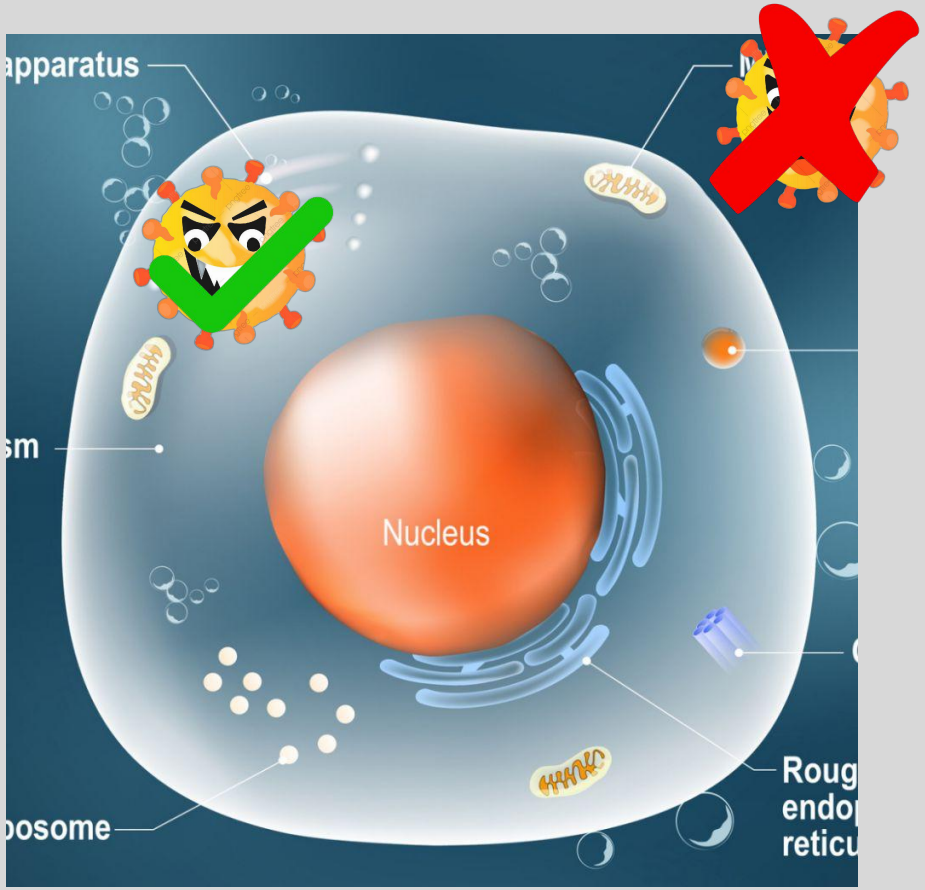
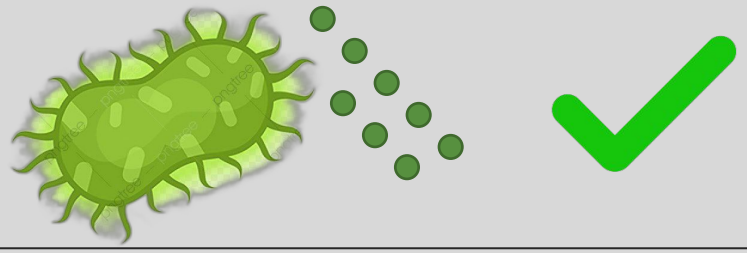
INTRODUCTION TO VIROLOGY (2)

Done by: Abdelhadi Okasha

Obligate intracellular



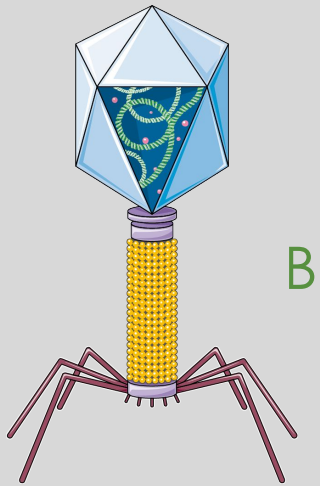


contain sole genome

DNA RNA

Both

Very diverse



Bacteriophages

Smallest infectious agent

(20-300 nm)

A: Nucleic acid

B: Capsid

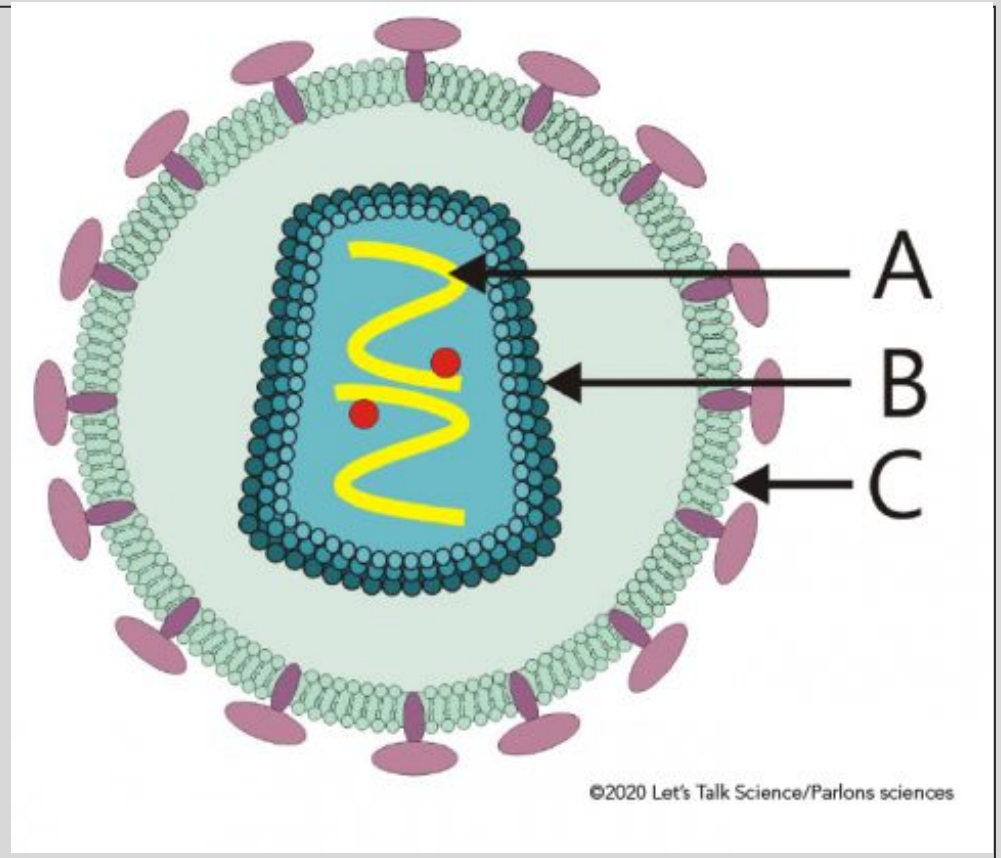
Protein coat

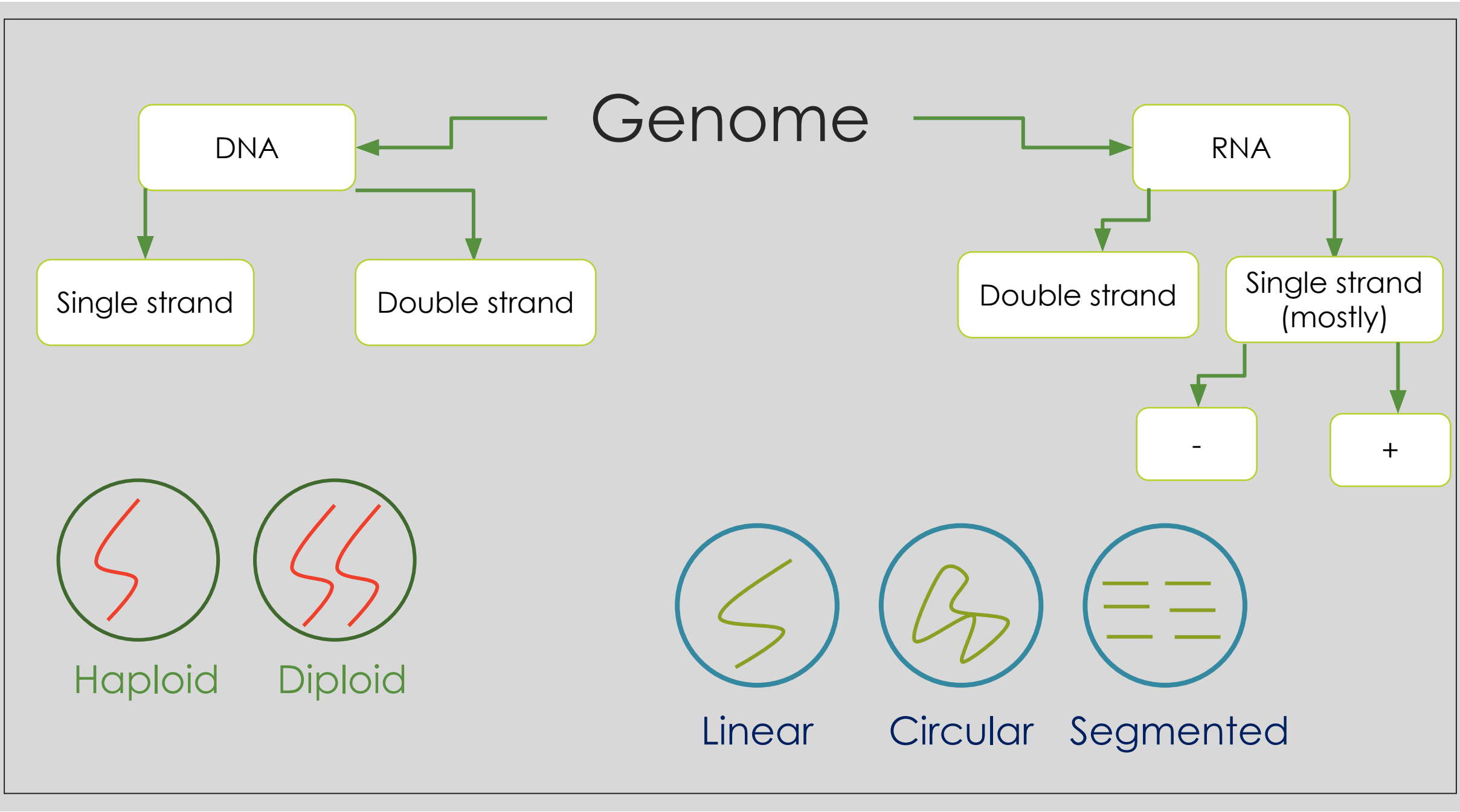
C: Envelope

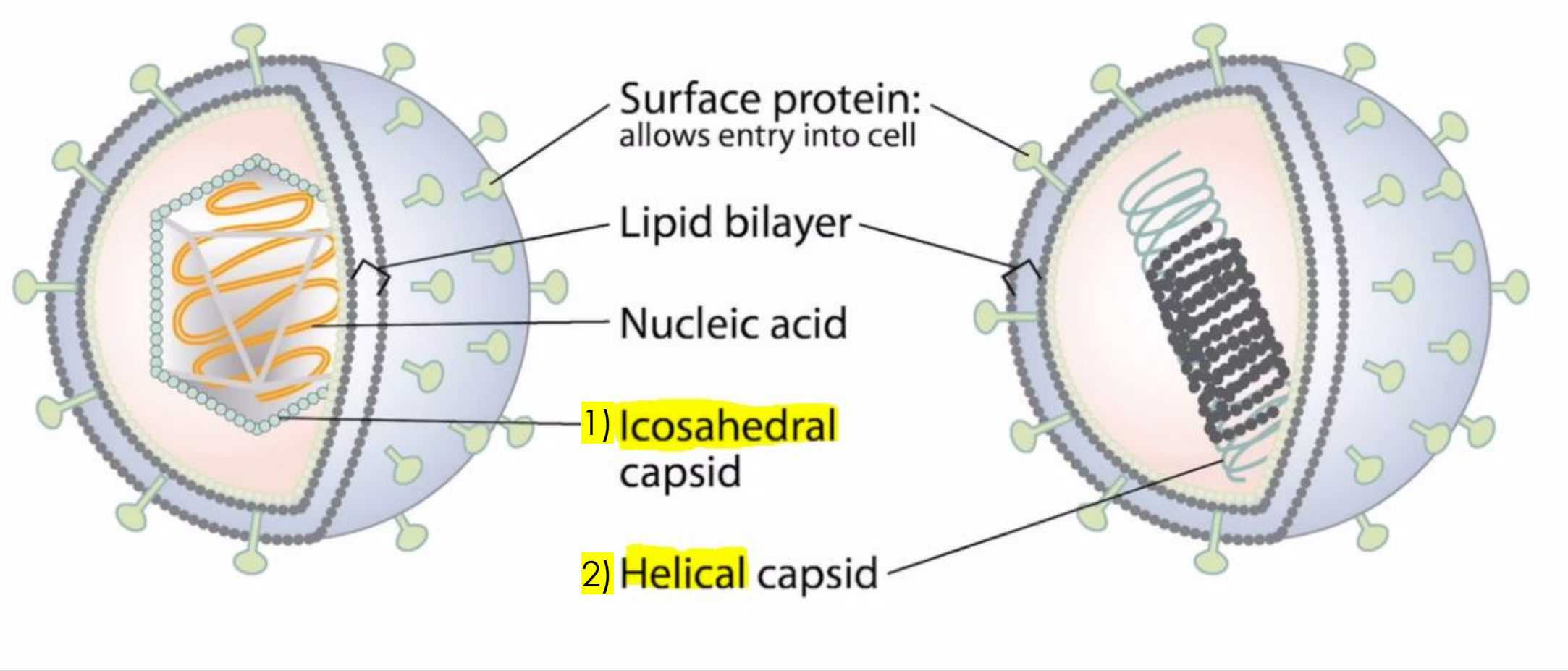
Lipid membrane

Not always

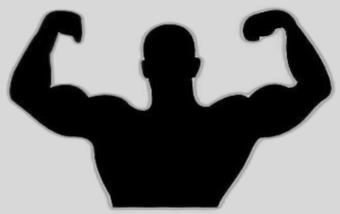
Contain some Proteins

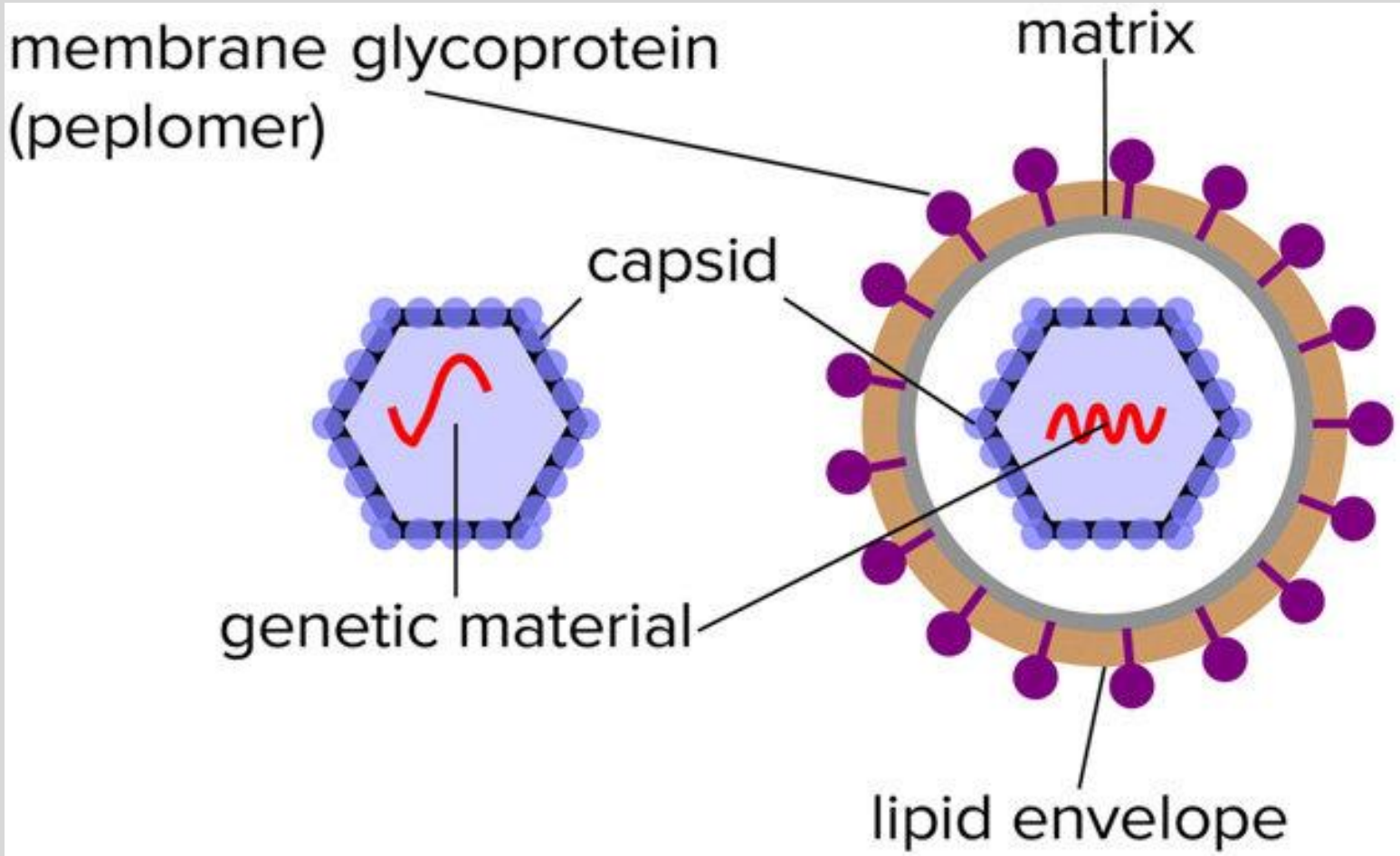



Genome
DNA
RNA
Single strand
Double strand
Double strand
Single strand (mostly)
-
+
Haploid
Diploid
Linear
Circular
Segmented



3) Complex capsid





Naked Enveloped

Topics discussed in this lecture

1- Cultivation and assay of viruses

- Types of cell culture: primary culture, secondary culture and continuous cell lines
- Signs of replication of viruses: developing cytopathic effects (e.g. inclusion bodies) , appearance of viral synthesized proteins, adsorption of cells into an infected cell, detection of nucleic acid, Viral growth in an embryonated chick egg

2- Quantitation of Viruses

- Titer: 50% infectious dose.
- Type of methods to detect the quantity: physical and biological.

3- Purification of Virus Particles

Done by: Concentration + separation

4- Effect of chemical and physical agents

- Agents that affect viruses: 1) Heat and Cold, 2) Adding salts, 3) pH, 4) High energy particles, 5) Some detergents, 6) Vital dyes and 7) Others
- We need to inactive viruses for many reasons (e.g. Vaccination)

5- Replication of viruses

- Types of Infections: Productive, abortive and latent
- eclipse: intense synthetic activity period
- Steps of replication: Adsorption/ Penetration/ uncoating/ Early transcription/ early translation/ DNA synthesis and late transcription/ late translation/ condensation/ assembly/ release

6- Genetics of animal viruses

- Some terms: Genotype, phenotype, mutation, genome, wildtype, field isolates
- Applications: Anti-virals, vaccines

7- Mapping of viral genomes: used to identify and classify viruses

8- Types of virus mutants: Have wide effects, may be benefit or no

12 –Emerging viral diseases

13 –Bioterrorism agents: using viruses as weapons (e.g smallpox)

9- Defective viruses

- A defective virus is one that lacks one or more functional genes required for viral replication
- Types: defective interfering virus particles, pseudovirions, Delta-viruses

10 -Interactions Among Viruses

- Types of interactions are: Recombination, Complementation and interference

11- natural history (ecology) and modes of transmission of viruses

- Viruses may be transmitted by:
  1) Direct transmission
  2) Indirect transmission
  3) Transmission from animal to animal with humans as accidental host
  4) Arthropod vector

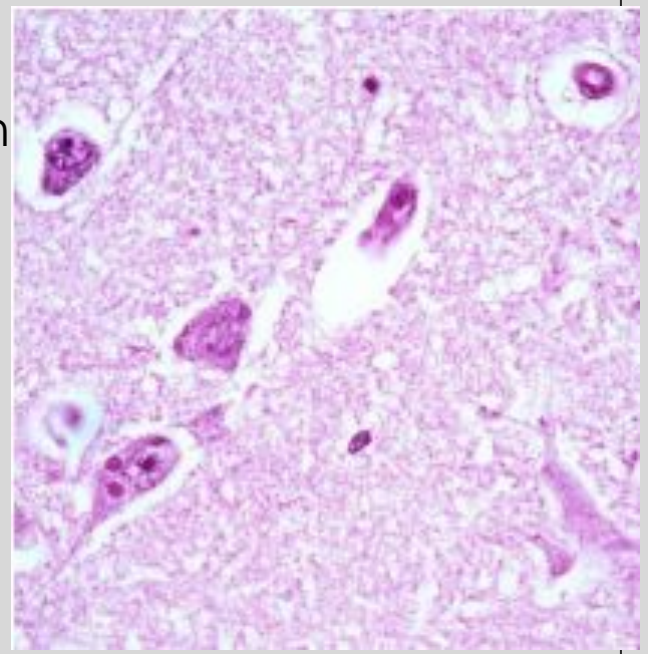
- Many factors that affect: Environmental changes ▪ Human behavior ▪ Socioeconomic and demographic phenomena .. Etc
- Examples: Ebola virus, Nipah virus, Hantavirus pulmonary disease, HIV, West Nile virus and Emerging Coronaviruses

1- Cultivation and assay of viruses

- Cells grown in the vitro are essential for cultivation and characterization of viruses (Remember: viruses are obligate intracellular pathogens)
- There are 3 types of cell cultures that viruses can grow in:
  1- Primary culture: made by dispersing cells (usually with trypsin) from freshly removed host tissues, and they are unable to grow for more than a few passages
  2- Secondary culture: formed from diploid cells that undergo changes but have a normal chromosome pattern, they can grow up to 50 patterns
  3- Continuous cell lines: a culture which is formed from malignant or diploid cells that have irregular number of chromosomes, they are capable of prolonged or even indefinite growth
- The type of cell culture used for viral cultivation depends on the sensitivity of a cell to a particular virus.

1- Cultivation and assay of viruses

- Multiplication of a virus can be recognized in a variety of ways:
- 1. Development of cytopathic effects (i.e., morphologic changes in the cells). Types of virus-induced cytopathic effects include cell lysis or necrosis, inclusion formation, giant cell formation, and cytoplasmic vacuolization. Most viruses produce some obvious cytopathic effect in infected cells, we use a direct method (e.g. light microscope to detect these changes.
- In the course of viral multiplication within cells, virus-specific structures called inclusion bodies may be produced. They become far larger than th individual virus particle and often have an affinity for acid dyes (e.g., eosin). They may be situated in the nucleus (herpesvirus), in the cytoplasm (poxvirus), or in both (measles virus). In many viral infections, the inclusion bodies are the site of development of the virions (the viral factories). Variations in the appearance of inclusion material depend largely on the tissue fixative used. The presence of inclusion bodies may be of considerable diagnostic aid. The intracytoplasmic inclusion in nerve cells (the Negri body) is pathognomonic for rabies.



1- Cultivation and assay of viruses

- 2. Appearance of a virus-encoded protein, such as the hemagglutinin of influenza virus. Specific antisera can be used to detect the synthesis of viral proteins in infected cells (so we use immuno-florescence essay).
- 3. Adsorption of erythrocytes to infected cells, called hemadsorption, caused by the presence of virus-encoded hemagglutinin (parainfluenza, influenza) in cellular membranes

1- Cultivation and assay of viruses

- 4. Detection of virus-specific nucleic acid. Molecular-based assays such as polymerase chain reaction provide rapid, sensitive, and specific methods of detection.
- 5. Viral growth in an embryonated chick egg may result in death of the embryo (e.g., encephalitis viruses), production of pocks or plaques on the chorioallantoic membrane (e.g., herpes, smallpox, vaccinia), or development of hemagglutinins in the embryonic fluids or tissues (e.g., influenza).

2- Quantitation of Viruses

- The titer is expressed as the 50% infectious dose (ID50), which is the reciprocal of the dilution of virus that produces the effect in 50% of the cells or animals inoculated (Plaque assay, IFA (Immuno-florescence assay)).
- To detect the quantity of viruses we can use 2 types of methods:

A) Physical Methods: PCR, RIA (radio immuno-assay) , EIA (Enzyme immuno-assay) , Agglutination/hemagglutination

B) Biologic methods: End-point biologic assays depend on the measurement of animal death, animal infection, or cytopathic effects in tissue culture at a series of dilutions of the virus being tested.

3- Purification of Virus Particles

- Done in 2 steps:
  1. Concentration by precipitation with ammonium sulfate, ethanol, or polyethylene glycol or by ultrafiltration.
  2. After concentration, virus can be separated from host materials by differential centrifugation, density gradient centrifugation, column chromatography, and electrophoresis.

4- Virus reaction to physical and chemical agents

1) Heat and cold

- Heat and Cold: There is great variability in the heat stability of different viruses.
- Icosahedral viruses tend to be stable, losing little infectivity after several hours at 37°C.
- Enveloped viruses are much more heat labile than naked viruses, rapidly dropping in titer at 37°C. Viral infectivity is generally destroyed by heating at 50–60°C for 30 minutes, although there are some notable exceptions (e.g., hepatitis B virus, polyomaviruses).
- Viruses can be preserved by storage at subfreezing temperatures, and some may withstand lyophilization and can thus be preserved in the dry state at 4°C or even at room temperature.
- Usually -20 C temperature is cold enough to store DNA viruses, while for RNA viruses we need to store them in colder environment (-70 / -80 C) as DNA is more stable than RNA
- Viruses are sensitive to repeated freezing and thawing (more than 3 times).

4- Virus reaction to physical and chemical agents

2) Salts

- Many viruses can be stabilized by salts in concentrations of 1 mol/L (i.e., the viruses are not inactivated even by heating at 50°C for 1 hour) and the mechanism by which the salts stabilize viral preparations is not known.
- Viruses are preferentially stabilized by certain salts. $MgCl_2$, 1 mol/L, stabilizes picornaviruses and reoviruses; $MgSO_4$, 1 mol/L, stabilizes orthomyxoviruses and paramyxoviruses; and Na_2SO_4, 1 mol/L, stabilizes herpesviruses.
- The stability of viruses is important in the preparation of vaccines. The ordinary non-stabilized oral polio vaccine must be stored at freezing temperatures to preserve its potency. However, with the addition of salts for stabilization of the virus, potency can be maintained for weeks at ambient temperatures even in the high temperatures of the tropics.

4- Virus reaction to physical and chemical agents

3) pH

- Viruses are usually stable between pH values of 5.0 and 9.0. Some viruses (e.g., enteroviruses) are resistant to acidic conditions. All viruses are destroyed by alkaline conditions. In hemagglutination reactions, variations of less than 1 pH unit may influence the result.

4) High energy particles

- Ultraviolet, x-ray, and high-energy particles inactivate viruses. The dose varies for different viruses. Infectivity is the most radiosensitive property because replication requires expression of the entire genetic contents. Irradiated particles that are unable to replicate may still be able to express some specific functions in host cells.

4- Virus reaction to physical and chemical agents

5) Detergents

- Ether susceptibility can be used to distinguish viruses that possess an envelope from those that do not.
- Non-ionic detergents (e.g., Triton X-100) solubilize lipid constituents of viral membranes. The viral proteins in the envelope are released (undenatured).
- Anionic detergents (e.g., sodium dodecyl sulfate) also solubilize viral envelopes; in addition, they disrupt capsids into separated polypeptides.

4- Virus reaction to physical and chemical agents

6) Vital dyes

- Viruses are penetrable to a varying degree by vital dyes such as toluidine blue, neutral red, and proflavine. These dyes bind to the viral nucleic acid, and the virus then becomes susceptible to inactivation by visible light.
- Neutral red is commonly used to stain plaque assays so that plaques are more readily seen. The assay plates must be protected from bright light after the neutral red has been added; otherwise, there is the risk that progeny virus will be inactivated and plaque development will cease.

4- Virus reaction to physical and chemical agents

7) Others

- Antibacterial antibiotics have no effect on viruses.
- Some antiviral drugs are available.
- Quaternary ammonium compounds are not effective against viruses.
- Organic iodine compounds are also ineffective.
- Larger concentrations of chlorine are required to destroy viruses than to kill bacteria, especially in the presence of extraneous proteins
- For example, the chlorine treatment of stools adequate to inactivate typhoid bacilli is inadequate to destroy poliomyelitis virus present in feces.
- Alcohols, such as isopropanol and ethanol, are relatively ineffective against certain viruses, especially picornaviruses.

4- Virus reaction to physical and chemical agents

- Viruses may be inactivated for various reasons, such as to sterilize laboratory supplies and equipment, disinfect surfaces or skin, make drinking water safe, and produce inactivated virus vaccines, for example:

1) Sterilization may be accomplished by steam under pressure, dry heat, ethylene oxide, and γirradiation.

2) Surface disinfectants include sodium hypochlorite, glutaraldehyde, formaldehyde, and peracetic acid.

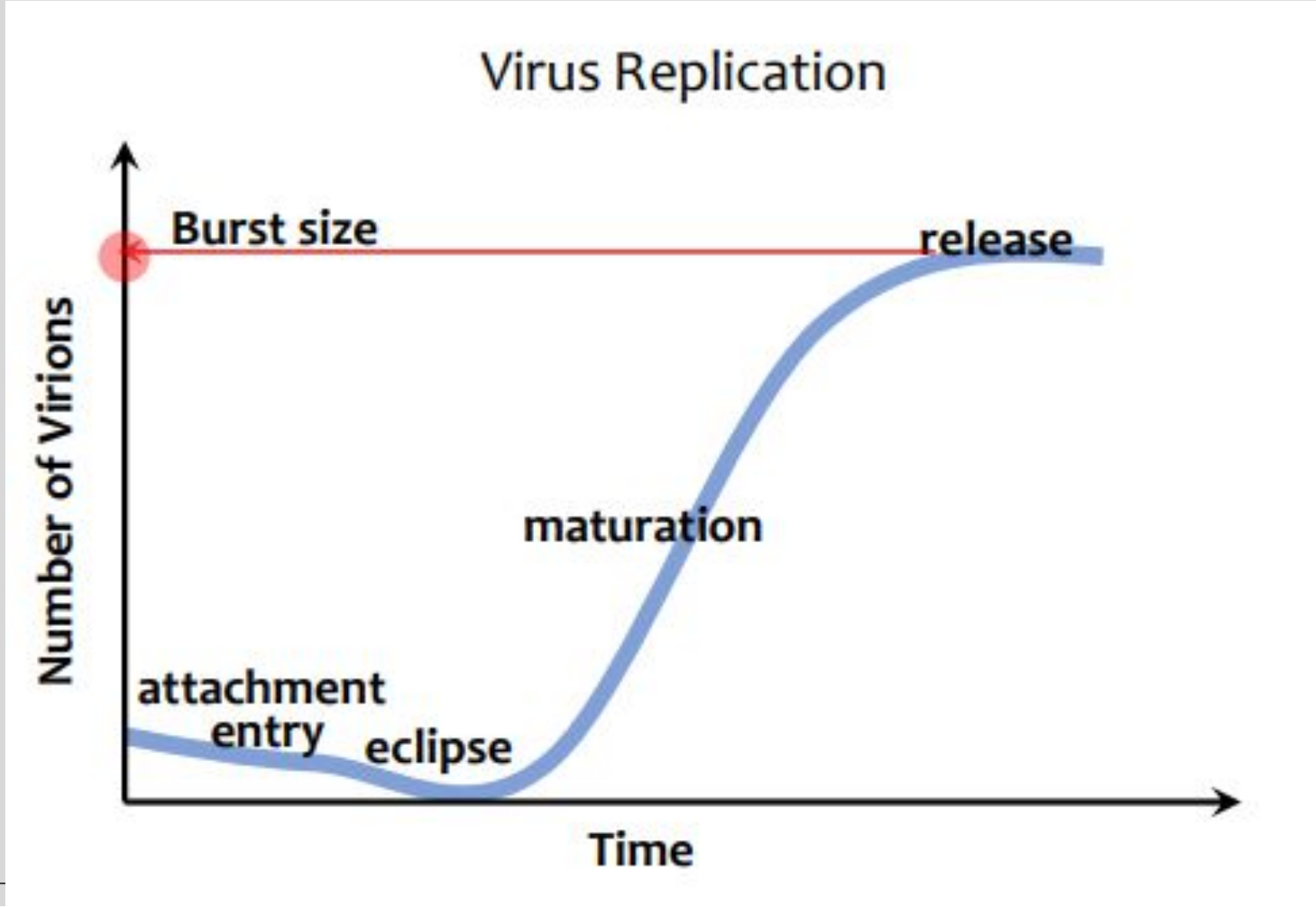
3) Skin disinfectants include chlorhexidine, 70% ethanol, and iodophores.

4) Vaccine production may involve the use of formaldehyde, β-propiolactone, psoralen + ultraviolet irradiation, or detergents (subunit vaccines) to inactivate the vaccine virus.

5- Replication of viruses

- Types of infections:
  1) Productive infections: occur in permissive cells and result in the production of infectious virus.
  2) Abortive infections: fail to produce infectious progeny, either because the cell may be nonpermissive and unable to support the expression of all viral genes or because the infecting virus may be defective, lacking some functional viral gene.
  3) A latent infection: may ensue, with the persistence of viral genomes, the expression of no or a few viral genes, and the survival of the infected cell. The pattern of replication may vary for a given virus, depending on the type of host cell infected.

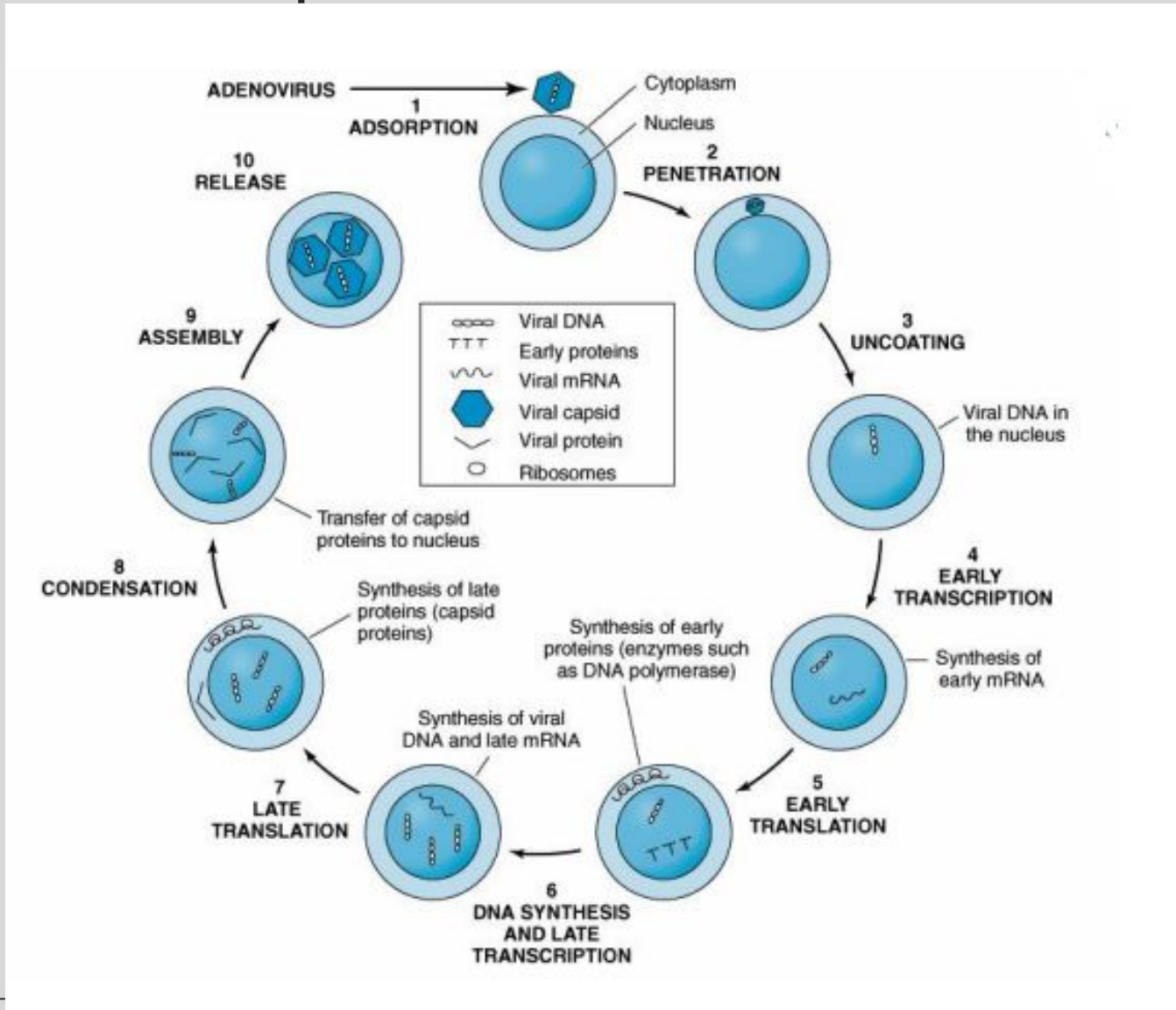
5- Replication of viruses

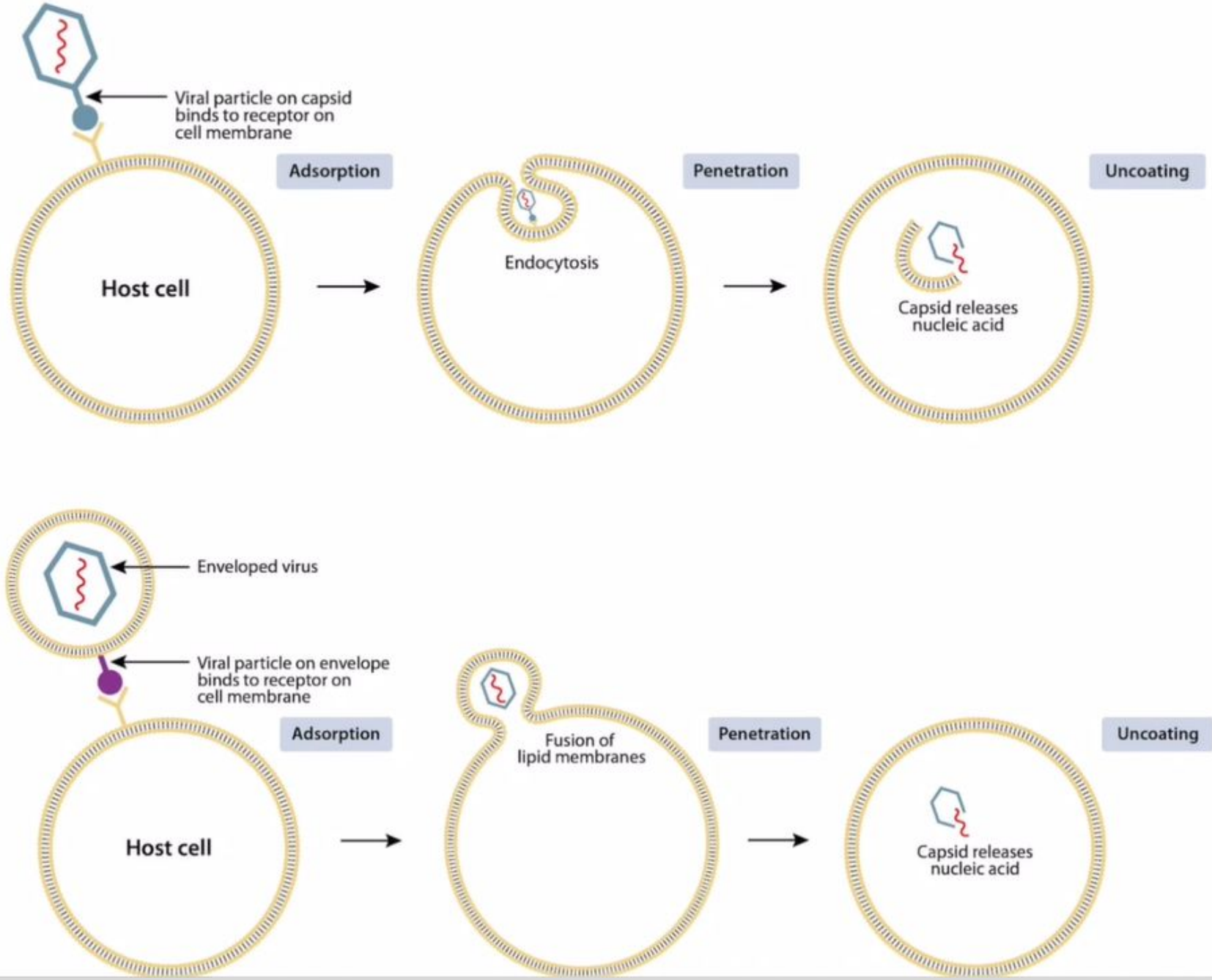


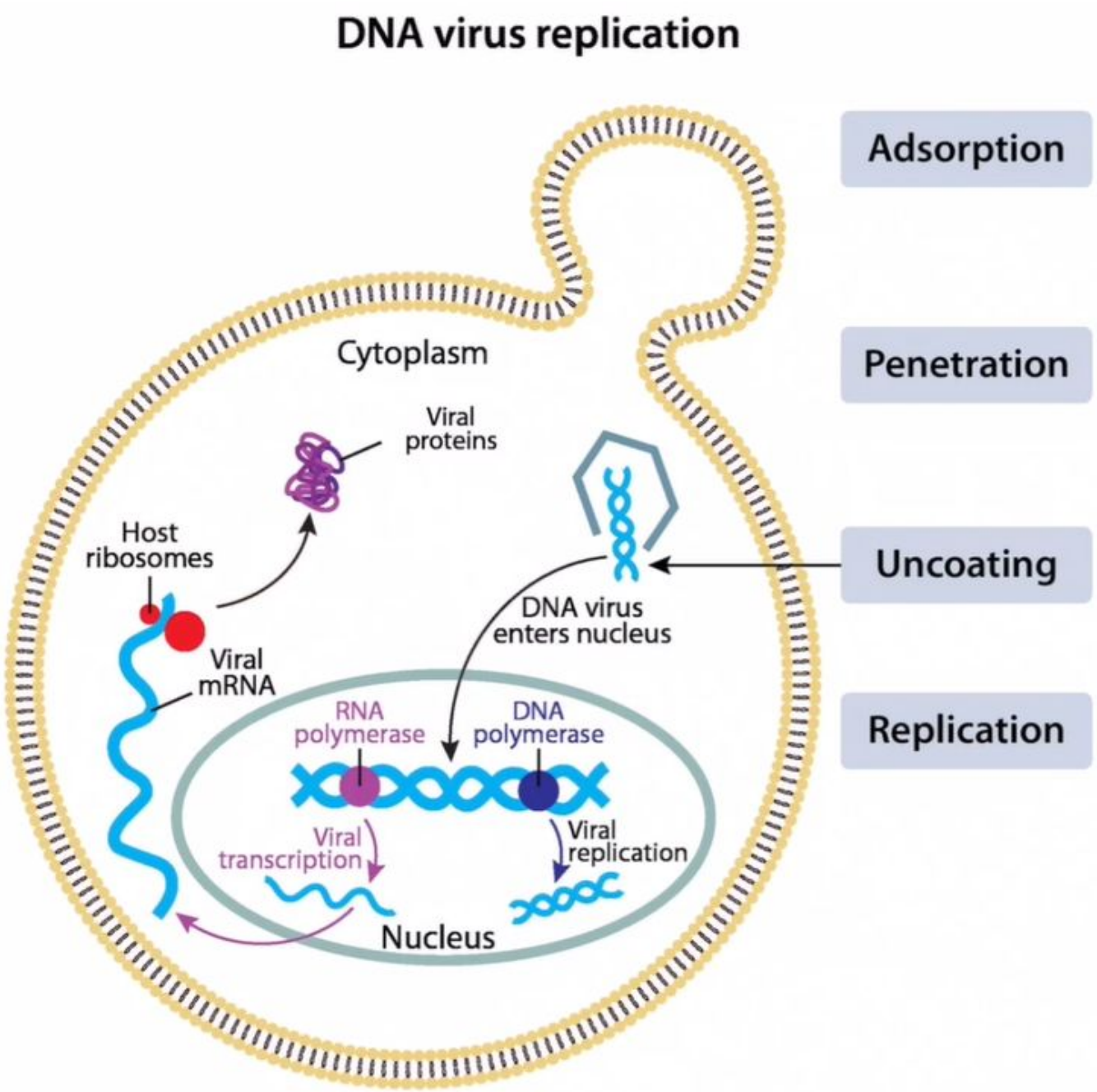
5- Replication of viruses

- The unique feature of viral multiplication is that soon after interaction with a host cell the infecting virion is disrupted and its measurable infectivity is lost.
- This phase of the growth cycle is called the eclipse period; its duration varies depending on both the particular virus and the host cell, and it is followed by an interval of rapid accumulation of infectious progeny virus particles.
- The eclipse period is actually one of intense synthetic activity as the cell is redirected toward fulfilling the needs of the viral "pirate."

5- Replication of viruses

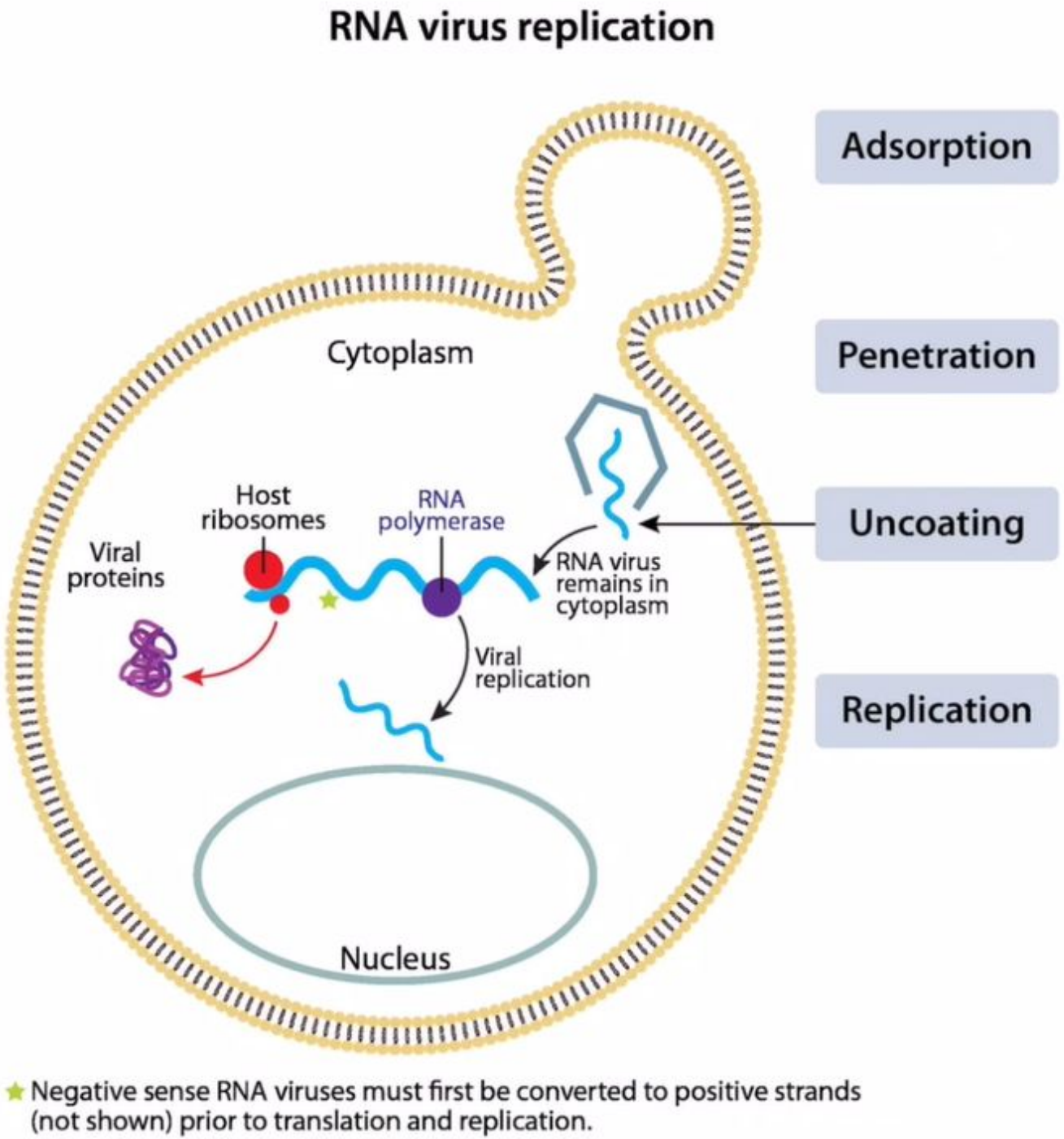



Viral particle on capsid binds to receptor on cell membrane
Adsorption
Host cell
Endocytosis
Penetration
Capsid releases nucleic acid
Uncoating
Enveloped virus
Viral particle on envelope binds to receptor on cell membrane
Adsorption
Host cell
Fusion of lipid membranes
Penetration
Capsid releases nucleic acid
Uncoating



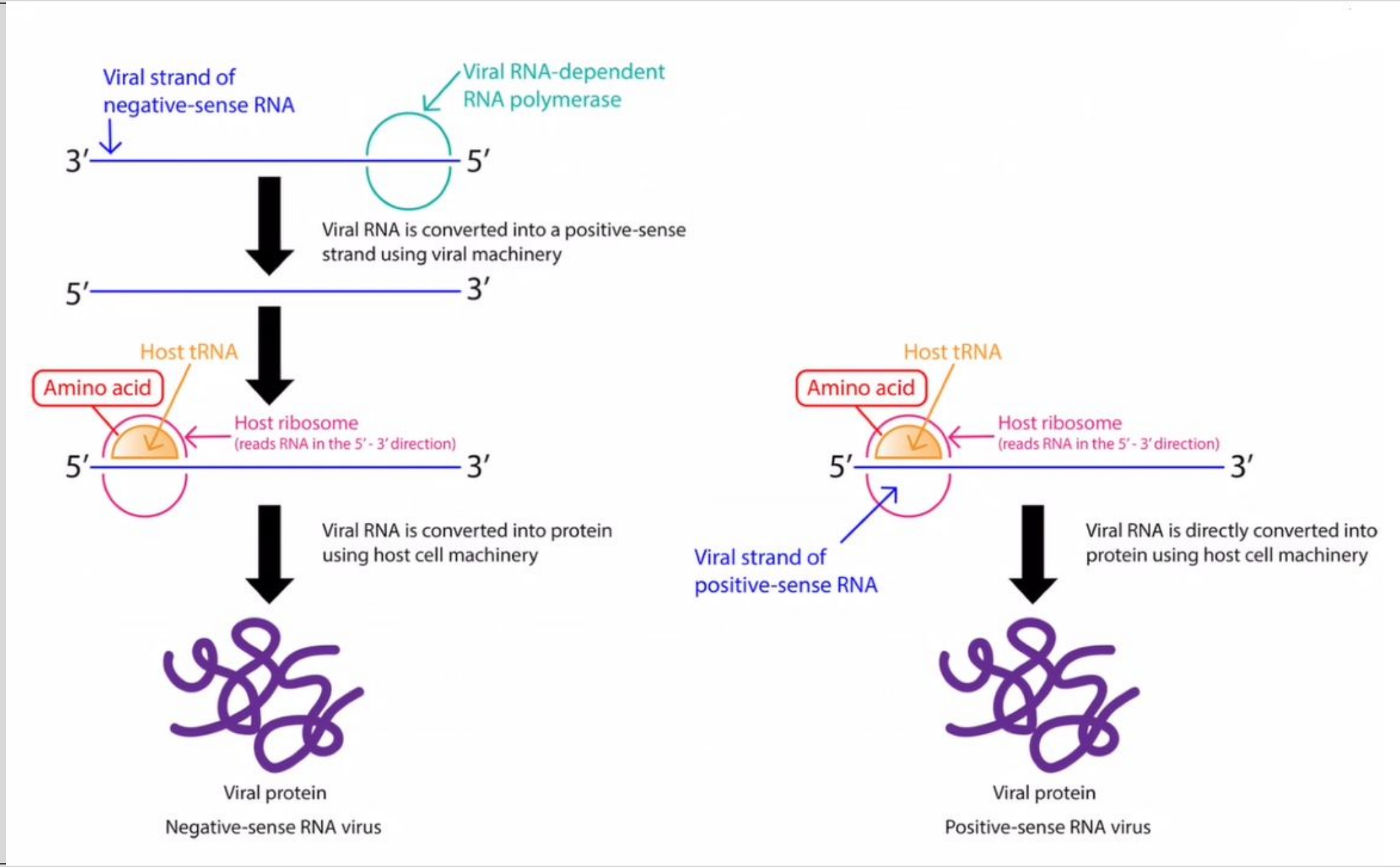
5- Replication of viruses

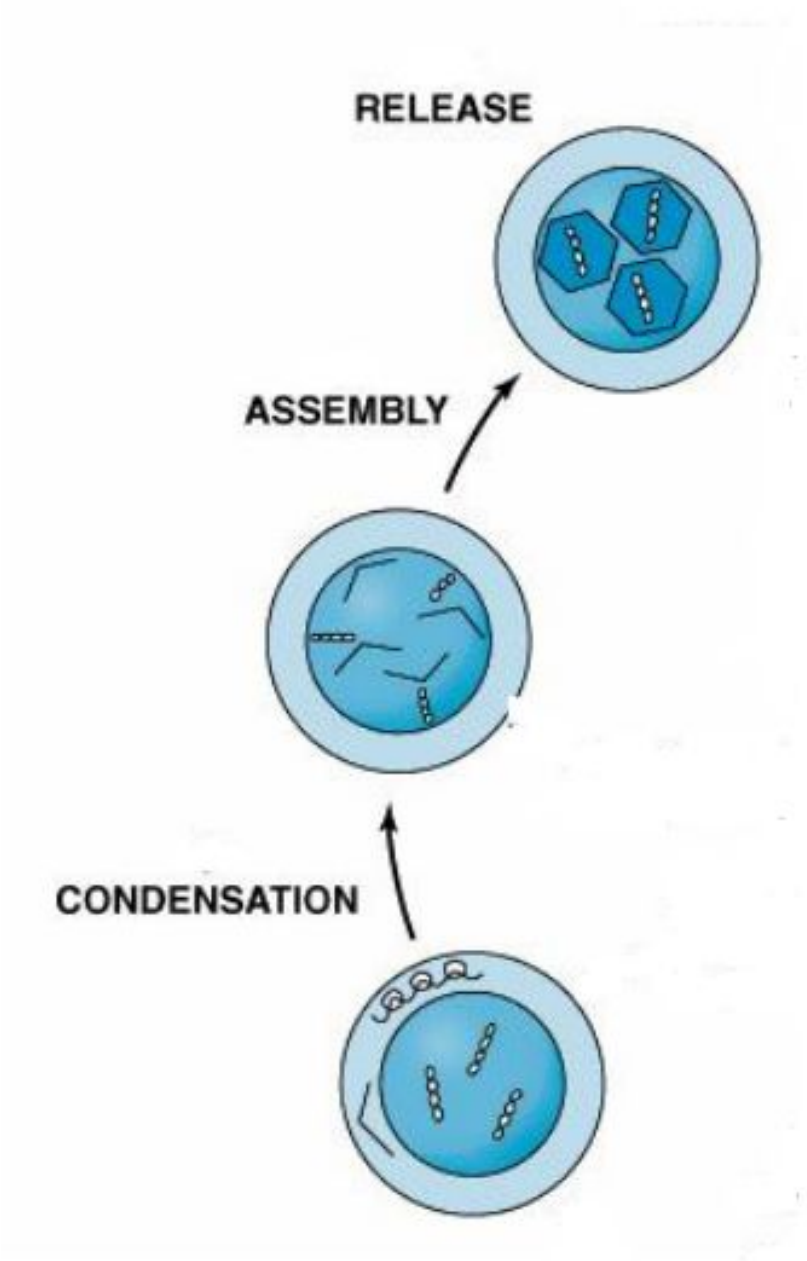
- All DNA viruses are Double stranded except Parvovirus B19 (From Parvoviridae family)
- All DNA viruses replicate their genome in the Nucleus except poxviridae family



5- Replication of viruses

- All RNA viruses are single stranded except reoviridae
- All RNA viruses replicate their genome in the cytoplasm except Retroviridae and Orthomyxoviridae


Viral strand of
negative-sense RNA
Viral RNA-dependent
RNA polymerase
3′
5′
Viral RNA is converted into a positive-sense
strand using viral machinery
5′
3′
Host tRNA
Amino acid
Host ribosome
(reads RNA in the 5′ - 3′ direction)
5′
3′
Viral RNA is converted into protein
using host cell machinery
Viral protein
Negative-sense RNA virus
Host tRNA
Amino acid
Host ribosome
(reads RNA in the 5′ - 3′ direction)
5′
3′
Viral strand of
positive-sense RNA
Viral RNA is directly converted into
protein using host cell machinery
Viral protein
Positive-sense RNA virus



5- Replication of viruses

- All Enveloped viruses gain their envelope from the cellular membrane except Herpesviridae, as they gain their envelope from the nuclear membrane.

5- Replication of viruses

- The yield of infectious virus per cell ranges widely, from modest numbers to more than 100,000 particles.
- The duration of the virus replication cycle also varies widely, from 6 to 8 hours (picornaviruses) to more than 40 hours (some herpesviruses).

TABLE 29-2 Pathways of Nucleic Acid Transcription for Various Virus Classes

Type of Viral Nucleic Acid	Intermediates	Type of mRNA	Example	Comments
± ds DNA	None	+ mRNA	Most DNA viruses (eg, herpesvirus, adenovirus)	
+ ss DNA	± ds DNA	+ mRNA	Parvoviruses	
± ds RNA	None	+ mRNA	Reoviruses	Virion contains RNA polymerase that transcribes each segment to mRNA
+ ss RNA	± ds RNA	+ mRNA	Picornaviruses, togaviruses, flaviviruses	Viral nucleic acid is infectious and serves as mRNA. For togaviruses, smaller + mRNA is also formed for certain proteins
− ss RNA	None	+ mRNA	Rhabdoviruses, paramyxoviruses, orthomyxoviruses	Viral nucleic acid is not infectious; virion contains RNA polymerase, which forms + mRNAs smaller than the genome. For orthomyxoviruses, + mRNAs are transcribed from each segment
+ ss RNA	− DNA, ± DNA	+ mRNA	Retroviruses	Virion contains reverse transcriptase; viral RNA is not infectious, but complementary DNA from transformed cell is

−, negative strand; +, positive strand; ±, a helix containing a positive and a negative strand; ds, double stranded; ss, single stranded.

TABLE 29-3 Comparison of Replication Strategies of Several Important RNA Virus Families

	Grouping Based on Genomic RNA[a]					
	Positive-Strand Viruses			Negative-Strand Viruses		Double-Stranded Viruses
Characteristic	Picornaviridae	Togaviridae	Retroviridae	Orthomyxoviridae	Paramyxoviridae and Rhabdoviridae	Reoviridae
Structure of genomic RNA	ss	ss	ss	ss	ss	ds
Sense of genomic RNA	Positive	Positive	Positive	Negative	Negative	
Segmented genome	0	0	0[b]	+	0	+
Genomic RNA infectious	+	+	0	0	0	0
Genomic RNA acts as messenger	+	+	+	0	0	0
Virion-associated polymerase	0	0	+[c]	+	+	+
Subgenomic messages	0	+	+	+	+	+
Polyprotein precursors	+	+	+	0	0	0

TABLE 29-4 Summary of Replication Cycles of Major Virus Families

Virus Family	Presence of Virion Envelope	Intracellular Location			Multiplication Cycle (Hours)[b]
		Replication of Genome	Formation of Nucleocapsid[a]	Virion Maturation	
DNA viruses					
Parvoviridae	0	N	N	N	
Polyomaviridae	0	N	N	N	48
Adenoviridae	0	N	N	N	25
Hepadnaviridae	+	N	C	M–E	
Herpesviridae	+	N	N	M	15–72
Poxviridae	0	C	C	C	20
RNA viruses					
Picornaviridae	0	C	C	C	6–8
Reoviridae	0	C	C	C	15
Togaviridae	+	C	C	M–P	10–24
Flaviviridae	+	C	C	M–E	
Retroviridae	+	N	C	M–P	
Bunyaviridae	+	C	C	M–G	24
Orthomyxoviridae	+	N	N	M–P	15–30
Paramyxoviridae	+	C	C	M–P	10–48
Rhabdoviridae	+	C	C	M–P	6–10

[a]The synthesis of viral proteins always occurs in the cytoplasm.

[b]The values shown for duration of the multiplication cycle are approximate; ranges indicate that various members within a given family replicate with different kinetics. Different host cell types also influence the kinetics of viral replication.

C, cytoplasm; M, membranes; M–E, endoplasmic reticulum membranes; M–G, Golgi membranes; M–P, plasma membranes; N, nucleus.

6- Genetics of animal viruses

- Genotype: the genetic constitution of an organism.
- Phenotype: the observable properties of an organism, which are produced by the genotype in cooperation with the environment.
- A mutation is a heritable change in the genotype.
- The genome is the sum of the genes of an organism.
- Wildtype virus denotes the original virus from which mutants are derived and with which the mutants are compared; the term may not accurately characterize the virus as it is isolated in nature. Fresh virus isolates from the natural host are referred to as field isolates or primary isolates.

6- Genetics of animal viruses

- Genetic analysis is a powerful approach toward understanding the structure and function of the viral genome, its gene products, and their roles in infection and disease.
- Viruses that have stable antigens on their surfaces (poliovirus, measles virus) can be controlled by vaccination. Other viruses that exist as many antigenic types (rhinoviruses) or change frequently (influenza virus A) are difficult to control by vaccination.
- Genetic analysis will help identify virus-specific processes that may be appropriate targets for the development of antiviral therapy.

7- Mapping of Viral Genomes

- Biochemical and physical mapping can be done much more rapidly than genetic mapping using classic genetic techniques.
- For isolates that can be cloned, sequence analysis and comparison with known viruses is often used.
- Restriction endonucleases can be used for identification of specific strains of DNA viruses.

8- Types of Virus Mutants

- Classic genetic studies with animal viruses require a sensitive and accurate quantitative assay method, such as a plaque assay for viral infectivity, and good mutants (resulting from single mutations) that are easily scored and reasonably stable.
- Some markers commonly used include plaque morphology, antibody escape or resistance to neutralizing antisera, loss of a virus protein, drug resistance, host range, and inability to grow at low or high temperatures.
- Conditional-lethal mutants are mutants that are lethal (in that no infectious virus is produced) under one set of conditions—termed nonpermissive conditions—but that yield normal infectious progeny under other conditions— termed permissive conditions.

9- Defective Viruses

- A defective virus is one that lacks one or more functional genes required for viral replication. Defective viruses require helper activity from another virus for some step in replication or maturation.
- One type of defective virus lacks a portion of its genome (i.e., deletion mutant).
- Spontaneous deletion mutants may interfere with the replication of homologous virus and are called defective interfering virus particles.
- DIPs have lost essential segments of genome but contain normal capsid proteins; they require infectious homologous virus as helper for replication, and they interfere with the multiplication of that homologous virus

9- Defective Viruses

- Another category of defective virus requires an unrelated replication-competent virus as helper.
- Examples include the adeno-associated satellite viruses and hepatitis D virus (delta agent), which replicate only in the presence of coinfecting human adenovirus or hepatitis B virus, respectively
- The essential helper function supplied by the helper virus varies, depending on the system.

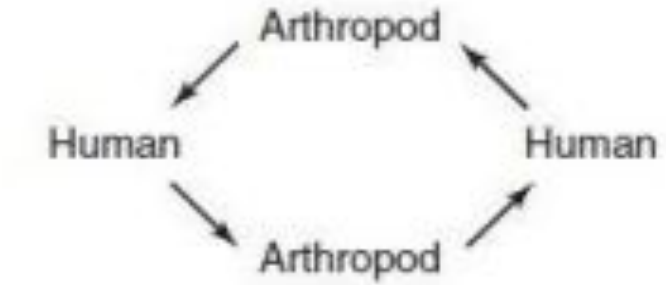
9- Defective Viruses

- Pseudovirions, a different type of defective particle, contain host cell DNA rather than the viral genome.
- During viral replication, the capsid sometimes encloses random pieces of host nucleic acid rather than viral nucleic acid.
- Such particles look like ordinary virus particles when observed by electron microscopy, but they are not able to replicate.

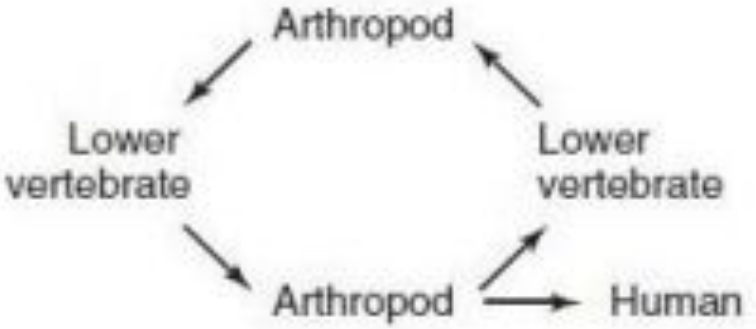
10 -Interactions Among Viruses

- Recombination results in the production of progeny virus (recombinant) that carries traits not found together in either parent. The classic mechanism is that the nucleic acid strands break, and part of the genome of one parent is joined to part of the genome of the second parent.
- Complementation is the interaction of viral gene products in cells infected with two viruses, one or both of which may be defective. It results in the replication of one or both under conditions in which replication would not ordinarily occur. The basis for complementation is that one virus provides a gene product in which the second is defective, allowing the second virus to grow.
- Infection of either cell cultures or whole animals with two viruses often leads to an inhibition of multiplication of one of the viruses, an effect called interference, this is seen in some respiratory infections.

E.g. Dengue fever

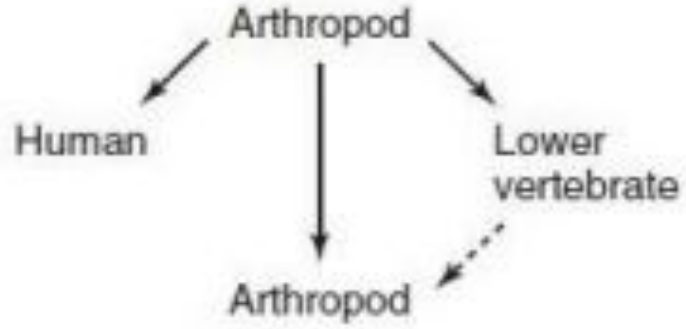


E.g. Sant – luis encephalitis



The host here is lower vertebrae organisms and human is an accidental host

E.g. La crosse encephalitis



The host here are lower vertebrae organisms and **arthropods**, human is an accidental host

11- natural history (ecology) and modes of transmission of viruses

- Viruses may be transmitted by the following ways:

1) Direct transmission
2) Indirect transmission
3) Transmission from animal to animal with humans as accidental host
4) Arthropod vector

12- Emerging Viral Diseases

Contributing factors

- Environmental changes
- Human behavior
- Socioeconomic and demographic phenomena
- Travel and commerce
- Food production
- Health care
- Microbial adaptation
- Public health measures

Examples of emerging viral infections

Ebola virus

Nipah virus

Hantavirus pulmonary disease

Human immunodeficiency virus

West Nile virus

Rift Valley fever

Emerging Coronaviruses

13- Bioterrorism Agents

- Microorganisms (or toxins) that could be used to produce death and disease in humans, animals, or plants for terrorist purposes.
- Potential bioterrorism agents are classified into risk categories based on the ease of dissemination or transmission from person to person, mortality rates, ability to cause public panic, and requirement for public health preparedness.
- Viral agents in the highest risk category are smallpox and the viral hemorrhagic fever