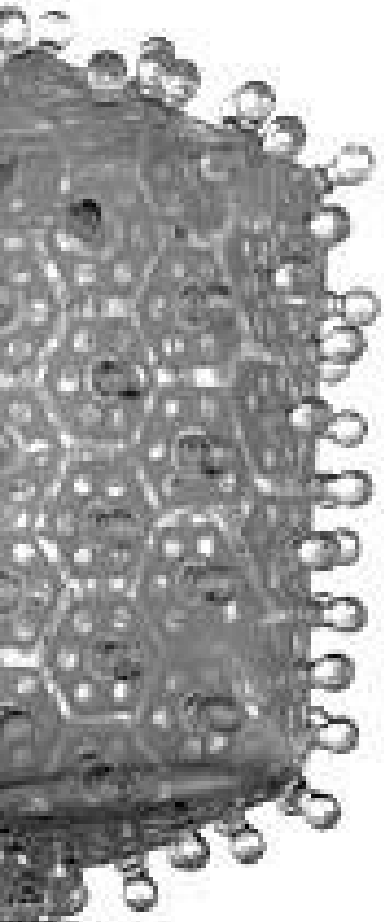

Microbiology

Lecture No:..6- Viro 2.....

Dr Name:..Hamed Al Zoubi

Done by:..Aya Naim Abusheikha

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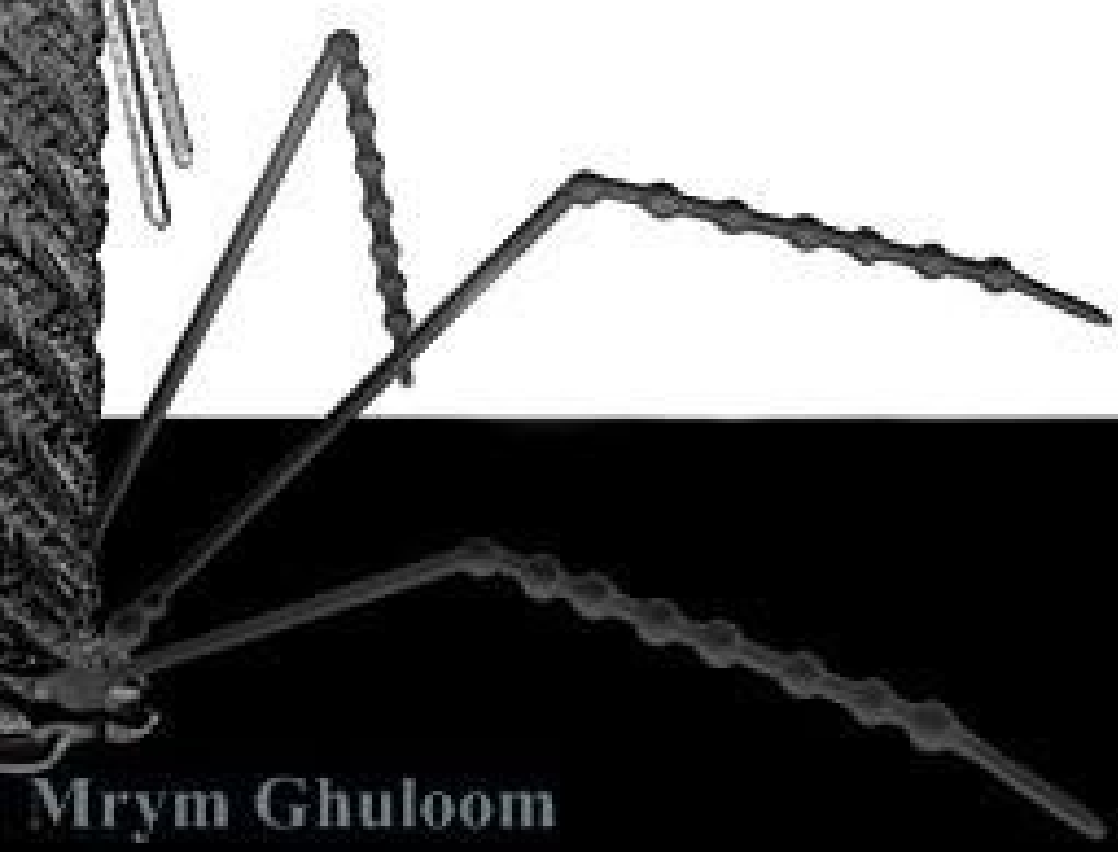

Mrym Ghuloom

VIROLOGY

REPLICATION AND GENETICS

In the previous lecture we talked about the history and development of viruses as well as viral structure. Today we'll be talking about the life cycle, replication and genetics of viruses.

Quick revision:

- **Positive RNA viruses:**

In positive RNA viruses, the viral RNA genome can be considered the viral mRNA and can be **immediately translated** by the host cell; the genome can be used directly to synthesize proteins.

- **Negative RNA viruses:**

The viral RNA is complementary to the viral mRNA and **cannot be translated into proteins directly.**

What is the difference between viroids , viruses and prions ?

- Viruses have capsid and genetic material (may have envelope) .
- Viroids have just genetic material (which is RNA), but no capsid .
- Prions are just proteins , and don't have a genetic material .

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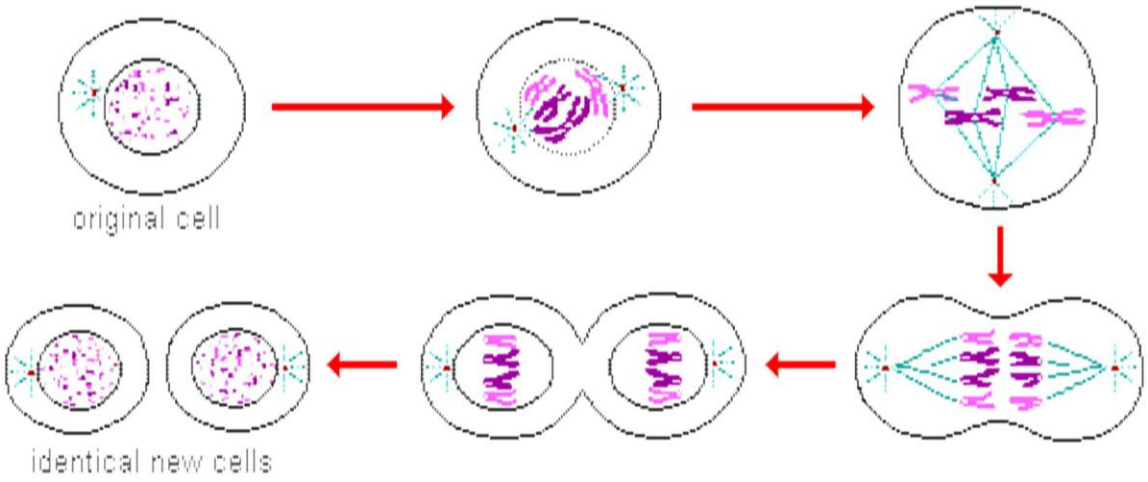
Bacteria replicate by binary fission, which is a type of asexual reproduction where a cell divides giving rise to two cells, which are clones of the parent cell; they have the complete genetic material and identical structures.



Viruses, however, don't reproduce by binary fission because they depend upon other cells to multiply; viruses don't have their own multiplication machines. When a virus enters the cell, it enters with nothing but its own genome which consists of only 20 genes compared to 100000 genes for a human cell, This is why it must rely so heavily on the host cell for the materials it needs for reproduction, it will diverse the mechanisms inside the cell to itself (KIDNAP).

Structure and general features of the human cell:

- An enveloped, perforated **nucleus** containing double-stranded DNA.

  Pores allow the entry and exit of materials to and from the nucleus.

- **Cytoplasm** that contains chemicals and organelles.
- **Golgi apparatus**
- **Lysosomes** for intracellular digestion
- **Ribosomes** for protein synthesis
- **The plasma membrane** which surrounds the cell and has receptors.

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Receptors on the plasma membrane bind hormones and chemicals which are necessary for the cell and are also recognized and utilized by viruses, this is the first step.

DNA replication

DNA is wrapped inside the cell and is made of two strands which are composed of subunits called nucleotides.

> ***Recall that the Adenosine nitrogenous base pairs with Thymine, whereas Cytosine pairs with Guanine.***

Each strand serves as a template for the production of a complementary strand leading to the formation of four strands which reassemble into two identical DNA molecules. This is called ***DNA replication***.

- **Steps of gene expression:**

1) **Transcription**; which is the process by which DNA is copied to mRNA, it will be not fully processed.
2) **Processing of mRNA** by the addition of the 5'guanine cap and 3'poly A tail.
3) **Removal of introns** which are parts of genes that don't code for proteins and therefore have to be excised. Introns are removed by ***small nuclear ribonucleoprotiens***.

The reading frame is the section of the nucleic acids that will be read and translated into an amino acid.

3 nucleotides=1 codon; it is a triplet of nucleotides that will code for amino acids.

Each codon will be later translated to an amino acid, so in the reading frame we have introns which are removed and will not be translated so the protein will be synthesized in the right conformational way, functional protein. This is where the importance of removal of introns stems from.

Exons, however, are nucleic acids portions that will be translated into proteins and remain present after introns have been removed.

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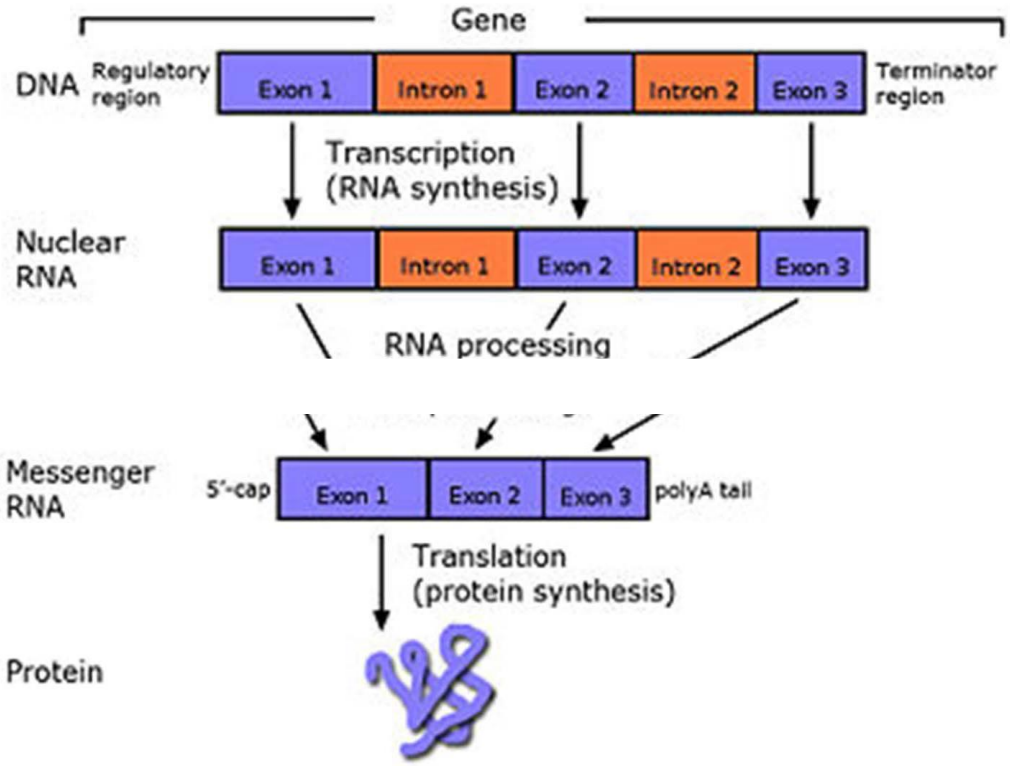


4) **Translation into proteins;** which is the process in which cellular Ribosomes (Protein factors) create proteins by translating the codons into amino acids which are the building units of a polypeptide.

Recall that the start codon is (AUG) and the stop codon could be one of three: UAG, UGA or UAA.

* Translation starts at the start codon and stops at the stop codon.

Viral replication and infection

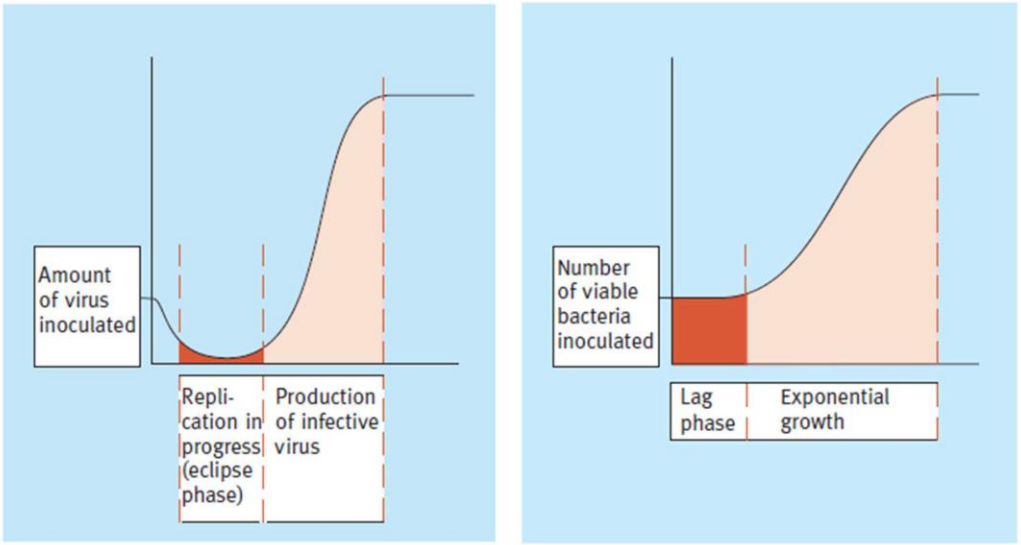
In general, infection is rather a hit or miss process simply as for a virus to enter a cell and replicate its genome it has to bind receptor, and since there is no locomotion , infection depends greatly upon chances of contact and the number of viral copies.

It is not mediated by locomotion; meaning viruses can't move. Viruses aren't motile because they lack cytoskeletons; so viruses have no actin filaments, intermediate filaments or microtubules.

Few viral particles are enough to initiate infection. Even one virus could cause an infection if the infection dose was down to one particle, because the number of receptors of the host cell ranges between 10000 and 100000, which means that there's a very high chance of contact between the virus and one of the receptors.

It has been mentioned in the previous lecture that needle stick injuries might damage your life. That tiny amount of blood transferred to you by the needle might have thousands copies of a very dangerous virus, and since one virus could initiate infection, you will for sure be infected.

THE DIFFERENCE BETWEEN VIRAL AND BACTERIAL REPLICATION



Starting with the graph on the right:

This graph illustrates two phases of bacterial replication: **The <u>lag phase</u> and the <u>exponential phase</u>** (there are other phases we aren't required to know).

- **Lag phase**: The phase in which bacteria will adapt and prepare themselves for replication utilizing the nutrients.

Notice that the number of bacteria and the infectivity of the bacteria stays the same during this phase.

- **Exponential phase**: The phase in which bacteria will be replicating very fast.

Moving to the graph on the left:

The graph illustrates two phases of viral growth: the **eclipse phase** and the **productive phase**.

- **The eclipse phase:** The period in which the virus gains control of the host's synthetic machinery and starts producing components required to assemble into a virus. ***No infectious viruses are present in this phase.*** No viruses are detected by the electron microscope at all in this phase because the virus lost its physical identity and structure.

So the eclipse phase starts with the entrance of the virus and ends with the appearance of the assembled virus inside the cell.

- **The productive phase:** The virus will assemble into a complete virus and will be released from the cell during this phase.

Read only:

Don't confuse the eclipse phase with the incubation period which is the time from the moment of exposure to an infectious agent until signs and symptoms of the disease appear.

To sum up

The main difference between the replication of viruses and bacteria is that bacteria don't lose their structure and infectivity throughout the growth cycle whereas viruses do.

STAGES OF THE VIRAL LIFE CYCLE

1) **Adsorption (attachment):** to the receptor**.**
2) **Penetration (internalization),** and as the name indicates it is the process by which the virus enters the host cell.

3) **Uncoating** in order to free the genetic material so that it'd be subjected to replication machinery inside the cell.
4) **Transcription to mRNA**
"If we are talking about DNA, then we are talking about trancription, If we are talking about RNA specially positive RNA then we are talking about translation."
5) **Translation into early (functional) and late (structural) proteins.**

- **Non-structural proteins** are often referred to as **early proteins** because they are produced early in the life cycle of the virus, and the reason is that the virus wants to translate what is very important to it which will function and help the virus to complete its life cycle like **enzymes**.

- **Structural proteins** are also called **late proteins** because they are produced during the late stages of the life cycle of the virus in the host cell. And as indicated by the name, structural proteins are found in the protein coat which surrounds the genetic material and is known as the capsid.

6) **Final assembly** of the virus in the cytoplasm, nucleus or membrane. Assembly of the virus includes the complement and the arrangement between the genetic material and the capsid.
7) **Release of the virus** by one of two methods: **lysis** or **budding**. Lysis damages the cell whereas budding takes a part of the membrane and leaves the cell intact.

The eclipse phase stretches from stage three to stage six because in this period you can't see the virus under the electron microscope.

These stages can be divided into three phases:

1) Initiation phase

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- Attachment
- Penetration
- Uncoating

2) Replication phase

- DNA Synthesis
- RNA Synthesis
- Protein synthesis

3) Release phase

- Assembly
- Maturation
- Exit from cell

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1) **Adsorption:** Is the attachment of a virus to a host cell's receptors through spikes, which act as receptor-binding proteins.

*Antireceptor is the part of the virus that will attach to the receptor.

Cellular receptors could be either glycoprotiens or glycolipids.

As mentioned earlier, viruses aren't motile; so adsorption is a random process determined by diffusion and by the concentrations of both the receptors and the viruses; infectious dose is important in determining the outcome.

Spikes attach to the receptors by a lock and key mechanism. If there is a mutation for genes that are responsible for a particular receptor the receptor shape might change and the virus won't be able to recognize it. On the other hand, if there is a mutation to genes responsible for making the part of the virus that attach with the receptor, the same thing might happen.

Examples:

- ***The Influenza virus***

The receptor for this virus is Sialic acid.

- ***HIV1 (Human immunodeficiency virus)***

This virus is responsible for acquired immunodeficiency syndrome (AIDS), and has two types of receptors:

1. **Primary receptors:** like CD4 which is found on the surface of T-cells. HIV1 recognizes this receptor and binds to it to attack and infect the T-cells and this is why the CD4 is counted for the diagnosis and treatment of AIDS disease.

2. **Secondary receptors:** CXCR4 and CCR5
   Some people like balkans don't have secondary receptors, so they are immune for HIV infection because both receptors are necessary for the virus to complete its life cycle.

So in order to become infected you must have the CD4, CXCR4 and CCR5 receptors. Absence of any of these receptors simply means that no infection will take place.

Logically, blocking the HIV secondary receptors (we can conclude that they are not so functional important as they are missed in some people) could be an idea to give a treatment for the attack of HIV1.

The ***neutralizing antibodies*** can be generated by our immune systems against the virus and if they are capable of blocking the receptors or anti-receptors, the infection will not continue.

2) **Penetration** could occur by one of three mechanisms:

I. **Direct membrane fusion (fusion from without)**
II. **Receptor-mediated endocytosis (viropexis)**
III. **Non-clathrin mediated endocytosis (Caveolae)**

Now all these strategies show how evil viruses are. All viruses have to do is trick the cell into thinking that the virus knocking at the door is nothing more than

nutrition. The cell, which naturally takes in resources from the environment by attaching them to surface receptors and bringing them into the cell, will engulf the virus.

- **Direct membrane fusion (fusion from without)**

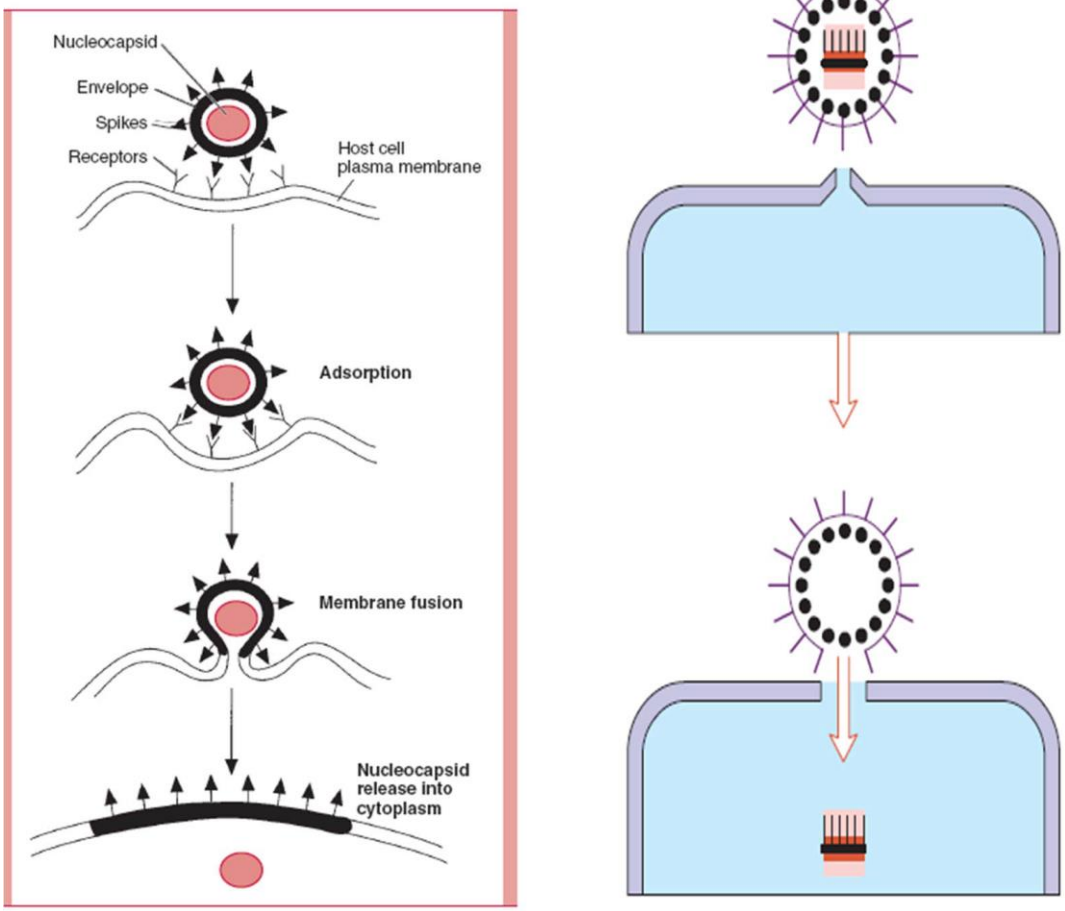
In this mechanism the envelope of the virus fuses with the membrane of the host cell and stays there, which means that the virus must be of the enveloped type. This mechanism is mediated by hydrophobic amino acids.

Examples:

***Paramyxoviruses** like Parainfluenza

***Retroviruses** like HIV1

*** Herpesviruses**



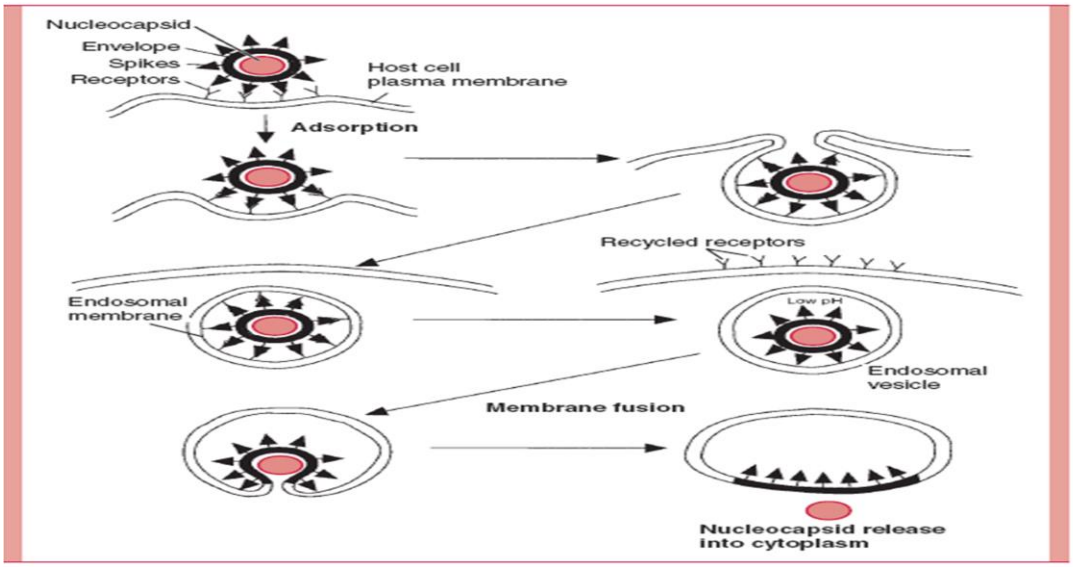
In the figure above, the receptor-binding proteins (spikes) of the virus bind the receptors at the surface of the host cell; this is called *adsorption*, the envelope itself will **fuse with the lipid membrane of the cell**, releasing the capsid to the cytoplasm; this is called *penetration*.

The viral envelope will remain in the the cellular envelope, and the capsid will later be degraded by proteases in the cytoplasm resulting in exposure of the genetic material.

Receptor-binding proteins are also called *fusion proteins*. After penetration, these proteins are recognized as foreign bodies and their presence in the host cell's membrane might activate and be targeted by the immune system (especially the T-lymphocytes) and it also enhances cell fusion between the infected and not infected cells.

- **Receptor-mediated endocytosis (viropexis)**

It is the second mechanism of penetration in which enveloped viruses other than Paramyxoviruses, Retroviruses and Herpesviruses penetrate the membrane by this mechanism. Naked viruses also utilize viropexis.



The virus will attach to a receptor and an invagination will take place as well as an inversion of the cellular membrane. A part of the cellular membrane will be pinched off forming a vesicle called the *endosomal vesicle*.

If the virus is enveloped, the envelope will fuse with the endosomal vesicle envelope and will release its capsids inside the vesicle.

Fusion in this mechanism occurs inside the cell as opposed to fusion in the first mechanism where fusion takes place on the cell's exterior.

If the virus is naked, it will utilize acidity changes in the endosomal vesicle resulting in the destruction of the capsid and release of the genetic material.

So basically the virus took a part of the membrane and entered the cytoplasm of the host cell then it fused its own membrane with the vesicle's membrane.

This process is *Clathrin-mediated*. Clathrin is a protein found in the cellular membrane and this protein forms rounded vesicles by gathering the part of the membrane at which receptors are attached to viruses, aiding in the budding and entry of these vesicles inside the cell.

Example:

- *The influenza virus*

Recall that influenza is an nonenveloped virus. The process is pH and Hemagglutinin mediated.

- **<u>Non-clathrin mediated endocytosis (Caveolae)</u>**

Caveolae are invaginations of the plasma membrane containing the protein Caveolin which is a cholesterol-binding protein. Increased binding of cholesterol leads to the expansion of the invagination and the formation of a vesicle.

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3) **Formation of viral mRNA**

mRNA is transcribed from double-stranded DNA.

Examples on dsDNA: Poxyvirus and Papilloma virus.

Positive ssRNA will be recognized just like the mRNA and will translated directly.

*__Examples__: Poliovirus and Flavivirus

Negative ssRNA viruses will not affect the cell directly because they need an enzyme to copy them into a mirror image in order to become a positive. In this case an antigenome must rise.

*__Examples:__ Rabies and Influenza.

Poxvirus is a DNA visrus but it replicated inside the cytoplasm not the nuclues.
There is no cellular machinery inside the cell that can cause the single-stranded DNA or the double-stranded RNA, there must be something inside the virus or inside the cell are able to deal with some exceptions.

"DO NOT GO WHERE THE PATH MAY LEAD, GO INSTEAD WHERE THERE IS NO PATH AND LEAVE A TRAIL."
— RALPH WALDO EMERSON