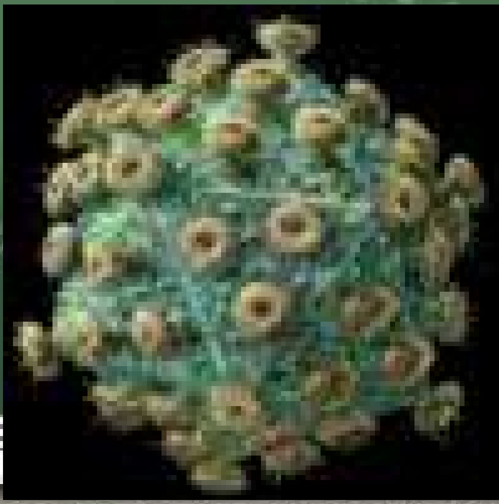
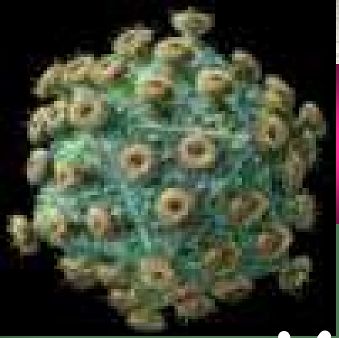
VIROLOGY

Prof. NAGWA Mohamed Amin AREF

511MICRO





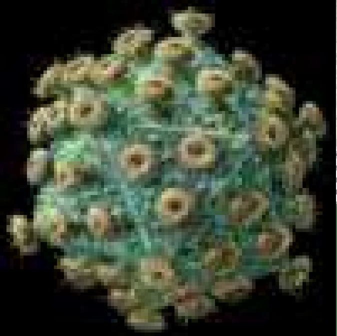
Introduction

Virus :

- a set of genes
- composed of either DNA or RNA
- packages in a protein containing coat

The resulting particle → Virion

Virus reproduction : virus particle infect a cell & program the cellular machinery to synthesize the constituents required for the assembly of new virions → considered an intracellular parasite

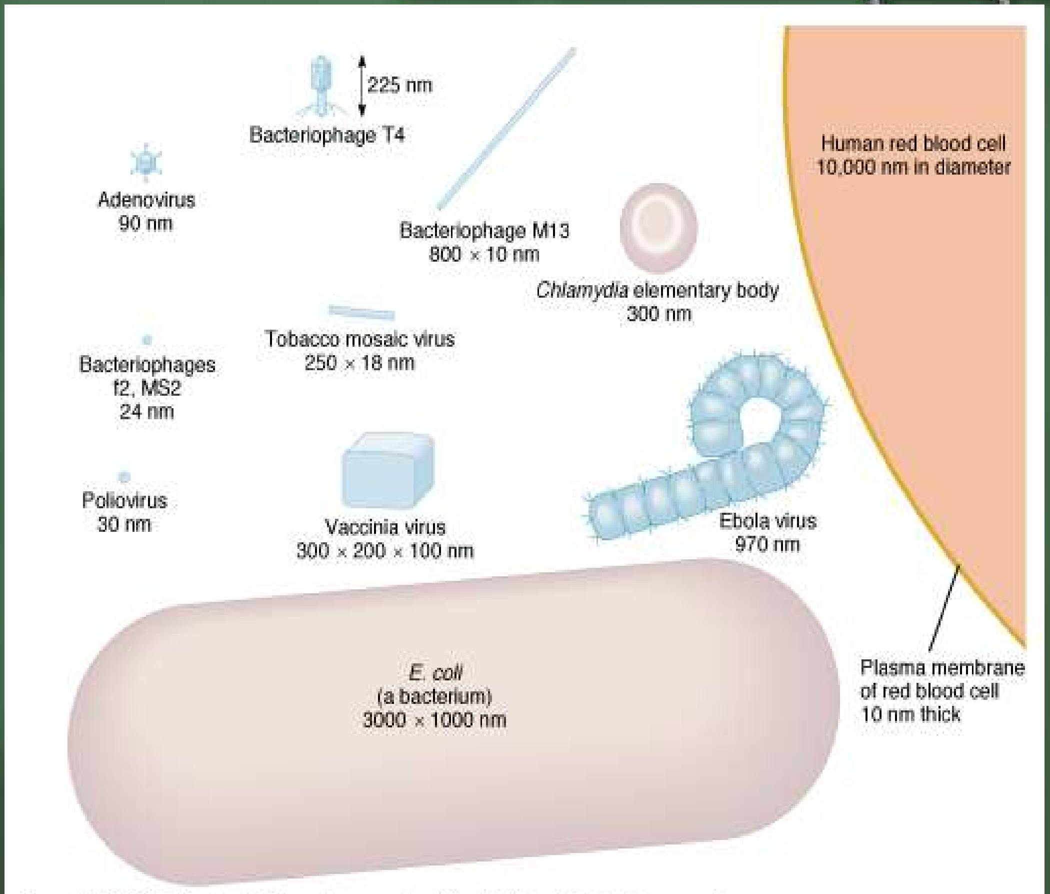


- Different viruses can have very different genetics structures → reflected in their replicative strategies
- Because of their small size, viruses have achieved a very high degree of genetic economy
- Viruses depend to great extent on host cell functions → difficult to combat medically They do exhibit unique steps in their replicative cycles that are potential targets for antiviral therapy

CHARACTERISTICS

- Small, ranging from about 20 – 300 nm diameter
- Totally depend upon a living cell, either eukaryotic or prokaryotic, for replication and existence
- Some viruses possess complex enzyme of their own : RNA or DNA polymerases but they cannot amplify & reproduce the information in their own genomes without assistance
- Have a component a receptor-binding protein for attaching to cells

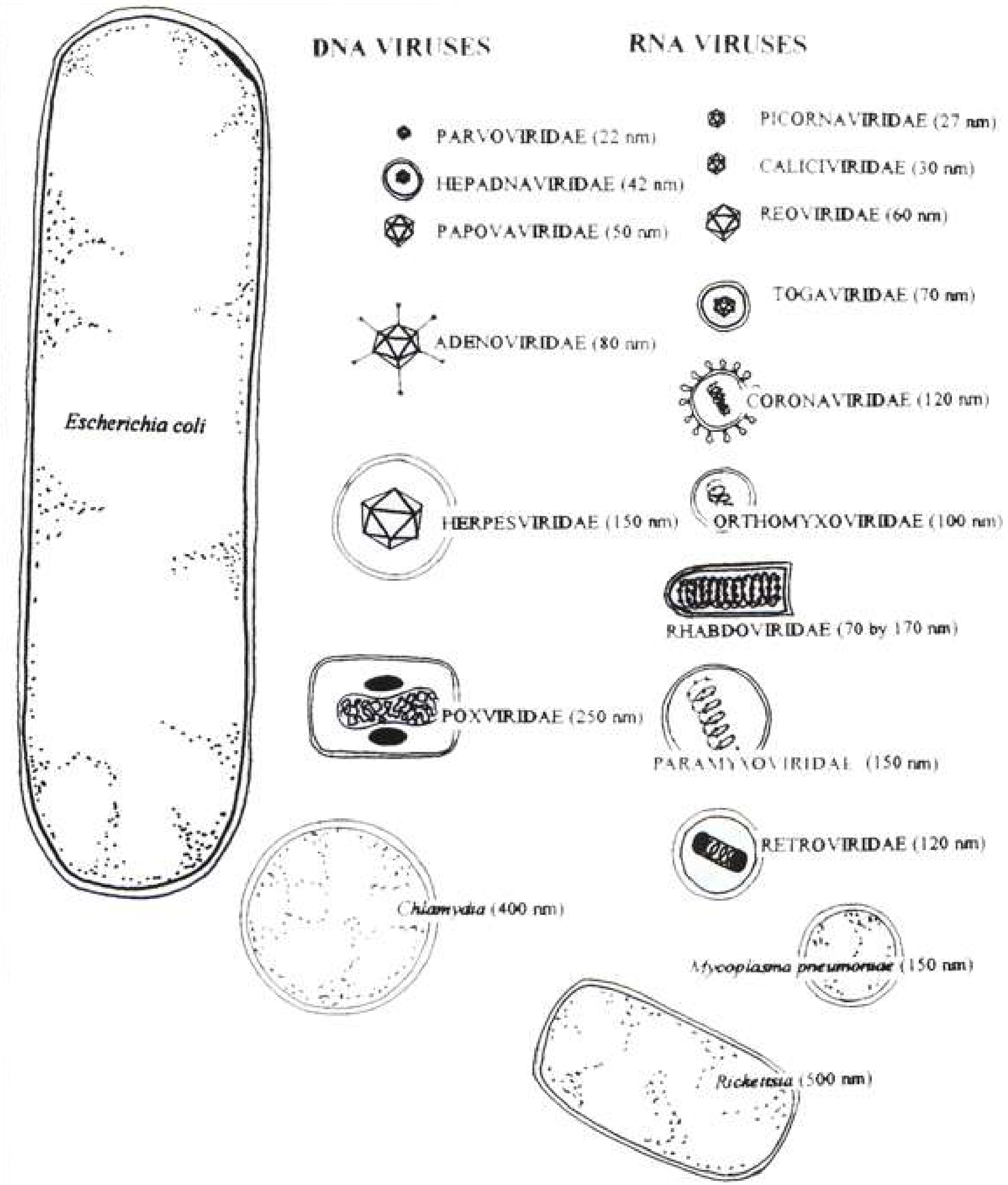
Viral Size

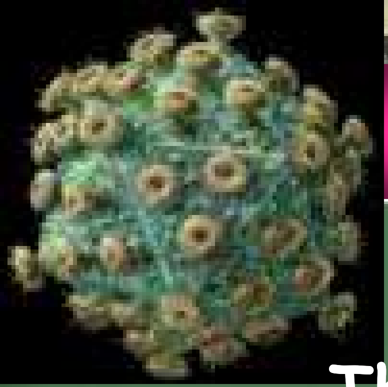


- Length 20 - 14,000 nm
- Much smaller than bacteria - therefore filterable (infectious agent that could pass through porcelain filter; many originated in animals)

CHARACTERISTICS

- To see the virus → electron microscope
- Growth → need living cells/ tissues
- Can not growth saprophytic
- Only have certain enzyme for metabolism and energy
- Easy mutated → changes antigenic property
- Multiplication different from bacteria


DNA VIRUSES
RNA VIRUSES
PARVOVIRIDAE (22 nm)
HEPADNAVIRIDAE (42 nm)
PAPOVAVIRIDAE (50 nm)
ADENOVIRIDAE (80 nm)
HERPESVIRIDAE (150 nm)
POXVIRIDAE (250 nm)
PICORNAVIRIDAE (27 nm)
CALICIVIRIDAE (30 nm)
REOVIRIDAE (60 nm)
TOGAVIRIDAE (70 nm)
CORONAVIRIDAE (120 nm)
ORTHOMYXOVIRIDAE (100 nm)
RHABDOVIRIDAE (70 by 170 nm)
PARAMYXOVIRIDAE (150 nm)
RETROVIRIDAE (120 nm)
Escherichia coli
Chlamydia (400 nm)
Mycoplasma pneumoniae (150 nm)
Rickettsia (500 nm)



Virus structure & Morphology

The basic design of all viruses places the nucleic acid genome on the inside of a protein shell

→ capsid

Two basic types of virions :

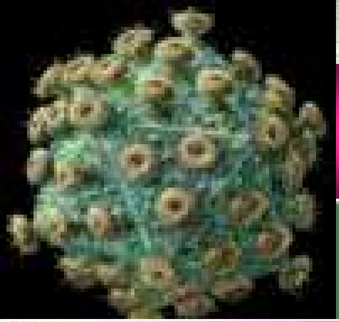
1. Enveloped viruses

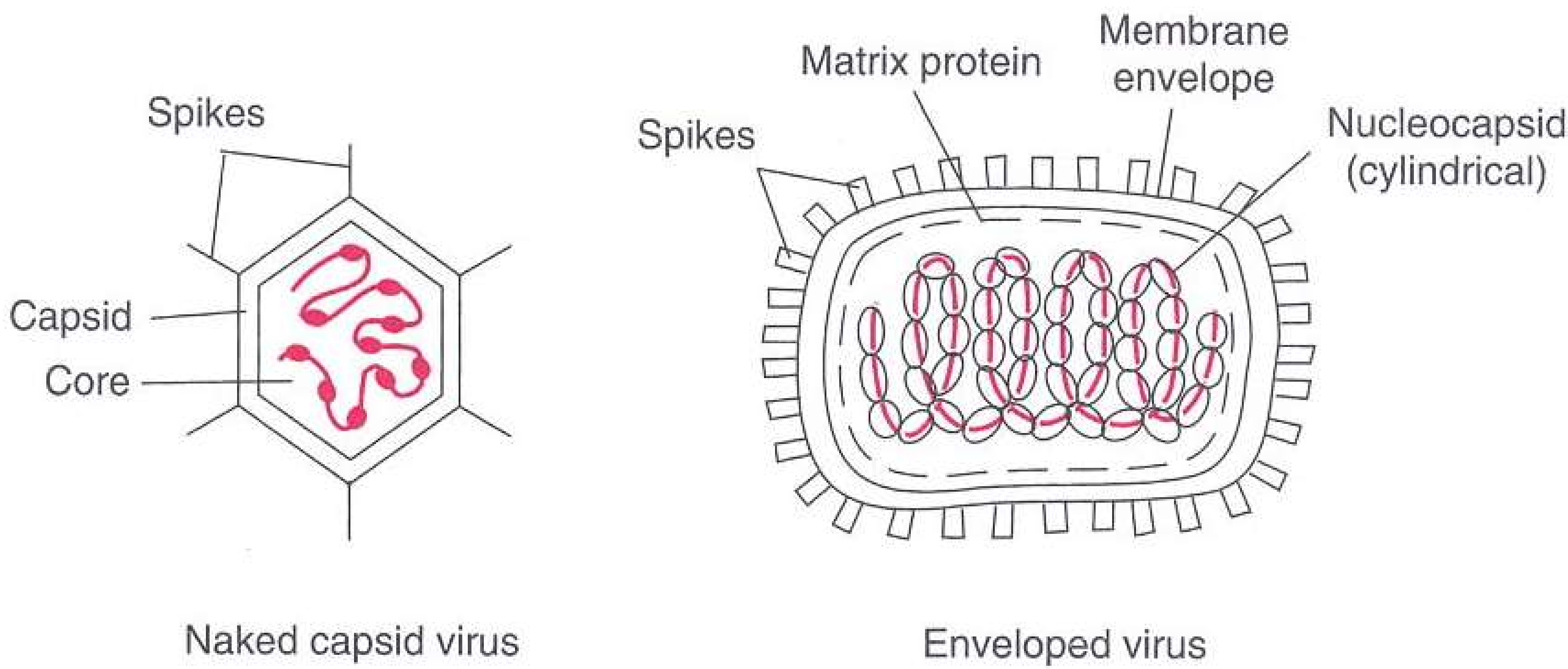
→ have a nucleocapsid of nucleic acid complexed to protein

2. Naked capsid viruses

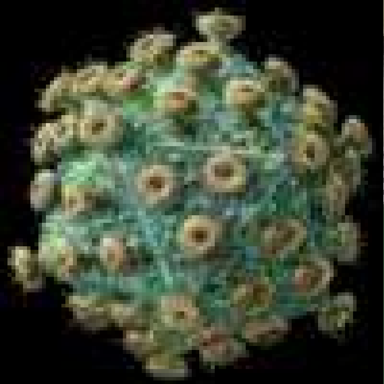
→ have a nucleic acid genome within a protein shell

Virus structure & Morphology





Schematic drawing of two basic type of virions



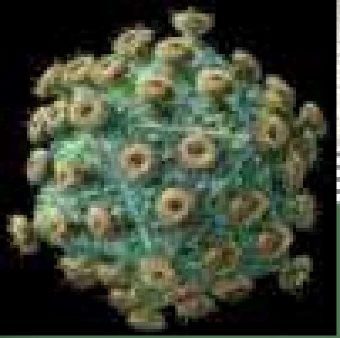
Two basic shapes of virions :

1. Cylindrical
2. Spherical

Some bacteriophages combine those 2 basic shapes

Functions of capsid or envelope of viruses :

1. To protect the NA genome from damage during extra-celullar passage of the virus from one cell to another
2. To aid in the process of entry into the cell
3. To package enzymes essential for the early steps of the infection process



Basic viral forms

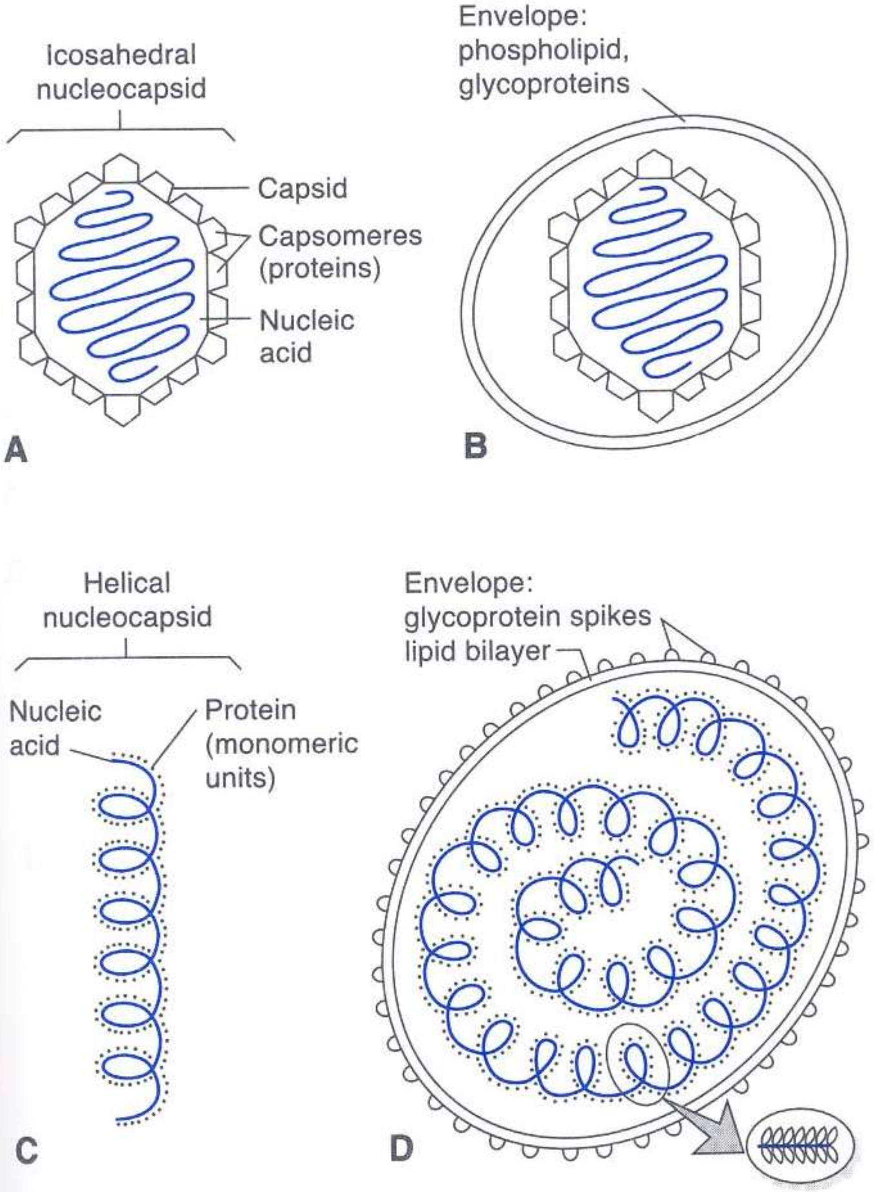
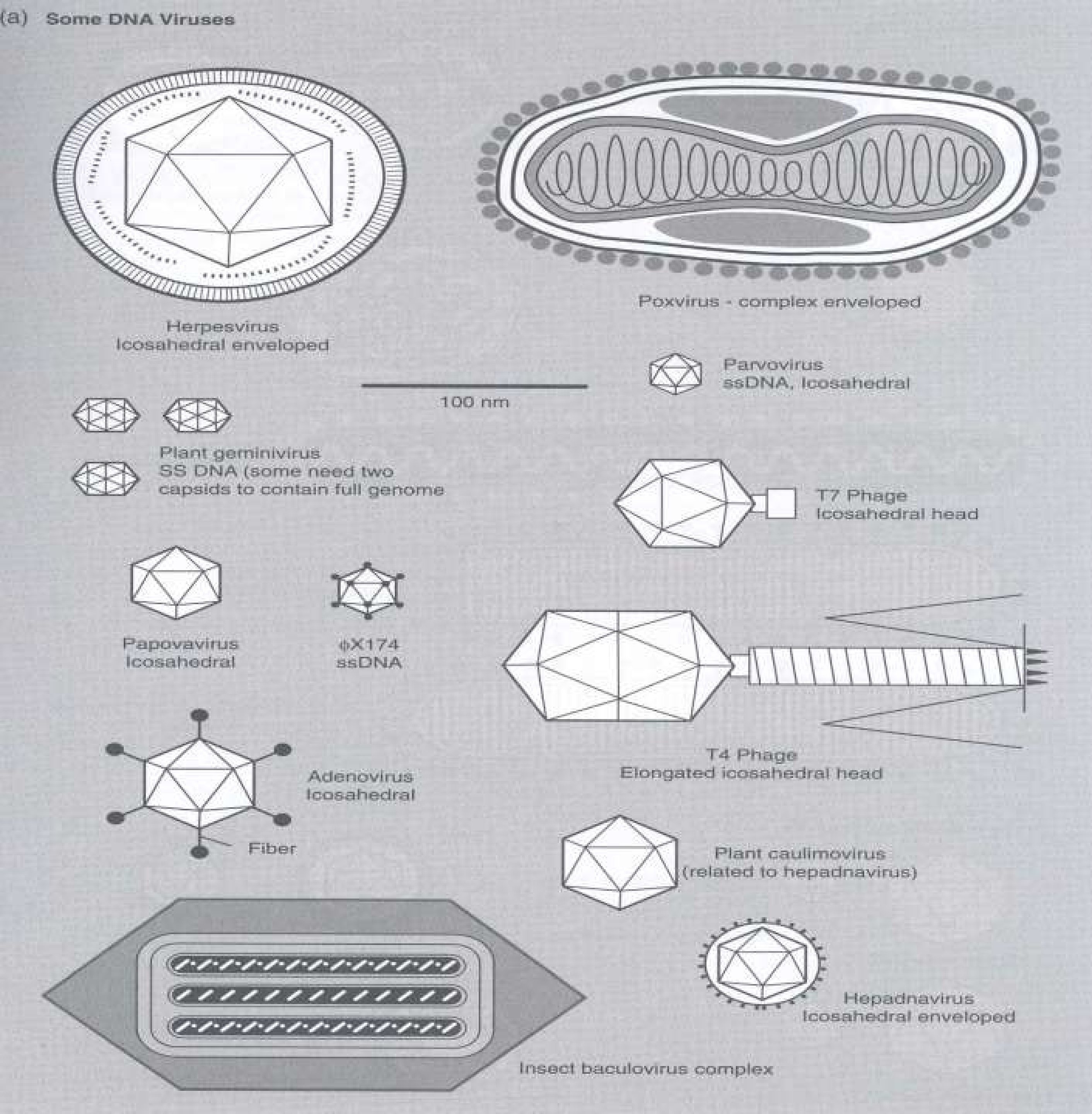
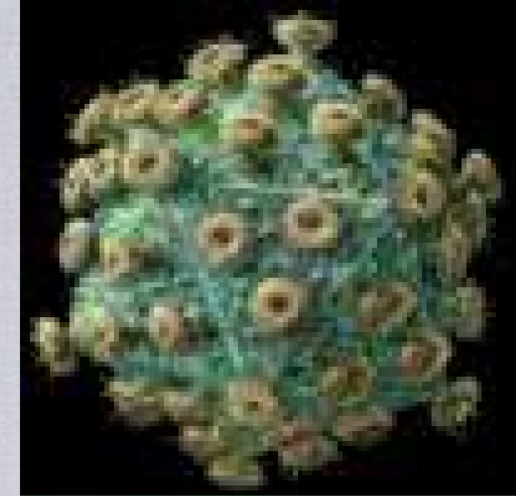
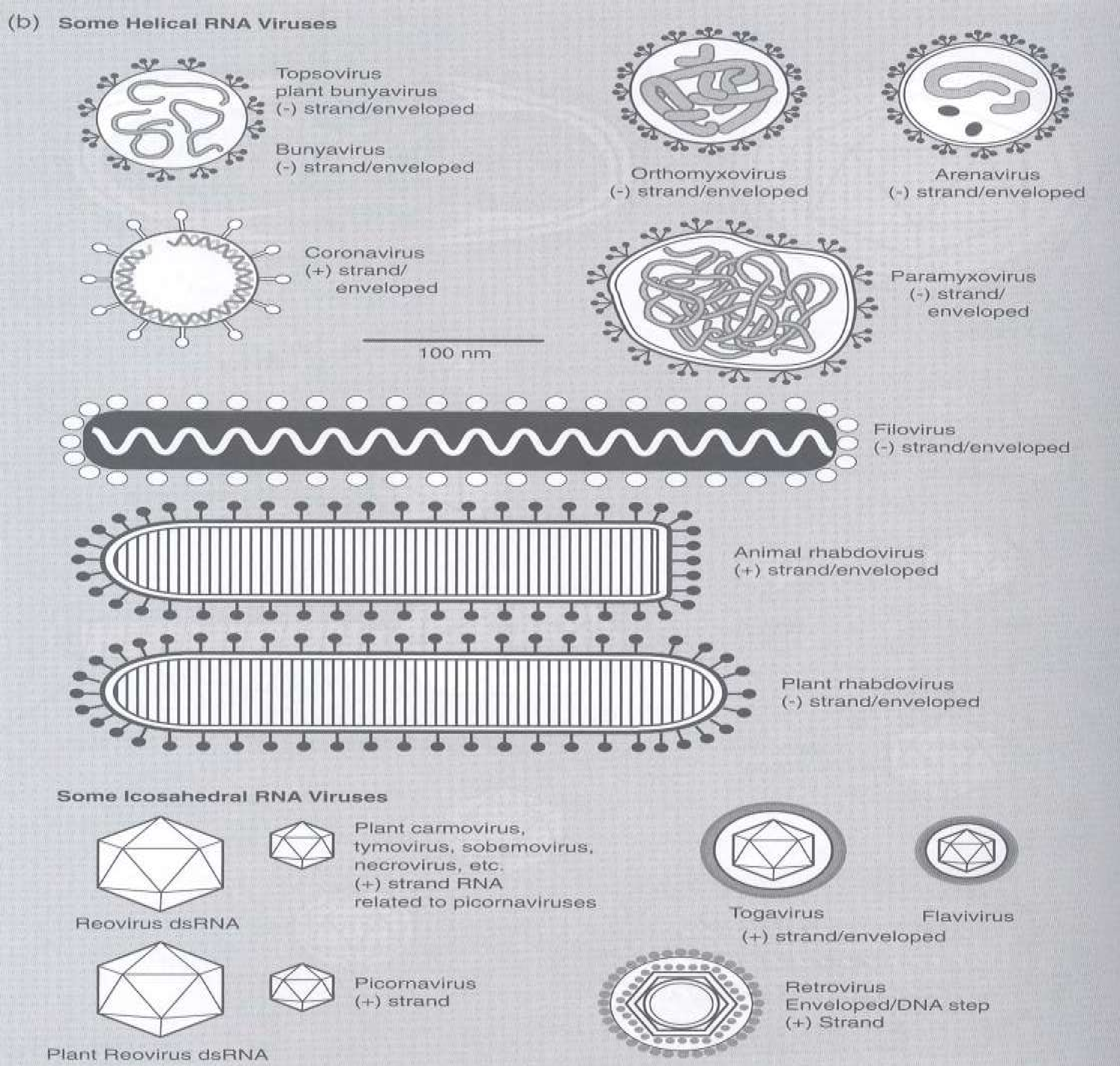


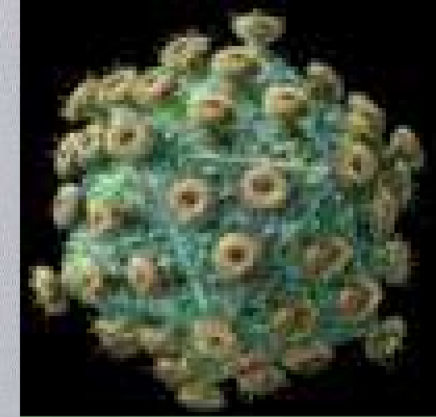
Figure 31.2. Basic viral forms. **A.** Icosahedral, nonenveloped; **B.** Icosahedral, enveloped; **C.** Helical, nonenveloped; **D.** Helical, enveloped. Inset depicts the nucleic acid–protein association.





The structure and relative sizes of a number of DNA





The structure and relative sizes of a number of RNA

DNA VIRUSES

ENVELOPED

- **Double – stranded**
  - Icosahedral
    - HERPES
    - HEPADNA
  - Complex
    - POX

NAKED

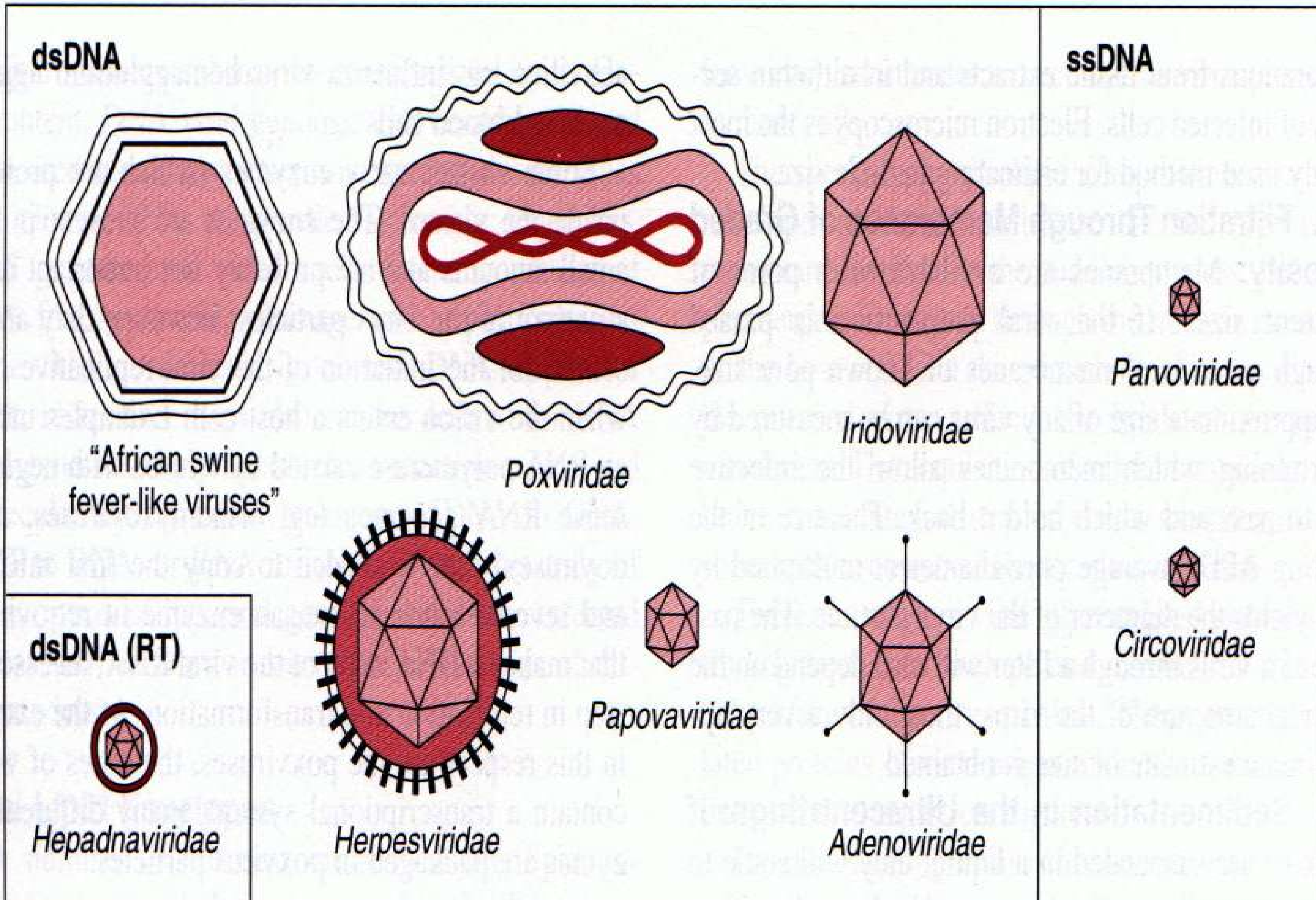
- **Double – stranded**
  - Icosahedral
    - PAPOVA
    - ADENO
- **Single – stranded**
  - Icosahedral
    - PARVO

RNA VIRUSES

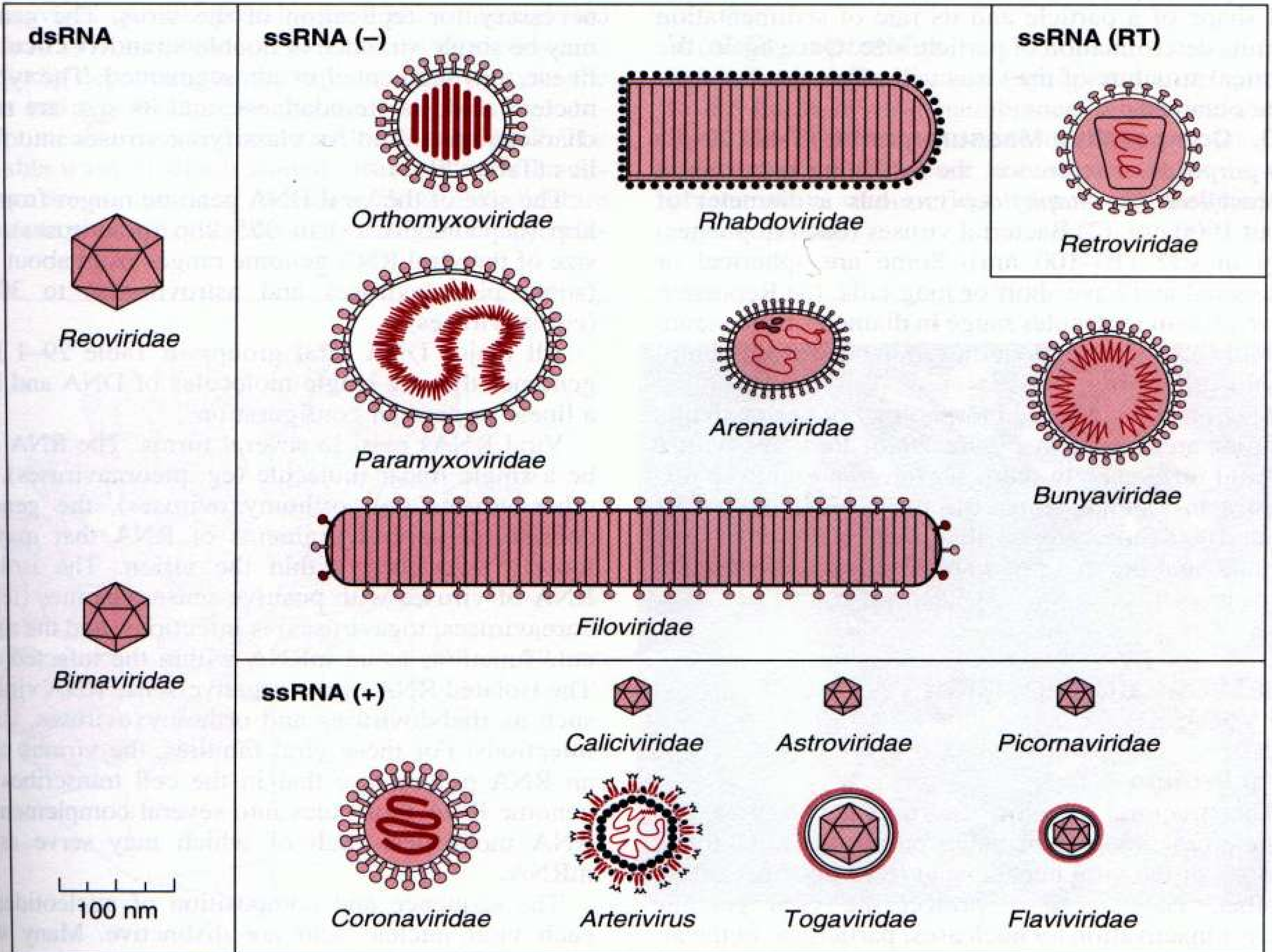
- Single – stranded
  - Positive – stranded (+)
    - Naked
      - *PICORNA
      - *CALICI
    - Enveloped
      - *TOGA
      - *FLAVI
      - CORONA
      - RETRO
  - Negative – stranded (-)
    - Enveloped
      - BUNYA
      - ORTHOMYXO
      - PARAMYXO
      - RHABDO
      - ARENA
      - FILO
- Double – stranded
  - Naked
    - *REO

* Icosahedral; all of the rest have helical symmetry

DNA viruses


dsDNA
"African swine fever-like viruses"
Poxviridae
Iridoviridae
ssDNA
Parvoviridae
Circoviridae
dsDNA (RT)
Hepadnaviridae
Herpesviridae
Papovaviridae
Adenoviridae

RNA viruses


dsRNA
Reoviridae
Birnaviridae
100 nm
ssRNA (–)
Orthomyxoviridae
Rhabdoviridae
Paramyxoviridae
Arenaviridae
Filoviridae
ssRNA (RT)
Retroviridae
Bunyaviridae
ssRNA (+)
Caliciviridae
Astroviridae
Picornaviridae
Coronaviridae
Arterivirus
Togaviridae
Flaviviridae

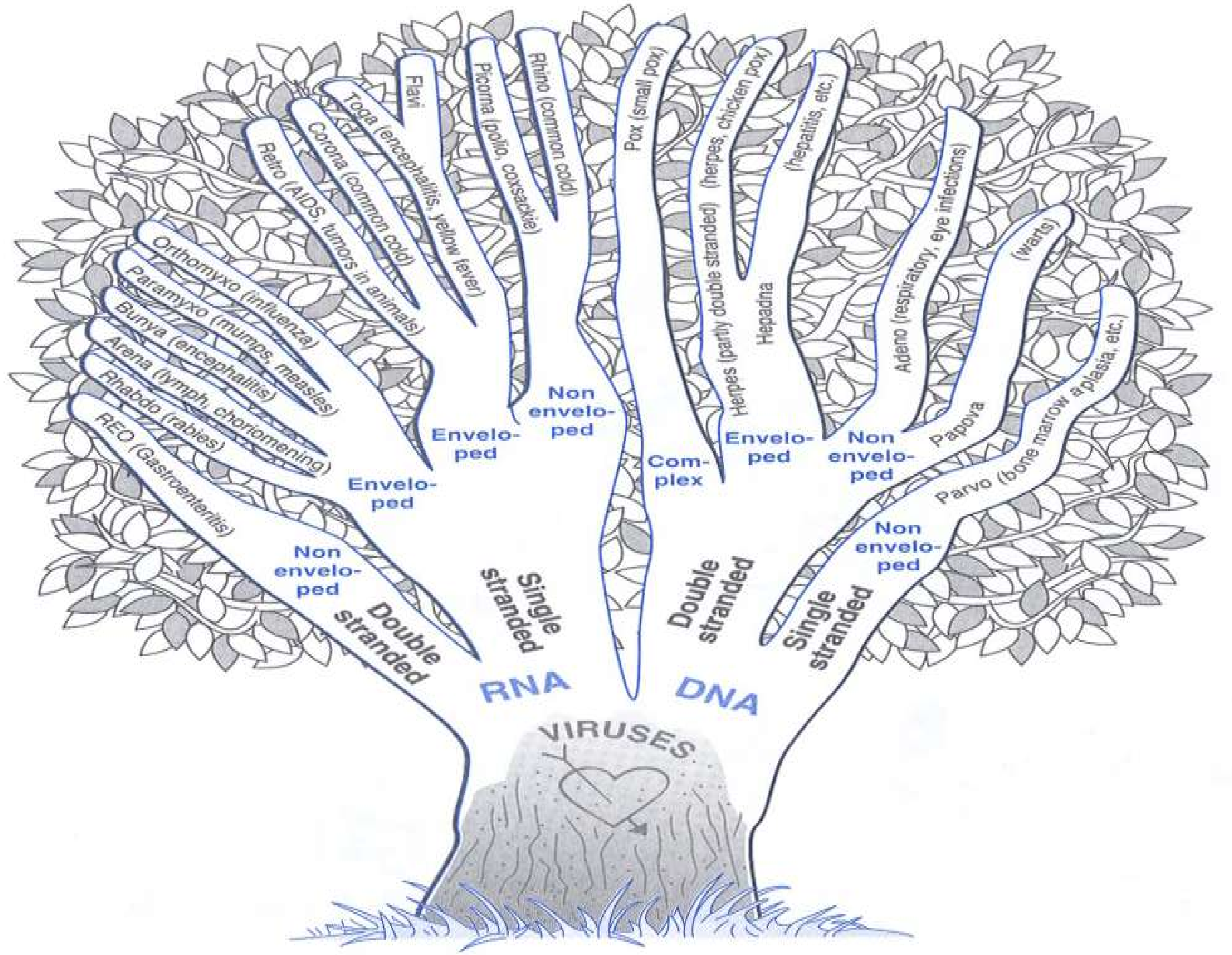
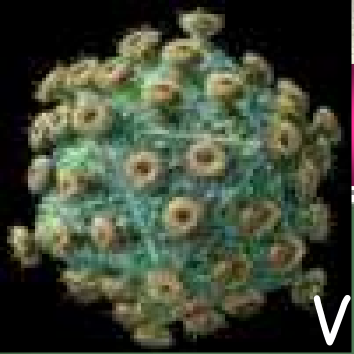


Figure 31.4. The main groups of human viruses. This is a practical representation and does not represent phylogenetic relationships.

The main groups of human viruses

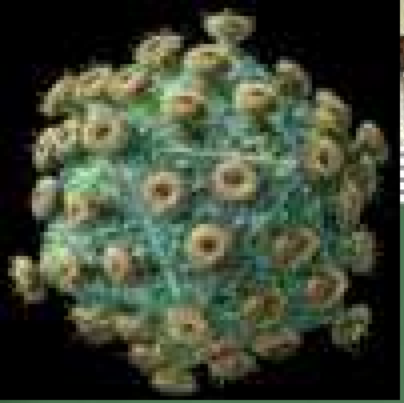


VIRAL REPLICATION

Virus multiplication cycle consist of 6 phases :

1. Attachment/ adsorption to the host cell
2. Penetration or entry
3. Uncoating to release the genome
4. Virion component production/ synthesis
5. Assembly/ maturation
6. Release from the cells

- Viral infections maybe productive/lytic response or nonproductive response (lysogeny)
- Lysogeny may be associated with oncogenic transformation by animal viruses



The outcome of the infection depend on :

the particular virus-host combination

other factors :

- the extracelullar environment
- multiplicity of the infection
- physiology & developmental state of the cell

ADSORPTION

Adsorption is the first step in every viral infection.

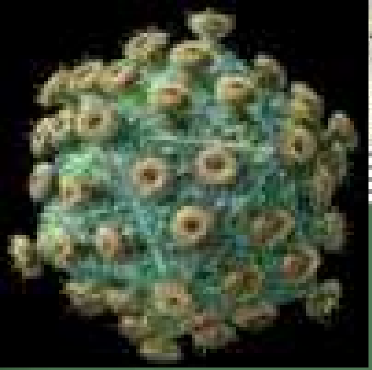
Adsorption involves :
- **virion attachment proteins**
- **cell surface receptor proteins**

Examples of Viral Receptors

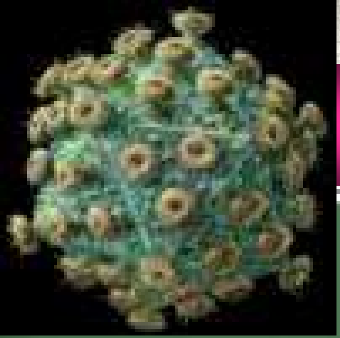
Virus	Receptor	Cellular Function
Influenza A	Sialic acid	Glycoprotein
Reoviruses	Sialic acid	Glycoprotein
	EGF receptor	Signaling
Adenoviruses	Integrins	Binding to extracellular matrix
Epstein–Barr	CR2	Complement receptor
Herpes simplex	Heparan sulfate	Glycoprotein
Human herpes 7	CD4	Immunoglobulin superfamily
HIV	CD4	Immunoglobulin superfamily
	CXCR4 and CCR5	Chemokine receptors
Human coronavirus	Aminopeptidase N	Protease
Human rhinoviruses	ICAM-1	Immunoglobulin superfamily
Measles	CD46	Complement regulation
Poliovirus	PVR	Immunoglobulin superfamily
Rabies	Acetylcholine receptor	Signaling
SV40	MHC I	Immunoglobulin superfamily
Vaccinia	EGF receptor	Signaling

Abbreviations: EGF, endothelial growth factor; HIV, human immunodeficiency virus; ICAM, intercellular adhesion molecule; MHC, major histocompatibility complex; PVR, poliovirus receptor.

Examples of viral receptors



- For some viruses co-receptors are involved in adsorption
  → HIV-1 : CD4 & chemokine receptors
- Viral spikes & phage tails carry attachment proteins
- In some case, a region of the capsid protein serve the function of attachment
- Adsorption is enhanced by presence of multiple attachment & receptor proteins.
- A particular kind of virus is capable to infecting only a limited spectrum of cell types → its host range
  Differences in host range & tissue tropism due to presence or absence of the receptors

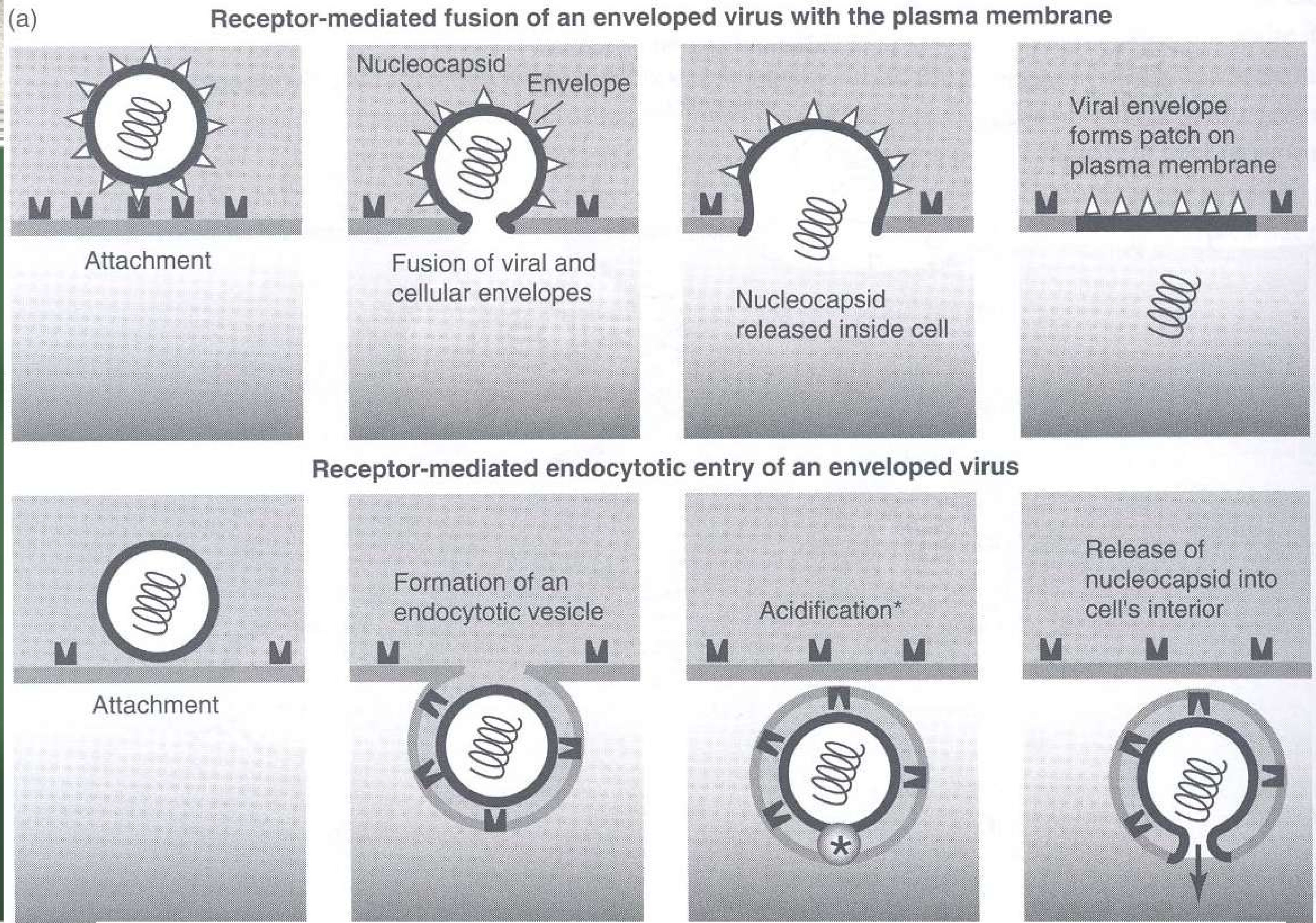


Entry & Uncoating

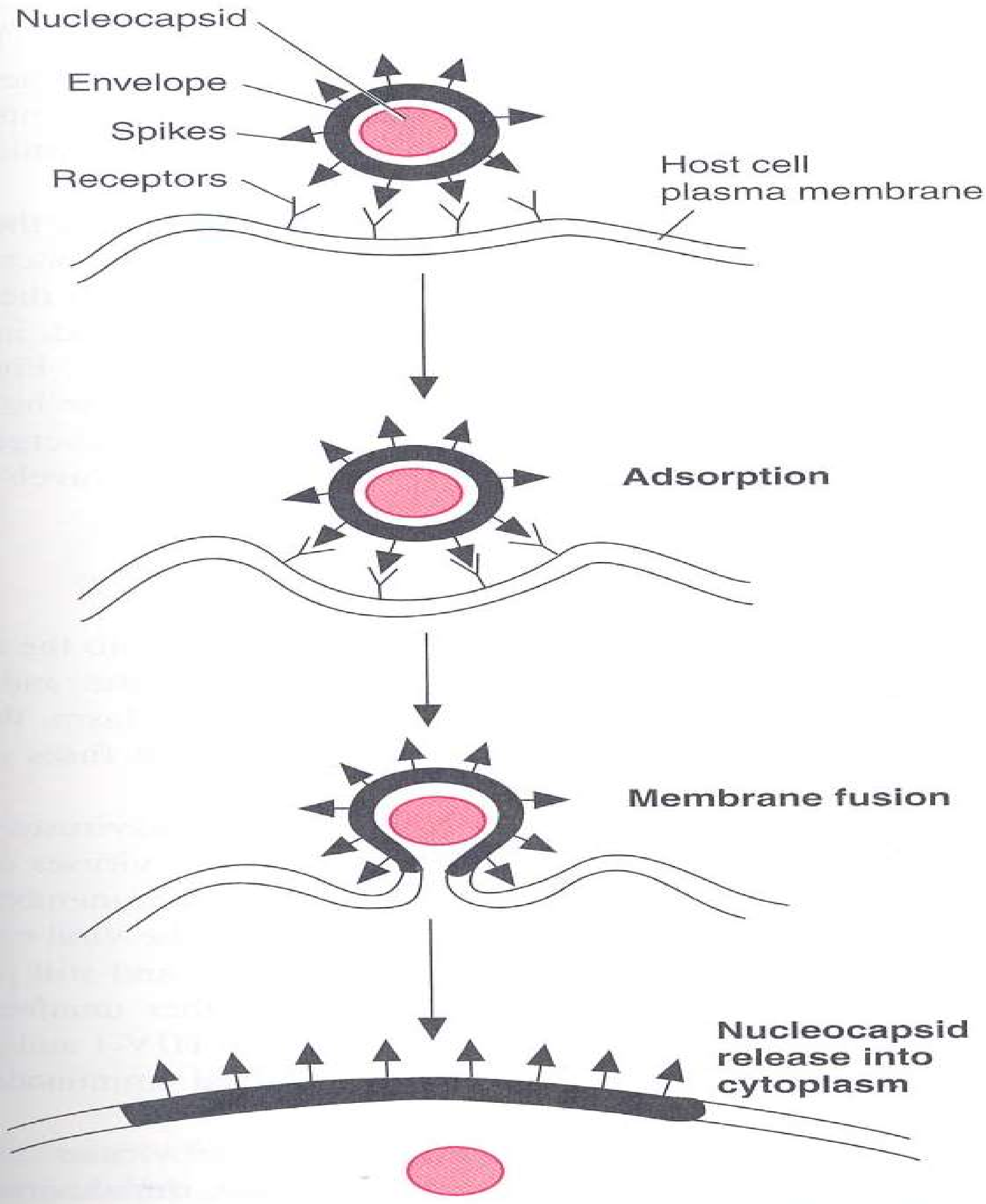
Enveloped Animal Viruses

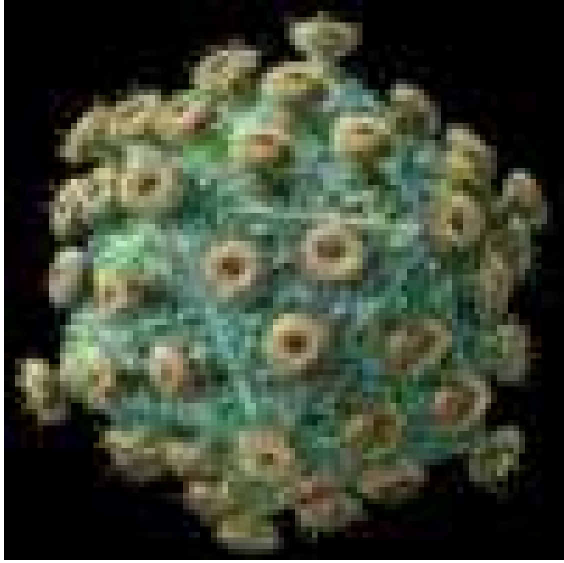
- Some enveloped viruses enter cells by **direct fusion** of plasma membrane & envelope, release the nucleocapsid directly into the cytoplasm.
  → Paramyxoviruses (eg. measles) retroviruses (eg. HIV-1) & herpesviruses

- Other enveloped & naked viruses are taken in by receptor-mediated endocytosis (viropexis).
  → orthomycovirus (eg. influenza viruses), togaviruses (eg. rubella viruses), rhabdoviruses (eg. rabies) & coronaviruses



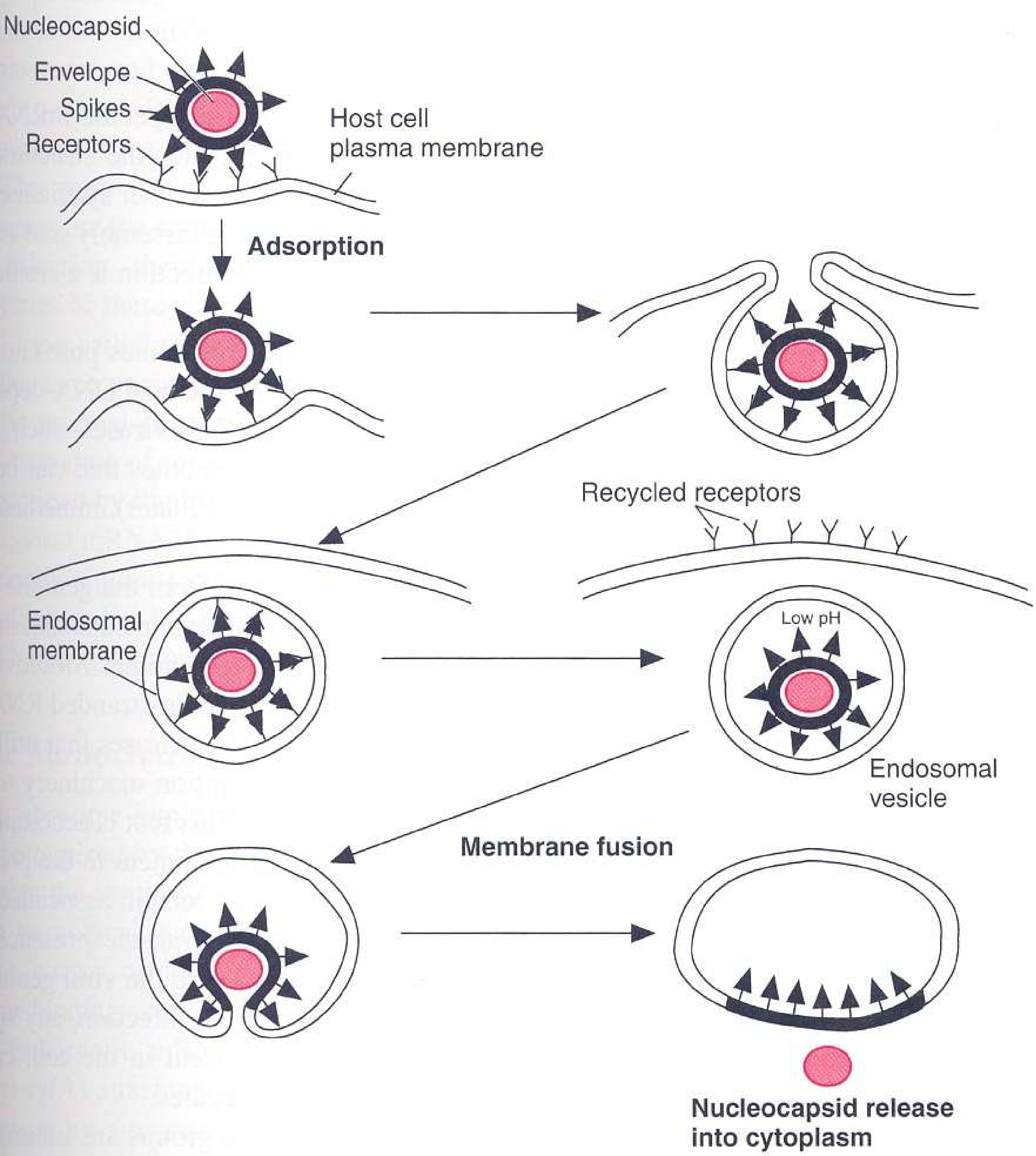
The two basic modes of entry of an enveloped animal virus into the host cell

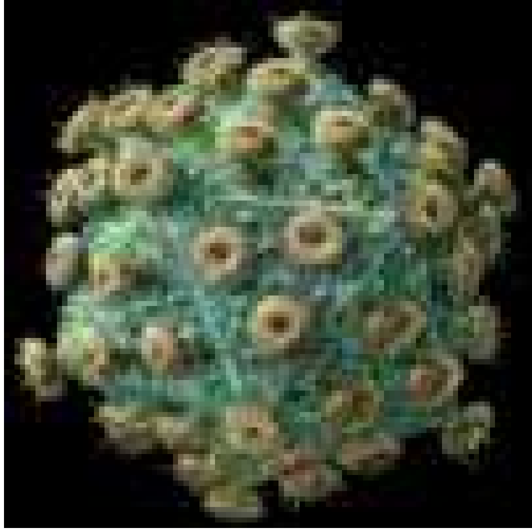




Entry by

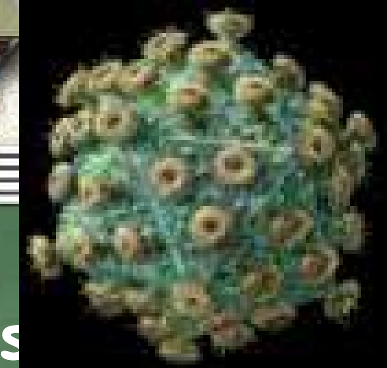
direct fusion


Nucleocapsid
Envelope
Spikes
Receptors
Host cell plasma membrane
Adsorption
Recycled receptors
Endosomal membrane
Low pH
Endosomal vesicle
Membrane fusion
Nucleocapsid release into cytoplasm

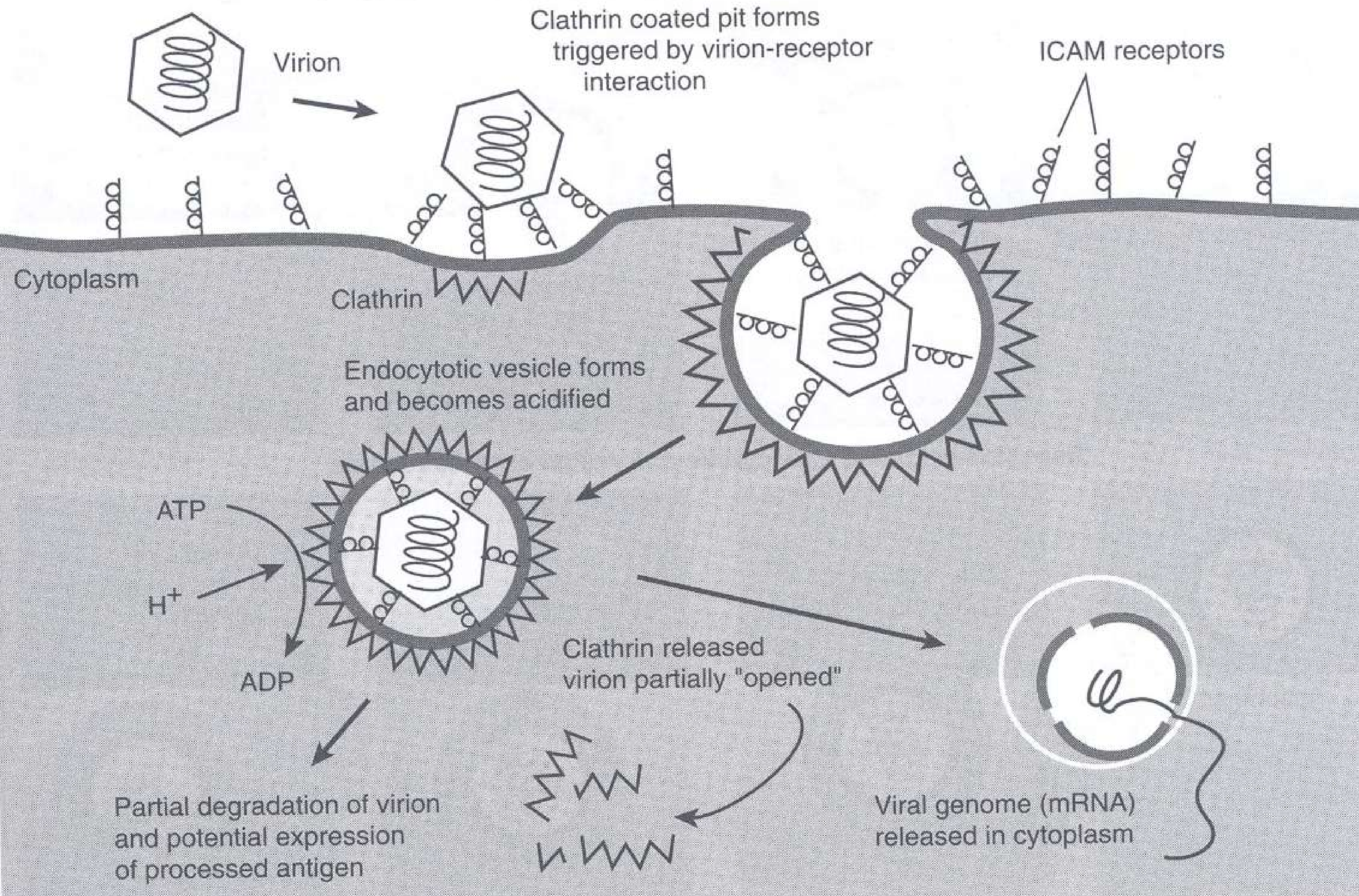


Viropexis

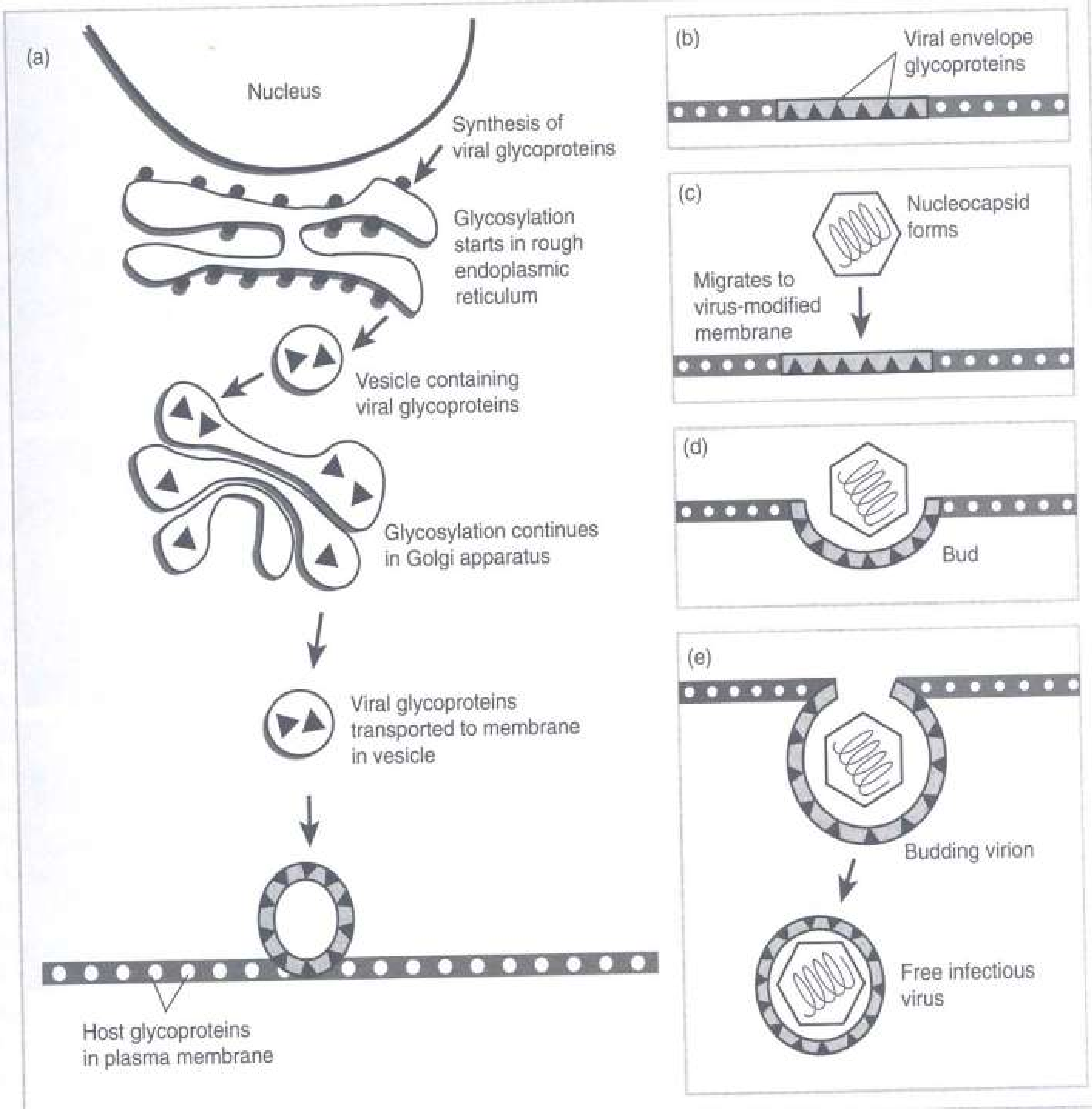
Naked Capsid Animal Viruses

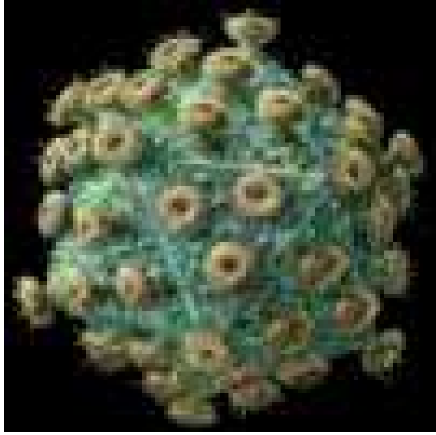


- Naked capsid viruses (eg. poliovirus, reovirus, adenovirus) also appear to enter by viropexis, but in this case, virus can't escape the endosomal vesicle by membrane fusion
- For poliovirus : acidified endosome expose hydrophobic domains → result in the binding of the virions to membrane & releases nucleocapsid to cytoplasm
- For reovirus : the content of the endosome are transferred to a lysosome → the lysosomal proteases strip away part of the capsid protein & active virions

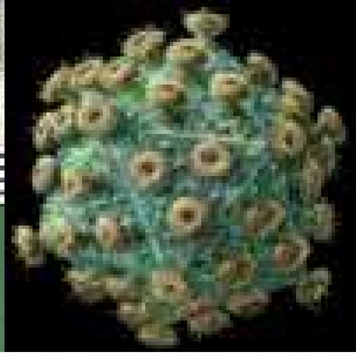


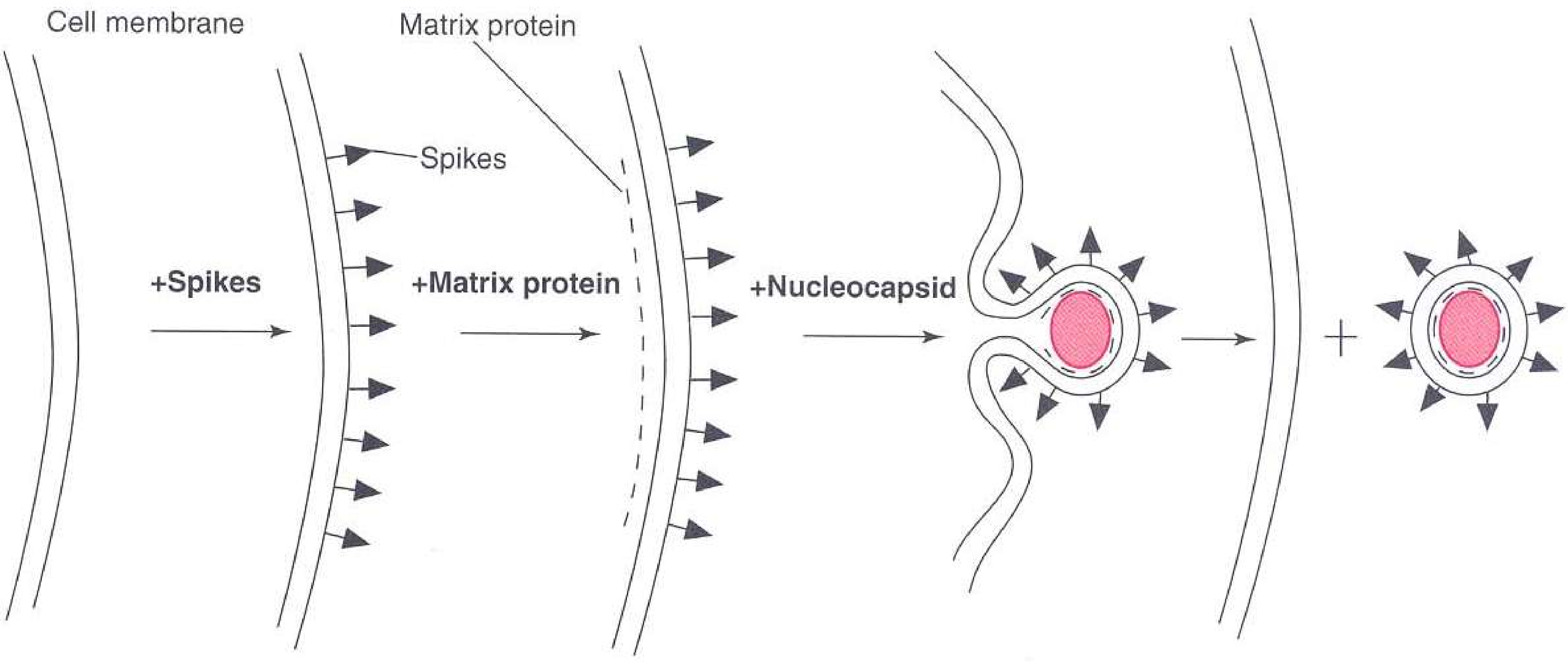
Schematic of receptor-mediated endocytosis ulitilized by poliovirus for entry into host cell



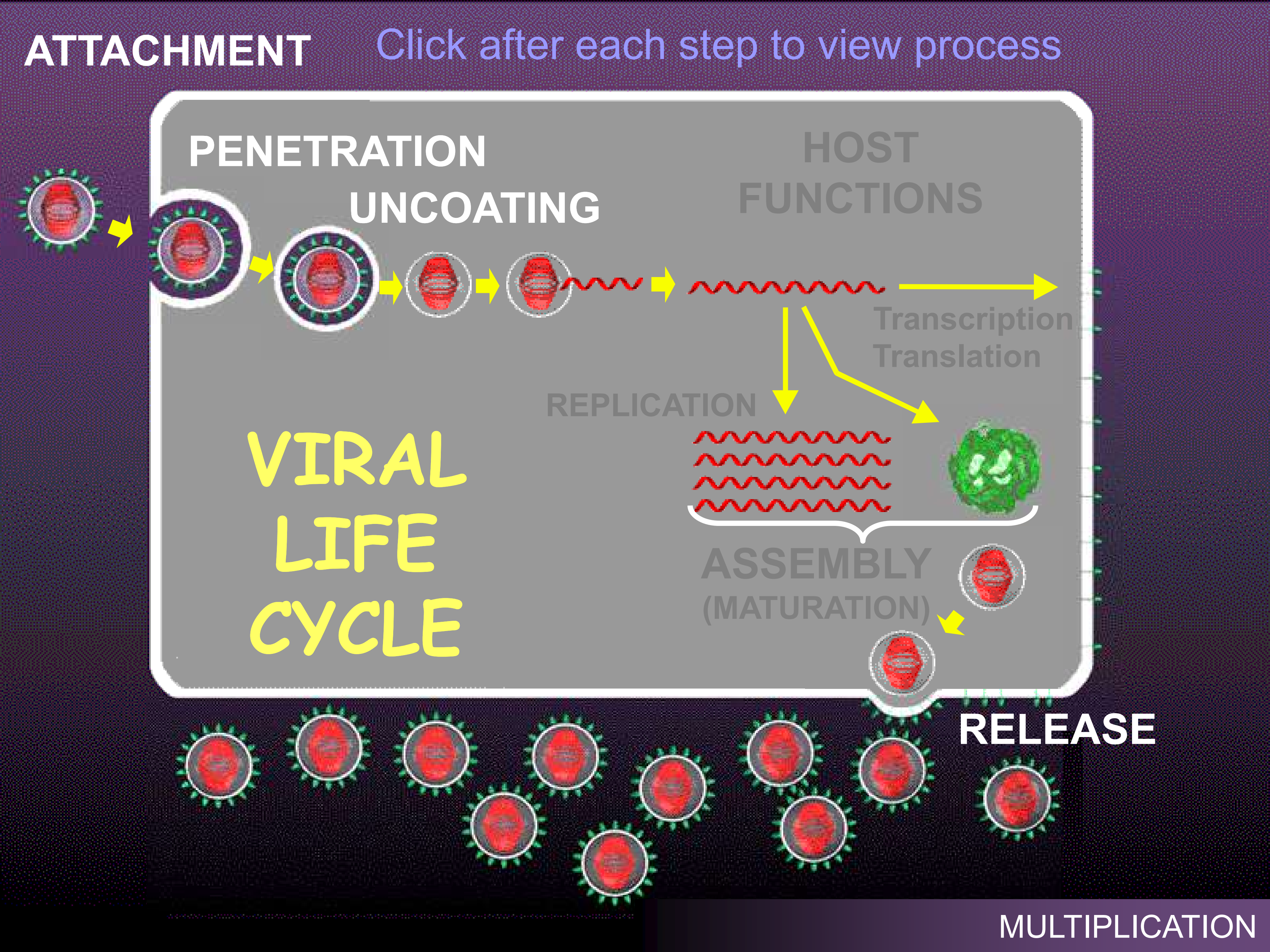


Insertion of glycoprotein into the cell's membrane structures and formation of the viral envelope

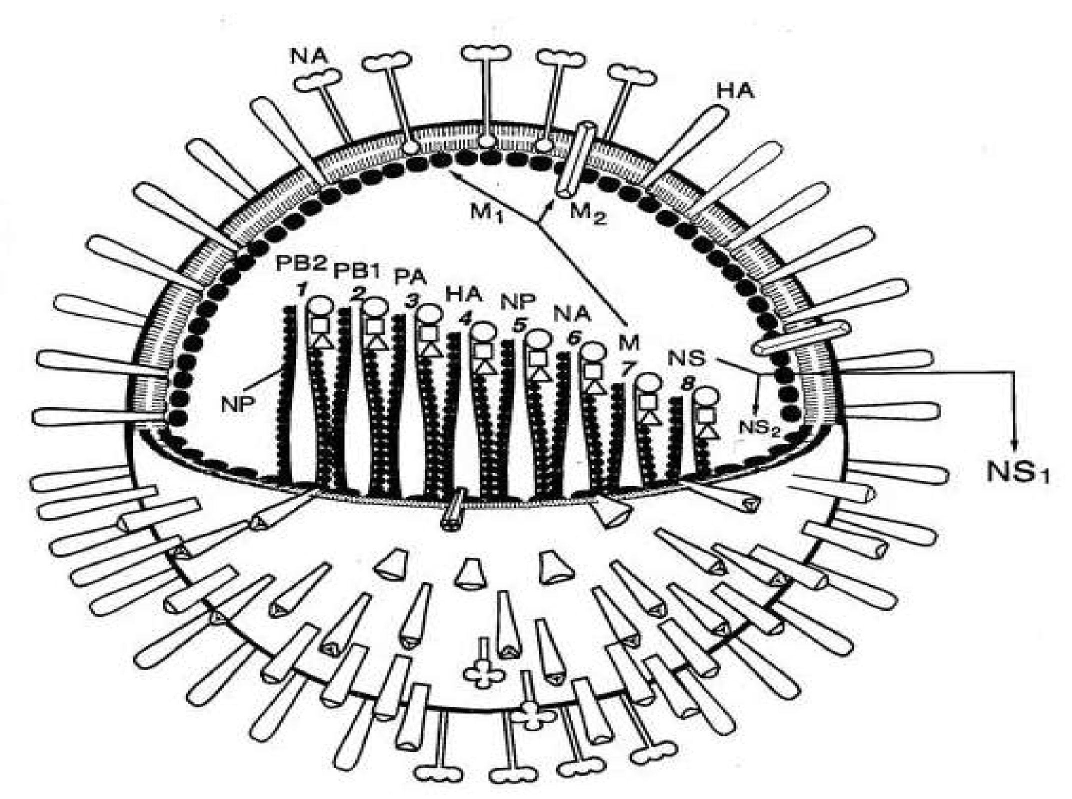




Viral release by Budding

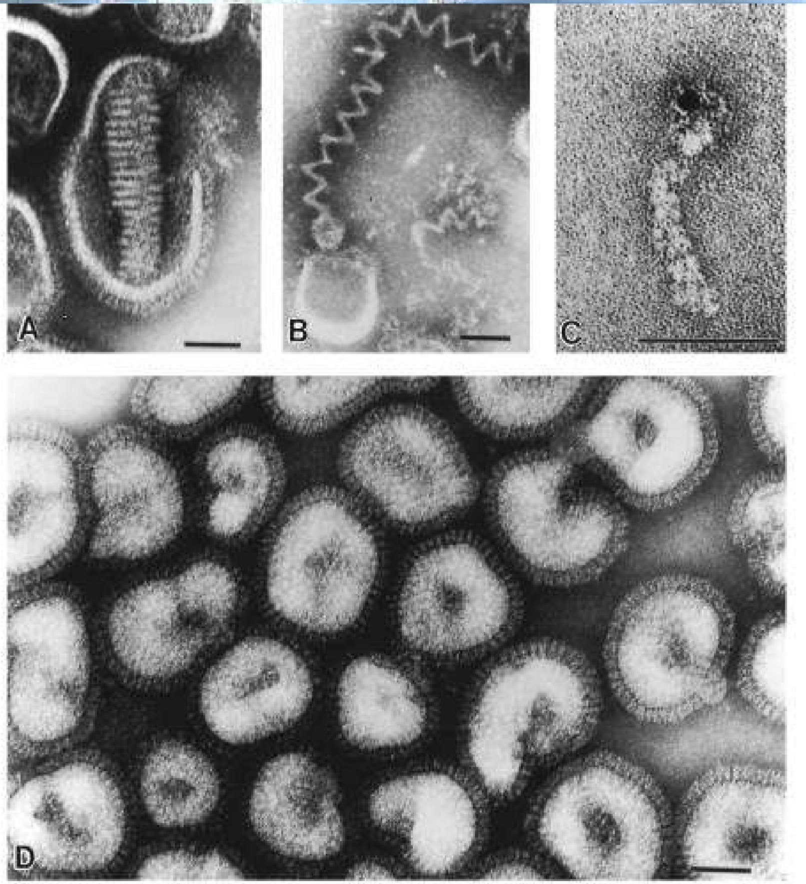

ATTACHMENT
Click after each step to view process
PENETRATION
UNCOATING
HOST
FUNCTIONS
Transcription
Translation
REPLICATION
VIRAL
LIFE
CYCLE
ASSEMBLY
(MATURATION)
RELEASE
MULTIPLICATION

Influenza virus structure



Structure of influenza virus. The diagram illustrates the main structural features of the virion. The surface of the particle contains three kinds of spike proteins: the hemagglutinin (HA), neuraminidase (NA), and matrix (M2) protein embedded in a lipid bilayer derived from the host cell and covers the matrix (M1) protein that surrounds the viral core. The ribonucleoprotein complex making up the core consists of at least one of each of the eight single-stranded RNA segments associated with the nucleoprotein (NP) and the three polymerase proteins (PB2, PB1, PA). RNA segments have base pairing between their 3´ and 5´ ends forming a panhandle. Their organization and the role of NS2 in the virion remain unresolved. (From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Fig. 47-2)

Influenza virus

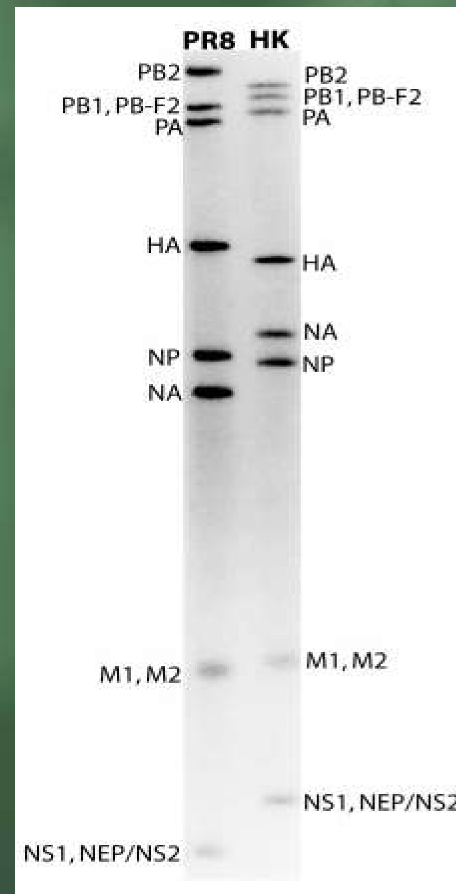


Electron micrographs of influenza virus. A–C: The structure of the internal components; (D) the external view. A substantial fraction (up to 50%) of influenza virions contain large helical internal components (A, B), which may contain individual ribonucleoprotein (RNP) segments (C) linked together. The individual RNPs each contain a binding site for the viral polymerase, as seen by the immunogold labeling of the end of the RNP segment (C). The external view of the virions (D) illustrates the pleomorphic appearance and the surface spikes. Bar in all figures equals 50 nm. (From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Fig. 47-2)

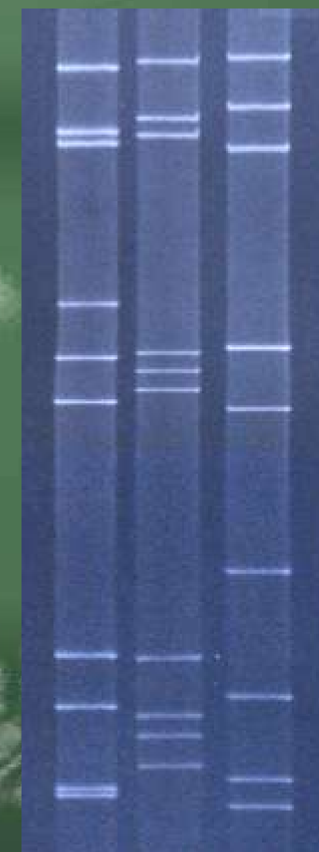
Some viruses have segmented genomes:

Allows “assortment”

Orthomyxoviridae



Reoviridae



Influenza gene functions

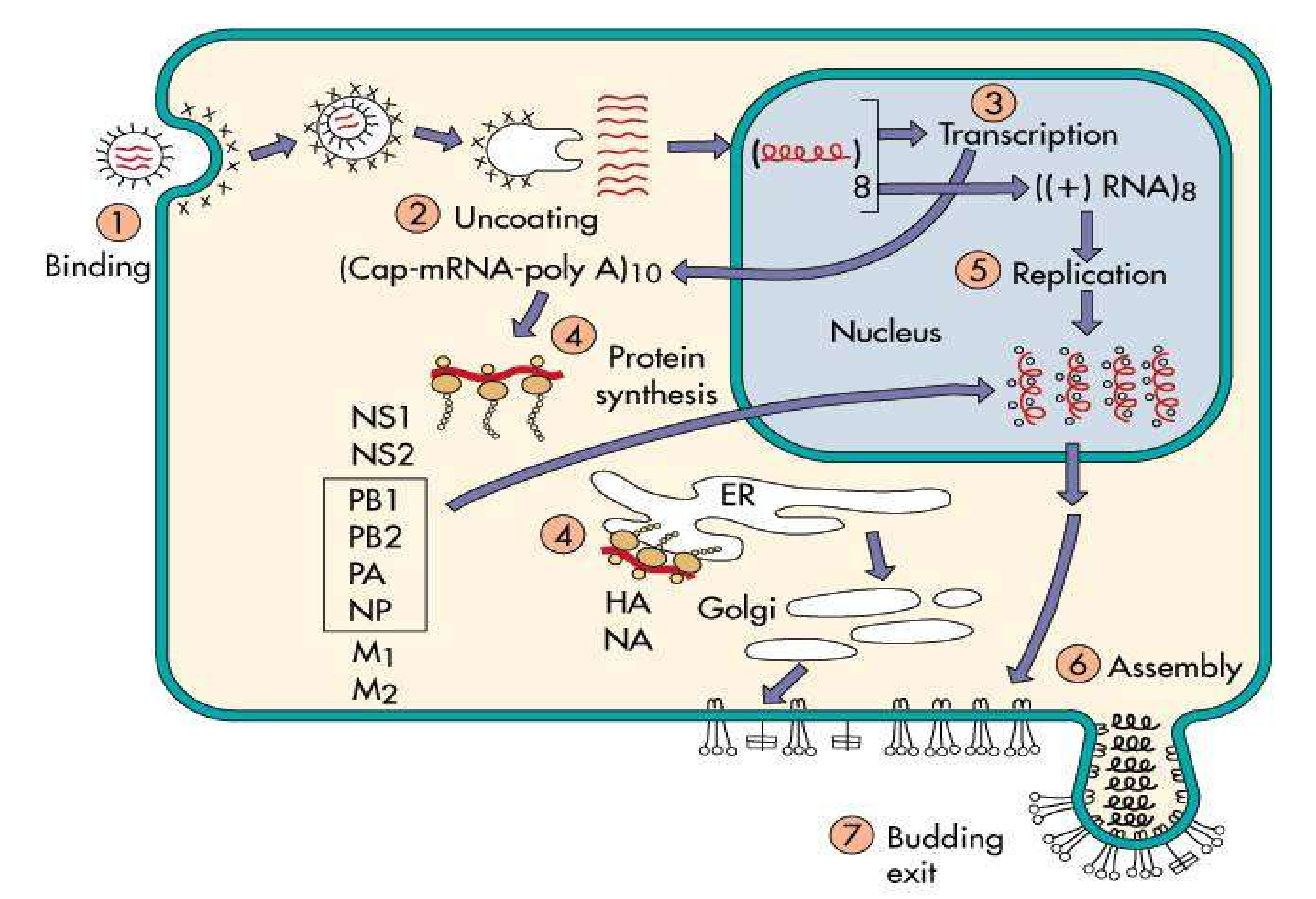
Segment*	Protein	Function
1	PB2	Polymerase component
2	PB1	Polymerase component
3	PA	Polymerase component
4	HA	Hemagglutinin, viral attachment protein, fusion protein, target of neutralizing antibody
5	NP	Nucleocapsid
6	NA	Neuraminidase (cleaves sialic acid and promotes virus release)
7†	M_1 M_2	Matrix protein: Viral structural protein (interacts with nucleocapsid and envelope, promotes assembly) Membrane protein (forms membrane channel and target for amantadine, facilitates uncoating and HA production)
8†	NS_1 NS_2	Nonstructural protein (inhibits cellular messenger RNA translation) Nonstructural protein (important but unknown function)

*Listed in decreasing order of size.

†Encodes two messenger RNAs.

From Medical Microbiology, 5th ed., Murray, Rosenthal & Pfaller, Mosby Inc., 2006, Table 60-1.

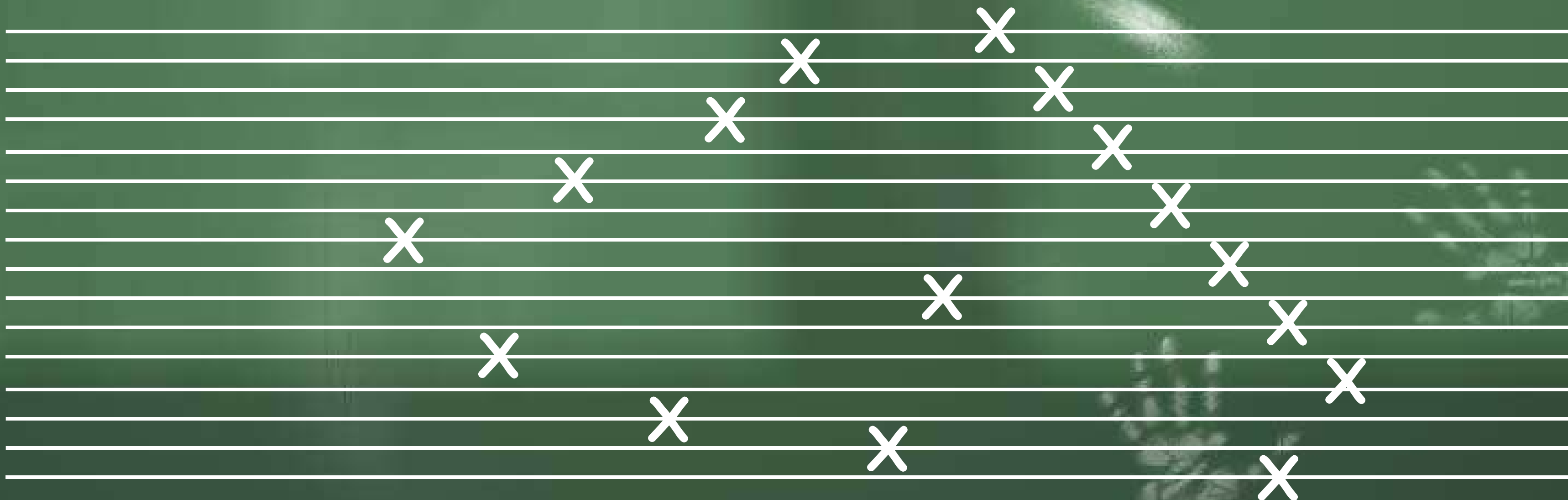
Influenza replication



Replication of influenza A virus. After binding (*1*) to sialic acid-containing receptors, influenza is endocytosed and fuses (*2*) with the vesicle membrane. Unlike for most other RNA viruses, transcription (*3*) and replication (*5*) of the genome occur in the nucleus. Viral proteins are synthesized (*4*), helical nucleocapsid segments form and associate (*6*) with the M_1 protein-lined membranes containing M_2 and the HA and NA glycoproteins. The virus buds (*7*) from the plasma membrane with 11 nucleocapsid segments. (-), Negative sense; (+), positive sense; ER, endoplasmic reticulum. (From Medical Microbiology, 5th ed., Murray, Rosenthal & Pfaller, Mosby Inc., 2005, Figure 60-2.)

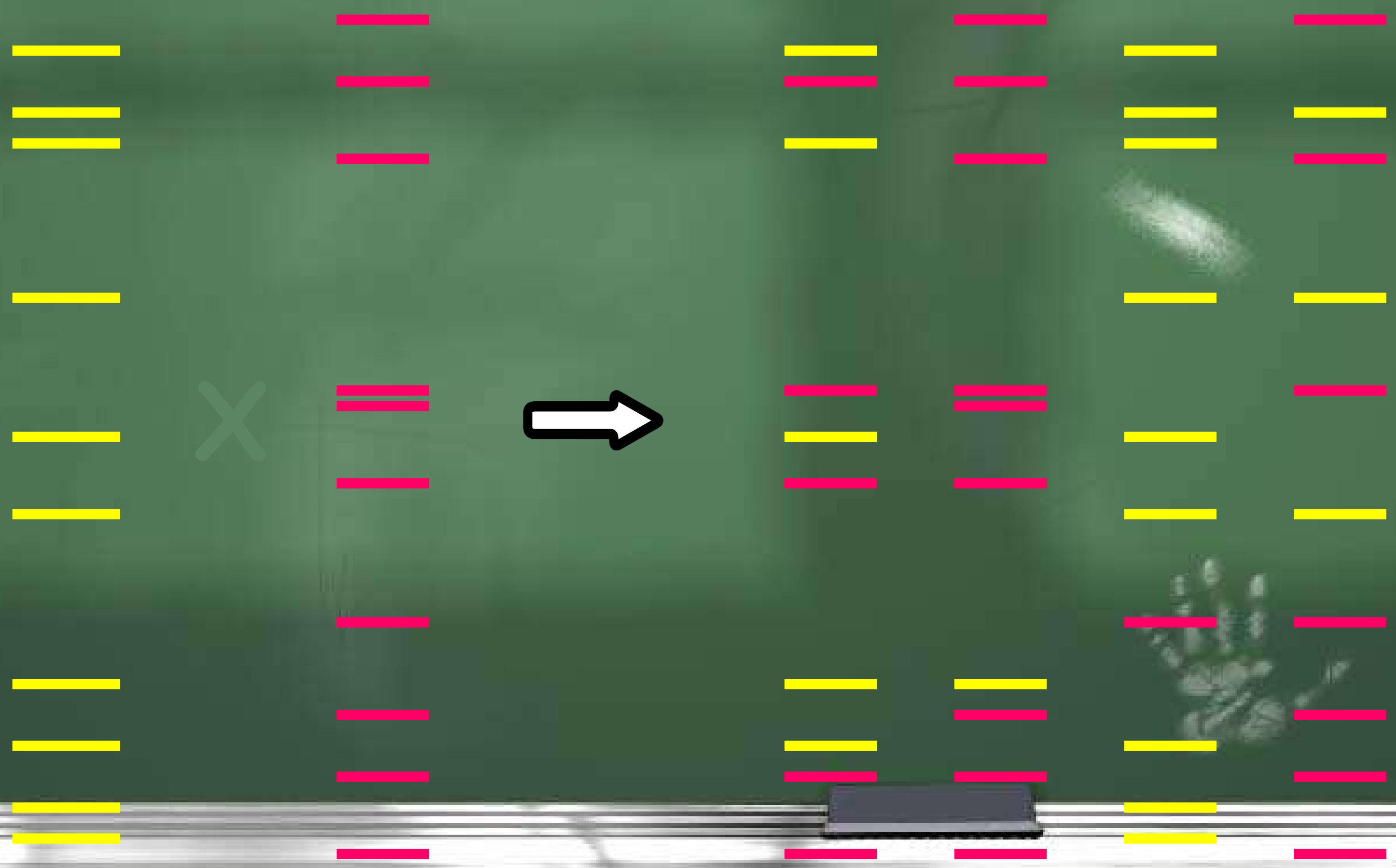
Implications of high mutation rate

10kb

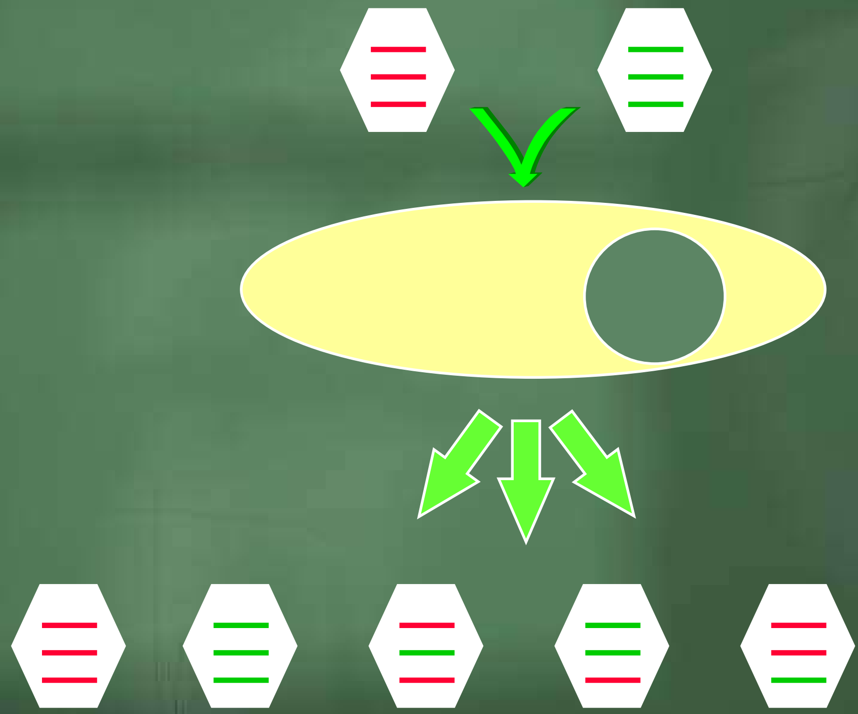


Quasispecies

Segmented Genomes Allow Assortment



Viruses with Segmented Genomes



. . . 2^n

Influenza (8 genes) = 256 possibilities

Rotavirus (11 genes) = 2048 possibilities

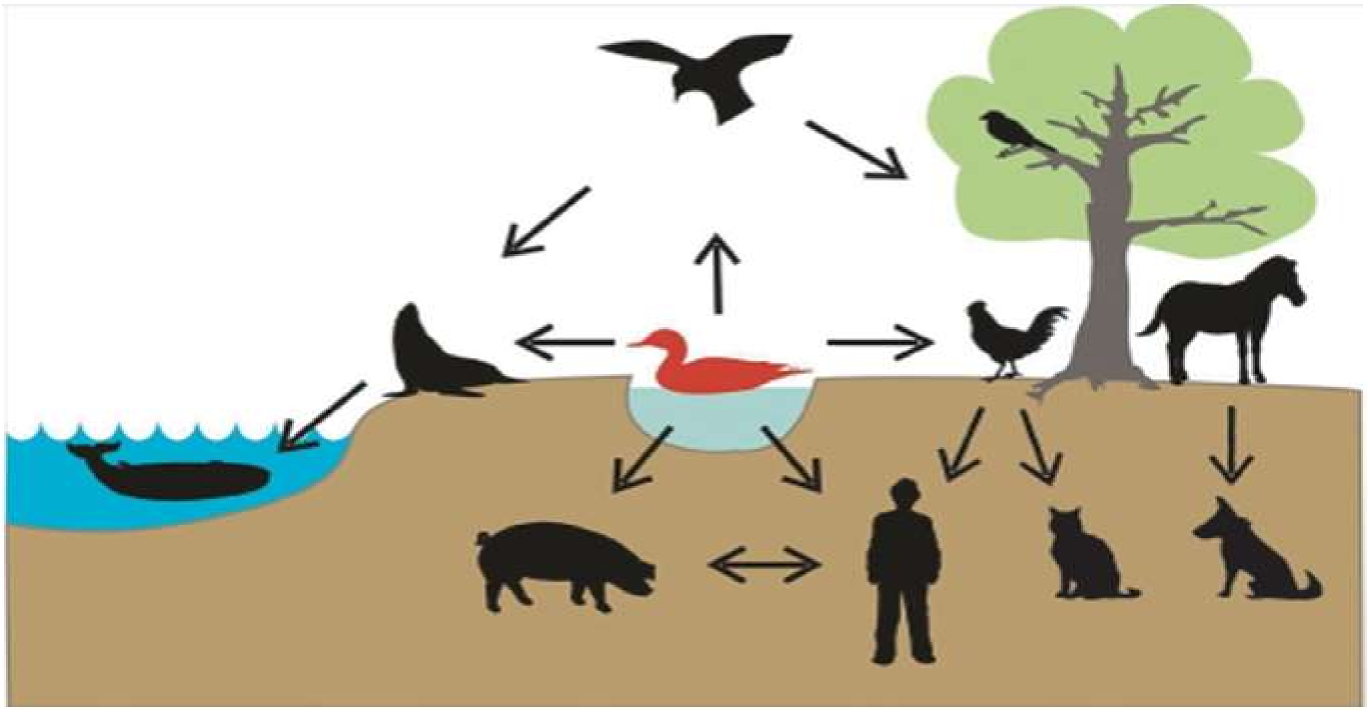
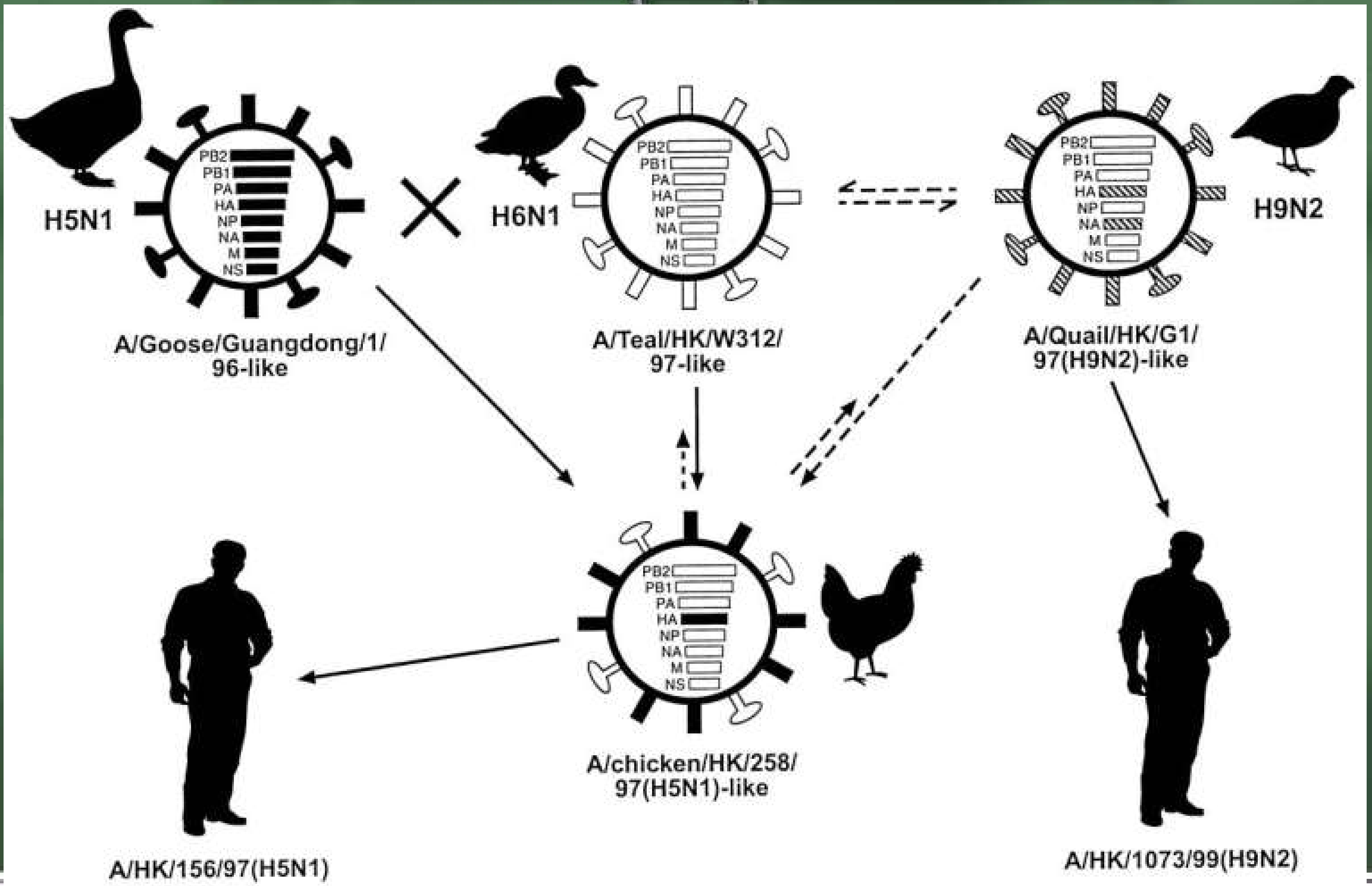
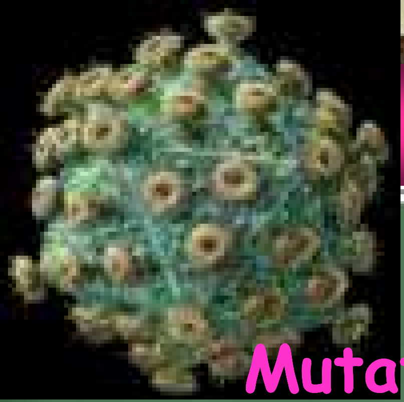


Figure 48.1 Influenza A virus reservoir. Wild aquatic birds are the main reservoir of influenza A viruses. Virus transmission has been reported from wild waterfowl to poultry, sea mammals, pigs, horses, and humans. Viruses are also transmitted between pigs and humans, and from poultry to humans. Equine influenza viruses have recently been transmitted to dogs.


H5N1
PB2
PB1
PA
HA
NP
NA
M
NS
A/Goose/Guangdong/1/96-like
H6N1
PB2
PB1
PA
HA
NP
NA
M
NS
A/Teal/HK/W312/97-like
H9N2
PB2
PB1
PA
HA
NP
NA
M
NS
A/Quail/HK/G1/97(H9N2)-like
PB2
PB1
PA
HA
NP
NA
M
NS
A/chicken/HK/258/97(H5N1)-like
A/HK/156/97(H5N1)
A/HK/1073/99(H9N2)



Mechanisms of genetic change

Mutation

- Many DNA viruses use the host DNA synthesis machinery for replicating their genomes
- However the largest animal viruses code for their own DNA polymerases, & these enzymes are not as effective at proofreading as the cellular polymerases
- The resulting higher error rates in DNA replication endow the viruses with the potential for a high rate of evolution, but they are partially responsible for the high frequency of defective viral particles
- High mutation rates permit adaptation to changed conditions

Recombination

Homologous recombination is common in DNA viruses

There are 2 mechanisms :

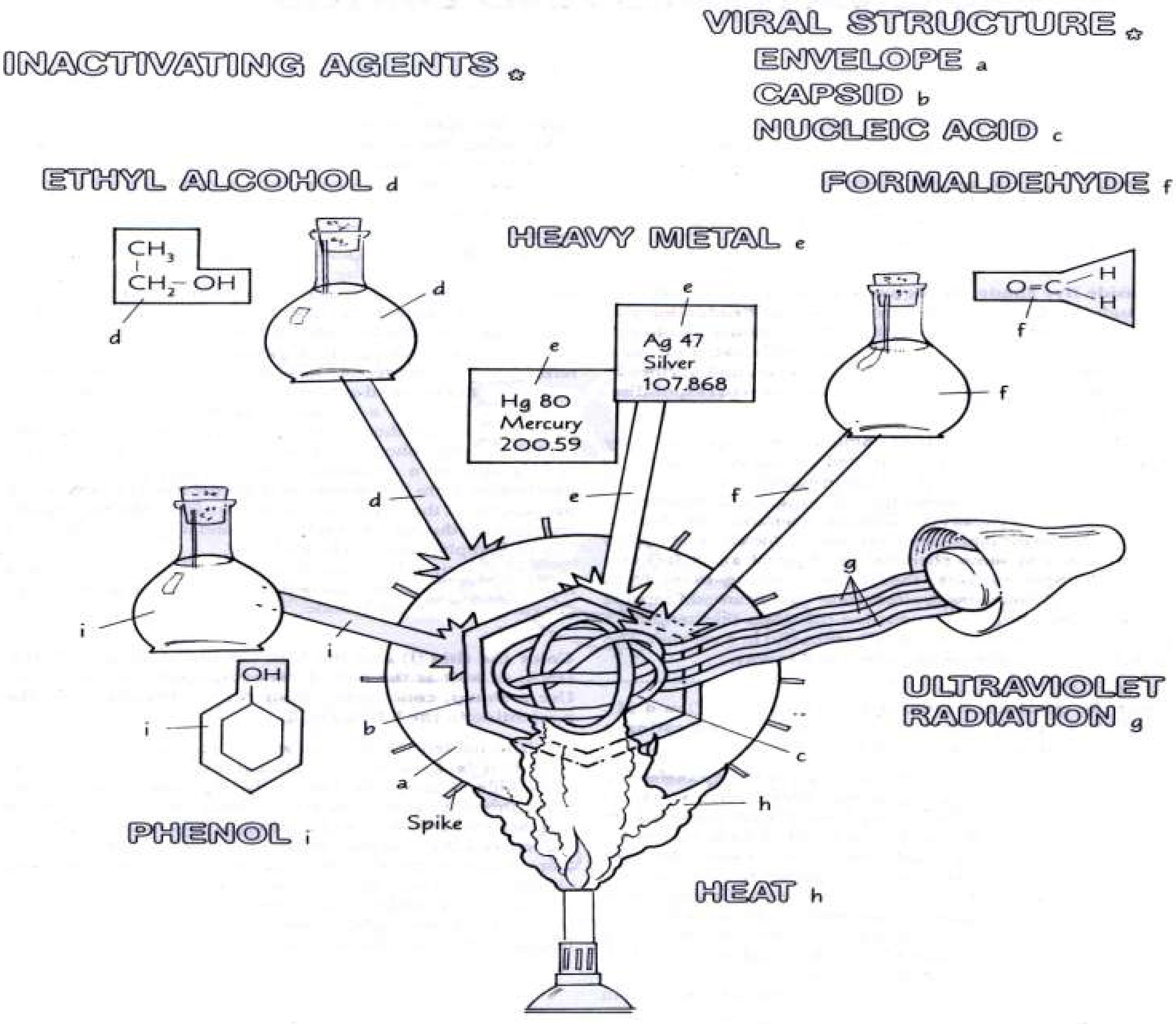
1. Which is unique to the viruses with segmented genomes, involves reassortment of the segments during a mixed infections involving 2 different viral strains.
2. Exemplified by the genetic recombination between different forms of poliovirus
   Recombination occurs during replication by a “copy choice” type of mechanism
   Poliovirus replicase switches templates to generate recombinants

COMMON METHODS OF INACTIVATING VIRUSES FOR VARIOUS PURPOSE

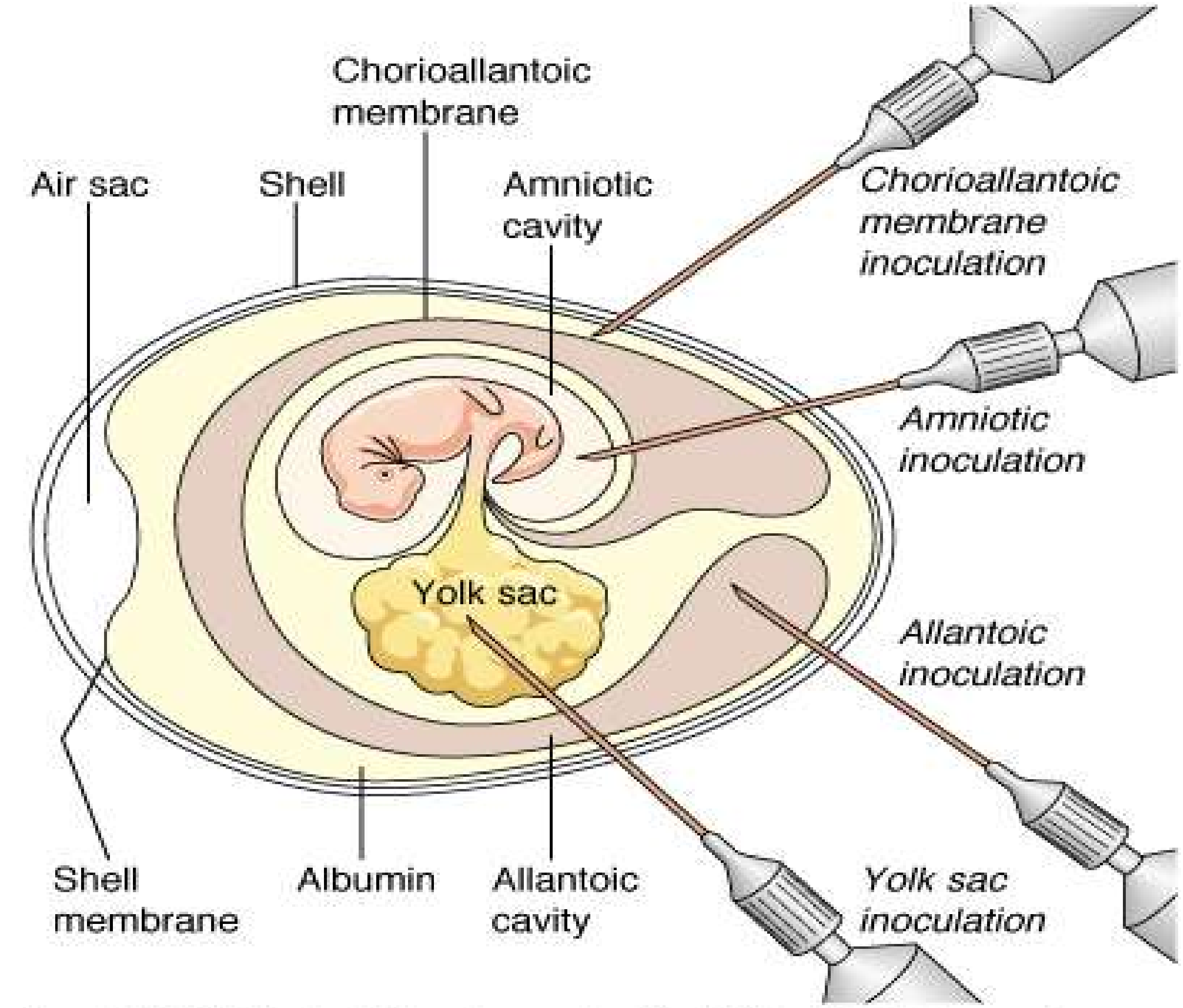
Virus may be inactivated for :

- Sterilize laboratory supplies
  - Sterilization : steam, dry heat, ethylene oxide, γ-irradiation
- Disinfect surfaces or skin
  - Surface disinfection : sodium hypochlorite, glutaraldehyde. formaldehyde, peracetic acid
  - Skin disinfection : chlorhexidine, 70% ethanol, iodophores
- Vaccine production :
  - formaldehyde, β-propiolactone, psoralen + uv irradiation, detergents (subunit vaccines)

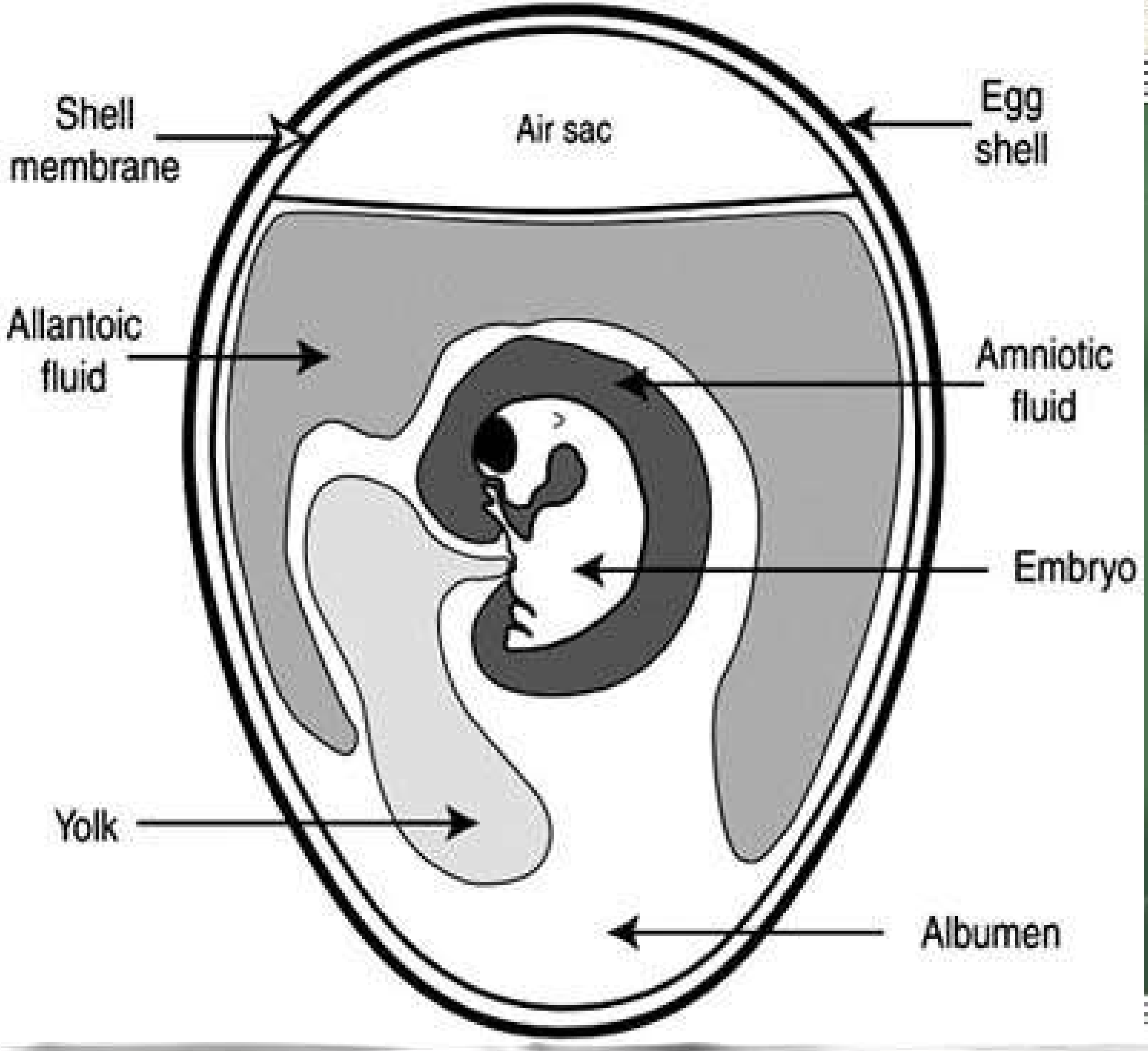
INACTIVATION OF VIRUSES



Cultivation in embryonated egg

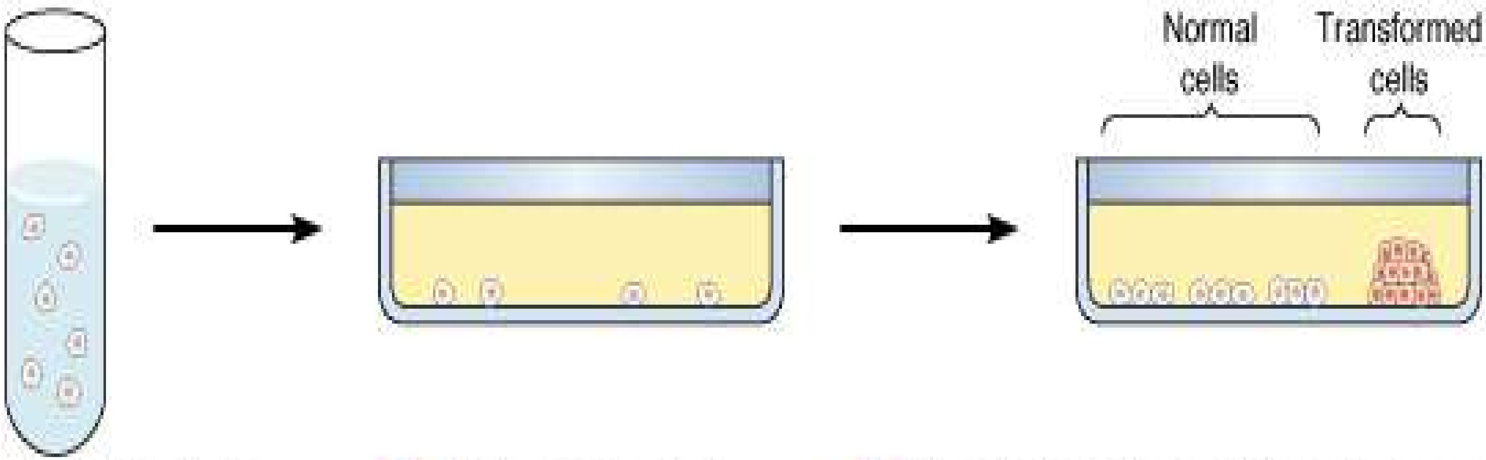


- Virus injected into appropriate region
- Method widely used for production of vaccines



Shell membrane
Air sac
Egg shell
Allantoic fluid
Amniotic fluid
Embryo
Yolk
Albumen

Cultivation in cell cultures



1. A tissue is treated with enzymes to separate the cells.
2. Cells are suspended in culture medium.
3. Normal cells or primary cells grow in a monolayer across the glass or plastic container. Transformed cells or continuous cell cultures do not grow in a monolayer.

Cultivation in cell culture

- Cytopathic effect - normal monolayer structure is disrupted by viral infection
- Cell lines developed from embryonic tissue
- Continuous cell lines (immortal) - HeLa
- Maintenance of cell culture lines is technically difficult; must be kept free of microbial contamination.

TRANSMISSION OF VIRAL INFECTION

- **DIRECT CONTACT**

  droplet/aerosol
  - influenza virus
  - morbilli virus
  - smallpox virus
- **GASTROINTESTINAL TRACT (ORALLY)**
  - enterovirus
  - Hepatitis A, E virus
  - poliomyelitis virus
- **ANIMAL BITE** : rabies virus
- **VECTOR** : dengue virus
- **PARENTERAL** : Hepatitis B, C virus, HIV

PATHOGENESIS OF VIRAL DISEASES

Viral pathogenesis :
interaction of viral and host factors leads to disease production

Virus pathogenic if : can infect and cause signs of disease of the host

Virus virulent : produce more severe disease

Steps in viral pathogenesis :

- **Viral entry & primary replication**
- **Viral spread and cell tropism**
- **Cell injury & clinical illness**
- **Recovery from infection**
- **Virus shedding**

DIAGNOSTIC METHODS OF VIRAL INFECTION

1. Clinical symptoms
2. Laboratory diagnosis

Typical clinical symptoms

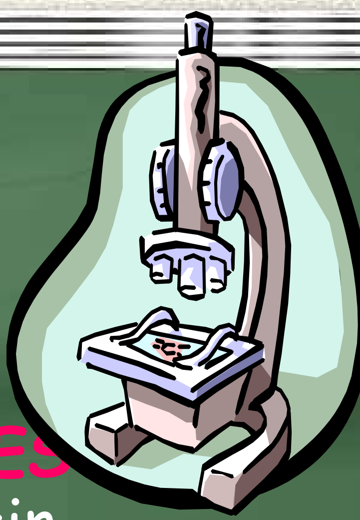
- Polyomyelitis
- Chicken Pox
- Measles
- Mumps

Laboratory diagnosis

1. Electron microscope → morphology
2. Light microscope → special staining

~ type of virus

inclusion bodies



- Variola virus

  Gispen staining : **PASCHEN BODIES**

- Rabies virus

  specimen : brain

  Sellers staining : inclusion bodies in nerve cells

  NEGRI BODIES

- Molluscum contagiosum virus skin nodule

  Lugol staining : inclusion bodies in cytoplasm of epithel cell

  MOLLUSCUM BODIES

Laboratory diagnosis

3. Culture

specimen : depend on the diseas

in vitro, in ovo, or in vivo

4. Serology

- Raise of antibody titer
- Antigen detection from the spe
- Viral type identification :

  agglutination, precipitation, complement fixation test, neutralization, inhibition haemagglutination, FAT, ELISA, RIA, LIA

PREVENTION AND TREATMENT OF VIRAL INFECTIONS

1. VIRAL VACCINES
   - Killed-virus vaccines
   - Attenuated live-virus vaccines
   - Future prospect :
     - attenuation of viruses by genetic mapping
     - avirulent viral vectors
     - purified proteins produced using cloned genes
     - synthetic peptides
     - subunit vaccines
     - DNA vaccines

2. INTERFERONS

IFNs :

- host-coded proteins of large cytokine family
- inhibit viral replication
- produced by intact animal or cell culture in response to viral infection or other inducers
- first line of defense against viral infection

3. ANTIVIRAL CHEMOTHERAPY

A. **Nucleoside analogs**

- Acyclovir & valacyclovir
- Didanosine
- Gancyclovir
- Idoxuridine
- Lamivudin (3TC)
- Ribavirin
- Stavudine (d4T)
- Trifluridine
- Vidarabine
- Zalzitabine (ddC)
- Zidovudine (AZT)

B. Nucleotide analogs

Cidofovir : active against CMV & HSV
inhibit s viral DNA polymerase

C. Nonnucleoside reverse transcriptase inhibitor

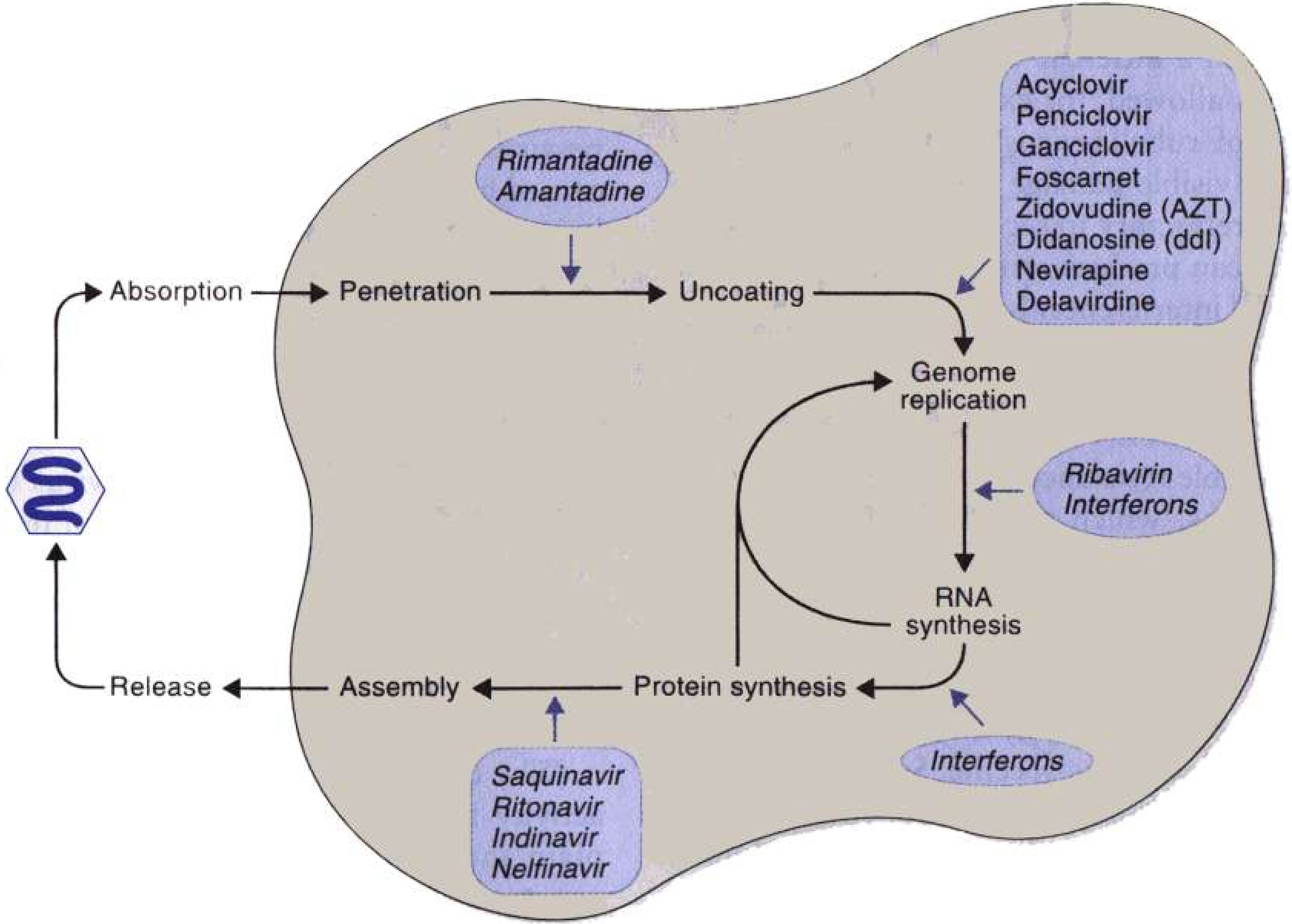
Nevirapine : inhibit reverse transcriptase of HIV

D. Protease inhibitors

Ritonavir, Saquinavir → HIV

E. Other types

Amantadine & rimantadine
Foscarnet
Methiasone



Schematic life cycle of viruses showing the steps at which replication can be inhibited by representative drugs. The gray area shows the intracellular space.

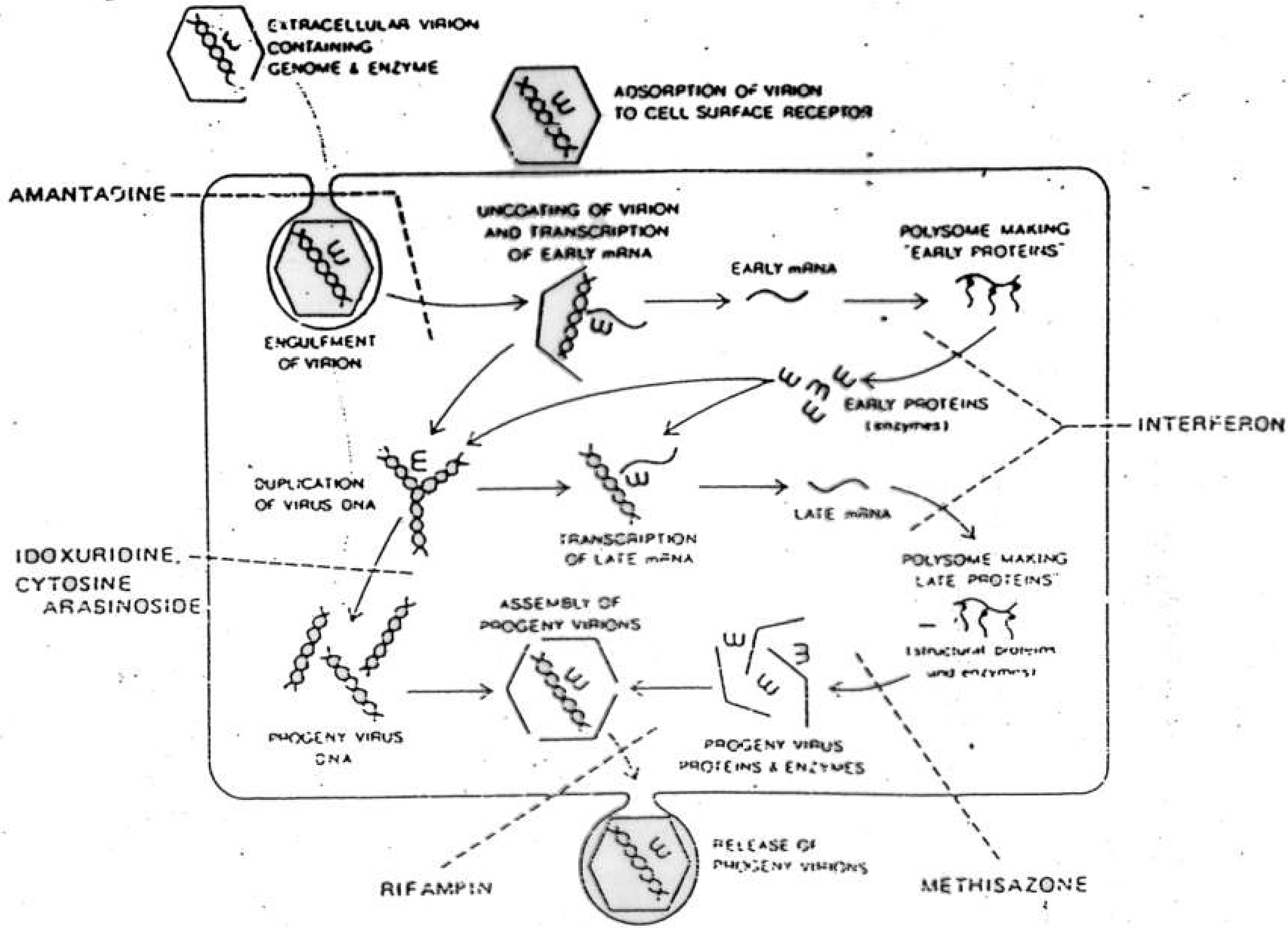
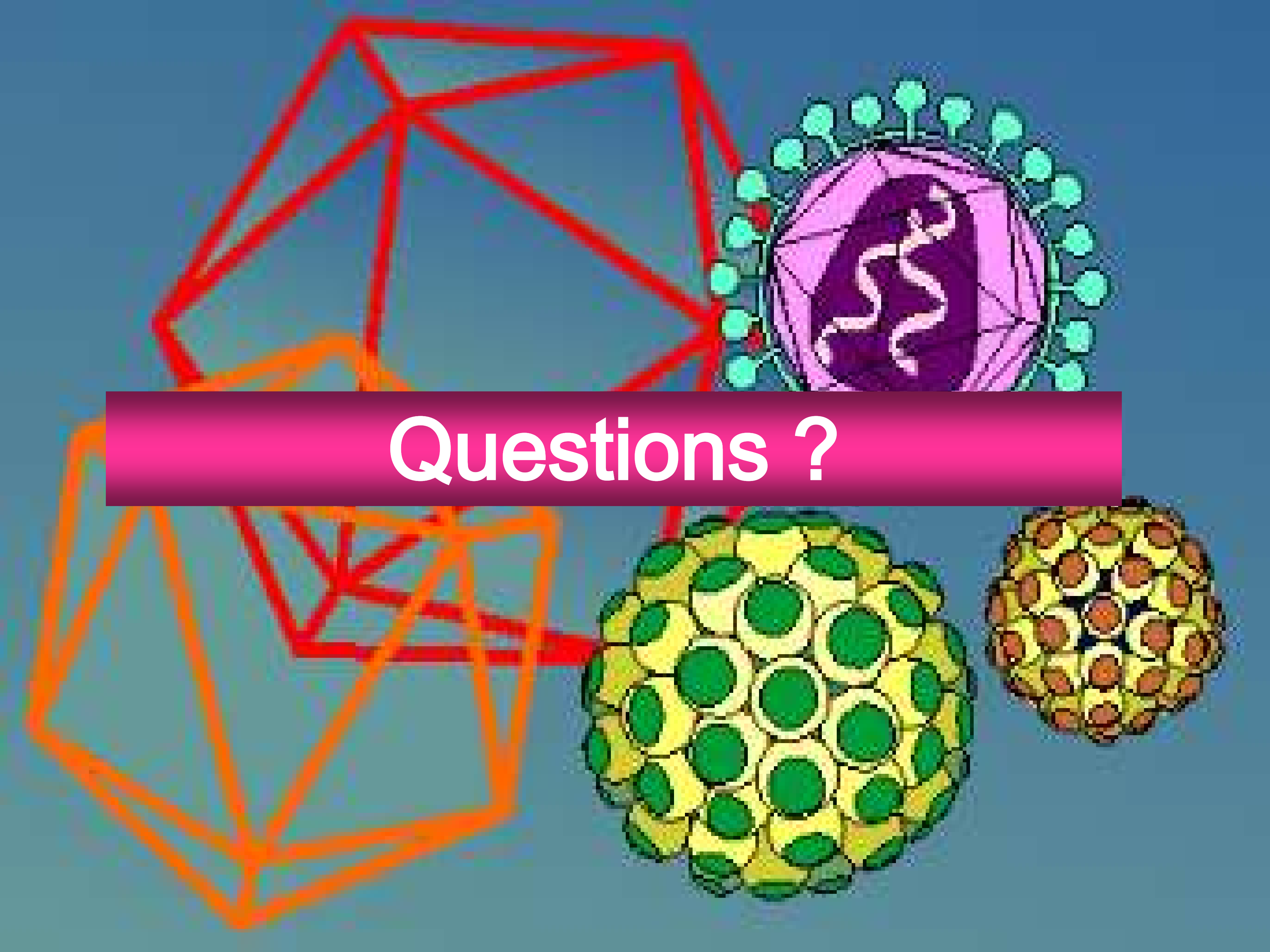


Figure 2. The possible interruption of viral replication by various antiviral agents.


Questions ?