BS20001 | Science of Living Systems | Spring 2022

Viruses: Life cycle, disease, therapy & diagnoses

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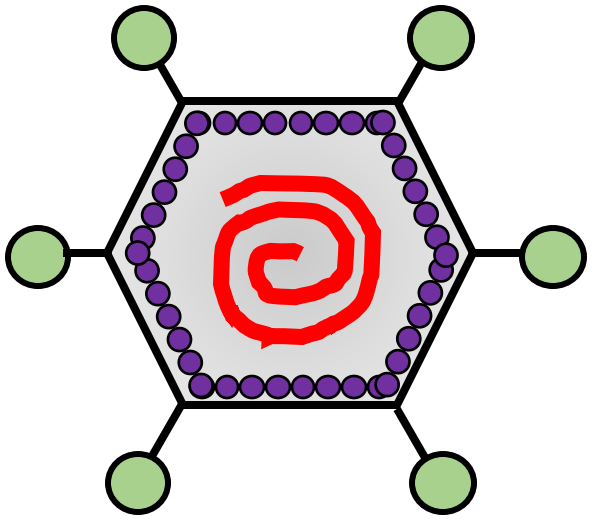
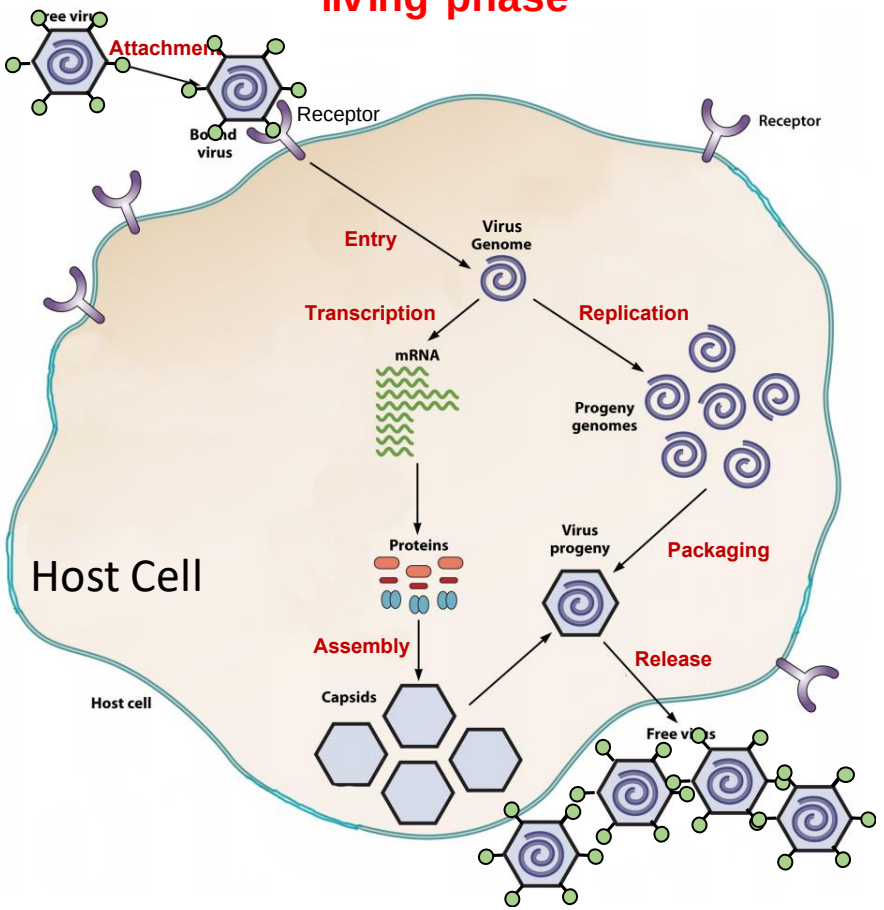
A virus is an organism with two phases

Nonliving phase



A virus particle

living phase

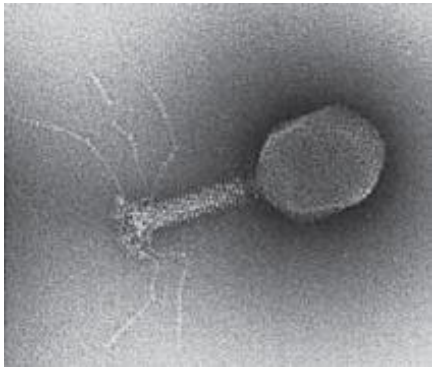


Viruses: a separate kingdom of life

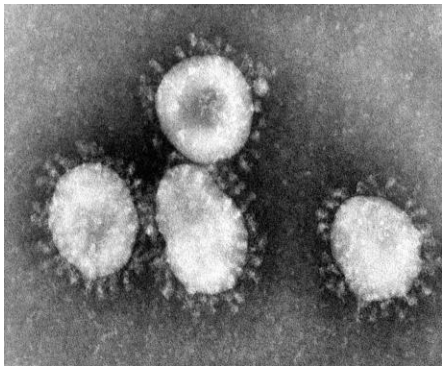
- Viruses could be considered as a simplest (hence ancient) *form of life* (?)
- Viruses are living only inside the host cell!
- Composed of **nucleic acids & proteins**
- A virus is an infectious, obligate, intracellular **parasite**
- Viruses can exploit all three domains of life (archaea, bacteria, eukaryotes) as their hosts

WHAT IS A VIRUS?

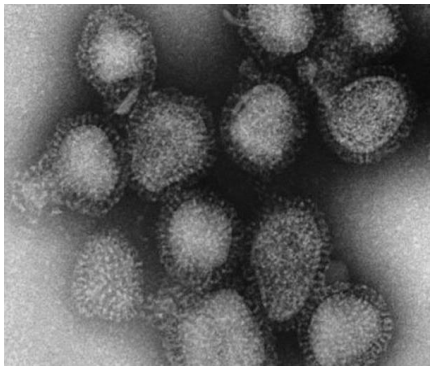
- A virus is a submicroscopic infectious particle that consists of an RNA or DNA genome enclosed in a protein shell.
- It is not able to reproduce on its own: it can only make more viruses by entering a cell and using its cellular machinery.
- Can only be visualized through **electron microscope**



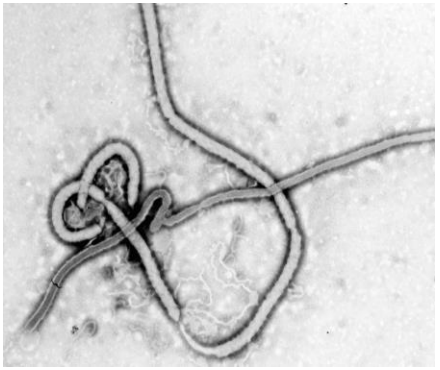
The bacteriophage T4



Corona virus



Influenza virus

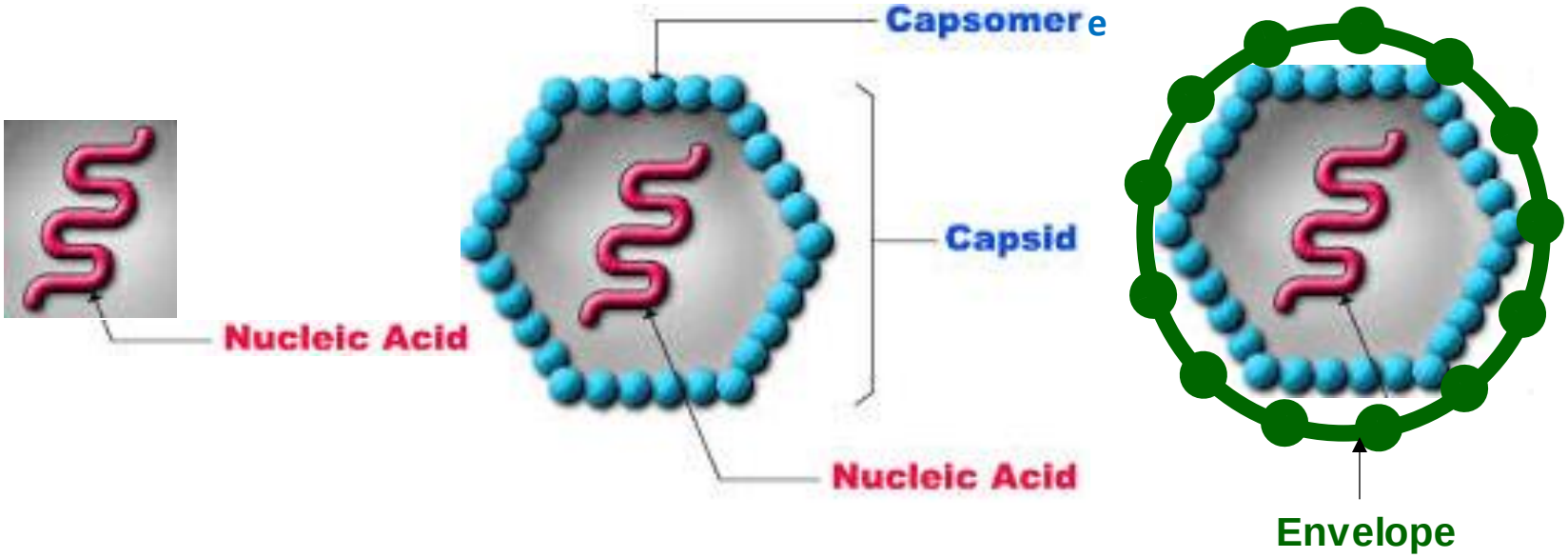


Ebola virus

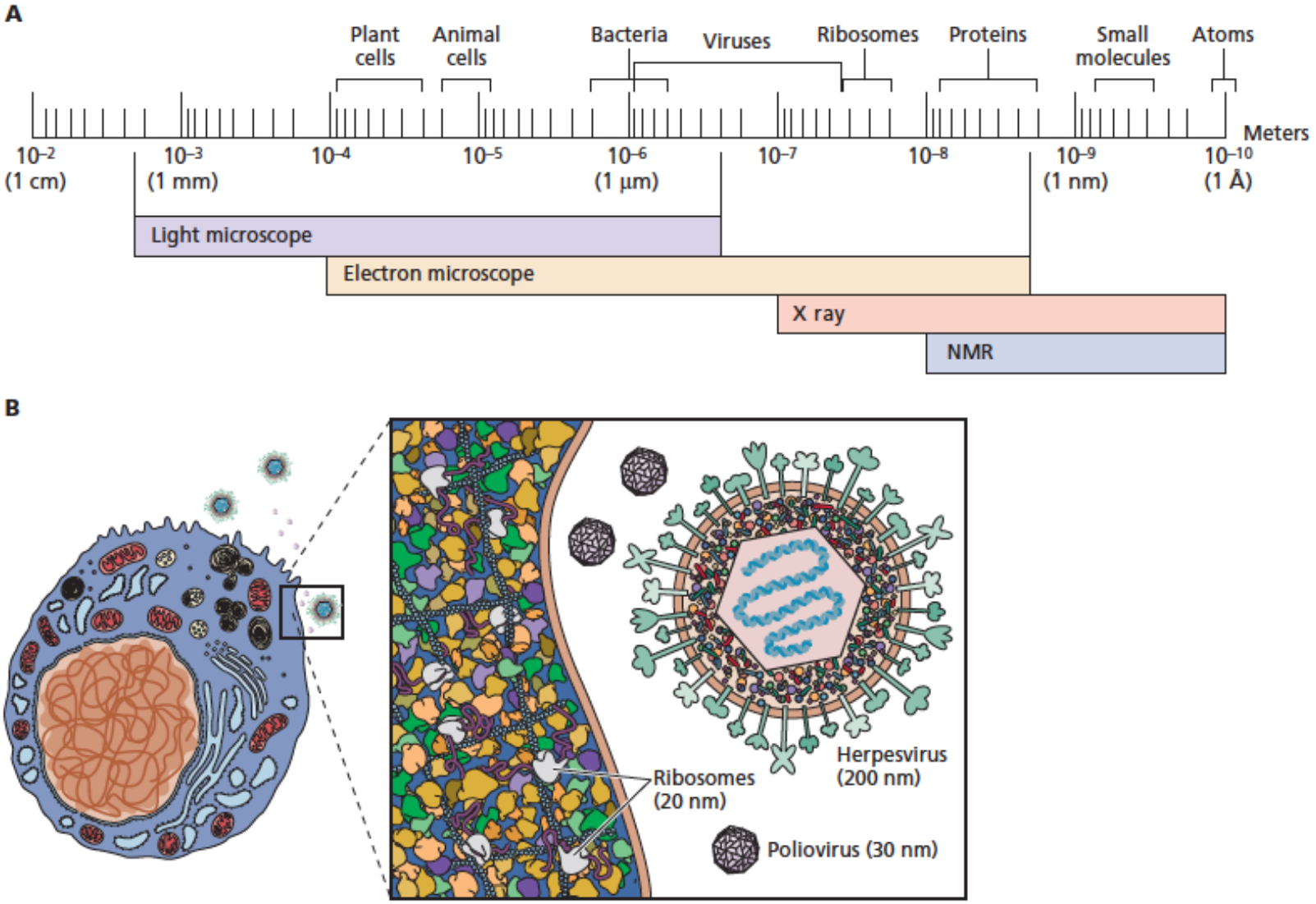
Transmission electron micrographs of virus particles

What is a Virus composed of?

- A virus is an obligate, intracellular parasite comprising of:
  - genetic material,
  - which is surrounded by a protein coat,
  - and/or an envelope derived from the host cell membrane



Virus is a submicroscopic particle



Flint's Principles of Virology

Unique Features of Virus

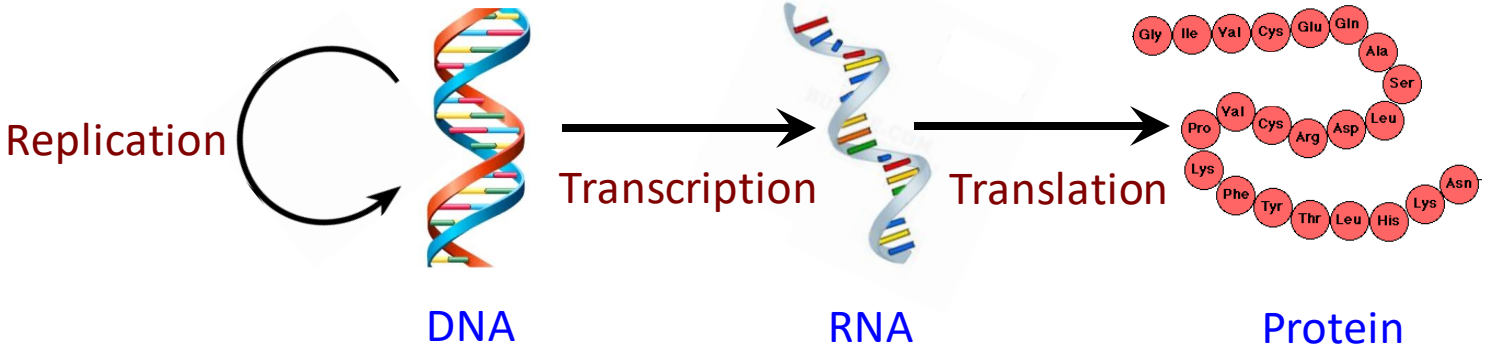
- Virus possesses **DNA** **or** **RNA** **as their genetic material !!!**
- **Lacks ribosomes** or any membrane bound organelles
- Virus **hijacks host-cell machineries** for their own good (to make more and more virus particles)

Unique Features of Virus

- Viruses are alive only when inside the host cell!!
- Only *organism* on Earth to contain RNA as their genomic material

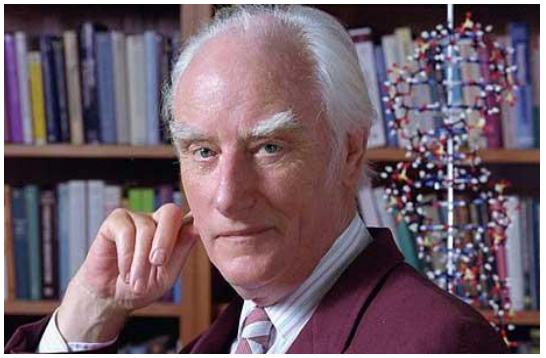
THEN

The biggest question is how the Flow of Genetic Information in Viruses fits with the Concept of Central Dogma of Molecular Biology



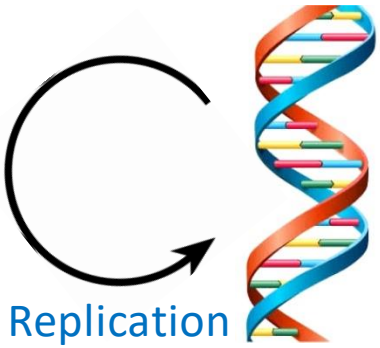
Flow of Genetic Information (Central Dogma)

Flow of Genetic Information: Updates



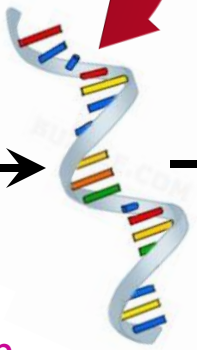
RNA Dependent
RNA Polymerase

RNA Replication

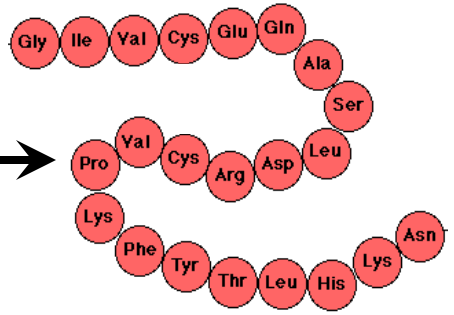


Replication

Transcription



Translation



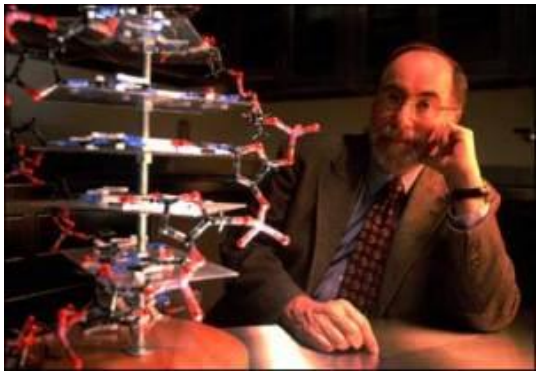
Reverse
Transcription

DNA

RNA

Protein

Reverse Transcriptase



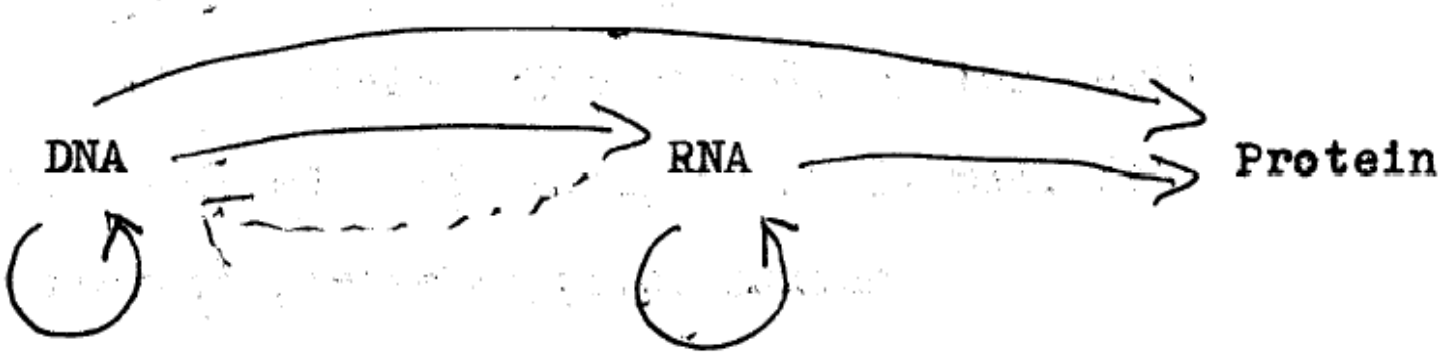
David Baltimore

Nobel Prize in 1975

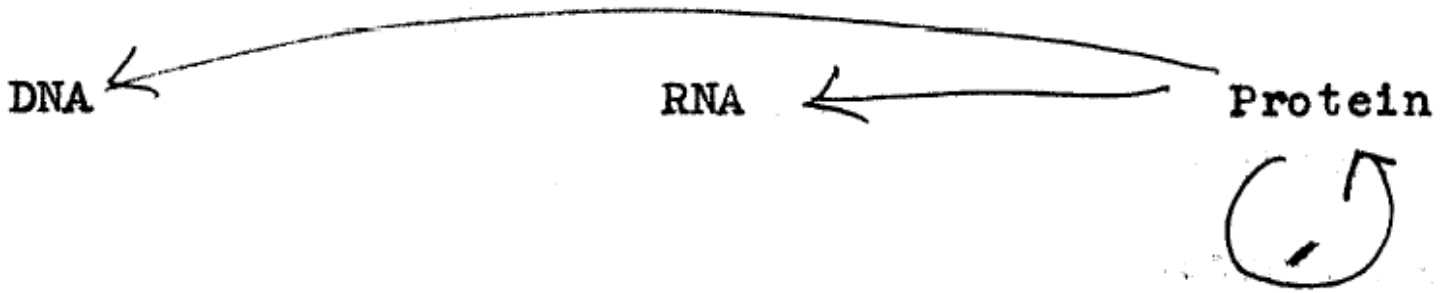
Ideas on Protein Synthesis (Oct. 1956)

Francis Crick

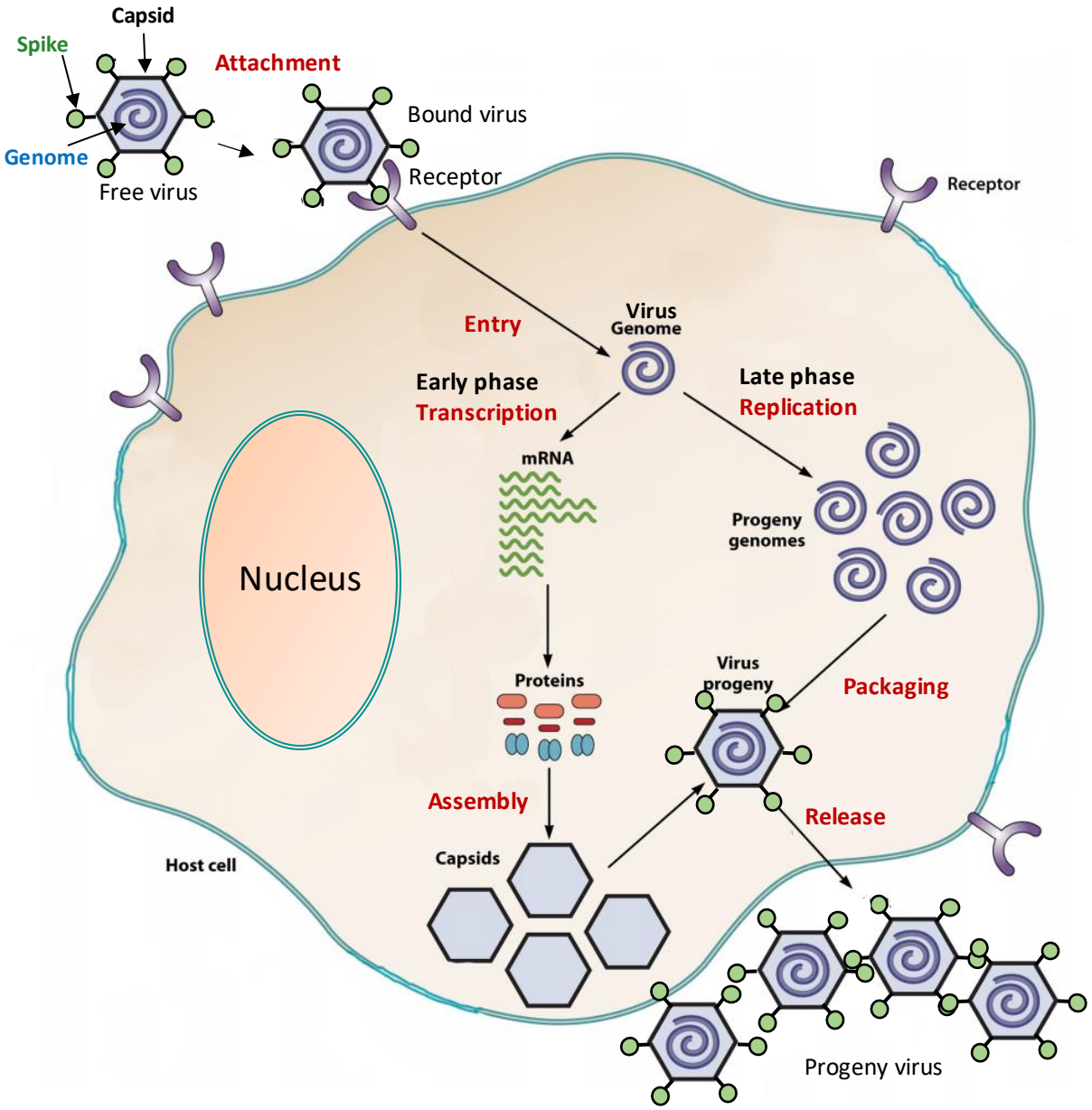
The Central Dogma: "Once information has got into a protein it can't get out again". Information here means the sequence of the amino acid residues, or other sequences related to it. That is, we may be able to have



but never



Virus Life cycle

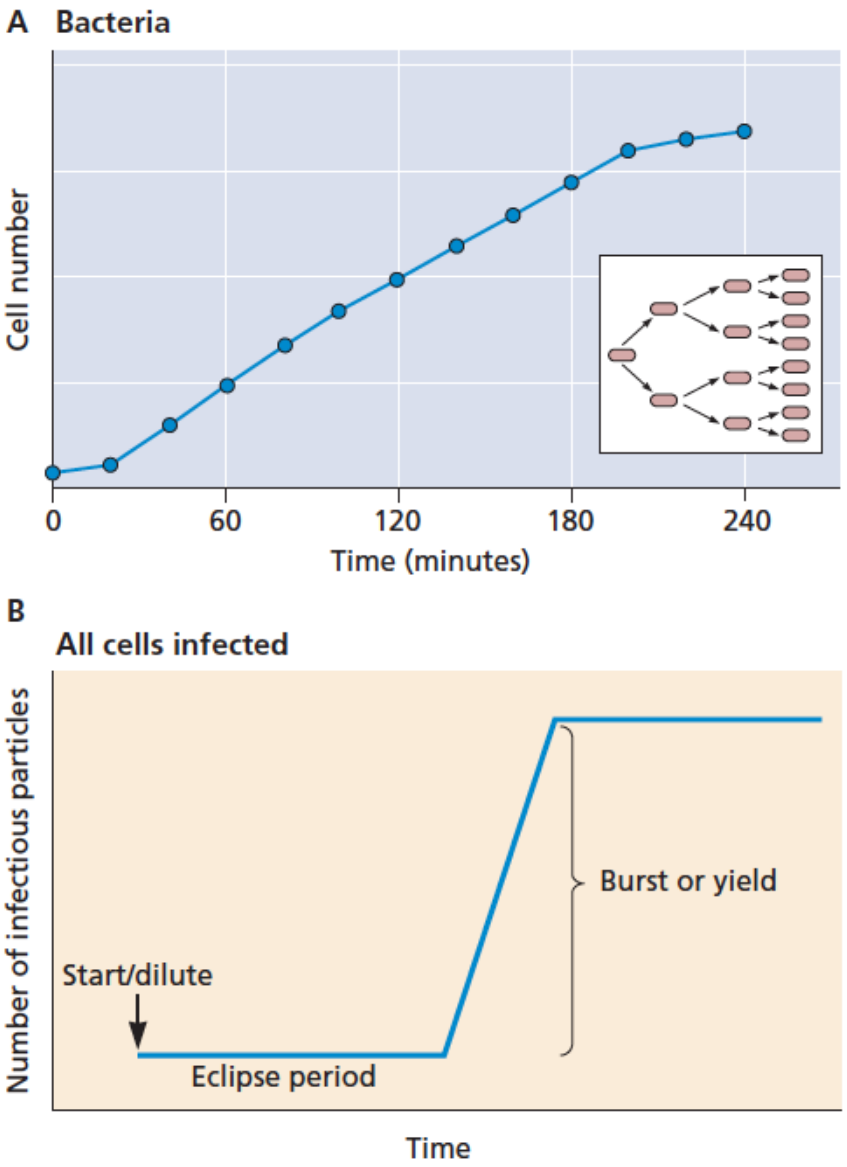


❑ **Steps of virus life cycle:**

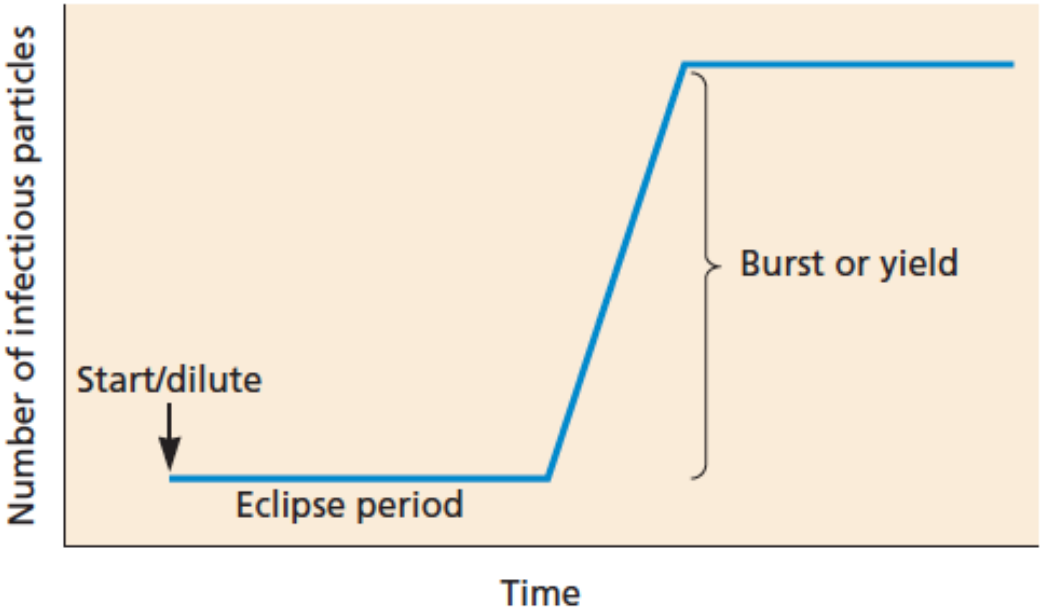
1. Attachment
2. Entry
3. Uncoating/ Disassembly
4. Translation
5. Replication
6. Packaging & Assembly
7. Release

Virus growth

- Unlike living organisms, viruses replicate by assembly of preformed components into many particles.
- Inside our cells, they make their parts and assemble into final product.
- Viruses use our cells as a factory to **make** different parts of their body and finally **assemble** them to produce large number of progeny virus particles



Virus growth



- **Eclipsed period is the lag between initiation of infection and the point when the first virus particle is produced.**
- During this initial lag phase viral proteins and genetic materials are produced; hence, practically there is no complete virus particles either inside or outside the cells during this time.
- Once sufficient amount of viral proteins and nucleic acids are produced they assemble into new virus particle and come out of it to infect new cells.

How to measure virus titer*?

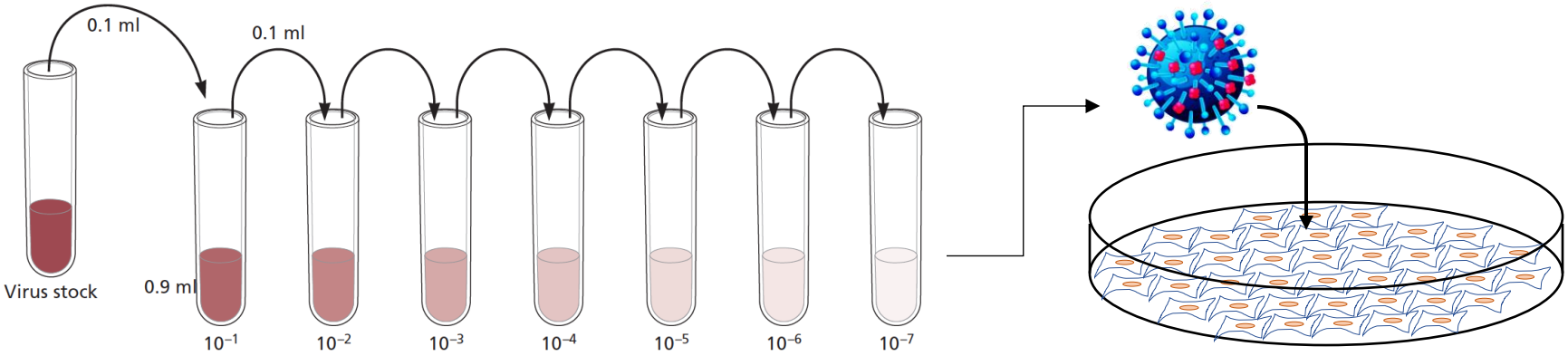
Plaque assay:

- Developed by Renato Dalbecco in 1952; Nobel prize in 1975
- Gives the measure of infectious virus units per milliliter of sample
- Depends upon the ability of viruses to infect and kill cells
- Does not account for the noninfectious or defective virus particles

*Titer or titre is a way of expressing concentration. It is the measurement of the amount or concentration of a substance in a solution. It usually refers to the amount of antibodies or viruses present in a person's blood/fluid

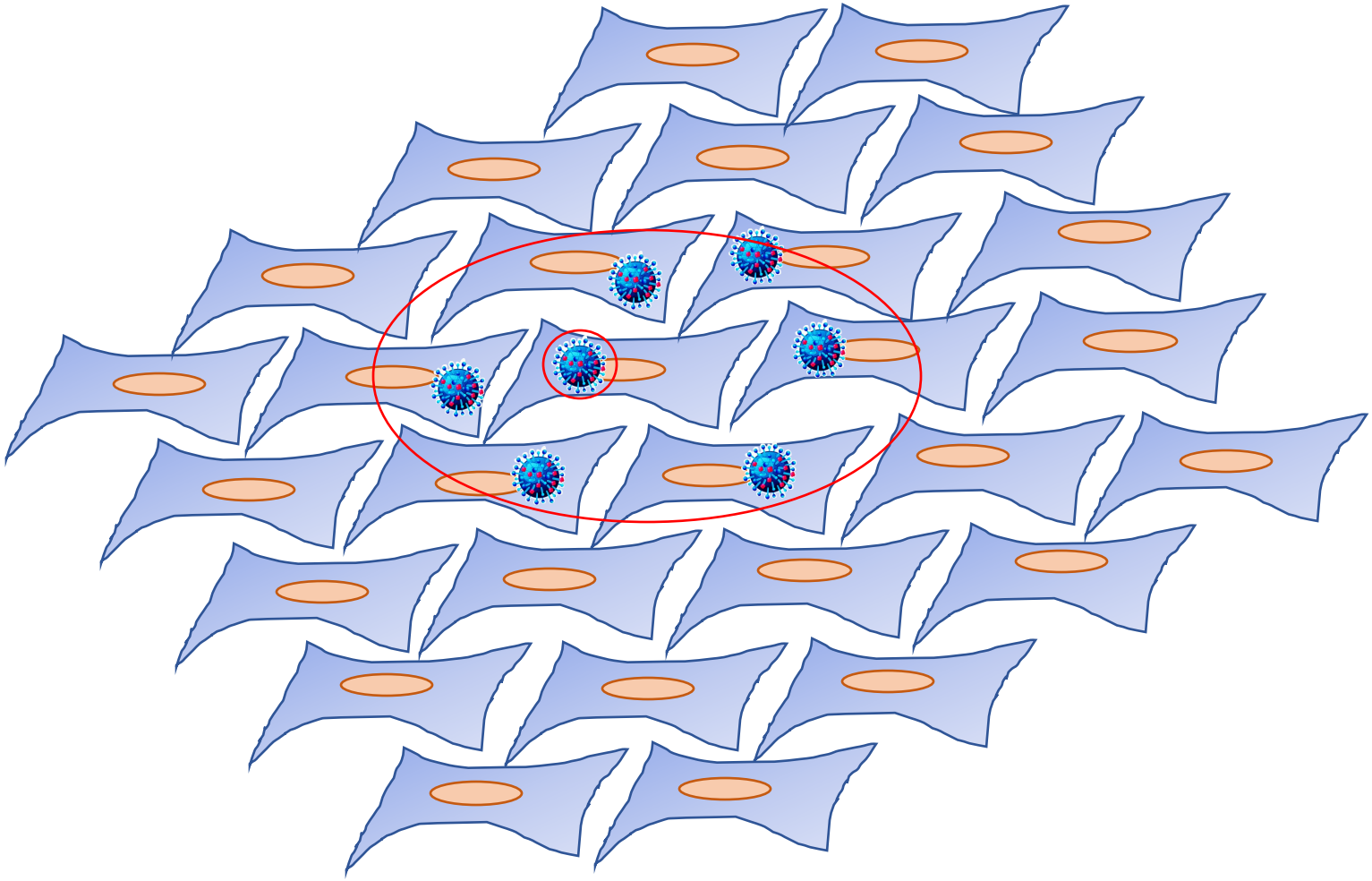
Plaque assay

- Cells are seeded into culture dishes so that they can form a monolayer
- Ten fold serial dilution of virus stock solution is prepared to infect the cells. Higher dilutions are used to make sure one cell get infected with one virus particle only



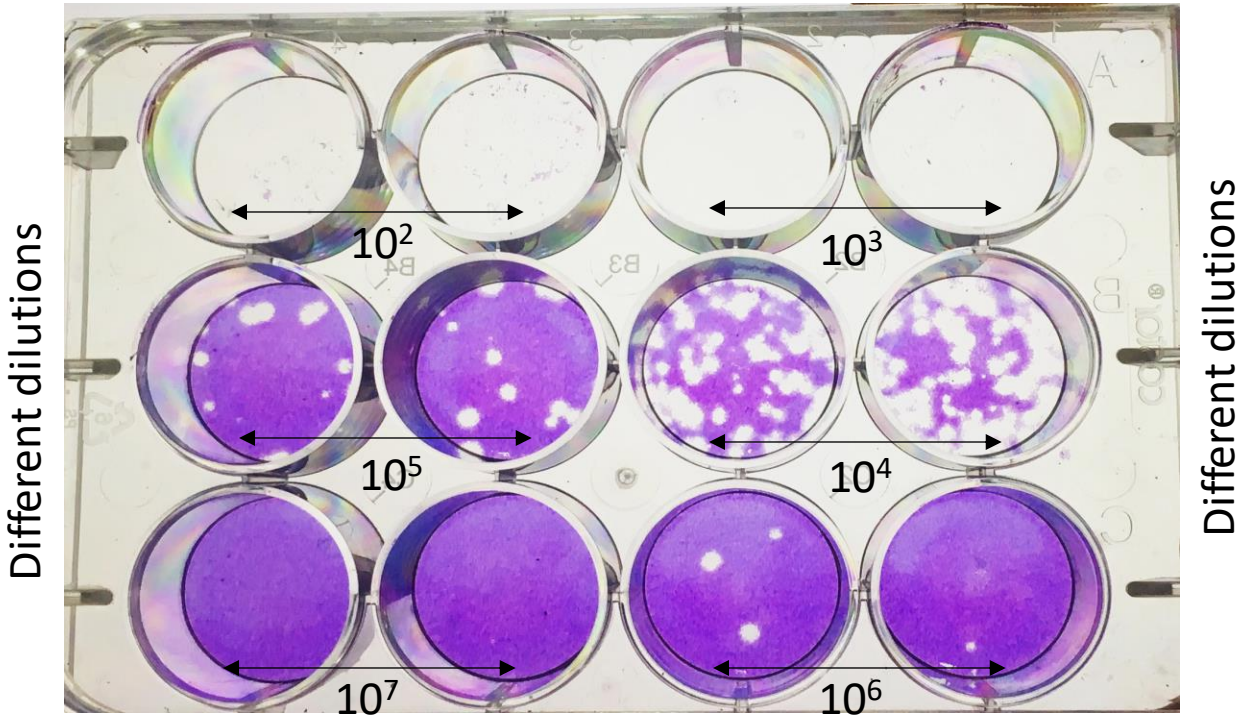
- After infection, the monolayer of cells is overlaid with agarose-media mixture that forms a semisolid medium. This inhibits newly formed viruses to infect cells located at a distance after coming out into the media.
- Only cells that are at the immediate neighborhood of the infected cell gets infected with the progeny viruses.

Plaque formation in a cell monolayer

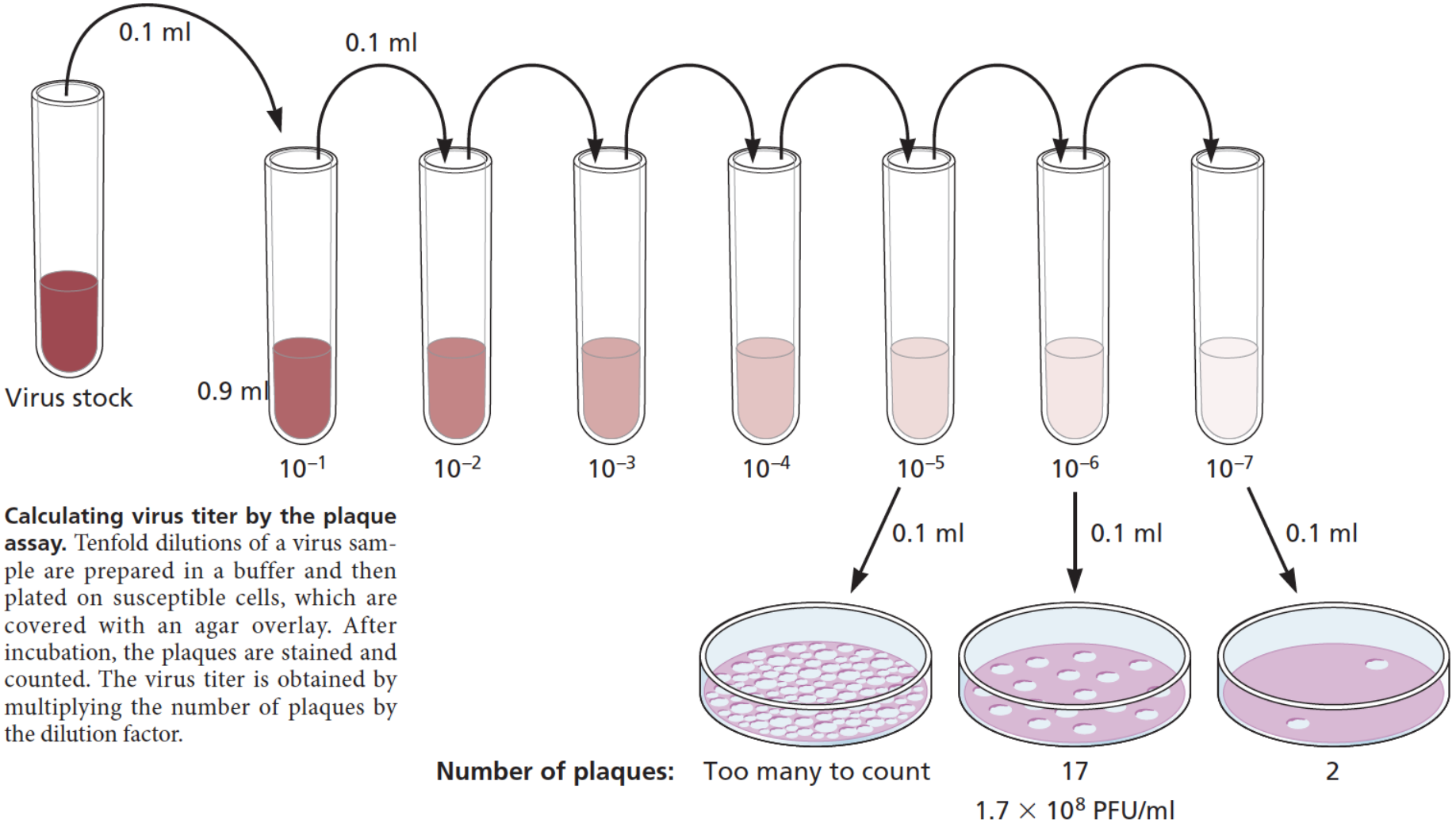


- This forms a foci of infected cells, which eventually die due to virus infection forming a hollo space in the cell monolayer known as **plaques**. Staining of live cells with crystal violet helps visualizing the plaques that appear as tiny holes in violet background.

Visualization of Plaques in the monolayer



Determining virus titer from the number of plaques



Calculating virus titer by the plaque assay. Tenfold dilutions of a virus sample are prepared in a buffer and then plated on susceptible cells, which are covered with an agar overlay. After incubation, the plaques are stained and counted. The virus titer is obtained by multiplying the number of plaques by the dilution factor.

Equilibrium and non-equilibrium viruses

- **Equilibrium viruses** have been long term parasites of the host species
  - They are usually non-lethal but spreads easily
  - Example: common cold

- **Non-equilibrium viruses** have recently jumped from another species
  - They are sometimes lethal, may spread poorly or easily
  - Represents most of the deadly viruses

Equilibrium and non-equilibrium viruses

❑ **Equilibrium viruses:**

- Polio virus
- Common cold (Adenovirus, Coronavirus*)
- Measles virus
- Herpes virus

❑ **Non-equilibrium viruses:**

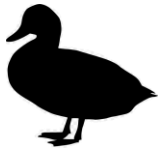
- SARS-coronavirus 2 (Bats?)
- Influenza virus (Birds)
- HIV (Chimps)
- Ebola virus (Bats)

➢ The Novel Coronavirus (also known as **SARS-Coronavirus 2**) is a non equilibrium virus which has jumped from another species (most probably bats) and cause the disease **COVID-19 (Coronavirus disease- 19)**

* There are a number of human Coronaviruses which cause common cold with mild flu like symptoms- ideal example of equilibrium virus

Non-equilibrium viruses

Natural host



Wild aquatic birds

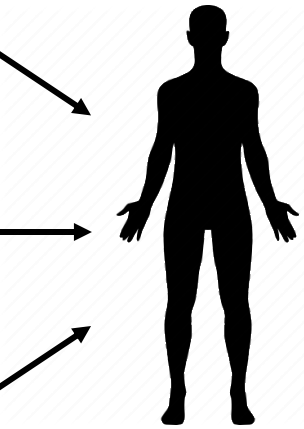
Influenza virus

Bats

SARS-CoV-2
Nipah
Ebola

Chimpanzee

Human Immunodeficiency Virus (HIV)



Zoonoses: Different animal viruses cross the species barrier and start infecting humans; major cause of emerging viral infections leading to epidemics/pandemics.

Corona Virus infections in humans

- Human Coronaviruses were first isolated in 1967
- Causes common cold with mild symptoms (soar throat, fever, headache etc.)
- Coronaviruses from other animals have recently started crossing the species barrier to infect human

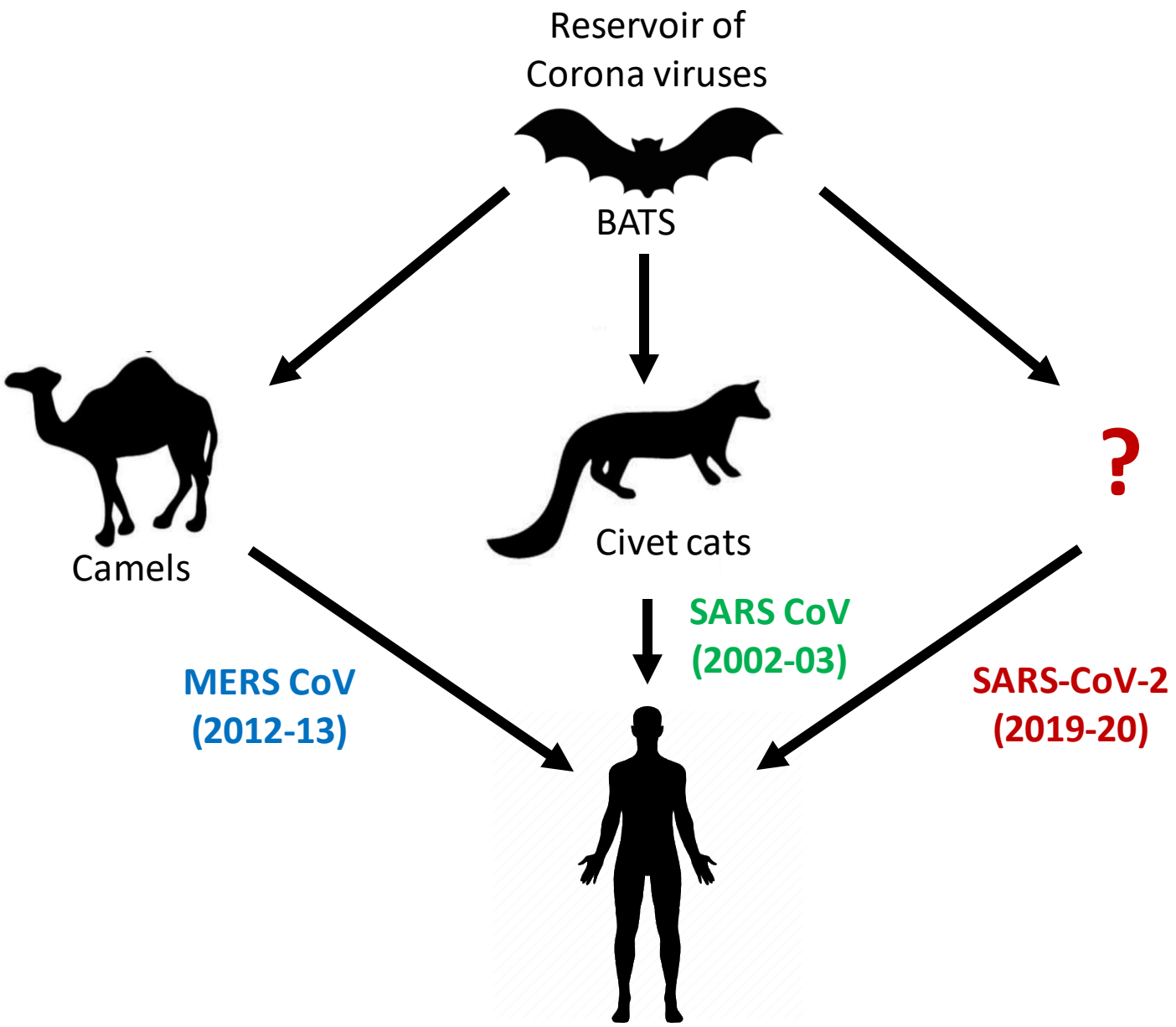
- **SARS Corona virus epidemic 2002-03**
  - Number of cases: 8,437
  - Number of deaths: 800
  - Fatality: 10-14%

- **MERS Corona virus epidemic 2012-13**
  - Number of cases: 2,495
  - Number of deaths: 858
  - Fatality: 35%

- **Novel Corona virus infection 2019-21**
  - Number of cases: 485,618,602
  - Number of deaths: 6,157,237
  - Fatality: 1-2%

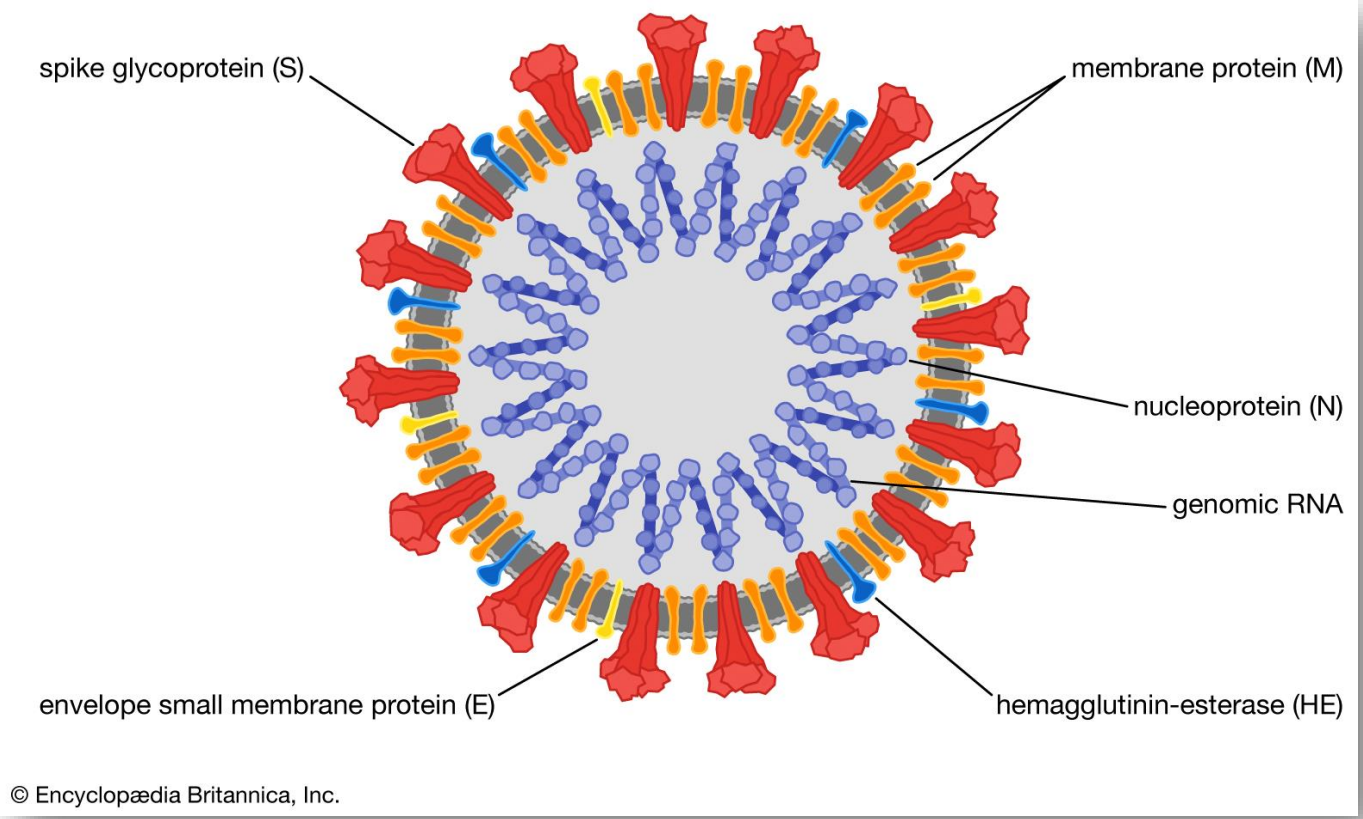
(as of 30th March 2022; Source: Worldometer)

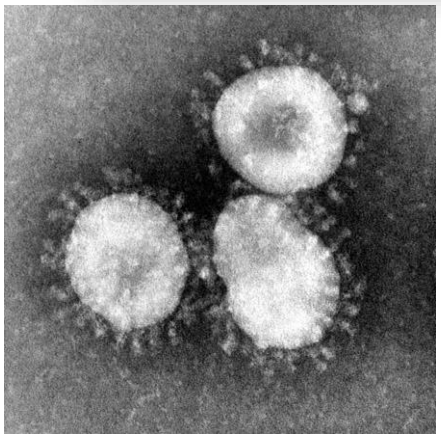
SARS-CoV-2: a non-equilibrium virus



Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

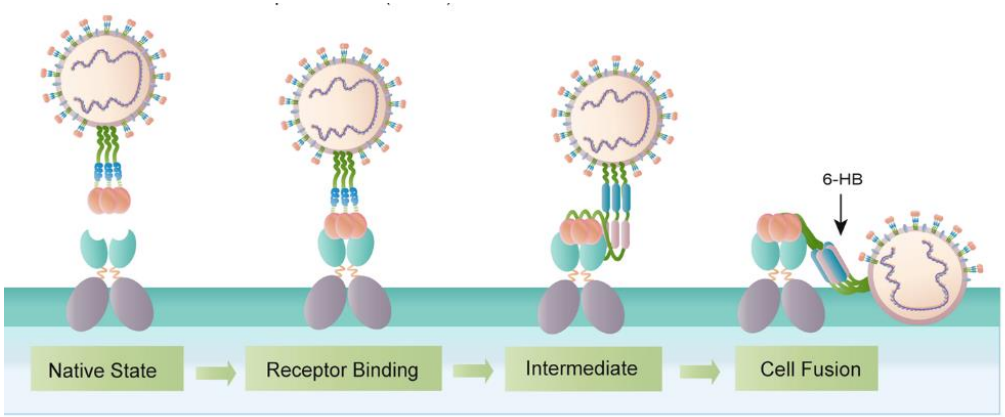
SARS-CoV-2 is a single-stranded RNA virus

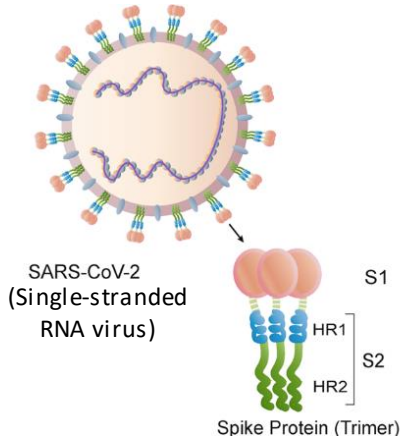


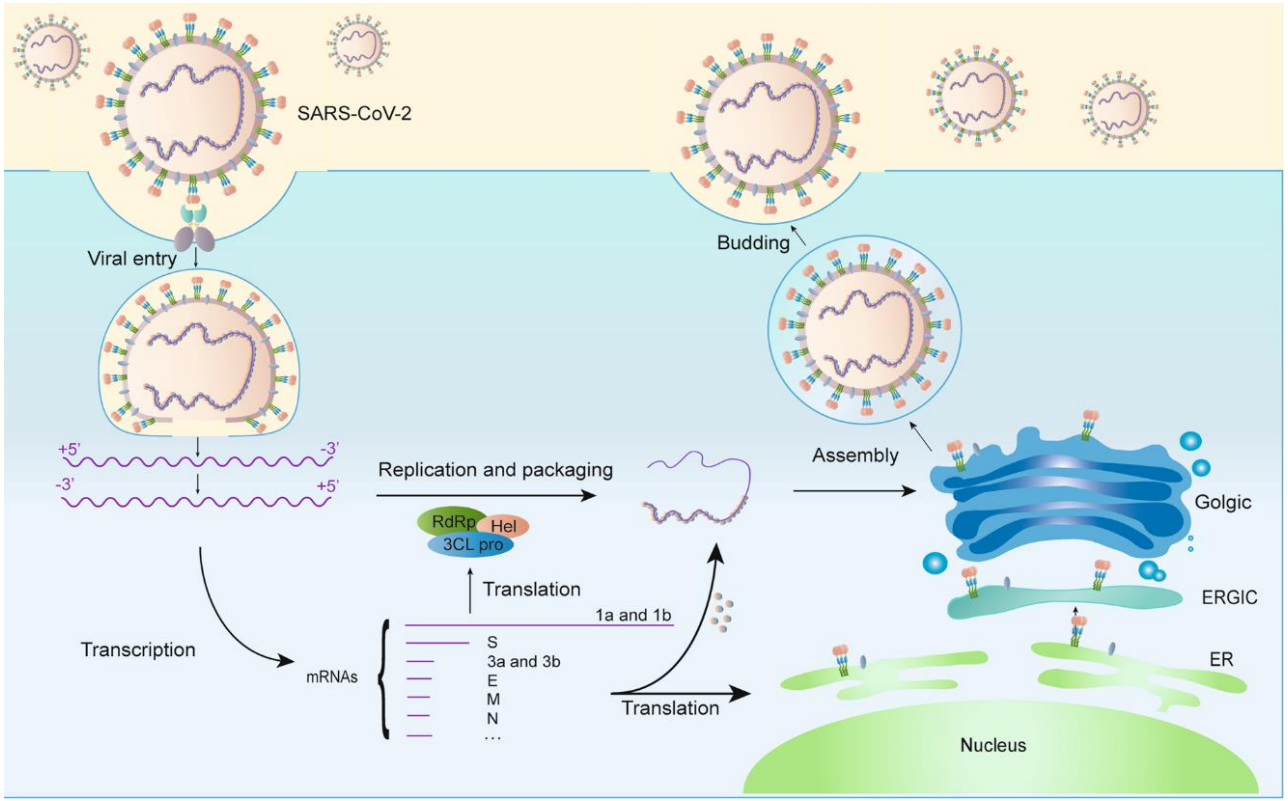


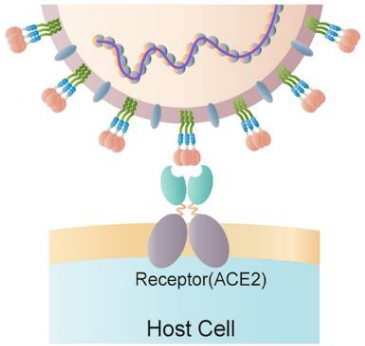
Under the electron microscope, virus particles with spike proteins projecting outwards form a crown-like appearance, leading to its name Corona (**corona** in Latin is **crown**).

How does SARS-CoV-2 infect human host?









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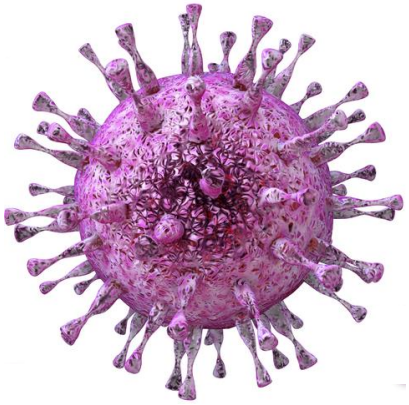
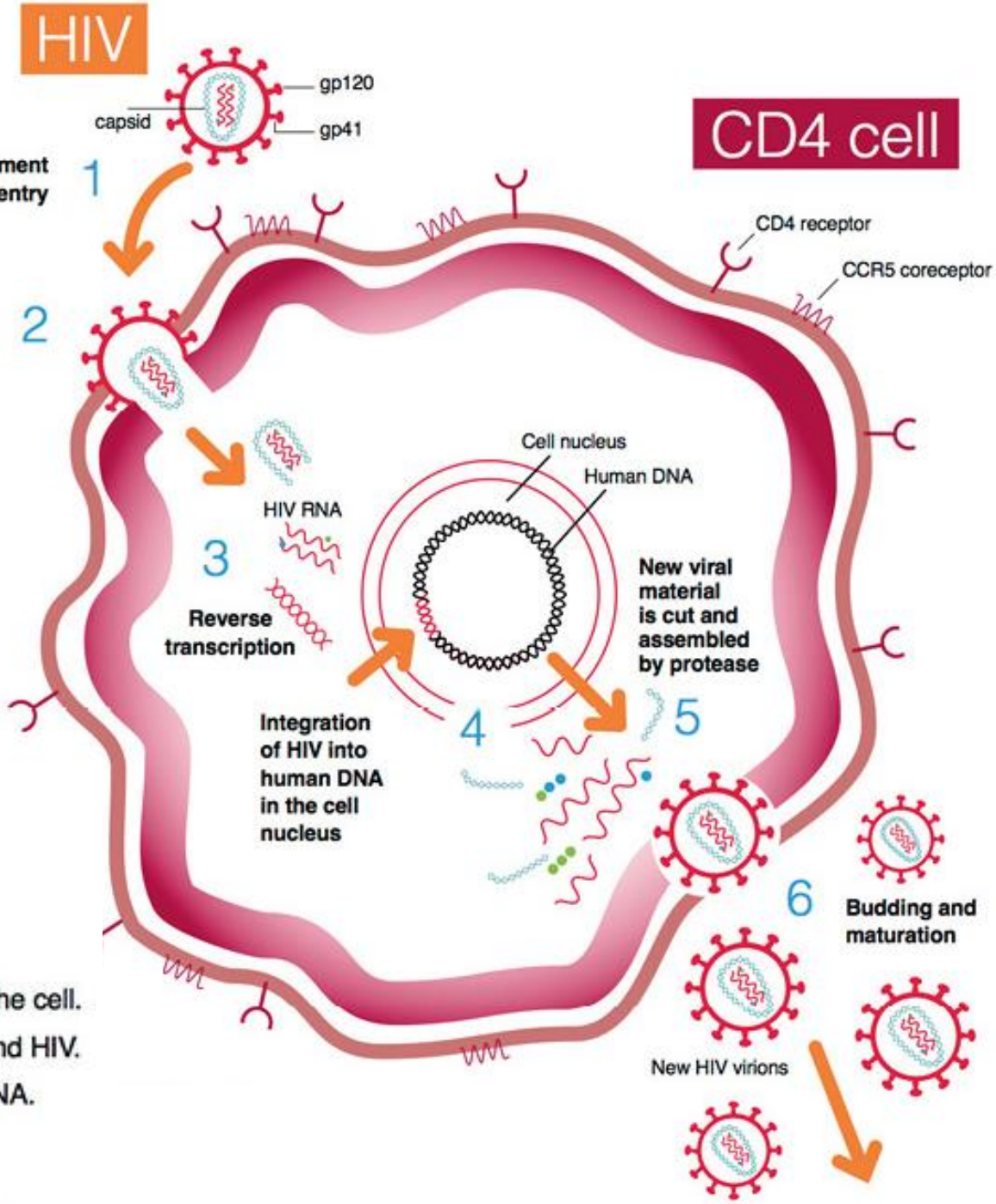
Human Immunodeficiency Virus (HIV)



- First appeared in humans in early 1980's
- Classic non-equilibrium virus
- Endemic in African monkeys and jumped into humans around 90 years ago
- It infects and kills **helper T cells** and and hence wipes out a major component of our immune system. It causes **AIDS (Acquired Immunodeficiency Syndrome)**.
- Gets integrated in our genome – infected person becomes a life long host for the virus

Life cycle of HIV



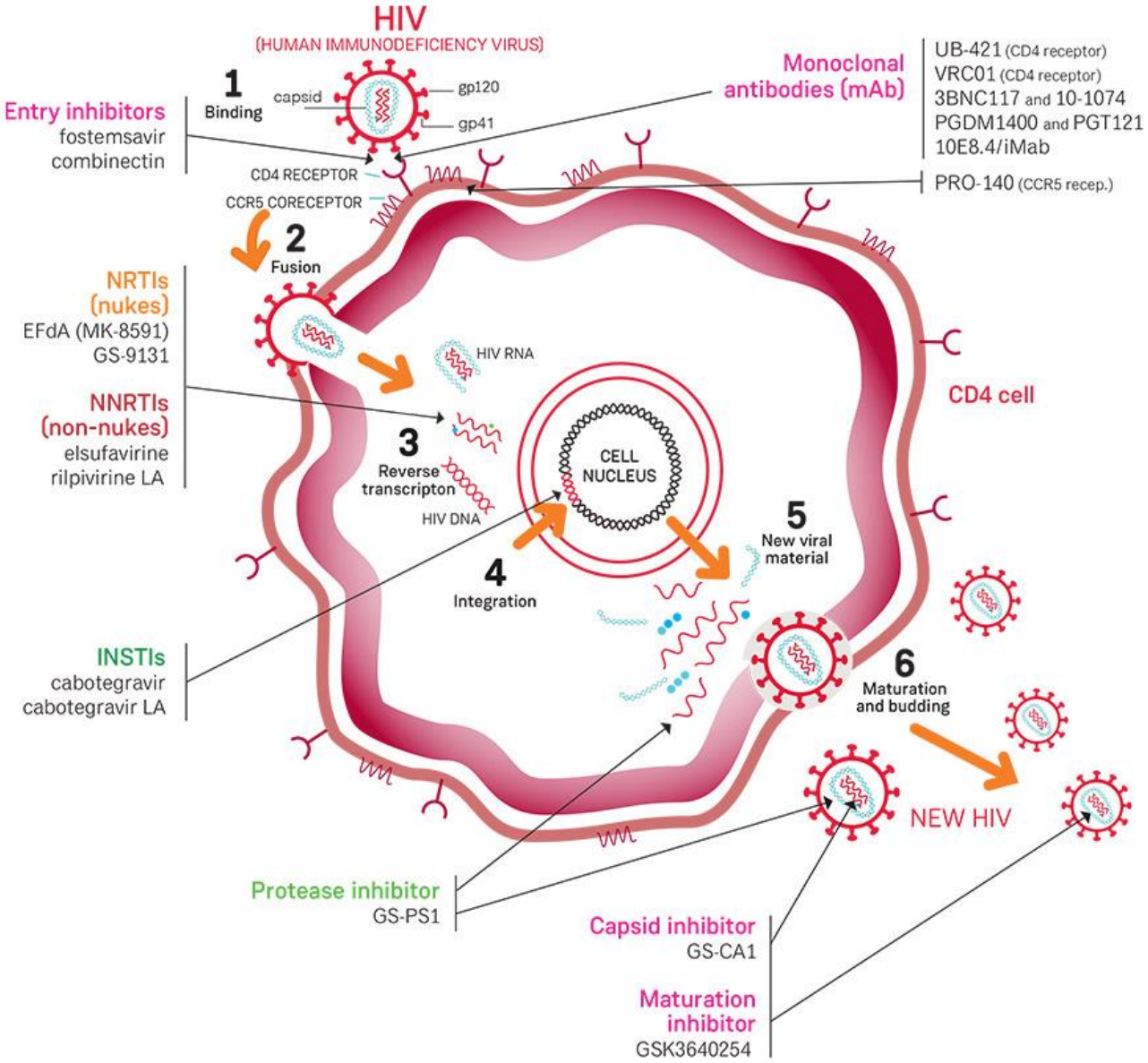
Stages of the HIV lifecycle

1. HIV attaches to the surface of a CD4 cell.
2. HIV proteins and enzymes are released into the cell.
3. Reverse transcription produces a double strand HIV.
4. Integrase enables HIV to link into the cell's DNA.
5. Protease cuts and reassembles new HIV.
6. Each cell produces hundreds of new virions.

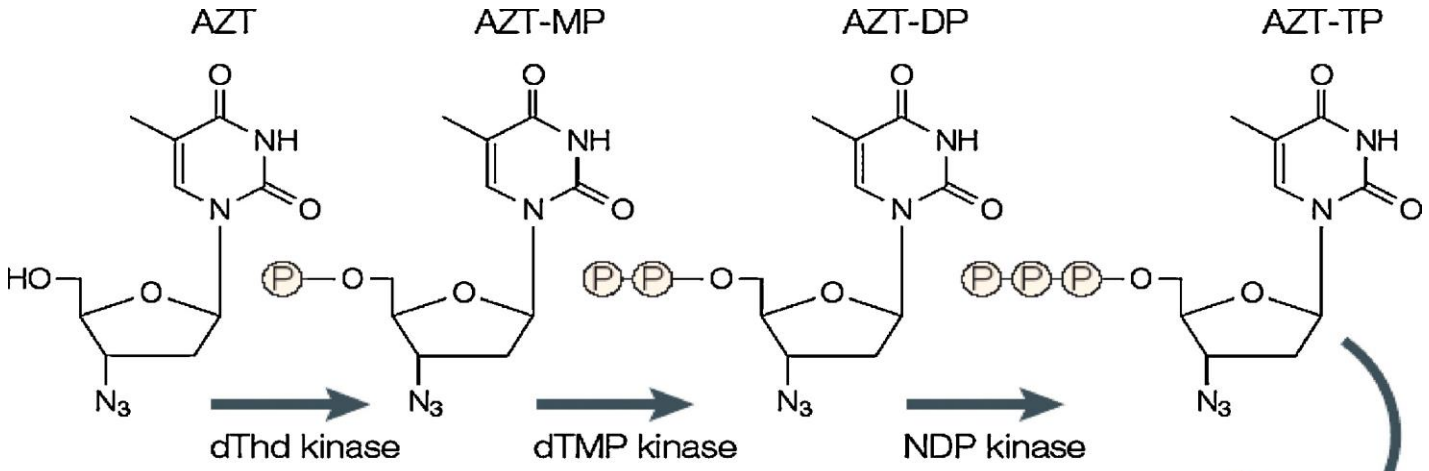
Hijacking the host - HIV

- Recognizes specific CD4 receptor found in the T helper cells. CCR5 acts as co-receptor
- Post entry viral enzyme, the reverse transcriptase performs the reverse transcription process - synthesize the proviral DNA
- Transported to the nucleus of the host cells
- Another viral enzyme, the Integrase integrates the proviral DNA into the host genome – establishment of **latency**
- The provirus can express viral proteins and RNA using host machinery to produce more virus – **lytic cycle**

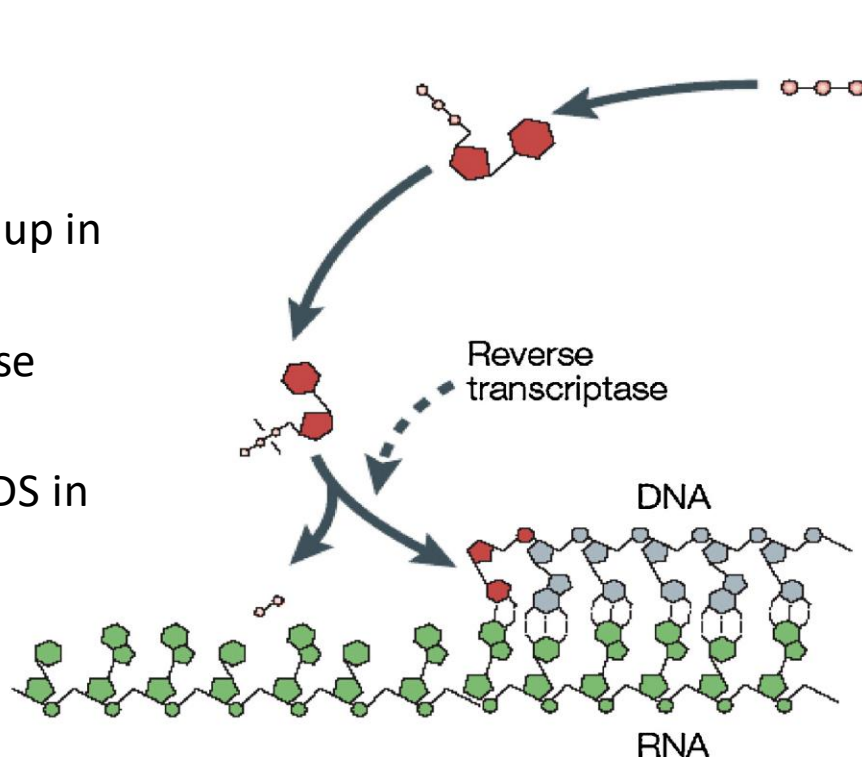
Antiviral drugs against HIV



Nucleoside Reverse Transcriptase Inhibitors (NRTIs)



- **AZT** (**azidothymidine)**
- Thymidine analogue
- Contains an azide (N3) group in place of the usual —OH
- Has high affinity for Reverse Transcriptase
- Delays development of AIDS in HIV +ve patients

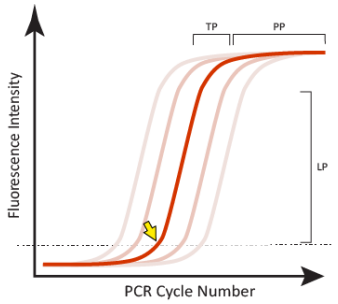


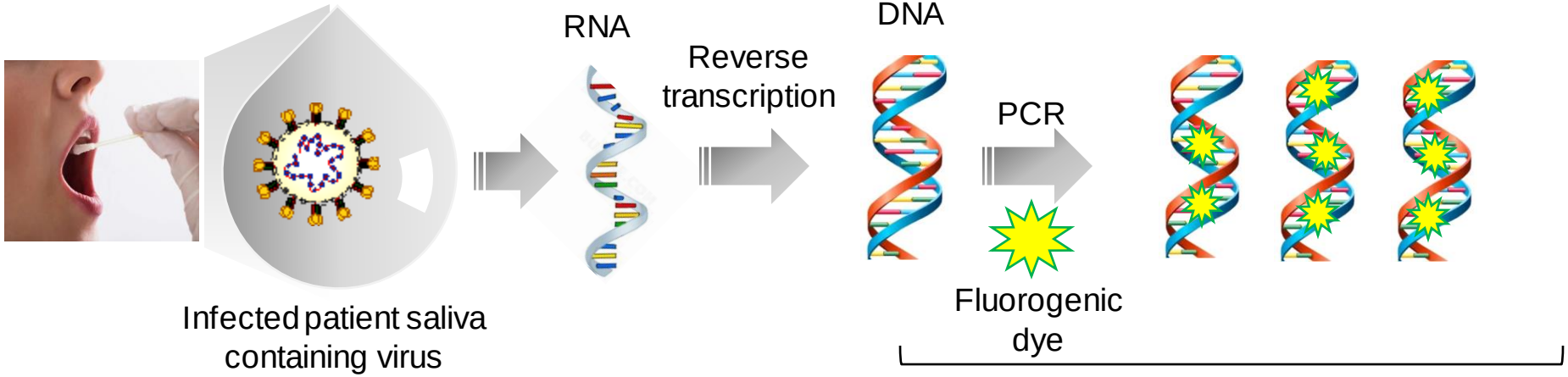
Antiviral drugs against HIV

- Attachment inhibitor: inhibits attachment of virus to the receptor/ co-receptor
- Fusion inhibitor: inhibits fusion of the virus and host cell membrane
- Non-nucleoside & nucleoside based reverse transcriptase inhibitors (NNRTI & NRTI): inhibits reverse-transcription
- Integrase inhibitor: inhibits integration of the viral dsDNA into the host chromosome
- Protease inhibitor: inhibits release and maturation of the progeny HIV particles

Diagnoses of virus infection: The RT-PCR test

Identification of the most fundamental signature of the virus - the genetic material.





STEPS:

- Extraction of viral RNA/ DNA from patient body fluid
- Reverse transcription (for RNA)
- PCR based amplification in presence of fluorogenic probe
- Realtime detection of the fluorescence intensity

HIV and AIDS
https://www.youtube.com/watch?v=ng22Ucr33aw