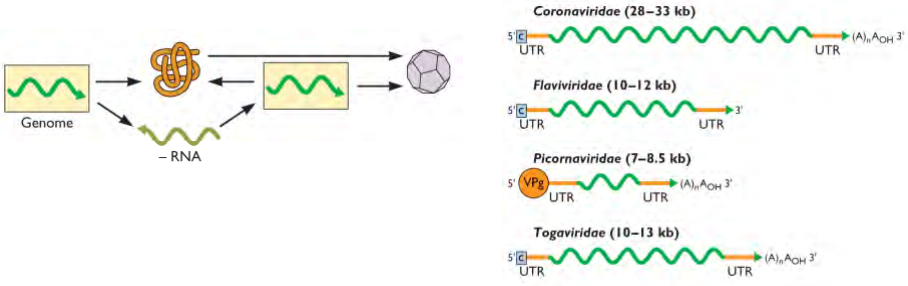
Single stranded RNA (ssRNA): (+) strand RNA

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

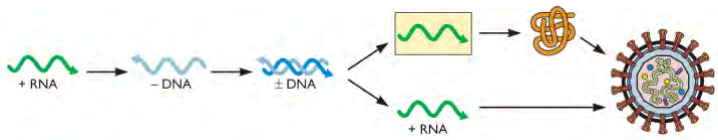
There is one viral family with viruses with (+) ssRNA genomes with DNA intermediate, *Retroviridae*

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

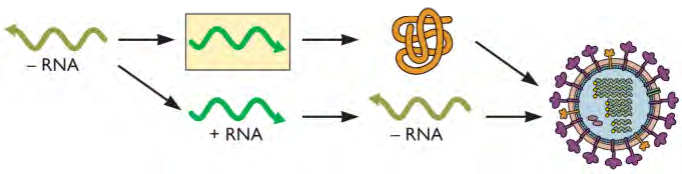
Paramyxoviridae (measles virus, mumps virus)
Rhabdoviridae (rabies virus)
Bornaviridae
Filoviridae (Ebola virus, Marburg virus)
Orthomyxoviridae (influenza virus)

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

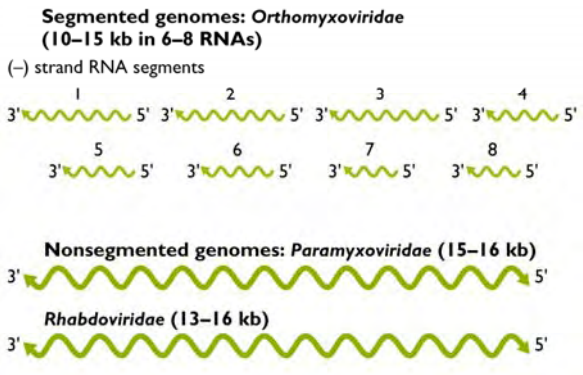
2. It also replicates the genome

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

- they are templates for making the genome

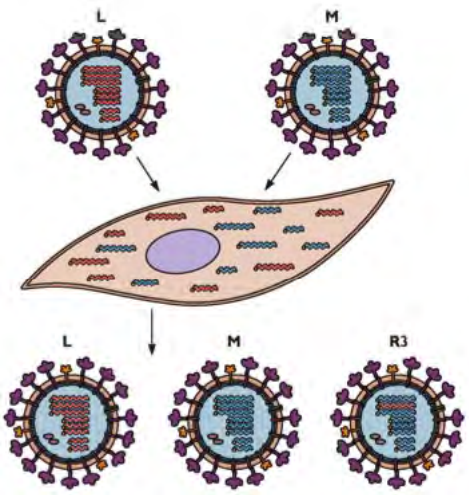
- these templates are copied to produce (-) strand genomes

Single strand RNA, (-) sense



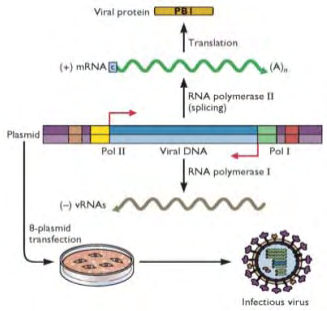
These (-)ssRNA genomes either can be single molecules (non-segmented) or segmented

Reassortment – a consequence of segmented genome



Infectious DNA clones

- DNA viruses
  - polyomavirus, papillomavirus, adenovirus, herpesvirus genomes cloned in bacterial vectors
- RNA viruses
  - achieved for (+), (-) strand, dsRNA viruses



Recovery of 1918 influenza virus

- Influenza virus was not identified until 1933
- In 2005, influenza RNA was isolated from formalin-fixed, paraffin-embedded lung tissue sample from autopsy of victim of influenza in 1918
- 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 was recovered by transfection of cells with 8 plasmids containing genome sequences

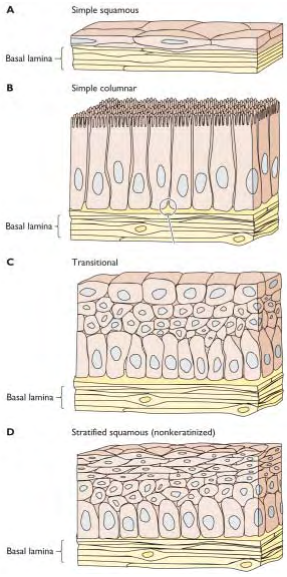
In vivo viral infections usually begin at exposed epithelial surfaces

Three topological surfaces:

Apical: presented to outside ("top")

Basal: presented to inside ("bottom")

Lateral: side-to-side cell contacts



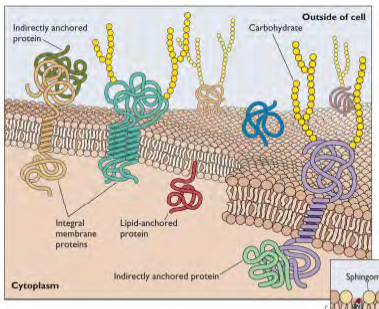
Simple Squamous: thin cells, blood vessels

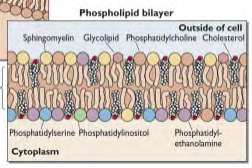
Simple columnar: thick, mucous secreting; GI tract

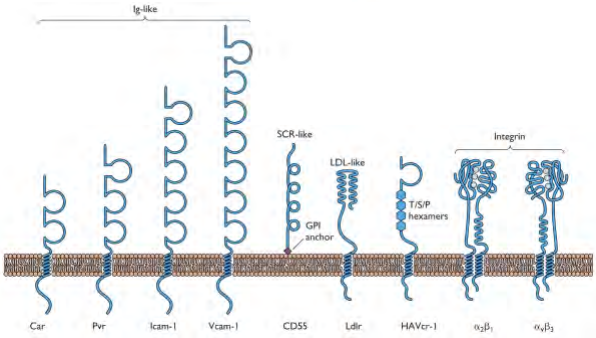
Transitional: Distinct layers: expandable

Stratified Squamous: Epidermal layer of skin, mouth, genitals

The plasma membrane

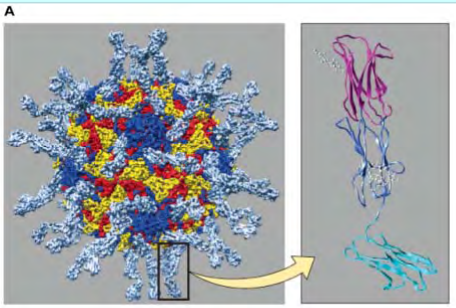


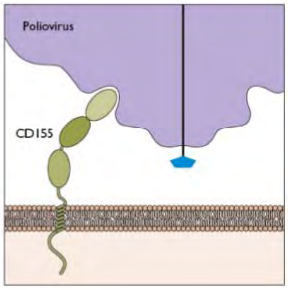


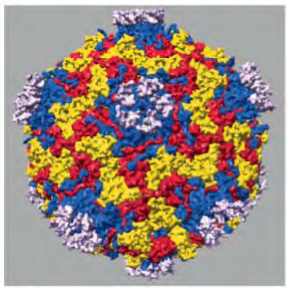


Some virus receptors

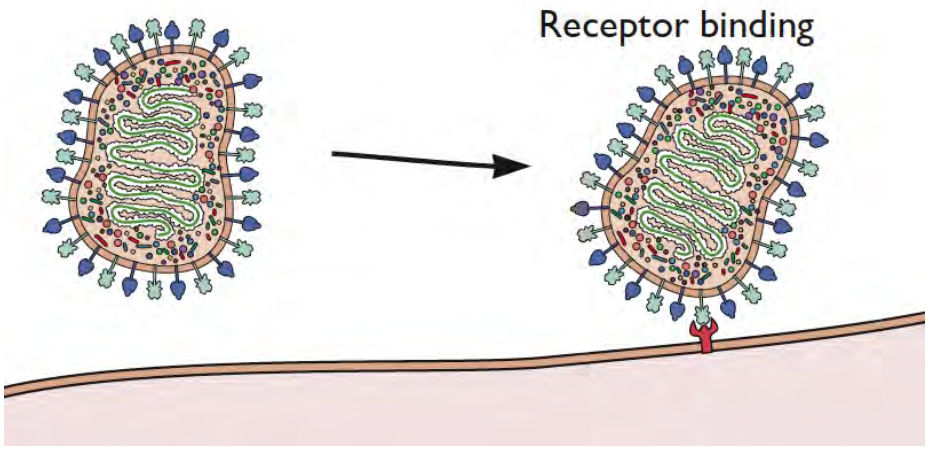
Attachment of icosahedral capsids to cell receptor: poliovirus and rhinovirus



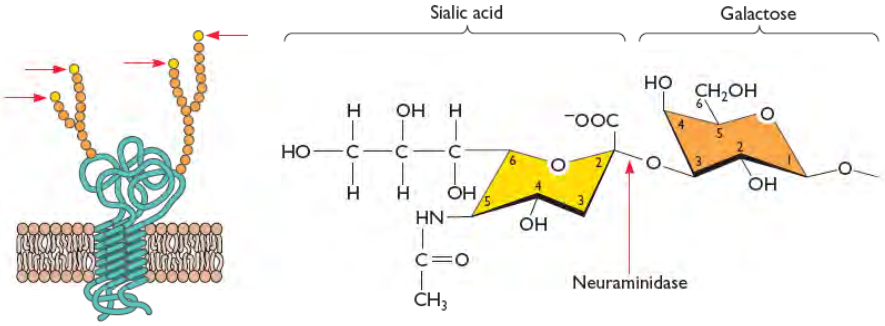




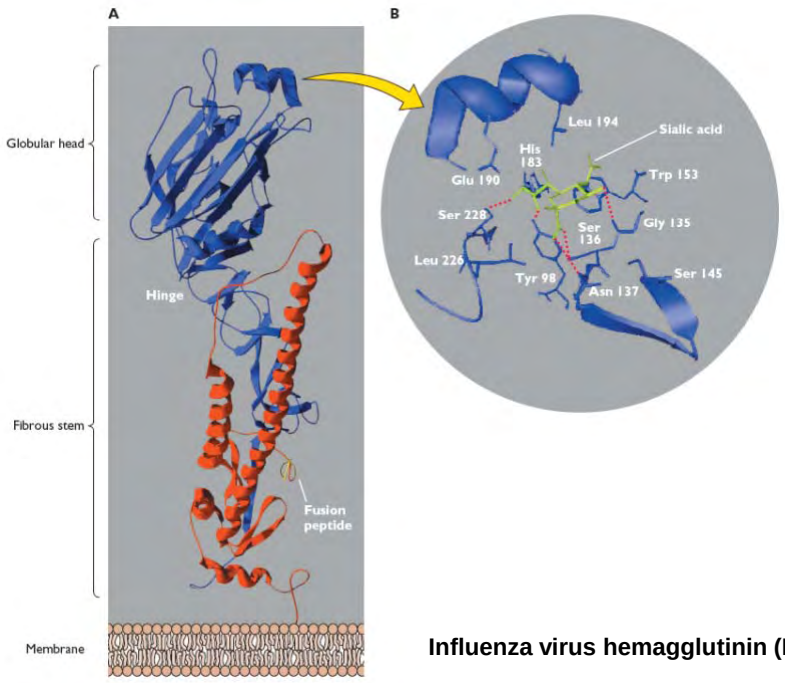
Enveloped viruses: receptor attachment is mediated by a viral glycoprotein



Sialic acid: cell receptor for influenza viruses

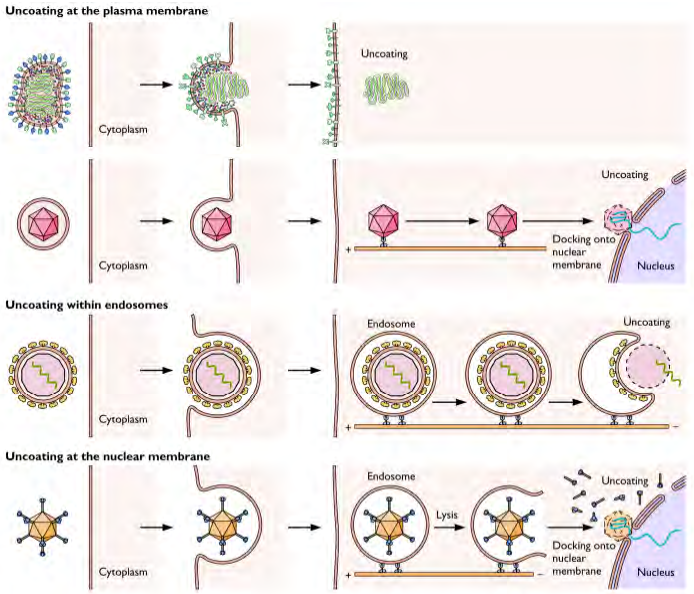


- α(2,3) shown is the major SA in the human respiratory tract
- α(2,6) SA bound preferentially by avian influenza viruses

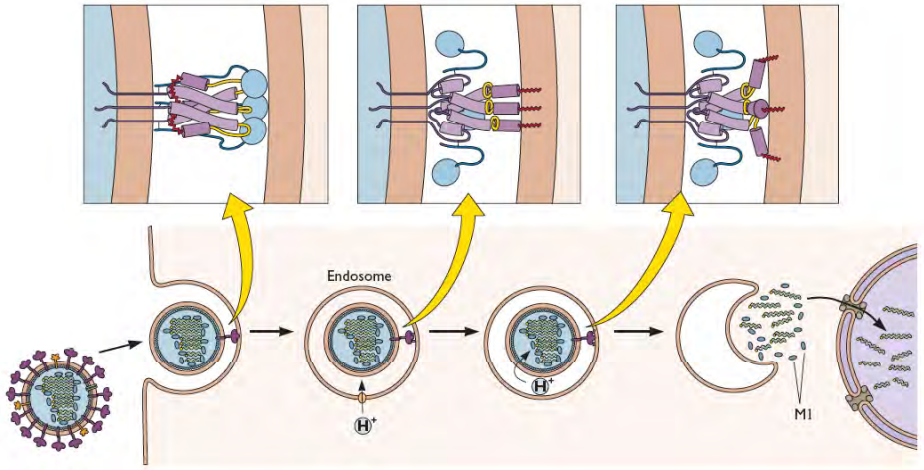


Influenza virus hemagglutinin (HA)

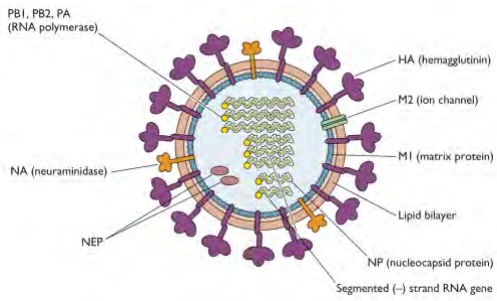
Three uncoating strategies

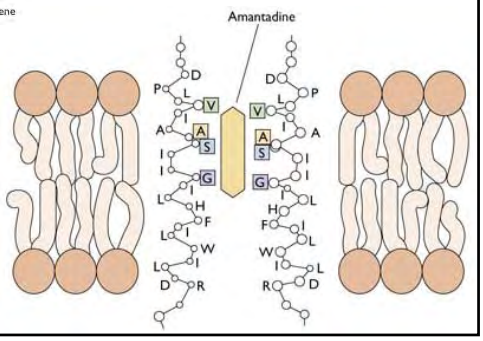


Influenza virus uncoating

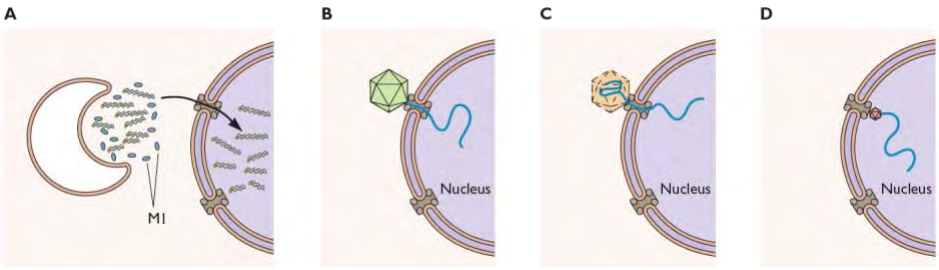


Uncoating: the target of the antiviral amantadine/rimantadine

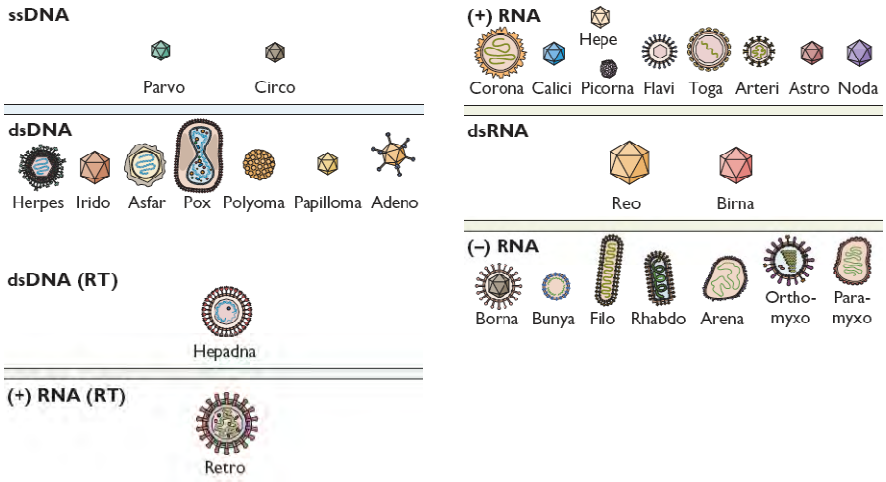




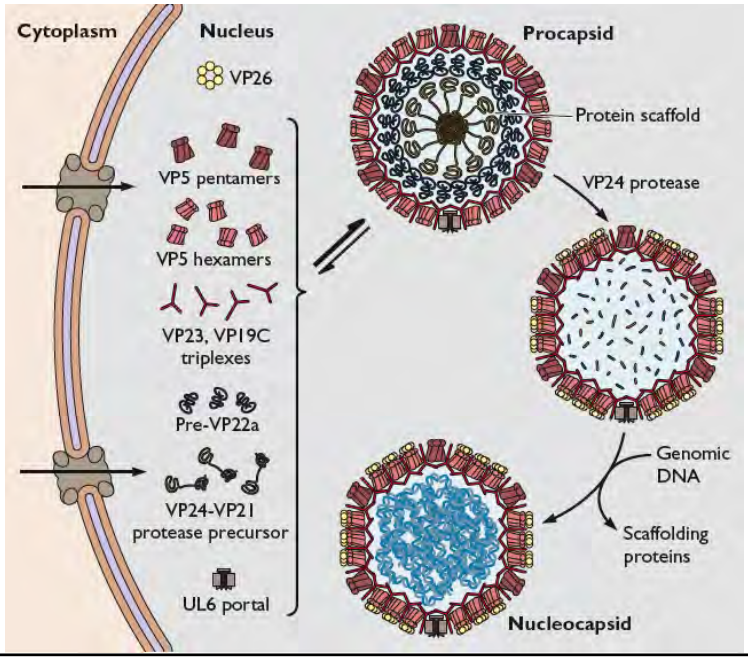
Strategies for entry of viral genomes into the cell nucleus



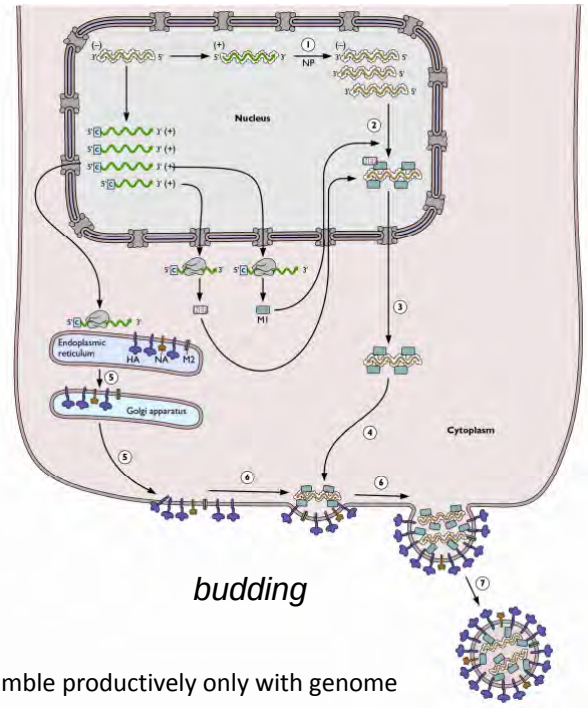
The structure of a virus determines the nature of the reactions by which it is formed



Sequential assembly – herpes simplex virus



Concerted assembly – influenza virus



Structural units of shell assemble productively only with genome

Virus release from cells

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

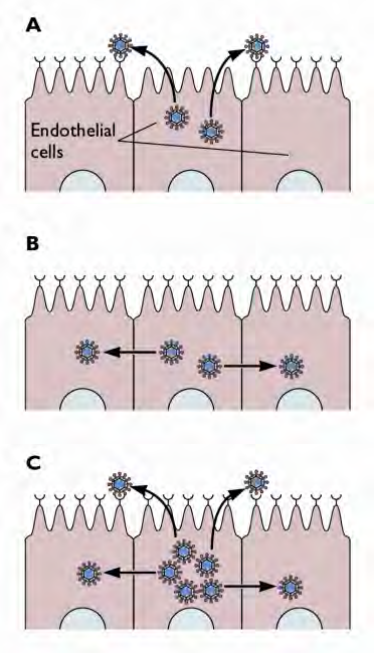
- release into the external environment upon budding from, or lysis of, the cell,
- move directly into a new host cell without physical release of particle (so-called "cell-cell" spread)

Extracellular and cell-to-cell spread

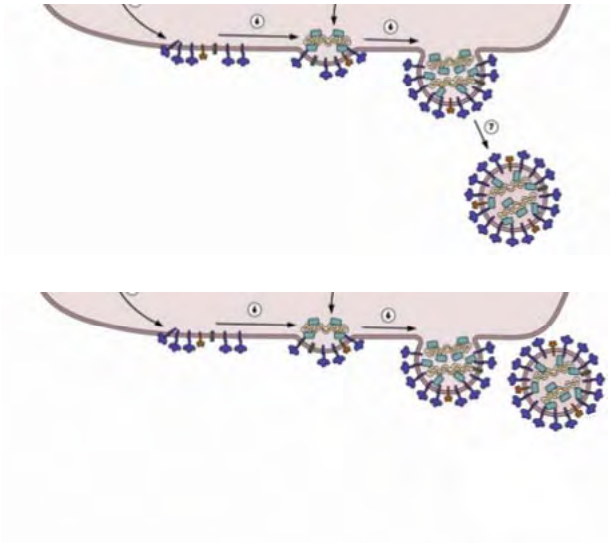
Extracellular spread

Cell-to-cell spread

Both extracellular and Cell-to-cell spread



Function of NA in viral release from cells



How inhibitors of NA (Tamiflu) work