National Pharmaceutical University
Department of Microbiology, Virology and Immunology

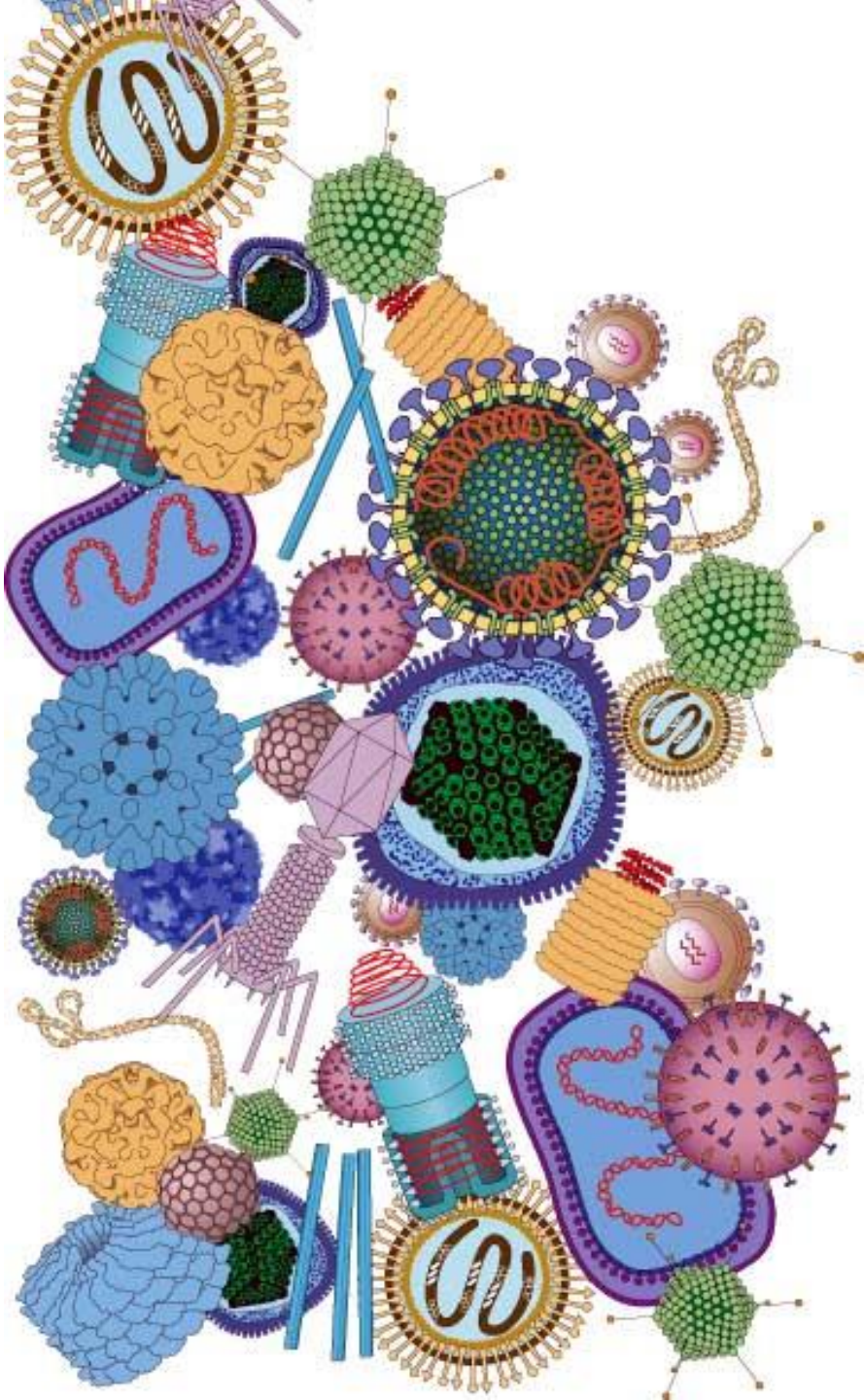
Lecture on Microbiology with immunology fundamentals specialty 226 Pharmacy

Viruses

Lecturer: associate professor Department microbiology virology and immunology Ph D. Dotsenko Roman Valeryevich

LECTURE PLAN

1. Definition of viruses.
2. Characteristics of viruses.
3. Structure of viruses.
4. Viral replication.
5. Classification of viruses.
6. Cultivation of viruses



Questions for self-examination:

1. *Cultivation of viruses*
2. *Detection of viruses*
3. *Cytopathic effect*

Recommended literature

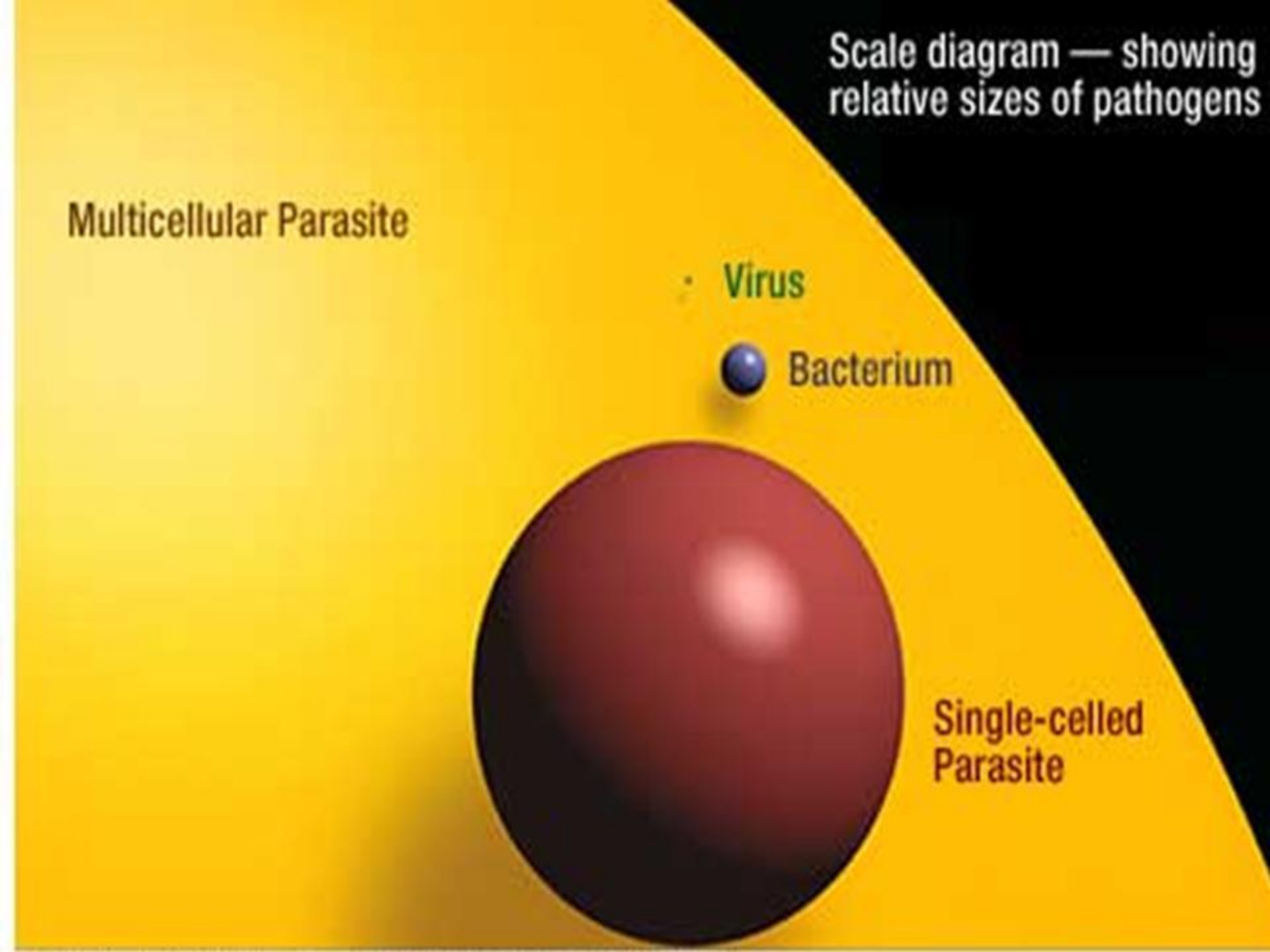
General microbiology: synopsis of lectures to laboratory classes /N. I Filimonova, M.M. Velika, N. Yu. Shevelyova. – Kharkiv : NUPh : Golden Pages, 2011. – 128 p.

Special microbiology in tables / N. I Filimonova, A. Bocharov. – Kharkiv : NUPh : Golden Pages, 2012. – 28 p.

Microbiology: Sub. for stud/ I. L. Dyky, I. Yu.Holupyak, N.Yu. Shevelev, and others. 2nd form. - X .: Professional, 2006. - 433 pp.

Microbiology: A Guide to Laboratory Lessons. Study a manual for students of higher educational institutions / IL Wild, I.I. Sidorchuk, I.Yu. Kholupiak, N.E. Shevelev, MM Great, N.A. Volkova, L.F. Silayeva, O.P. Strilec, O.G. Heyderich, V.E. Litarov - Kh.: Publishing house of NfaU; Golden Pages, 2002. 444 p.

Microbiology Methodical recommendations for students of pharmaceutical higher educational institutions / IL Wild, I.Yu. Kholupiak, MM Great, NE Shevelev and others - X., 2004. - 144 p.


Scale diagram — showing relative sizes of pathogens
Multicellular Parasite
Virus
Bacterium
Single-celled Parasite

Virology (the study of viruses) – is such a significant part of microbiology

A Virus is a non-cellular particle made up of genetic material and protein that can reproduction only in the living cells

A complete virus particle as it exists outside the cell called virion (or viral particle)

Viruses can infect all types of cells: bacteria, protozoa, plants, animals, human.

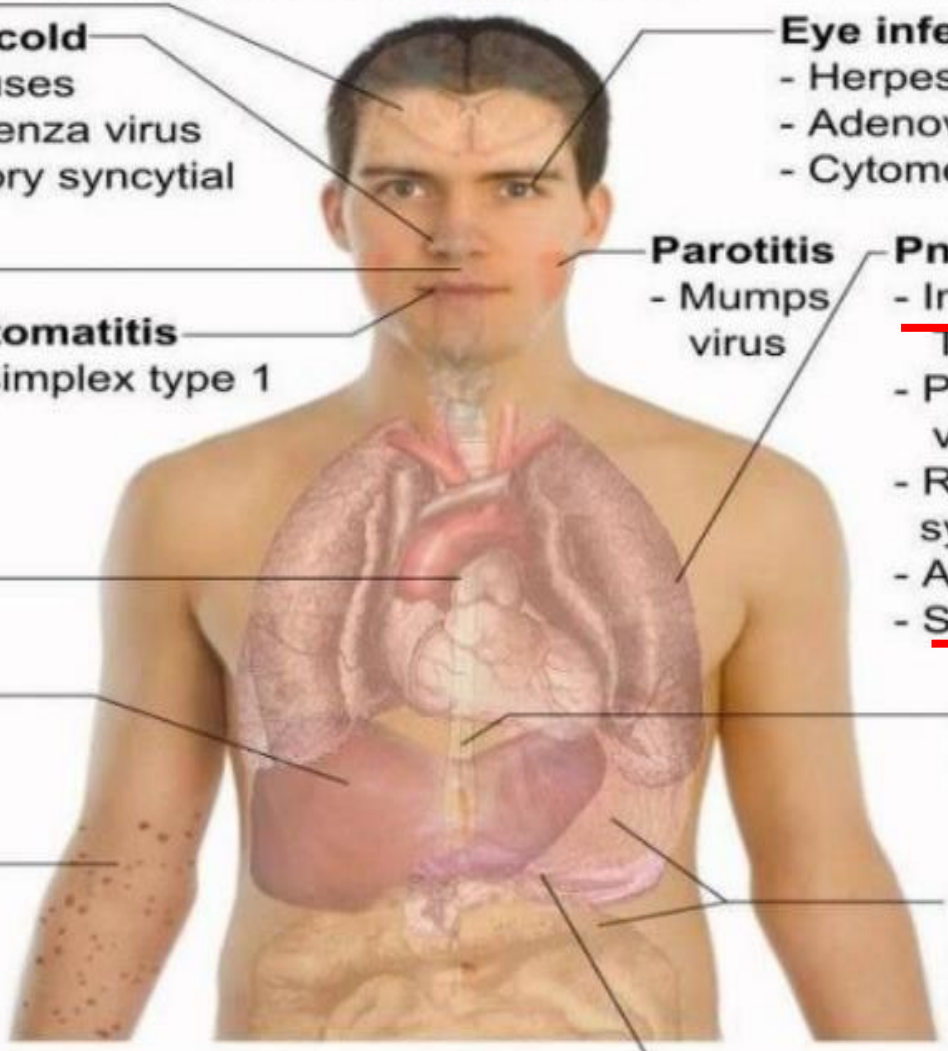
Prokaryotes Vs. Eukaryotes Vs. Viruses

- No membrane bound nucleus
- Has a cell wall
- Only a few organelles or none at all.
- Has a capsule surrounding it
- Three main types.

- Nucleus with membrane
- Only plants have cell wall
- Contains many organelles
- Has a lipid bi-layer membrane surrounding it.
- Specialized by thousands of different sizes and shapes.

- No nucleus
- No membranes
- No organelles
- Cannot reproduce on its own
- Generally not considered alive by most standards

Overview of
Viral infections



Encephalitis/ meningitis
- JC virus
- Measles
- LCM virus
- Arbovirus
- Rabies

Common cold
- Rhinoviruses
- Parainfluenza virus
- Respiratory syncytial virus

Eye infections
- Herpes simplex virus
- Adenovirus
- Cytomegalovirus

Parotitis
- Mumps virus

Pneumonia
- Influenza virus, Types A and B
- Parainfluenza virus
- Respiratory syncytial virus
- Adenovirus
- SARS coronavirus

Pharyngitis
- Adenovirus
- Epstein-Barr virus
- Cytomegalovirus

Gingivostomatitis
- Herpes simplex type 1

Cardiovascular
- Coxsackie B virus

Hepatitis
- Hepatitis virus types A, B, C, D, E

Myelitis
- Poliovirus
- HTLV-I

Skin infections
- Varicella zoster virus
- Human herpesvirus 6
- Smallpox
- Molluscum contagiosum
- Human papillomavirus
- Parvovirus B19
- Rubella
- Measles
- Coxsackie A virus

Gastroenteritis
- Adenovirus
- Rotavirus
- Norovirus
- Astrovirus
- Coronavirus

Sexually transmitted diseases
- Herpes simplex type 2
- Human papillomavirus
- HIV

Pancreatitis
- Coxsackie B virus

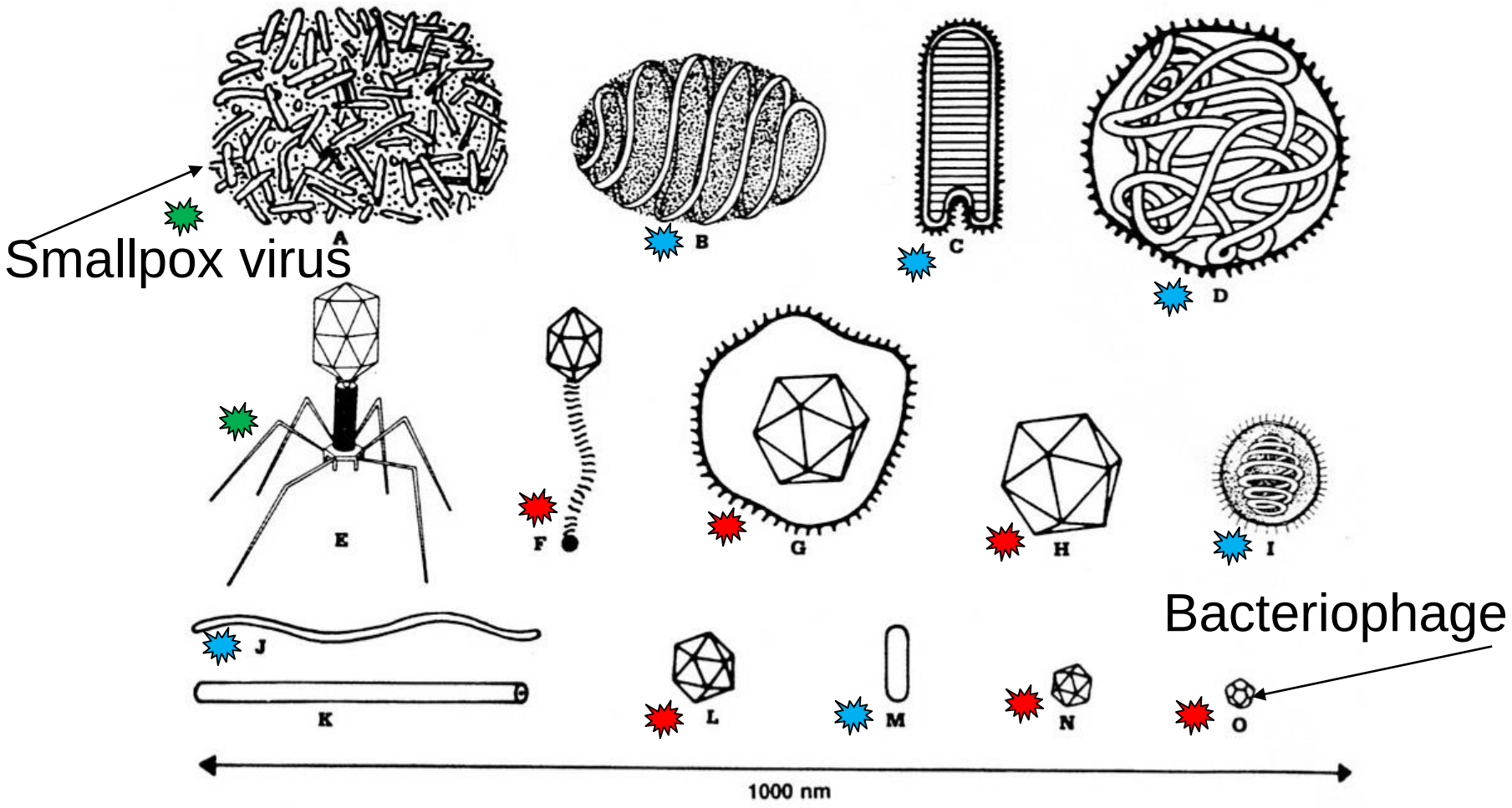
General characteristics of Viruses

1. **small size**: cannot be viewed with a light microscope, range of size = 30-400 nm
2. **characteristic shapes** - spherical (complex), helical, rod or polyhedral, sometimes with tails or envelopes. Most common polyhedron is the icosahedron which as 20 triangular faces.
3. **obligate intracellular parasites:** Viruses do not contain within their coats the machinery for replication. For this they depend upon a host cell and this accounts for their existence as obligate intracellular parasites. Each virus can only infect certain species of cells. This refers to the virus **host range**.
4. **no built-in metabolic machinery:** Viruses have no metabolic enzymes and cannot generate their own energy.

General characteristics of Viruses

5. **no ribosomes:** Viruses cannot synthesize their own proteins. For this they utilize host cell ribosomes during replication.
6. **only one type of nucleic acid:** Viruses contain either DNA or RNA (never both) as their genetic material. The nucleic acid can be single-stranded or double stranded.
7. **do not grow in size:** Unlike cells, viruses do not grow in size and mass leading to a division process. Rather viruses grow by separate synthesis and assembly of their components resulting in production of a "crop" of mature viruses.

Comparative size and shape of various groups of viruses



Viruses have fundamentally three morphologies:

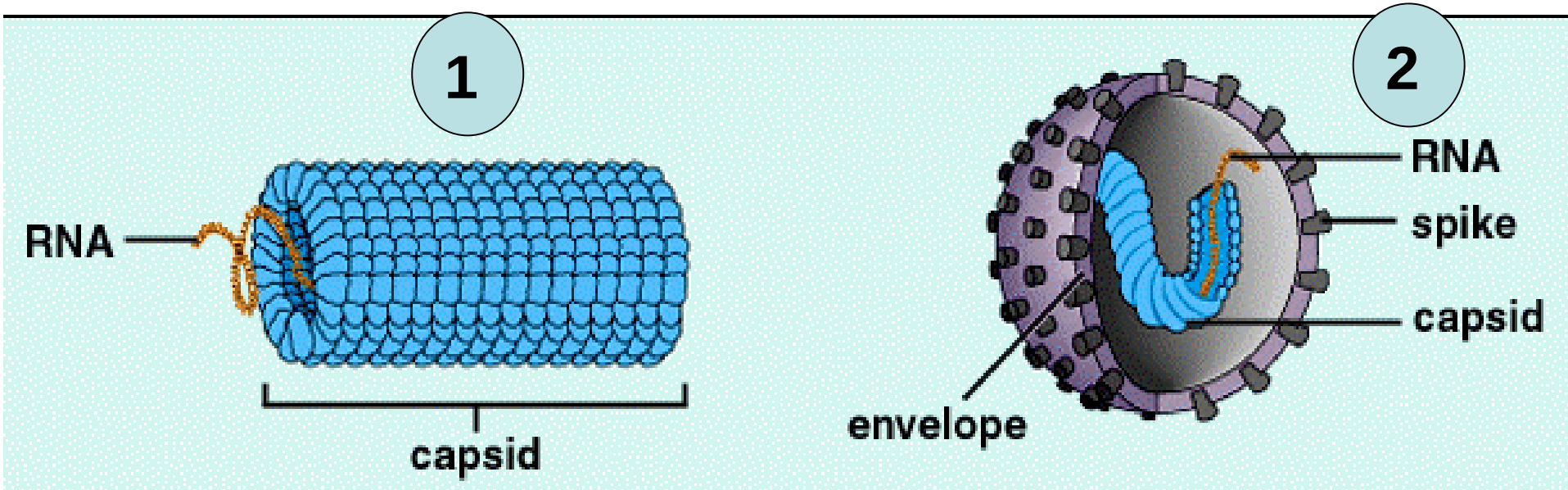
1. icosahedron (E, F, G, H, L, N);

2. helical (D, I, J, K, M; B is controversial); 3. **complex** (A).

Virus structure

Viruses contain with nucleic acid and protective protein coat. There are 2 large groups of viruses, which differ one from another morphologically.

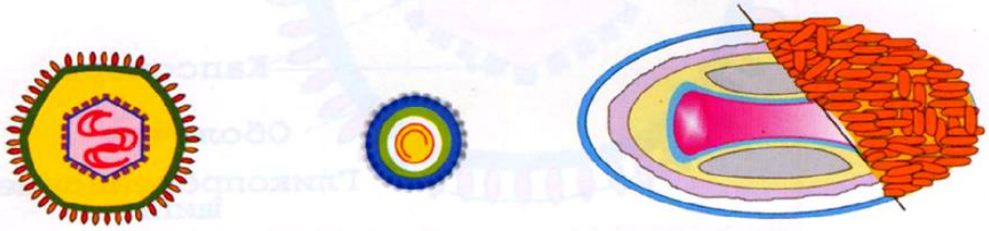
- The first group is naked viruses or simple viruses,
- The secod – envelope viruses or complex viruses.



Scheme of the structure of viruses

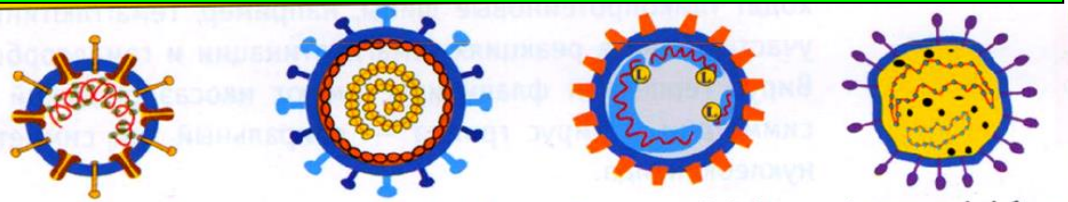
Envelope viruses

Double-stranded DNA



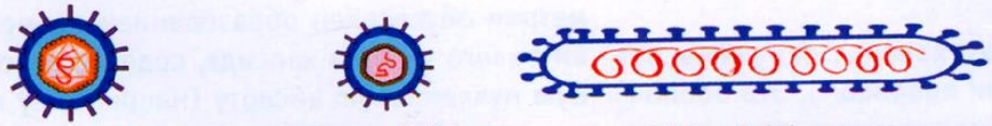
Herpesviridae Hepadnaviridae Poxviridae

Single-stranded RNA



Coronaviridae Paramyxoviridae Bunyaviridae Arenaviridae

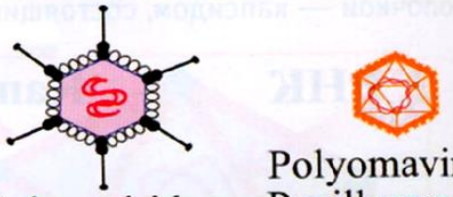
Orthomyxoviridae Retroviridae Rhabdoviridae



Togaviridae Flaviviridae Filoviridae

Nonenvelope viruses

Double-stranded DNA



Adenoviridae Polyomaviridae Papillomaviridae

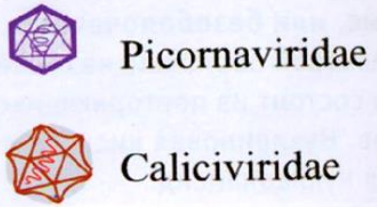
Single-stranded DNA

Parvoviridae Circinoviridae

Double-stranded RNA

Reoviridae

Single-stranded RNA



Picornaviridae Caliciviridae

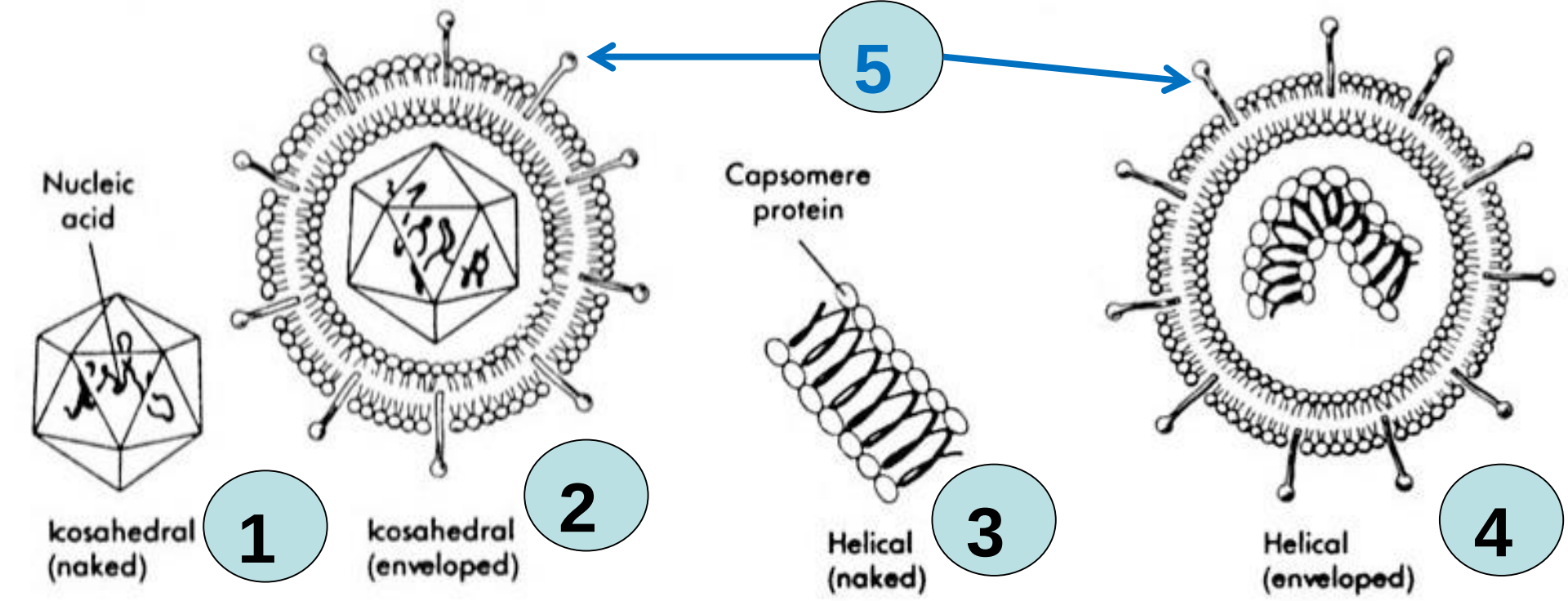
Inner Core - contains genetic information in the form of RNA or DNA (never both)

Capsid (Protein Coat) – serves to protect genetic information inside viral particle and permit attachment to appropriate host.

Outer Envelope - complex viruses also have a quasi-membrane or envelope (proteins and phospholipids) around the Capsid to enhance adhesion to an appropriate host

Capsid Construction - made of identical protein subunits termed capsomeres

The protein **spikes** on viruses surface that allow it to bind and fuse with host cells.



The most common viral morphologies. Left to Right.

A naked icosahedral virus (1), an enveloped icosahedral virus (2), a naked helical virus (3) and an enveloped helical virus (4).

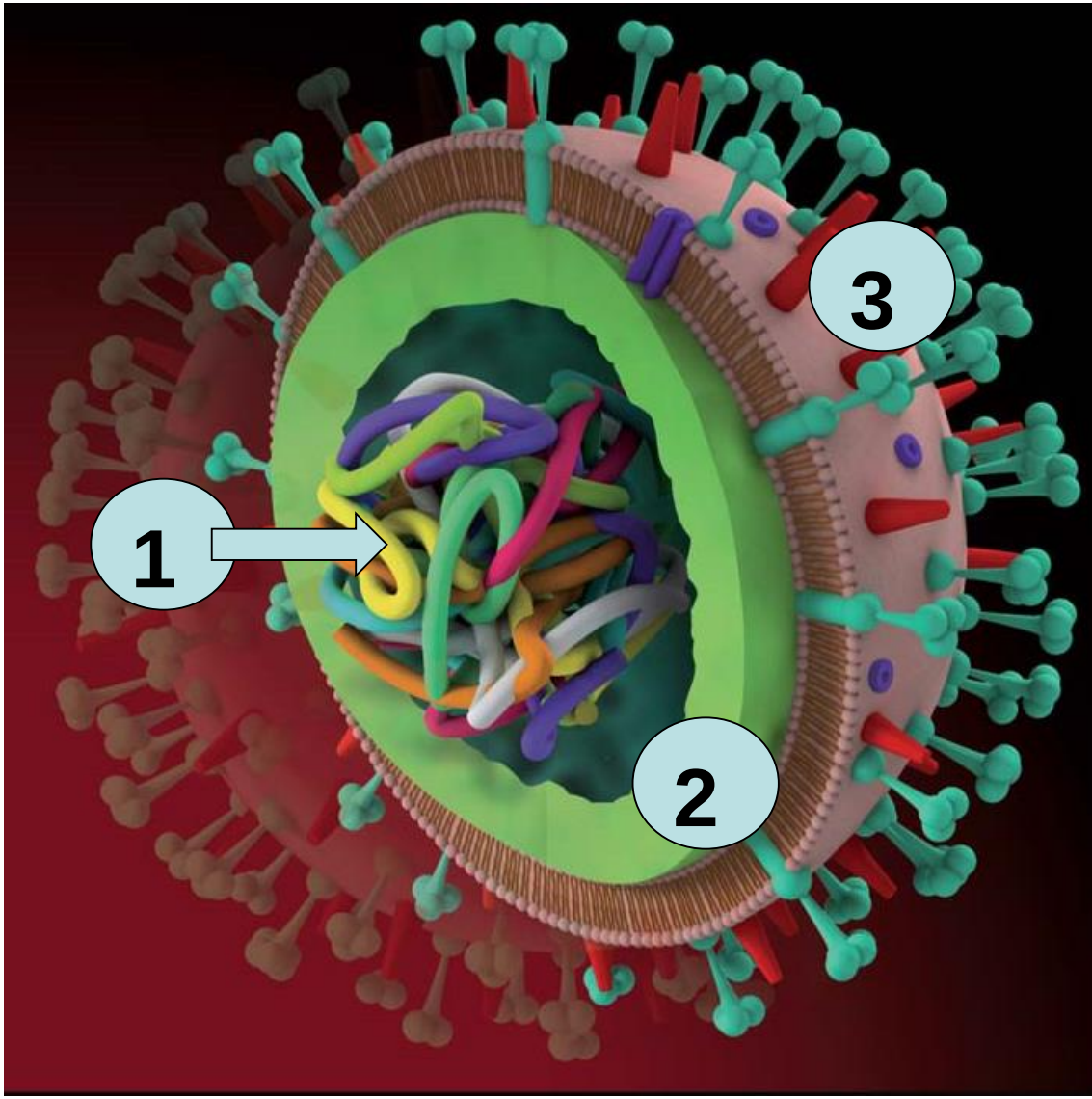
Some yuman viruses also contain an envelope, which contains unique viral proteins drawn here as "spikes“ (5).

Envelope Virus structure

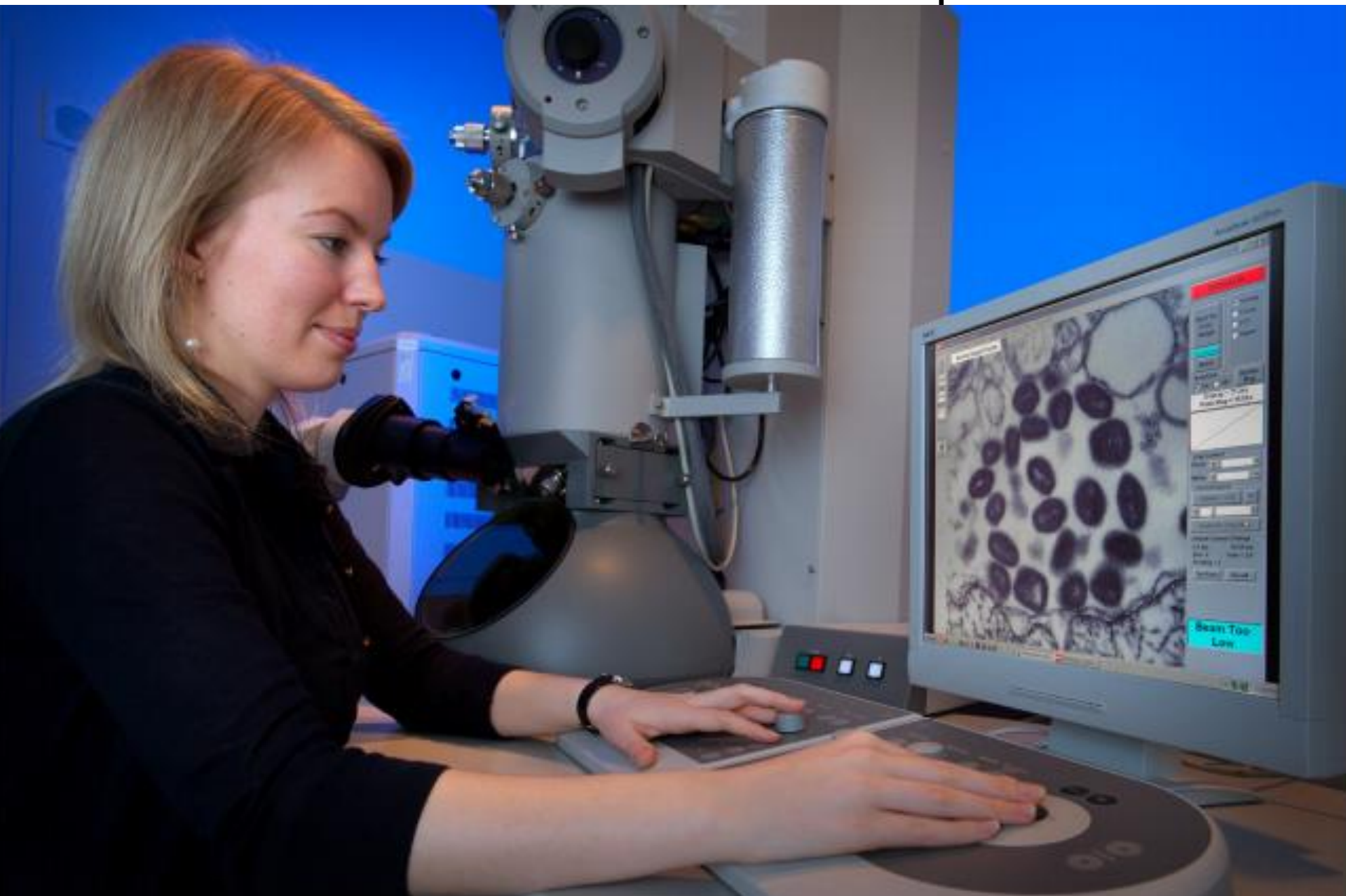
1 – genetic information

2 – Capsid

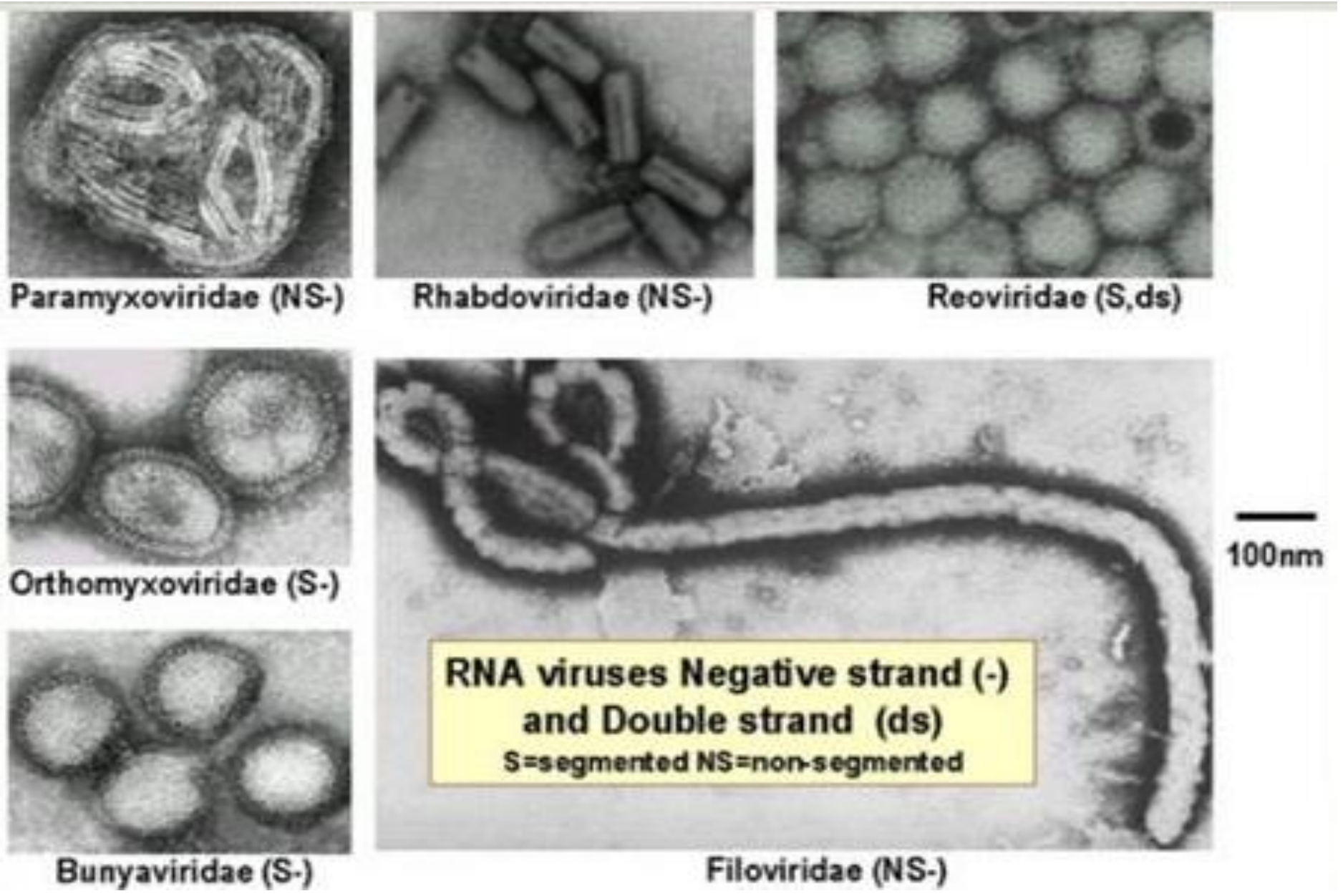
3 –Envelope with spikes

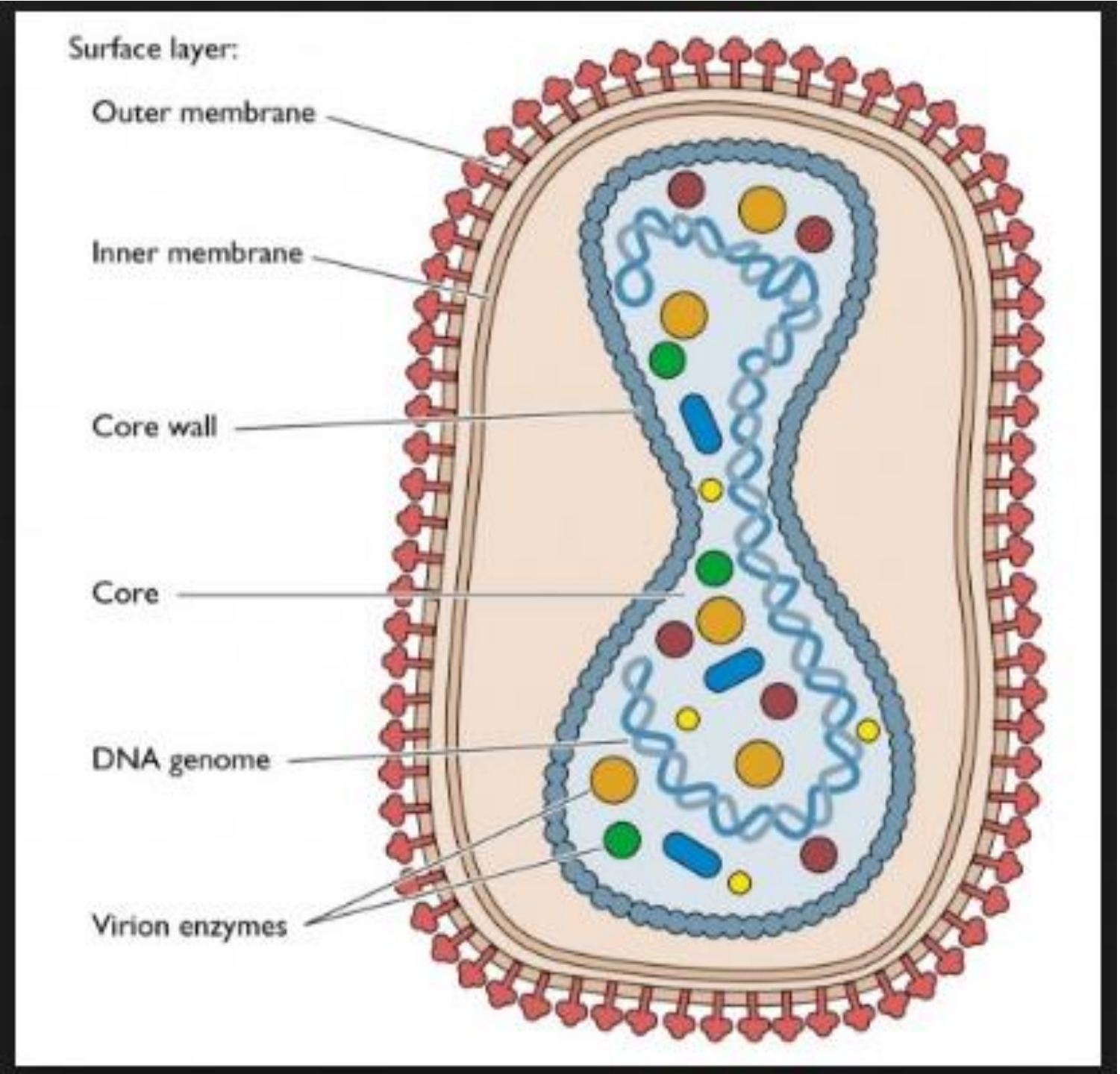


Study of the structure of the variola virus with an electron microscope



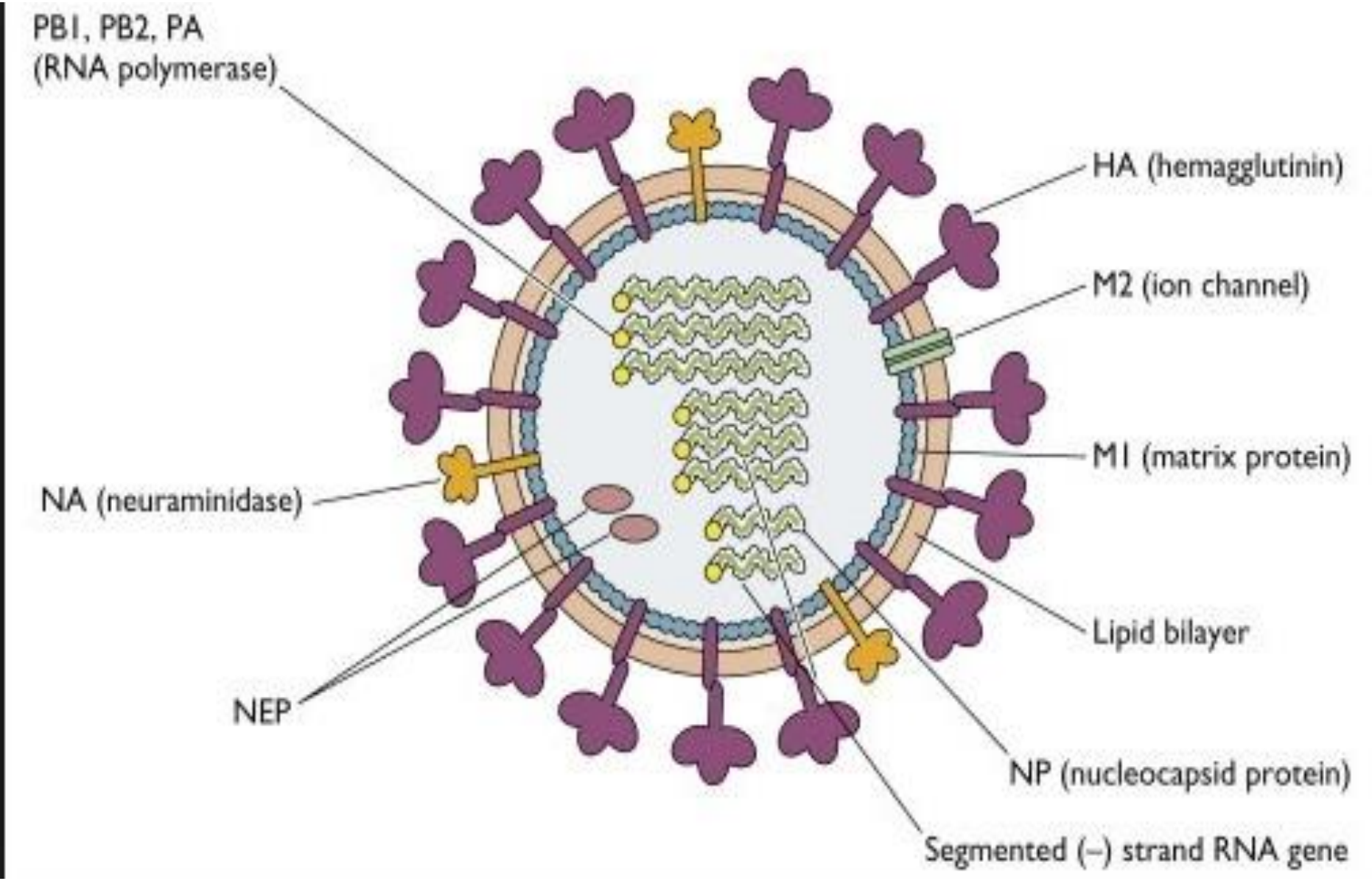
Photos of viruses

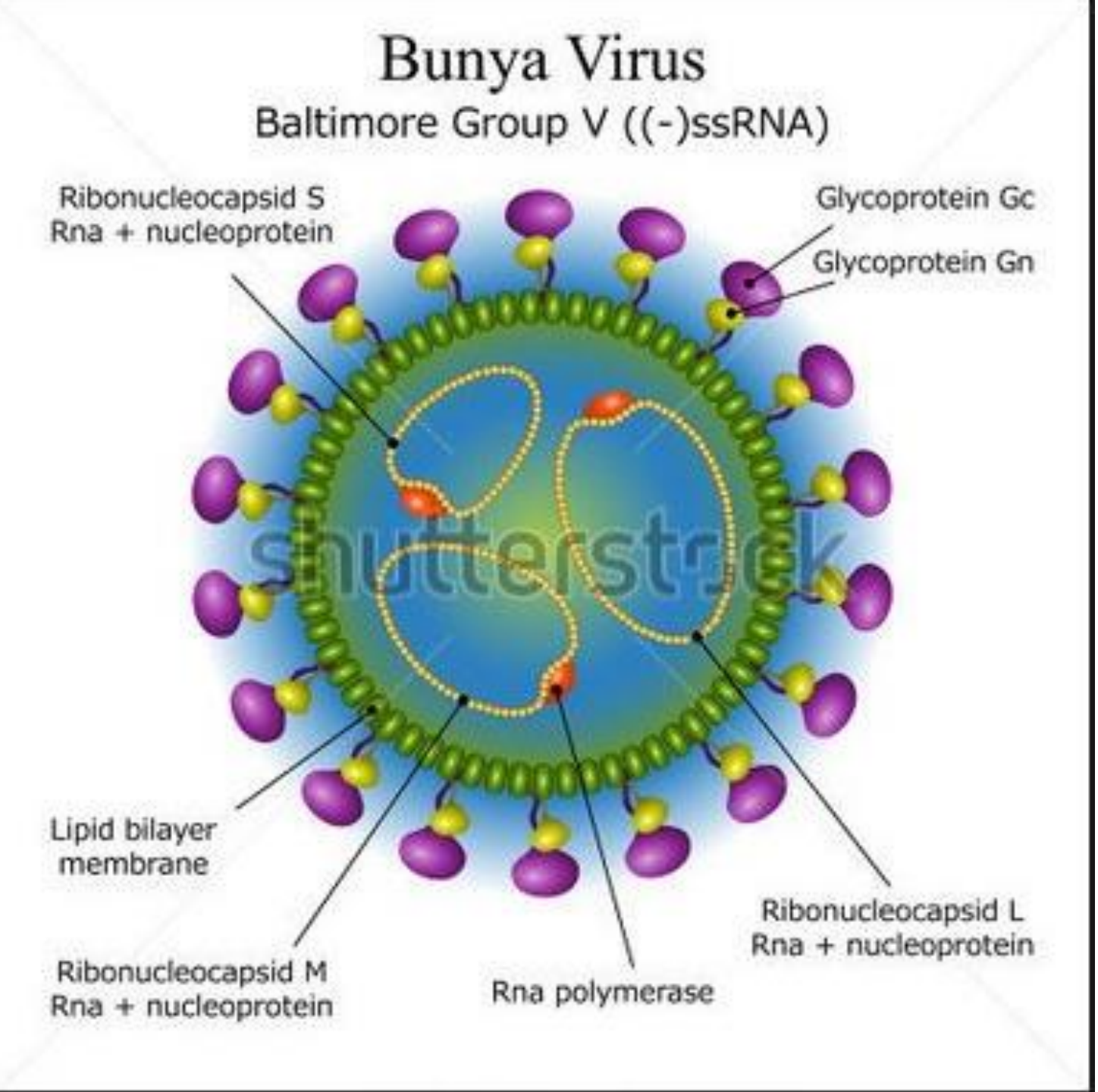




Virus structure

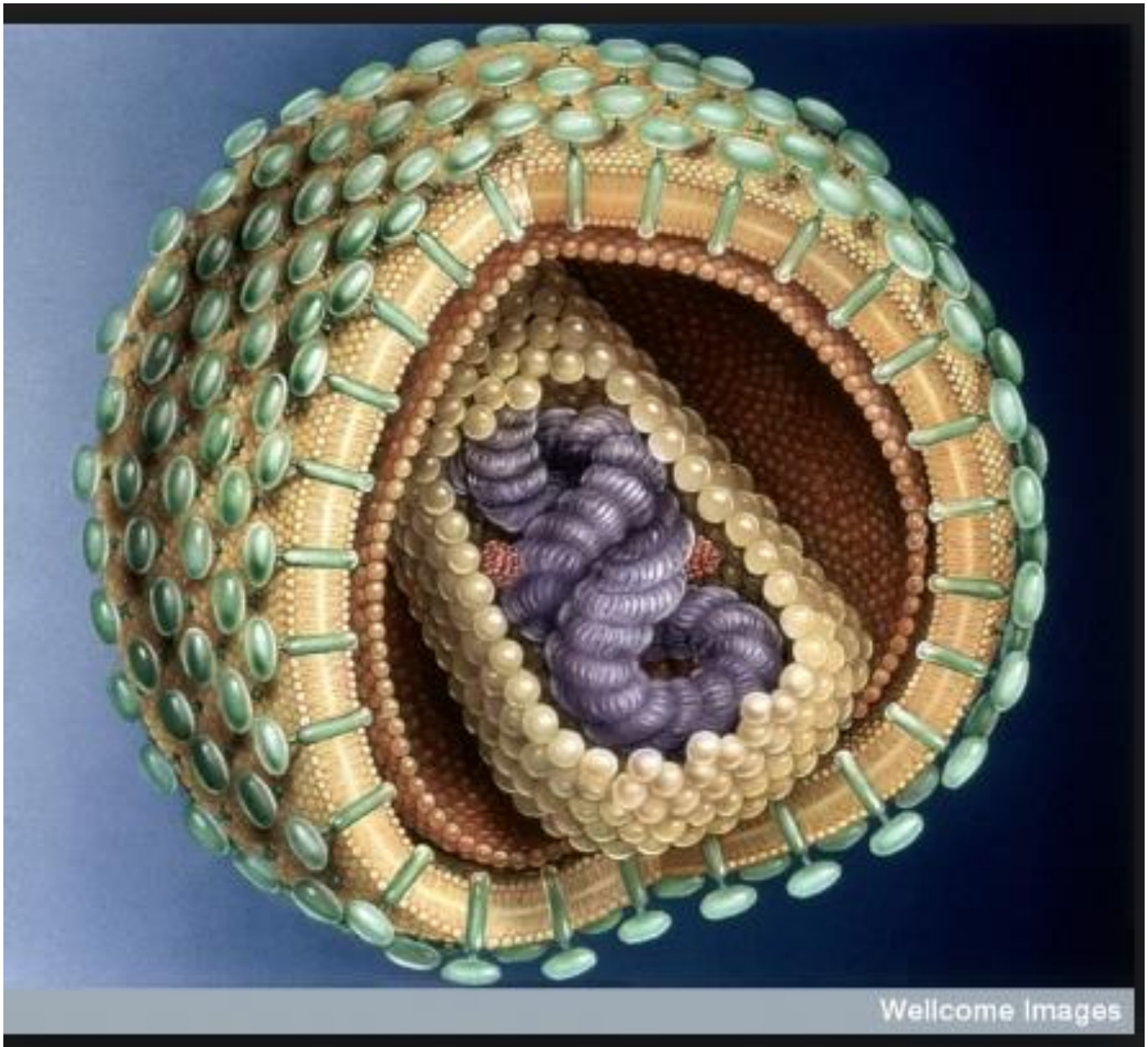
Virus structure





Virus structure

Virus structure (HIV)



Viral Replication

Viruses require living cells for reproduction. The cell that may be infected named host cell or target cell. Viruses are obligate intracellular parasites that are dependent on cellular energy production and cellular machinery for synthesis of their components.

Viruses have unique replication strategies – disjunctive mode of reproduction. This mode consists of separable synthesis of viral components in a host cell and spontaneous macromolecular interaction for their maturation.

There are permissive and nonpermissive cells in human organism. The **permissive** cell has a suitable cell receptors and intracellular requirements are sufficient for supporting the replication of a particular virus. A productive viral infection proceeds in permissive cells with form new virion. Virus replication is not supported in the **nonpermissive** cell because it does not have specific receptors. The virus cannot replicate itself in nonpermissive cell.

Viral life cycles in cells is terms **viral repduction** (**replication**)**.** Our knowledge of viral replication is now very detailed and is expanding rapidly. Every viral family has a different strategy of replication. Process of viral replication may be short – 4-12 hours or very long – for all the organism life.

An understanding of viral replication provides a basis for understanding pathogenesis, immunity, chemotherapy, and role of viruses in cancer.

There are two main ways that viruses reproduce or multiply

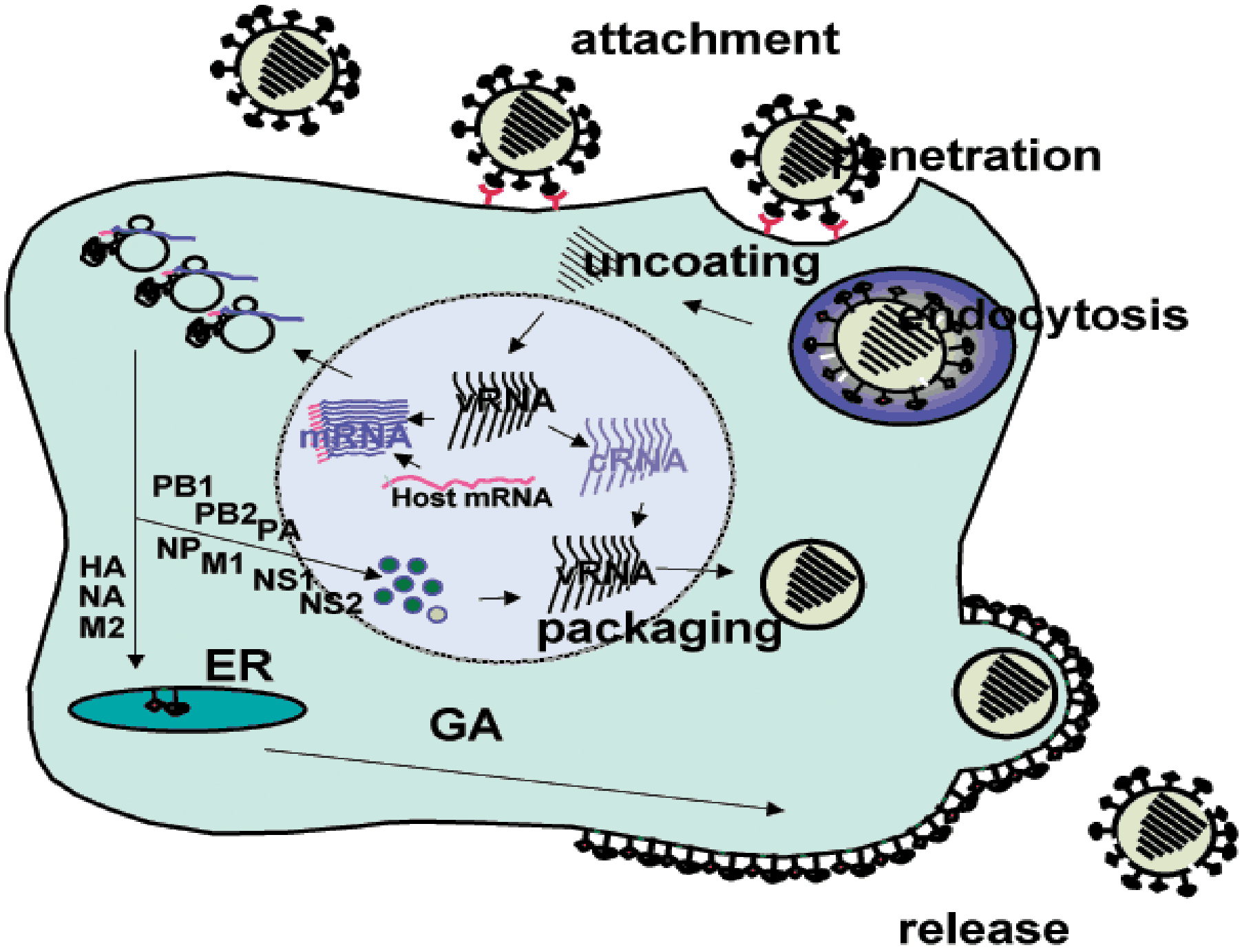
1. Lytic Cycle – the virus enters the cell, replicates itself hundreds of times, and then bursts out of the cell, destroying it.

2. Lysogenic Cycle – the virus DNA integrates with the host DNA and the host's cell helps create more virus DNA. An environmental change may cause the virus to enter the Lytic Cycle.

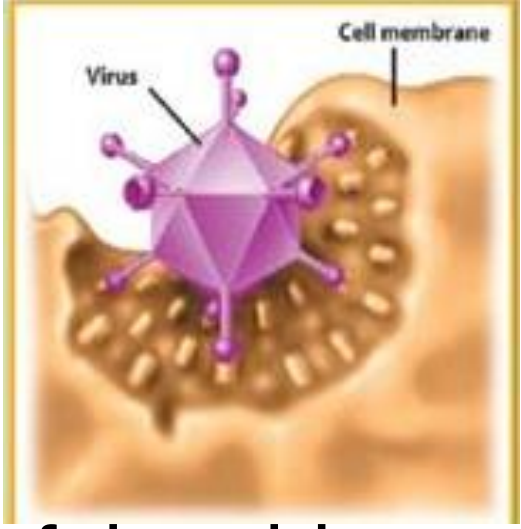
Viral Replication - Lytic Cycle

Lytic - the virus may be "lytic" (assembling new viral particles from the host's biomolecules) and eventually lysing the host to release the newly-assembled viral population. Lytic stages include:

- **Adsorption** - virus attaches to host's cellular membrane
- **Penetration** - virus injects genetic material through membrane
- **Uncoating** - viral coat digested releasing viral genetic material
- **Synthesis** - virus commences cellular take-over and assembly
- **Release** - newly-made viral particles released, cell lysed.


attachment
penetration
uncoating
endocytosis
vRNA
mRNA
cRNA
Host mRNA
PB1
PB2
PA
NP
M1
NS1
NS2
HA
NA
M2
vRNA
packaging
ER
GA
release

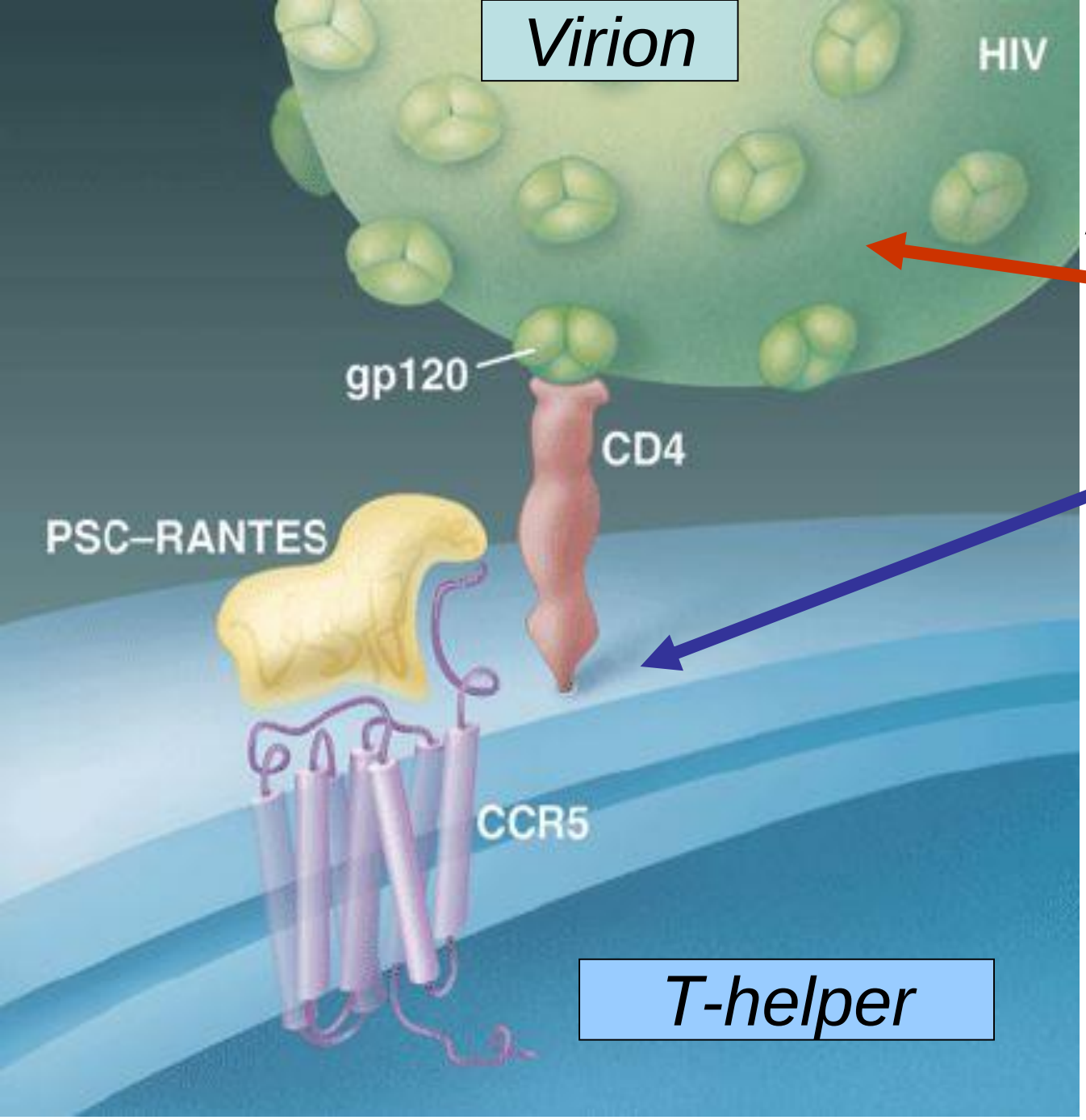
*1.**Attachment (Adsorption**) This is the first step in viral replication.*



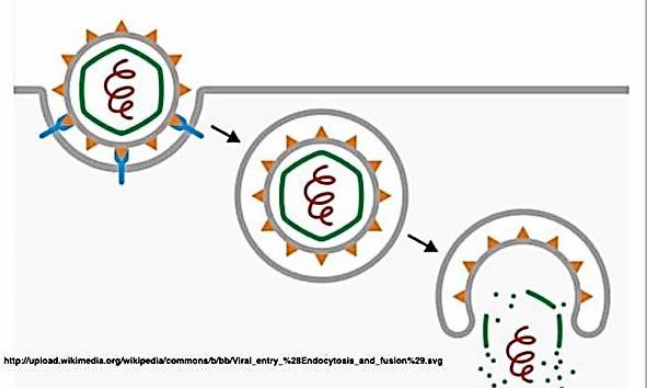
- Specific molecules on the surface of the virions attach specific receptors on the plasma membrane of cell. Specific-binding proteins of the virus interact with specific receptors on the target cell surface. Many kinds of virus receptors are now known. Adsorption occurs to specific cellular receptors. Some receptors are glycoproteins, others are phospholipids or glycolipids.

*1.**Attachment (Adsorption)***

- Some viruses have a very narrow host range, meaning that they can only infect one or a small number of cell types. For example, HIV infects only human T cells, because the surface protein interacts with CD4 receptors on the surface of the T cell.
- Some complex viruses have broad host ranges, meaning that they can infect a large number of different cell types, for example, Influenzavirus and Herpesviruses.
- The expression of receptors on the surface of cells largely determines the TROPISM of viruses. Tropism is the type of cell in which viruses are able to replicate.



Adsorption of HIV on the surface of T-helper



2.**Penetration**

- The virus must enter the cell. They can penetrate cells in a variety of ways. Electron microscopic show that virions can enter cells by different mechanisms.
- Many envelope viruses penetrate into cell with help of **fusion** with the cell membrane when the envelope of viruses to fuse directly with the plasma membrane, and the nucleocapsid to be released into the cytoplasm.
- **Endocytosis** is a specific process of the uptake of essential macromolecules. Virus enters into intracellular vacuoles, and then into the cytoplasm.

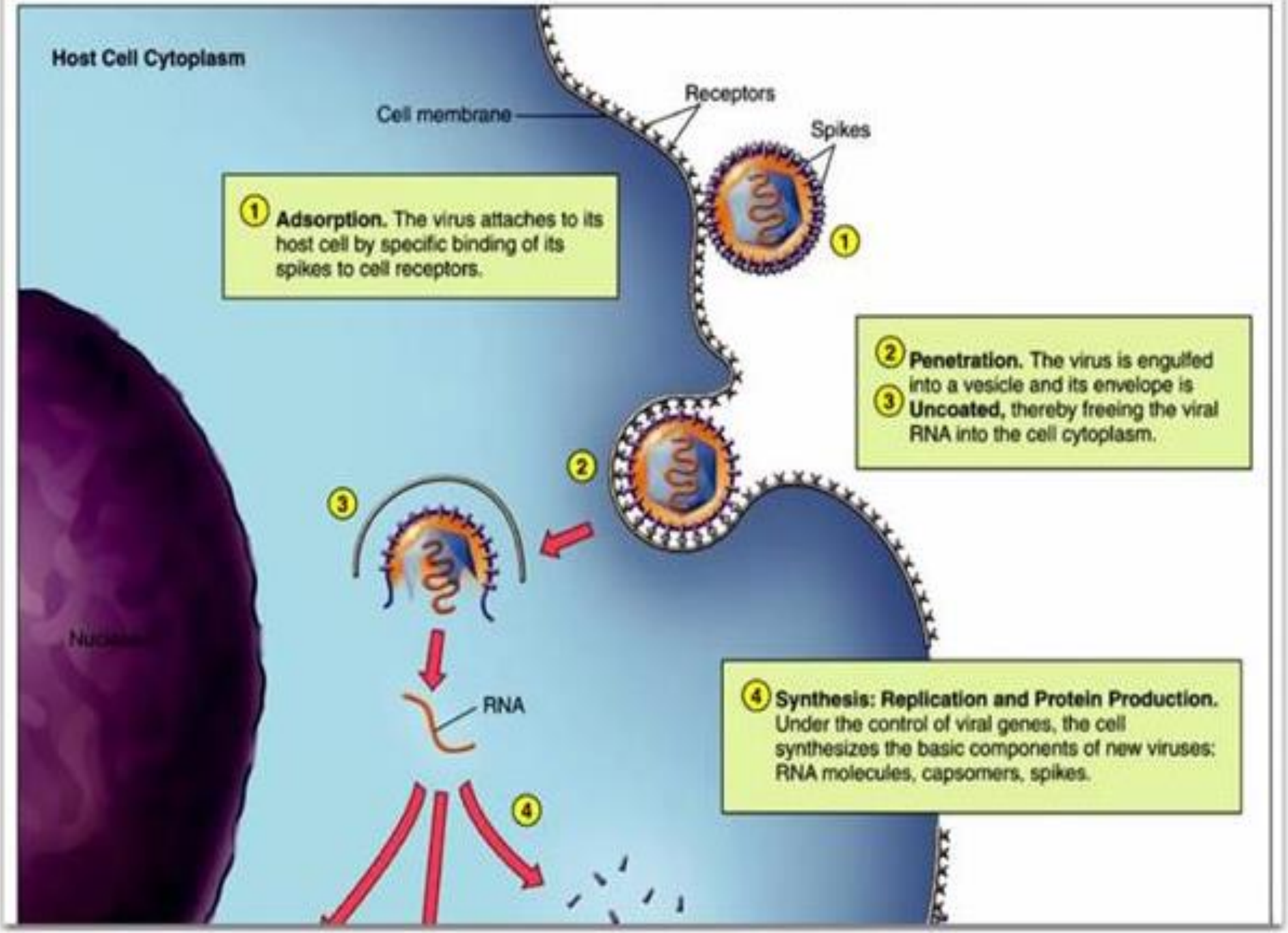
3.**Uncoating**

- **Uncoating** is the step in which viral enzymes degrade the virus capsid; that then exposes the viral genome to permit multiplication of the virus.

4.**Strategies of Viral Replication, Gene expression (biosynthesis)**:

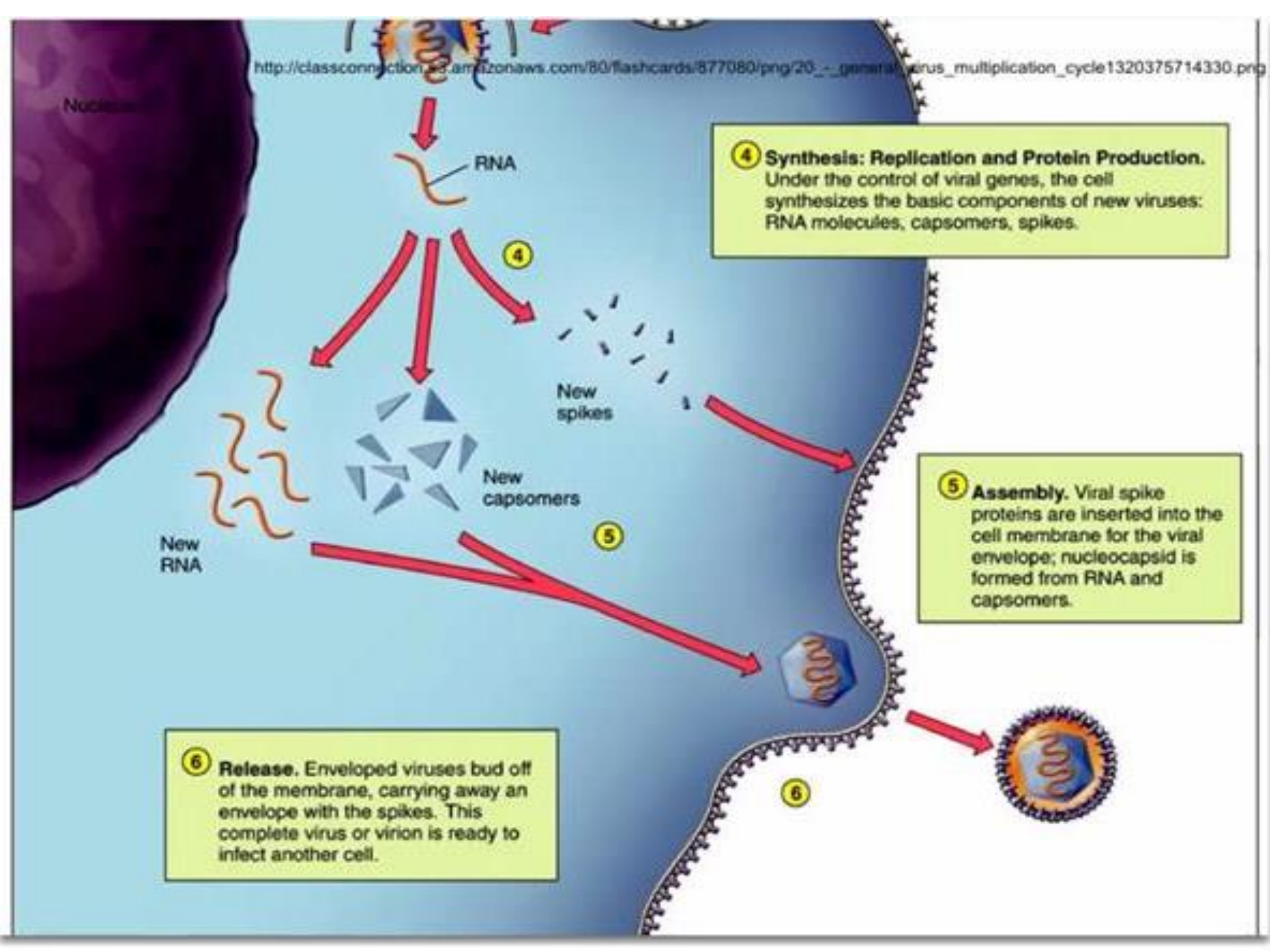
- synthesis of viral mRNA (transcription), synthesis of viral proteins (translation), and genome replication.
- The viruses must use host cellular machinery to replicate and make functional and structural proteins. Strategies for genomic expression for different taxonomic group of viruses are different.
- All virulent viruses shut off cellular protein synthesis and disaggregate cellular polyribosomes, favoring a shift to viral synthesis.

http://classconnection.s3.amazonaws.com/80/flashcards/877080/png/20_-_general_virus_multiplication_cycle1320375714330.png



5.Virion assembly (maturation)

- The process assembly of the new components into virions.
- The process of virion assembly involves bringing together newly formed viral nucleic acid and the structural proteins to form the nucleocapsid of the virus. There are basically two strategies that viruses employ.
- For example, for many nonenveloped viruses, the viral capsid is partially assembled, the newly synthesized capsid proteins associate together into a capsid-like structure. The viral genome is then inserted into the capsid to form a nucleocapsid.

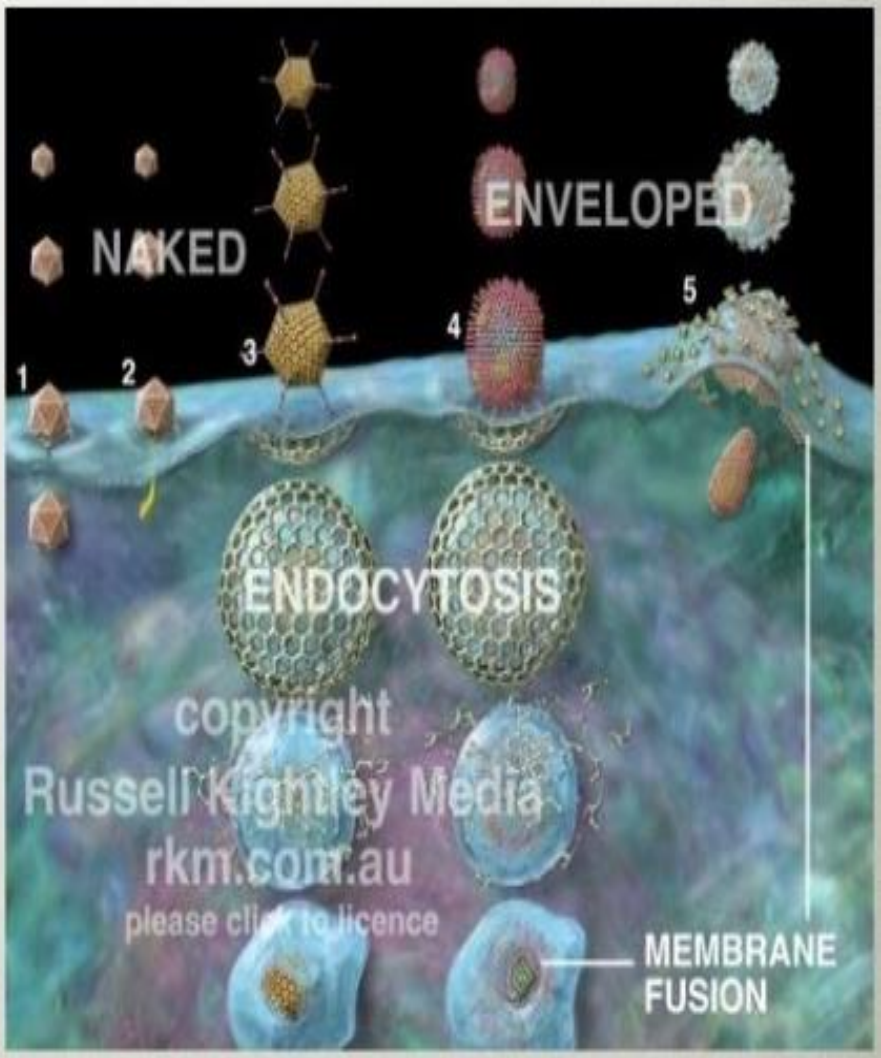

RNA
4
New spikes
New capsomers
New RNA
5
6
4 Synthesis: Replication and Protein Production. Under the control of viral genes, the cell synthesizes the basic components of new viruses: RNA molecules, capsomers, spikes.
5 Assembly. Viral spike proteins are inserted into the cell membrane for the viral envelope; nucleocapsid is formed from RNA and capsomers.
6 Release. Enveloped viruses bud off of the membrane, carrying away an envelope with the spikes. This complete virus or virion is ready to infect another cell.

6.Release

- of new infectious virus. There are basically two mechanisms for the release of mature virions from the infected cell: cytopathogenic or noncytopathogenic.
- Most nonenveloped viruses accumulate within the cytoplasm or nucleus. Release occurs only when the cell lysis or until the cell slowly degenerates and dies.
- Most enveloped viruses are released by budding from the plasma membrane. It's a process which can occur over a prolonged period without much damage to the cell.
- Viruses can replicate tens of thousands of times within only a few hours.

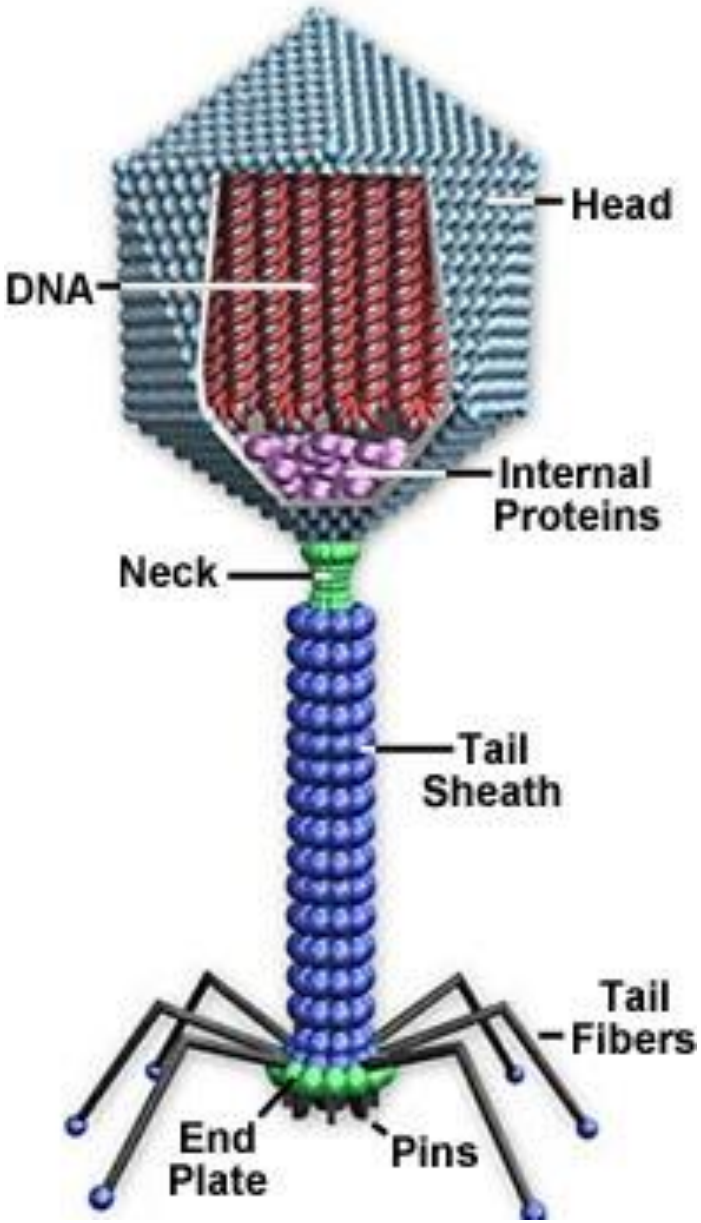
Release of envelope viruses

Enveloped viruses do not necessarily have to kill their host cell in order to be released, since they can **bud out of the cell** - a process that is not necessarily lethal to the cell - hence some budding viruses can set up persistent infections



Structure of Bacteriophages

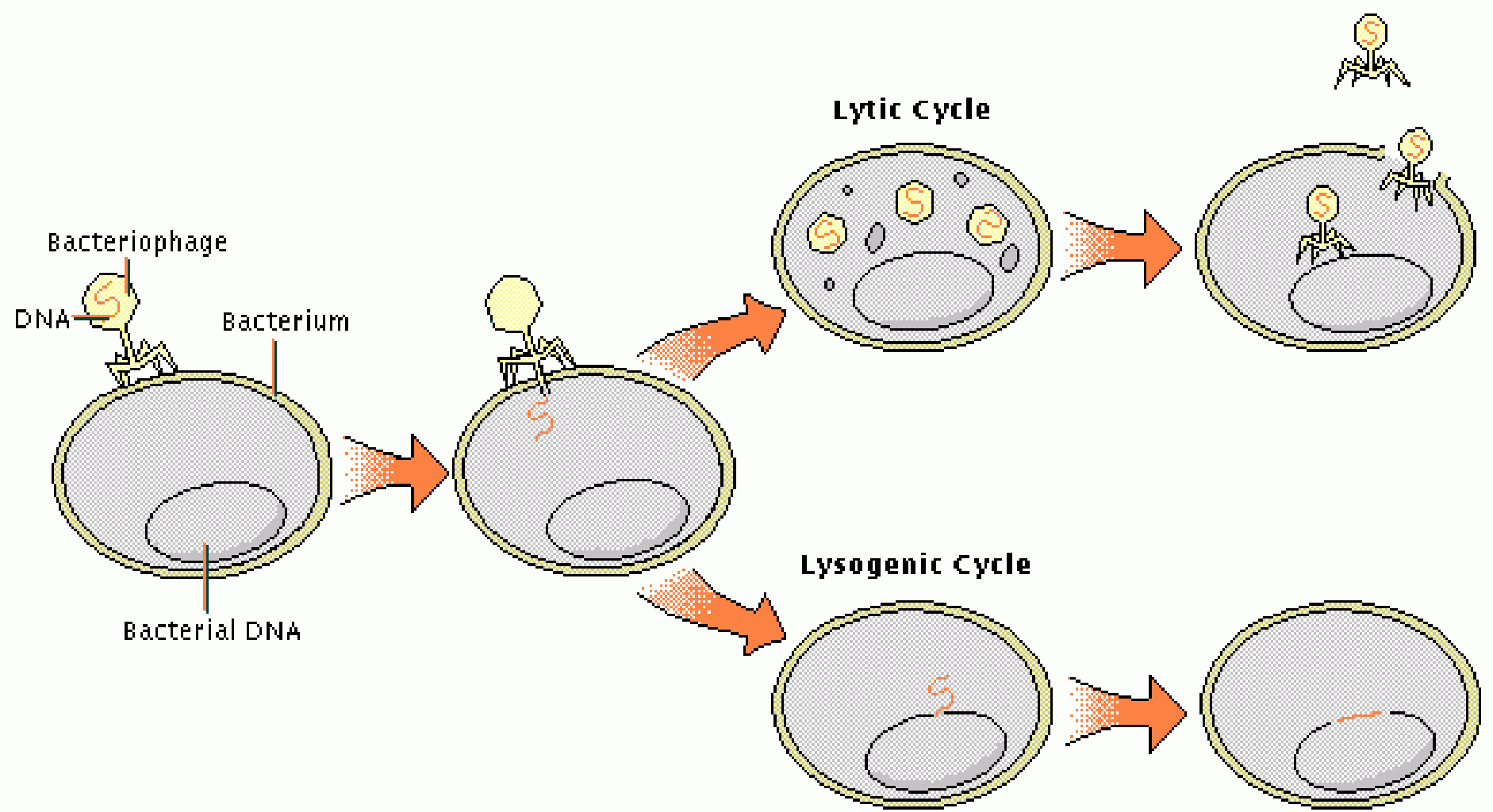
1. **Size**: Most phages range in size from 24-200 nm in length.
2. **Head or Capsid**: Some are icosahedral others are filamentous. The head is composed of proteins. Inside the head is found the nucleic acid. The head acts as the protective covering for the nucleic acid.
3. **Tail**: Many but not all phages have tails attached to the phage head. The tail is a hollow tube through which the nucleic acid passes during infection. In the more complex phages the tail is surrounded by a contractile sheath which contacts during infection of the bacterium. At the end of the tail the more complex phages have a base plate and one or more tail fibers attached to it. The base plate and tail fibers are involved in the binding of the phage to the bacterial cell.



All bacteriophages have a *lytic* or infectious cycle, in which the virus, incapable of replicating itself, injects its genetic material into a bacterium. By pirating its host's enzymes and protein-building capacities, the virus can reproduce and repackage, making about 100 new copies before it bursts from and destroys the bacteria.

Some bacteriophages, however, behave differently when they infect a bacterium. The injected genetic material instead integrates itself into its host DNA, passively replicating with it to be inherited by bacterial daughter cells. In about 1 in 100,000 of these *lysogenic* cells, the viral DNA spontaneously activates and starts a new lytic cycle.

Lytic and Lysogenic Cycle of Bacteriophages



- Lytic cycle: Phage causes lysis and death of host cell.
- **Lysogenic cycle: Prophage DNA incorporated in host DNA.**

How are viruses named?

1. Named after the diseases
   eg. Measles virus, smallpox virus

2. Name after the places where the disease first reported
   eg. Newcastle disease virus, Ebola virus, Norwalk virus, Bunyaviridae

3. Host and signs of disease
   eg. Tobacco mosaic virus, cauliflower mosaic virus brome mosaic virus

How are viruses named?

4. Latin and Greek words
   eg. Coronaviridae – "crown"
   Parvoviridae – "small"

5. Virus discovers
   eg. Epstein-Barr virus

6. How they were originally thought to be contracted
   eg. dengue virus ("evil spirit"), influenza virus (the "influence" of bad air)

7. Combinations of the above
   eg. Rous Sarcoma virus

How are viruses named?

Before discovery

- *Dermotropic* – infected skin cell
- *Neurotrophic* – infected nerve cell
- *Viscerotropic* – infect organ of digestive tract
- *Pneumotropic* – infected respiratory system

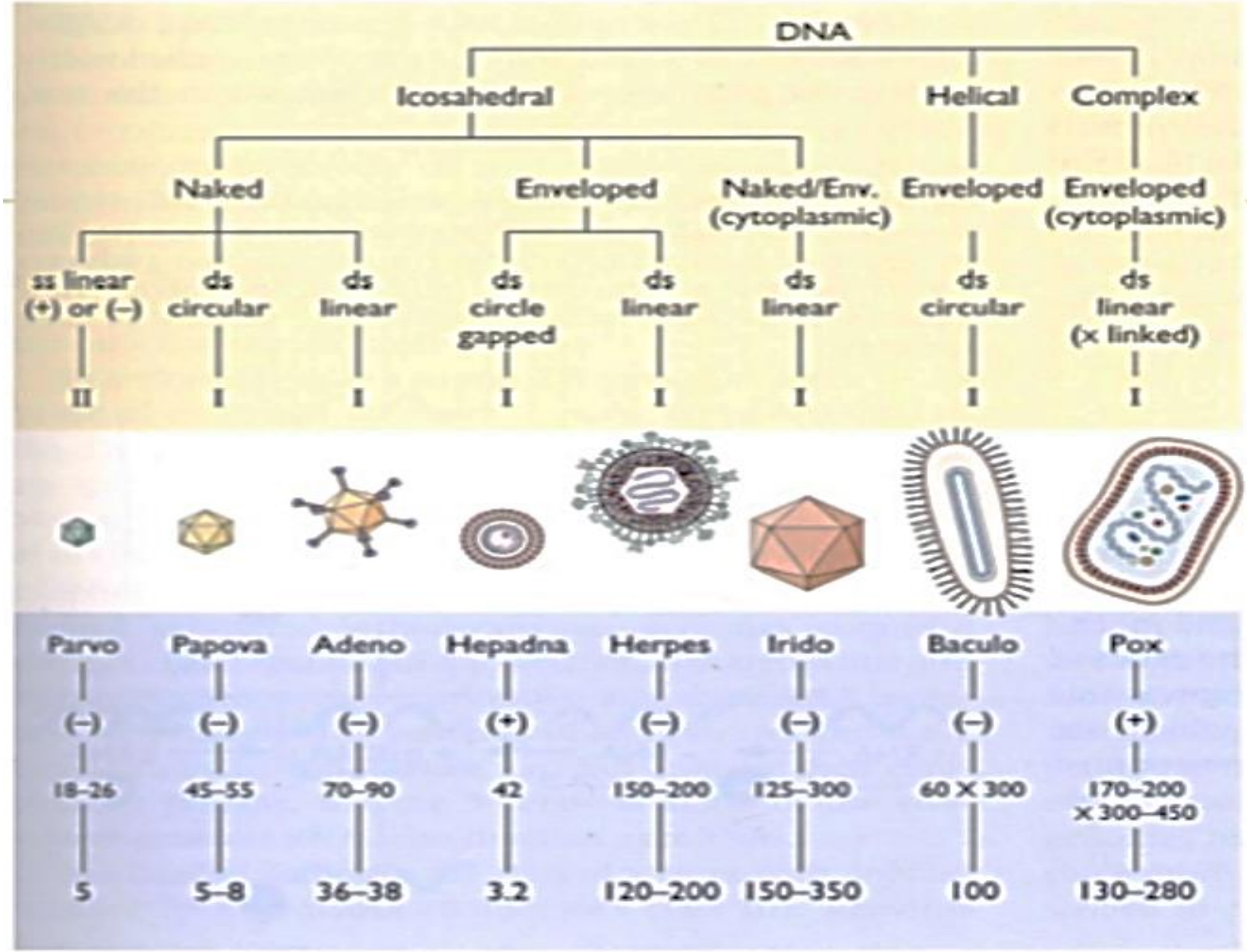
Classification of Viruses

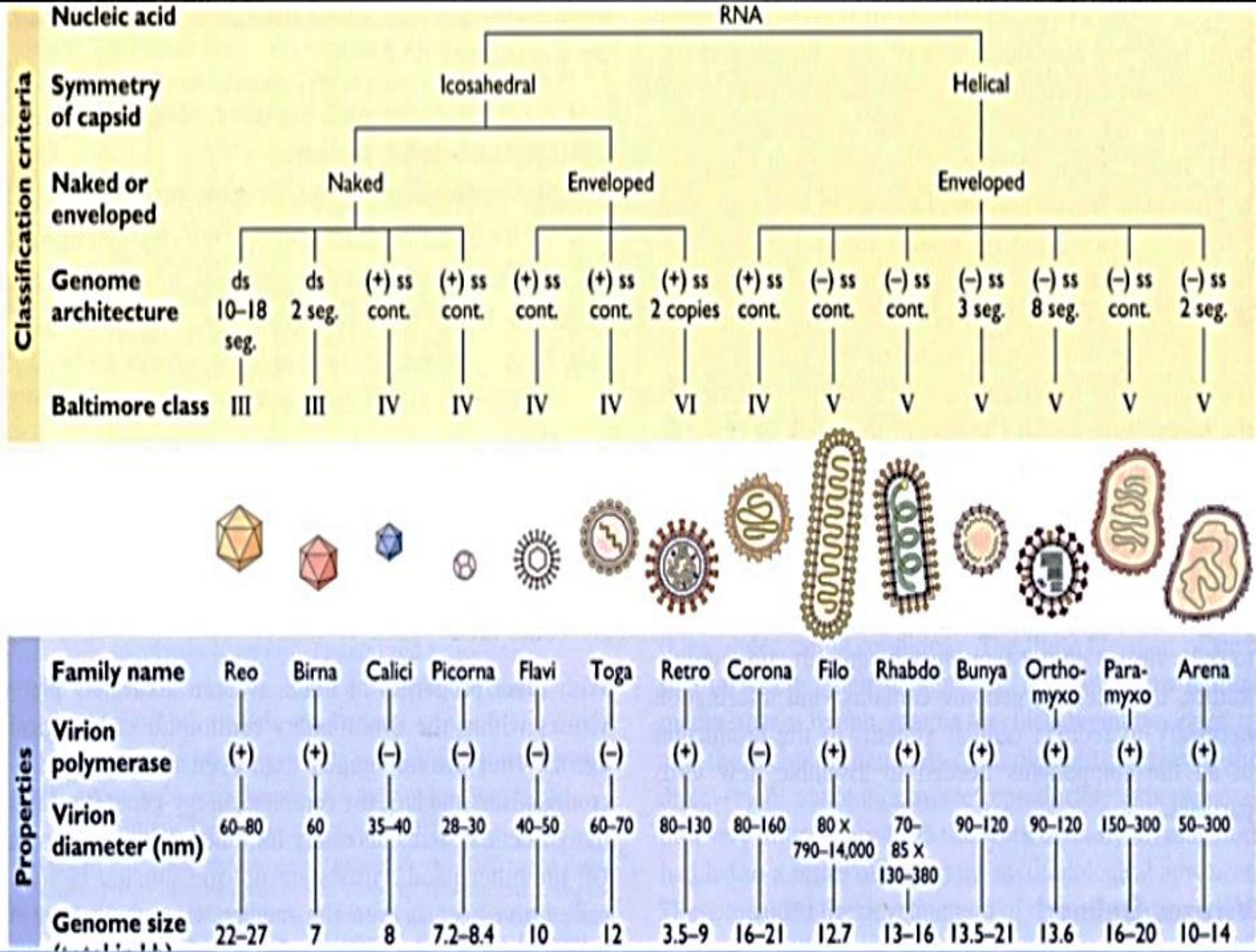
Viruses are classified on the basis of **host range**, morphology (size, shape), type of nucleic acid (DNA, RNA, single-stranded, double-stranded, linear, circular, segmented, etc.) and occurrence of auxilliary structures such as tails or envelopes.

International Committee on Taxonomy of Viruses names them based on three characteristics:

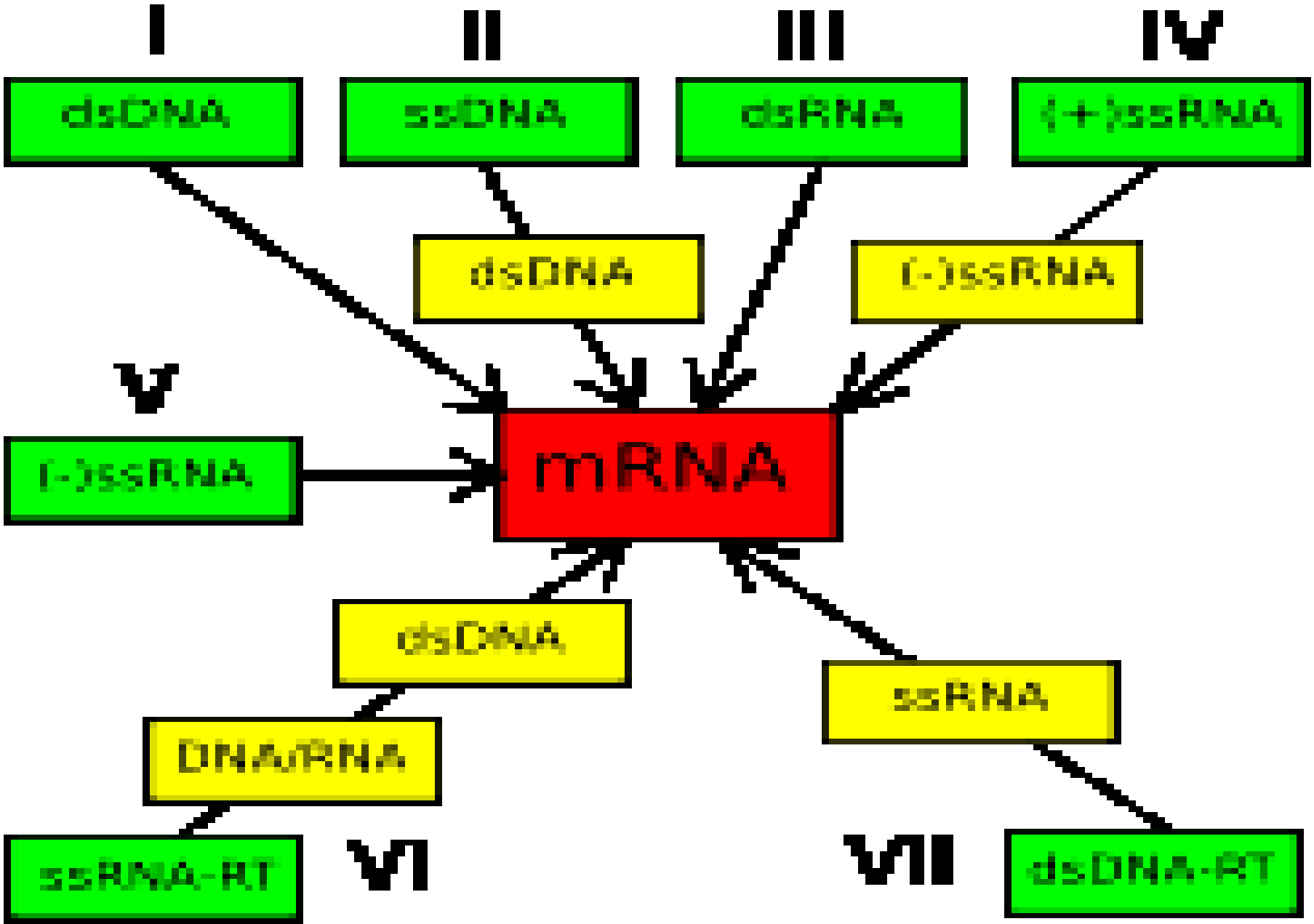
1. Type of nucleic acid;
2. Is the nucleic acid double or single stranded:
3. Presence or absence of nuclear envelope.

Classification of Viruses




Classification criteria
Nucleic acid
RNA
Symmetry of capsid
Icosahedral
Helical
Naked or enveloped
Naked
Enveloped
Enveloped
Genome architecture
ds 10–18 seg.
ds 2 seg.
(+) ss cont.
(+) ss cont.
(+) ss cont.
(+) ss cont.
(+) ss 2 copies
(+) ss cont.
(–) ss cont.
(–) ss cont.
(–) ss 3 seg.
(–) ss 8 seg.
(–) ss cont.
(–) ss 2 seg.
Baltimore class
III
III
IV
IV
IV
IV
VI
IV
V
V
V
V
V
V
Properties
Family name
Reo
Birna
Calici
Picorna
Flavi
Toga
Retro
Corona
Filo
Rhabdo
Bunya
Ortho-myxo
Para-myxo
Arena
Virion polymerase
(+)
(+)
(–)
(–)
(–)
(–)
(+)
(–)
(+)
(+)
(+)
(+)
(+)
(+)
Virion diameter (nm)
60–80
60
35–40
28–30
40–50
60–70
80–130
80–160
80 x 790–14,000
70–85 x 130–380
90–120
90–120
150–300
50–300
Genome size
22–27
7
8
7.2–8.4
10
12
3.5–9
16–21
12.7
13–16
13.5–21
13.6
16–20
10–14

BALTIMORE CLASSIFICATION SYSTEM



CULTIVATION OF VIRUSES:

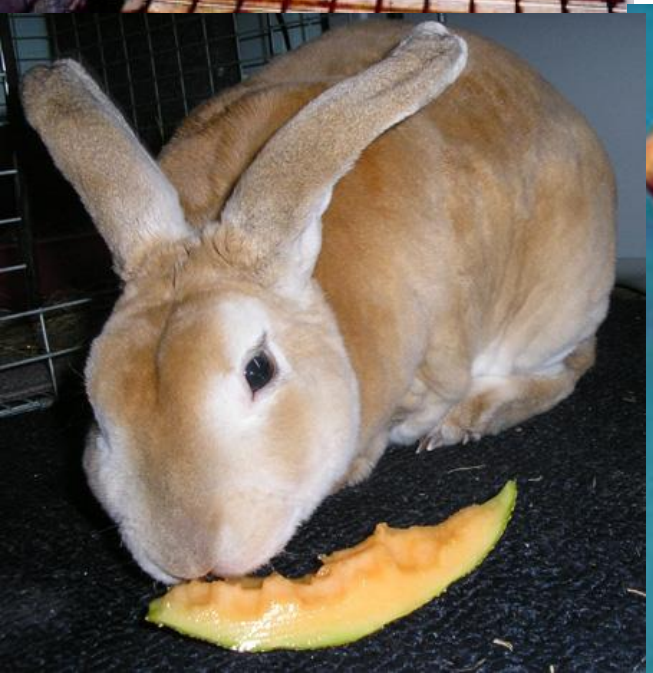
1. ***Laboratory animals***

2. ***Embryonated eggs***

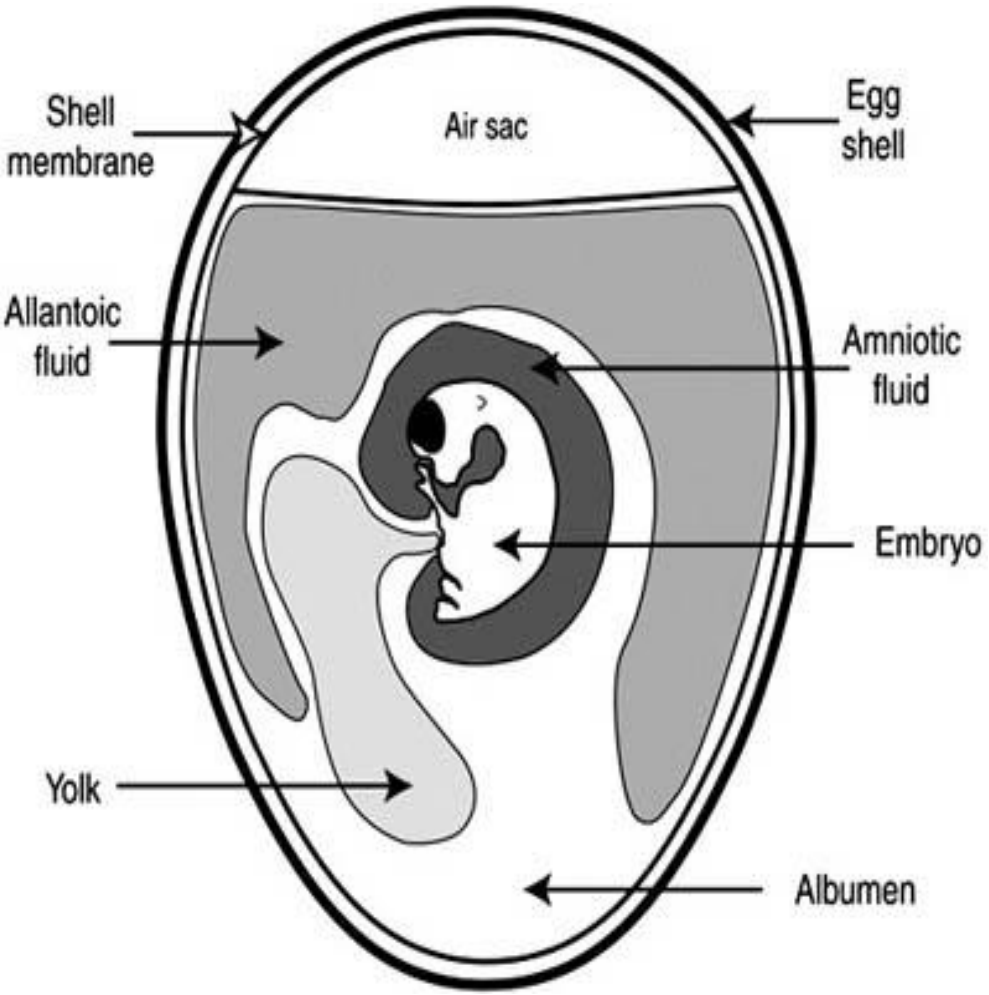
3. ***Cell culture***
   - » Primary cell cultures
   - » Diploid cell strains
   - » Continuous cell lines

Laboratory animals are used for primery isolation of certain viruses and for studies of the pathogenesis of viral diseases.

They are inoculated by intracerebral or intranasal routes. Specimens may also be inoculated by intraperitoneal and subcutaneous routes.



Growth of virus on embryonated eggs



This method is used for the cultivation of more than 30 species of viruses.
The material containing the virus is inoculated into the amnion, allantois, yolk sac and others.
After inoculation of the chick embryo, it is incubated and examined daily for virus growth.

The virus is inoculated into the allantois of the embryonated egg

Microbiologist is inoculating 10-day of emryonated chicken eggs. CDC.

The cell culture method is employed nowadays for identification and cultivation of viruses. Cell suspension is distributed in tubes, bottles or Petri dishes.

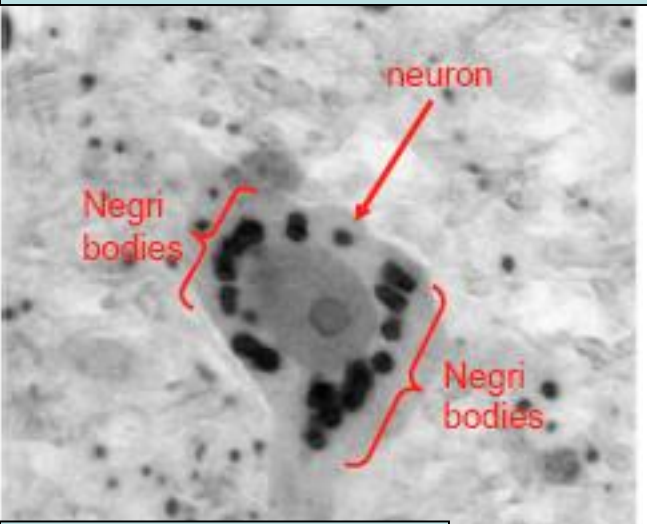
Cytopathic effect (CPE)

The development of the cytopathic effect is an indicator of the replication of the virus in cell cultures.

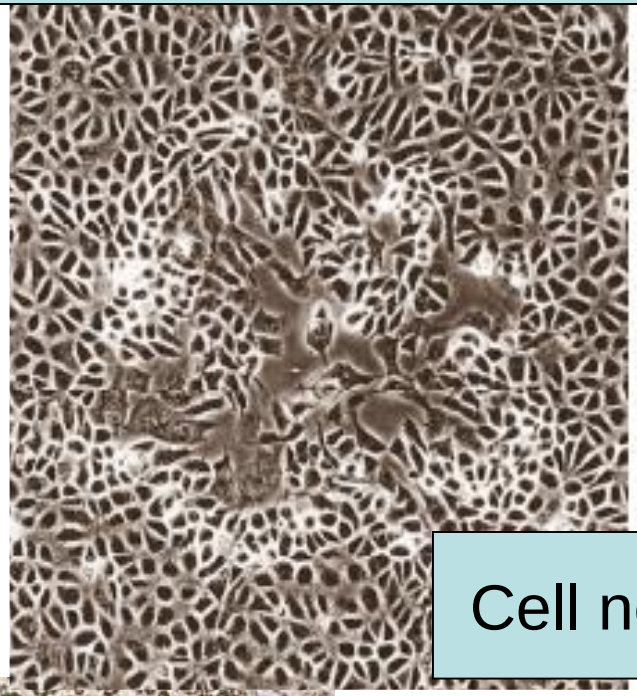
CPE are of the following types:

- total destruction of the monolayer sheet (cell necrosis and lysis), e.g. enteroviruses;
- syncytium (multinucleated giant cells) formation, e.g. measles virus;
- cellular clumping, e.g. adenovirus;
- inclusion bodies;
- discrete focal degeneration, e.g. herpes virus;
- transformation, e.g. oncogenic virus.

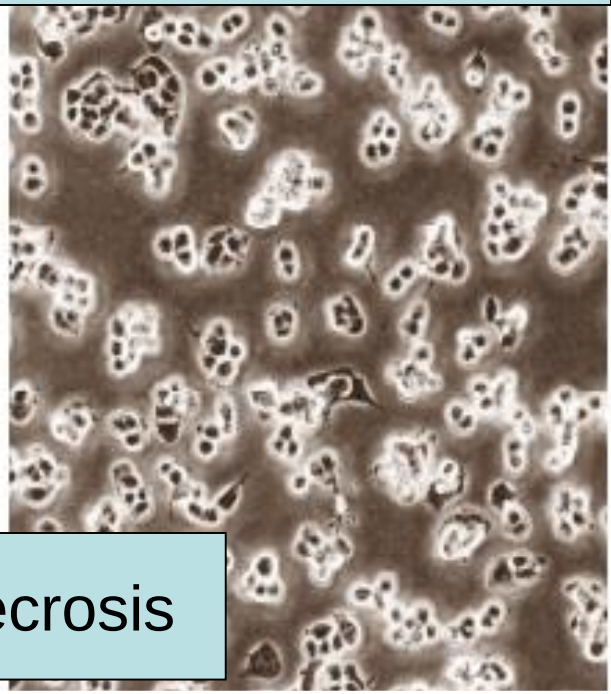
Cytopathic effect (CPE)



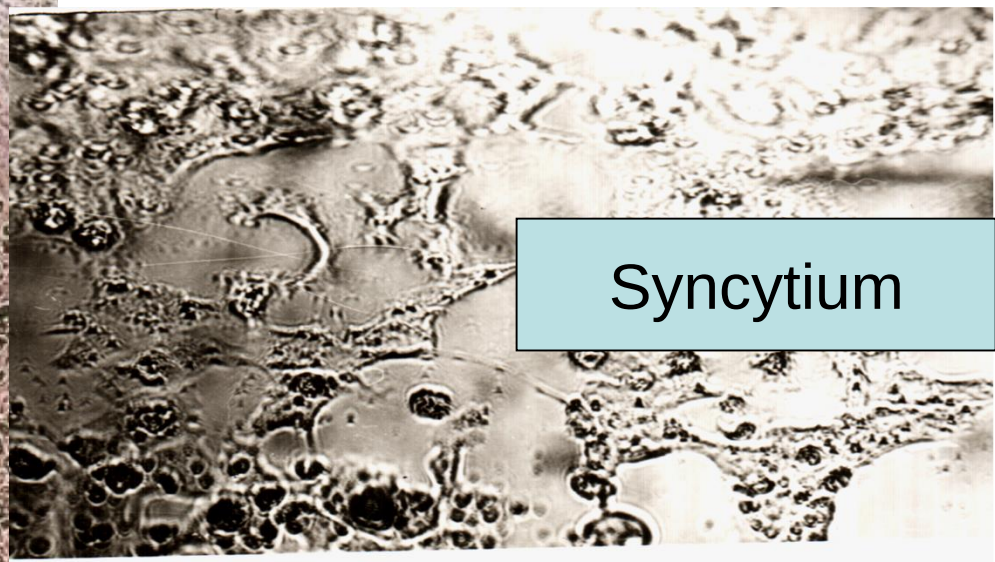
Inclusion body



Cell necrosis



Hemadsorbtion



Syncytium

Conclusions:

Thank you for attention