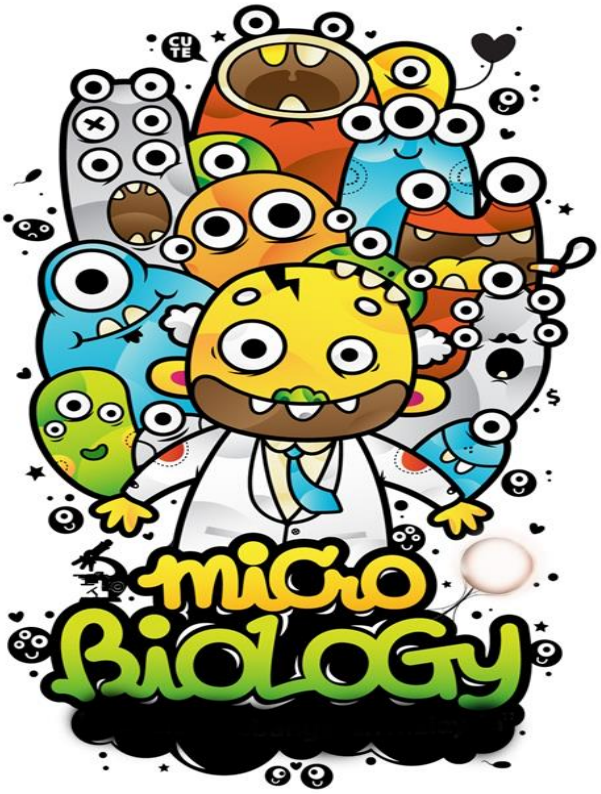

VIROLOGY

Subject: Viral Replication I

Lec. No.: 1

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The structure of viruses (a recap)

Viruses are classified according to their:

1- Nucleic acid
   a. DNA or RNA
   b. Double-stranded or single-stranded

- Viral genome is *always* either DNA or RNA, **NEVER** both together.

- The genome (DNA or RNA) is surrounded by a protein coat, called the *capsid*.

2- Capsid
   a. Icosahedral
   b. Helical
   c. Complex

- The genome, along with the capsid, make up the *nucleocapsid*.

3- Envelope
   a. Naked (non-enveloped)
   b. Enveloped

- Non-enveloped viruses, aka *naked* viruses, are those made up of the nucleocapsid only.

- Enveloped viruses are those with an envelope surrounding the nucleocapsid.

- Are all nucleocapsids surrounded by an envelope? No, not necessarily (i.e. some viruses are enveloped while others are not).

- General rule: *ALL* DNA viruses have icosahedral capsids.

- If present, the envelope has spikes and glycoproteins embedded within its structure. These bind to receptors on target cells to initiate the entry of the virus particle to the cell.

- RECALL: Viruses are obligate intracellular parasites: they can survive outside living cells for short periods of time. However, they *require a living host cell* to reproduce (replicate and proliferate) by taking over the cell's machinery.

- The glycoproteins mentioned previously form the outermost proteins within the structure of a virus particle and are therefore the most antigenic part of the virus. They are detected by the immune system, resulting in the activation of the humoral immune response, i.e. the production of **antibodies** against these foreign spikes/glycoproteins.

- Antibodies neutralize the virus by binding to the envelope glycoproteins/spikes, preventing the virus from binding to receptors on target cells and as a consequence, the entry of the virus to the cell is inhibited.

00:00 – 11:00

Viral replication terminologies

Before we start talking about viral replication in details, we need to be familiar with certain terminologies.

1- Plaque forming unit (PFU) – a measure of the number of particles (which, in this case, are virus particles) that are capable of forming plaques per unit volume. (not required)
2- Multiplicity of infection (MOI) – the ratio of infectious agents to infection targets. Put in other words, it is the ratio of the number of viruses to the number of cells.

This term is more often used in terms of viral laboratory work. In the lab, a virus titration is carried out to find out the number of viruses required to infect a known number of cells (recall: *viruses are obligate intracellular parasites*). These cells are put in a flask, and are titrated by a virus titre with a known amount (number) of viruses per unit volume. The volume of the titre eventually obtained is used to find out the MOI.

Assuming there is a hundred thousand cells in the flask, and the virus titre contains ten thousand virus particles, then the MOI is 10,000/100,000 → MOI is 0.1.

If a hundred thousand virus particles are added to ten thousand cells, then the MOI is 100,000/10,000 → MOI is 10.

Clinical significance: Once virus particles enter our bodies, does every cell become infected with a single virus particle only, or is it infected by more than

one? If the viral load (infectious dose) of the virus is high (a million virus particles entered), then the chance of one cell being infected by multiple virus particles is high. On the contrary, if the viral load is low (a hundred virus particles in this case), then there is a greater chance that one cell is going to be infected with one virus particle only.

3- Phases of viral replication:

(Note: do not worry about the time in hours because the time varies for different viruses; some have slow proliferation rates whereas others have fast proliferation rates.)

The simplest definition for viral replication is **disassembly followed by reassembly**.

a. Eclipse phase: the period during which the input virus becomes uncoated (dismantles or disassembles); 10-12hrs. This period starts with the entry of the virus to the cell. Once it has entered the cell, the virus will begin to disassemble.

Uncoating: the disassembly of the capsid and the release of the genomic material into the cytoplasm of the infected cell.

During this phase, if you assay for the presence of intact virus particles inside the cell you will find none, because they have already entered the cell and started to disassemble, resulting in the absence of intact virus particles (no complete virions).

b. Synthetic phase: the period during which new virus particles are assembled; 4-6hrs. This phase is divided into two phases; first, the reproduction of the genome and second, the production of proteins. Protein production is as well divided into two stages: early and late.

Late stage protein synthesis involves the synthesis of *structural* proteins, including capsid proteins and the spikes/glycoproteins of the envelope (if present). These are late stage proteins since they are needed by the virus at later stages, i.e. when it will start to reassemble.

Early stage protein synthesis involves the synthesis of *non-structural* proteins, including viral enzymes and proteins. These are produced at the beginning as the virus needs them for the whole replication step.

c. Latent phase: the period during which no extracellular viruses can be detected. During this period, the newly-formed virus particles are still inside the cells (intracellular) and have not been released to the outside yet. This period *ends* as the new viruses are *released* from the infected cells.

The latent phase includes the eclipse phase. *The latent period starts at the entry of virus particles into cells, and continues until before the release of new complete virus particles from the cells.* In other words, it is the period of time that separates between the viruses being intracellular (upon entry) and extracellular (upon release).

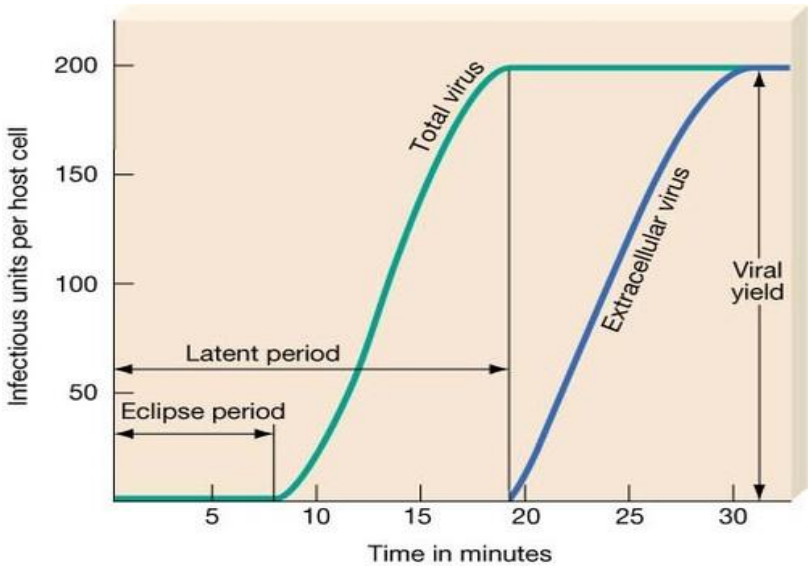

Fig 1: One-step virus growth curve

As can be seen from the curve above, the number of virus particles increases and then plateaus.

The maximum number of viruses produced from a single cell is called the ***burst size***.

4- Burst size: the amount of infectious virus produced per infected cell. So if a single cell produces 10,000 new virions, then the burst size is 10,000 and so forth.

-Going back to fig 1, why does the number of new viruses produced by a single cell reach a maximum and then plateau? The ultimate fate of most virally-infected cells is death. Once the virus enters the cell, it takes over the cell's reproductive machinery for its own benefit: to replicate the virus and produce new viral material. Since the cell has limited resources for replication, these resources are eventually going to be consumed by the virus, so the virus finally reaches a point where it is unable to produce more of itself.

The replication cycle

- The viral replication cycle is divided into eight or seven stages (depending on the resource you are reading from). Regardless of their hosts, all viruses must undergo each of these stages in some form to complete their replication cycle.

- There are eight steps in the cycle, and each step is going to be discussed as a separate entity (for purposes of simplification and clarification). However, not all the steps described here are detectable as distinct stages for all viruses. This means that during the replication of a single virus particle, multiple steps can occur simultaneously, and this is especially true during the synthetic phase, during which the replication of the genome and the synthesis of protein occur together. Taking the cell with multiple virus particles as a whole unit, all the steps can occur at a time, with a particle just entering and uncoating, while there is reassembly of another particle, and so on.

- An overview of the steps of viral replication:

Attachment/adsorption of receptor on host cell and spikes/glycoprotein on the virus particle → **penetration/entry** of the virus into the cell → **uncoating** which involves the disassembly of the capsid and the release of the genetic material into the cytoplasm of the infected cell → **synthetic phase** where the replication of the genome (DNA or RNA) and early and late protein synthesis occur → **assembly** of the newly-produced viral genome and proteins to make new virions → **release and maturation** where the virus leaves the cell either having already matured inside, or is still going to mature outside the cell (depends on the virus)

Keep in mind that a *mature* virus is a virion capable of infecting cells. Maturation is just a matter of time because certain proteins need time to take the final shape and

become fully functional, so maturation can occur inside the cell prior to the release of viruses or outside the cell after the viruses have been released.

20:00 – 30:20

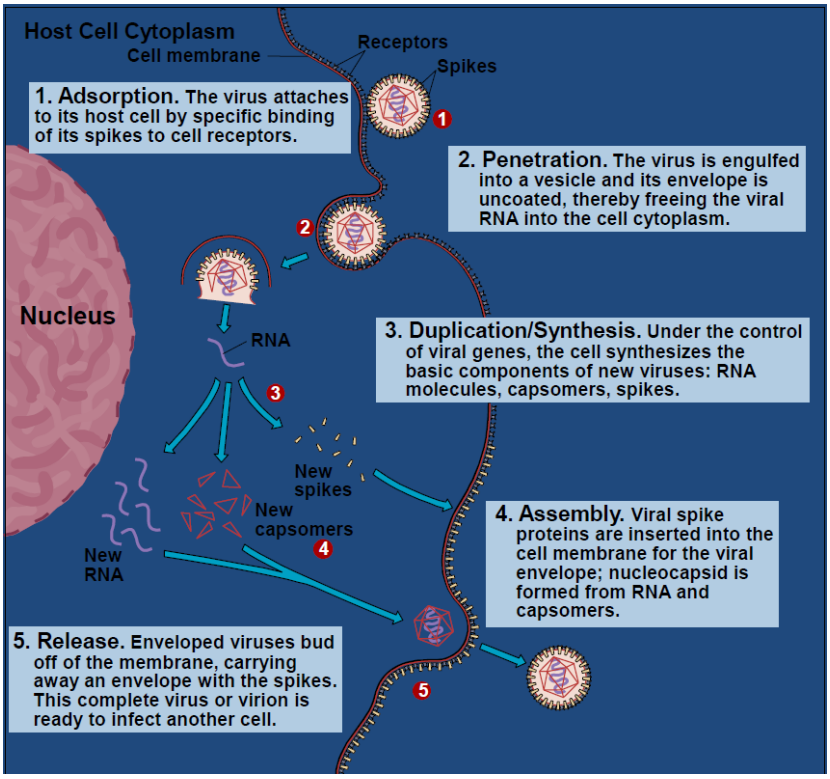

Fig 2: Life cycle of an animal virus

1- ATTACHMENT/ADSORPTION

- Virus attachment consists of specific binding of a virus-attachment protein (or 'antireceptor') to a cellular receptor molecule. In other words, the interaction occurs between the spikes/glycoproteins on virus particles and cellular receptor molecules.
- Target receptor molecules on cell surfaces may be proteins (usually glycoproteins), or the carbohydrate residues present on glycoproteins or glycolipids.
- Some complex viruses (e.g. poxviruses, herpesviruses) use more than one receptor and have alternative routes of uptake into cells.

- The glycoprotein molecules present on the virus particles can be composed of a single unit or multiple units (2, 3 …). Glycoproteins of the Picornaviridae virus have 3 units, for example. HIV glycoproteins are made of 2 units, with one embedded within the envelope (transmembrane unit) and the other one on top of it (subunit), and they are called GP41 and GP120 respectively.

- What about the receptors? Is only one receptor on the target cell enough to initiate the virus entry? Most of the time yes. Are there any exceptions? Yes, we have exceptions. Certain viruses require attachment between the glycoprotein and more than one cellular receptor in order to initiate viral entry. Other viruses, such as the HIV, need a *co-receptor* or a *chemokine receptor.*
  Regarding HIV,
  Which cells does it target? T-cells.
  What's the receptor on T-cells? CD4.
  HIV is composed of two glycoprotein units: GP120 and GP41.
  The GP120 attaches to the CD4 on the T-cells, and then falls off, allowing the transmembrane unit (GP41) to interact with the co-receptor (chemokine receptor), which is either CCR5 or CXCR4.

- The picornavirus is an example on viruses with multiple glycoprotein units. The poxvirus, herpesvirus and hepatitis C are all examples of viruses which require attachment between the glycoprotein and more than one receptor on the target cell. HIV is an example of a virus that requires a receptor and a co-receptor in order to initiate virus entry into the cell.

- ENVELOPED VIRUSES vs NAKED VIRUSES, in terms of attachment/adsorption:

    - Enveloped viruses have glycoproteins/spikes embedded within the structure of the envelope. These attach to receptors on target cells so the virus enters into the cell.
    - Naked viruses lack the envelope as part of their structure, and therefore have no glycoproteins. However, they still need to attach

to receptors on target cells to initiate virus entry. In fact, *both enveloped and naked viruses require receptor attachment to initiate virus entry to the target cell.* The question now is, how is that possible for naked viruses? Instead of having the glycoproteins/spikes, some naked viruses have surface proteins on the capsid. Others have slits/grooves within the capsid itself, still requiring the binding to a receptor on a target cell for the initiation of viral entry into the target cell.

- Recall: glycoproteins are the most antigenic part of the virus. Keeping this in mind, which is more superior in terms of escaping the immune system, enveloped or naked viruses? **Naked viruses**. Generally, surface proteins are immunogenic (i.e. relating to or producing an immune response). However, there are naked viruses which lack surface proteins and have slits or grooves within the capsid instead. This makes it difficult for the immune system to access these slits/grooves and recognize them. On the other hand, if the naked virus has surface proteins, it can be better recognized by the immune system. So, naked viruses with surface proteins on the capsid are at a higher risk of being recognized by the immune system than those with slits and grooves, but are still superior to enveloped viruses in terms of *evasion* from the immune system.

- ***Host range:*** the collection of hosts that an organism can utilize as a partner. It refers to the spectrum of hosts that a virus can infect, that is the *species*, including humans, animals, plants … The influenza virus infects humans and birds (swine flu). In general, there are viruses that only infect humans, and others that only infect animals, for example. However, there's the swine flu which can infect humans. *(to be discussed later)*

- ***Cellular (tissue) tropism:*** the cells and tissues of a host which support the growth of a particular virus.

    - HIV targets T-lymphocytes
    - Hepatitis A, B and C target hepatocytes

- Influenza virus targets cells of the upper respiratory tract (URT)

30:20 – 40:00

Tables (6-5) and (6-5) in the slides are not to be memorized. Only highlighted rows are required.

Virus	Target Cell	Receptor
HIV	Helper T-cell	CD4 molecule and chemokine receptor (CCR5 and CXCR4)
Influenza A	Epithelial cells	Sialic acid

Table 1: examples of viral receptors

Virus Family	Virus	Viral attachment proteins (VAP)
Orthomyxoviridae	Influenza A	HA
Paramyxoviridae	Measles virus	HA
	HIV	GP120

Table 2: examples of viral attachment proteins. HA is hemagglutinine

- Can more than one virus use the same receptor for entry? Yes. Examples include the influenza virus and the rotavirus which both use sialic acid receptors. The *specificity* of the receptors is still there: the receptors are *composed of* sialic acid, but don't necessarily have the same shape or structure in each case.

- Viral attachment proteins can be composed of a single or multiple units, such as in the case of picornaviridae (3 subunits) and HIV (2 subunits).

- ***Glycoprotein-receptor complexes***: one complex is not adequate to initiate the entry of the (enveloped) virus into the cell. More than one complex is required, e.g. HIV requires from 3-5 glycoprotein-receptor complexes to initiate viral entry into the cell.

- Once the virus has its glycoproteins attached to the cellular receptor, the entry/penetration step starts.

2- PENETRATION/ENTRY

- Penetration of the target cell normally occurs a very short time after attachment of the virus to its receptor in the cell membrane. Unlike attachment, cell penetration is generally an energy-dependent process (i.e. the cell must be metabolically active for this to occur). Three mechanisms are involved. However, only two of these are important for viruses.

  a. *Receptor-mediated endocytosis; where a receptor is required for all viruses to enter the cells.

  Receptor-mediated endocytosis occurs with both, naked and enveloped viruses.

  In the case of naked viruses, the nucleocapsid enters into an endocytic vesicle so is now surrounded by a vesicular membrane. As for enveloped viruses, the virus, with its envelope, become as well surrounded by the endocytic vesicle membrane.

  How are the viruses then released into the cytoplasm of the cell? The triggering mechanism is a drop in the pH (acidification) of the endocytic vesicle (endosome).

  - For naked viruses, the drop in pH will lead to the exposure of hidden domains within the capsid proteins (structure of the capsid changes, exposing the previously hidden domains). These hidden domains are going to interact (come into close proximity) with the endocytic vesicle and eventually either release the nucleocapsid (as a whole) or the viral genome with the viral enzymes into the cytoplasm.

Viropexis is another name for receptor-mediated endocytosis of naked viruses.

Another mechanism for the release of naked viruses is the lysis of the endocytic vesicle membrane.

- For enveloped viruses, the drop in pH will lead to the fusion of the envelope with the endocytic membrane and the release of the nucleocapsid into the cytoplasm of the target cell.

b. ****Fusion;*** where the viral envelope fuses with the plasma membrane of the cell.

Once attachment (formation of the glycoprotein-receptor complex) occurs, the virus starts to approximate its envelope to the plasma membrane of the cell → fusion of the two membranes. The viral envelope is then left to become a part of the cellular membrane, and the nucleocapsid is directly deposited in the cytoplasm of the cell.
Fusion only occurs with enveloped viruses.

c. Translocation; it is the attachment of a molecule to a receptor on the target cell and then flipping over to the cytoplasmic side of the cell. Translocation is not seen in viruses.

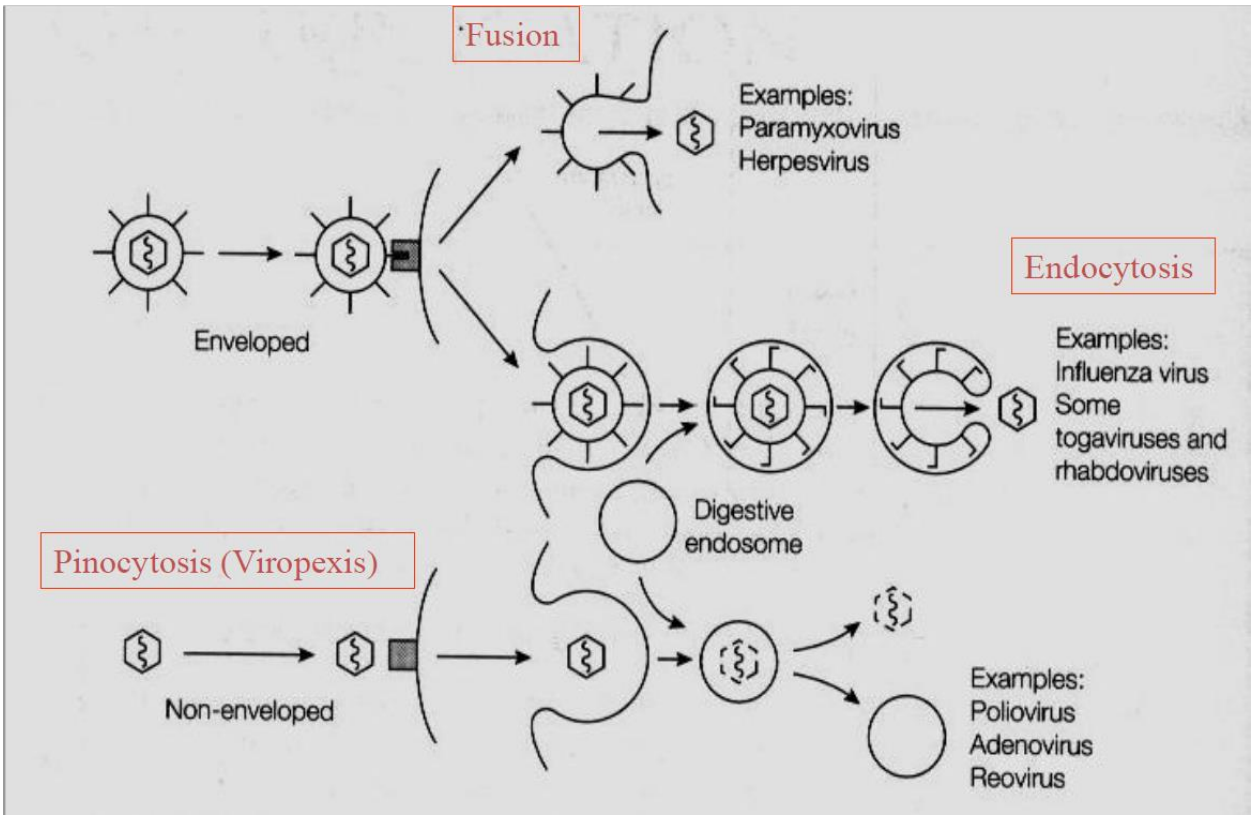

Fig 3: mechanisms of virus entry

Please refer to the slides for extra details regarding mechanisms of penetration.

Dr. Ashraf went through the examples listed in fig 3 above, please do likewise.

3- UNCOATING
4- SYNTHETIC PHASE
5- ASSEMBLY
6- RELEASE

Best of luck!