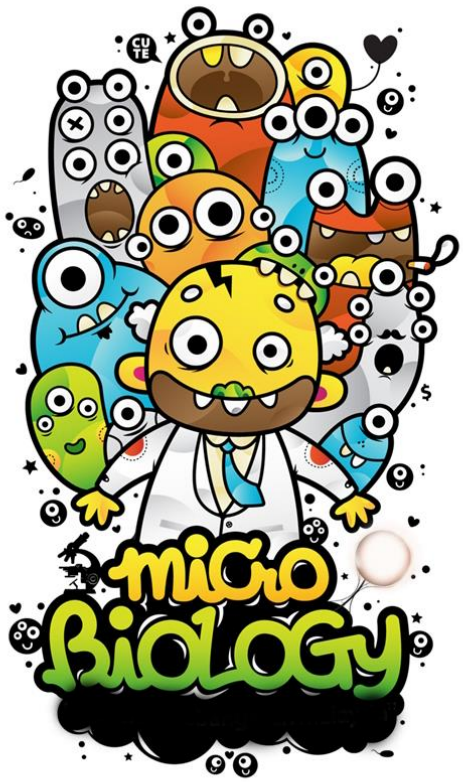

Virology

Subject: Viral Replication III

Lecture no: 3

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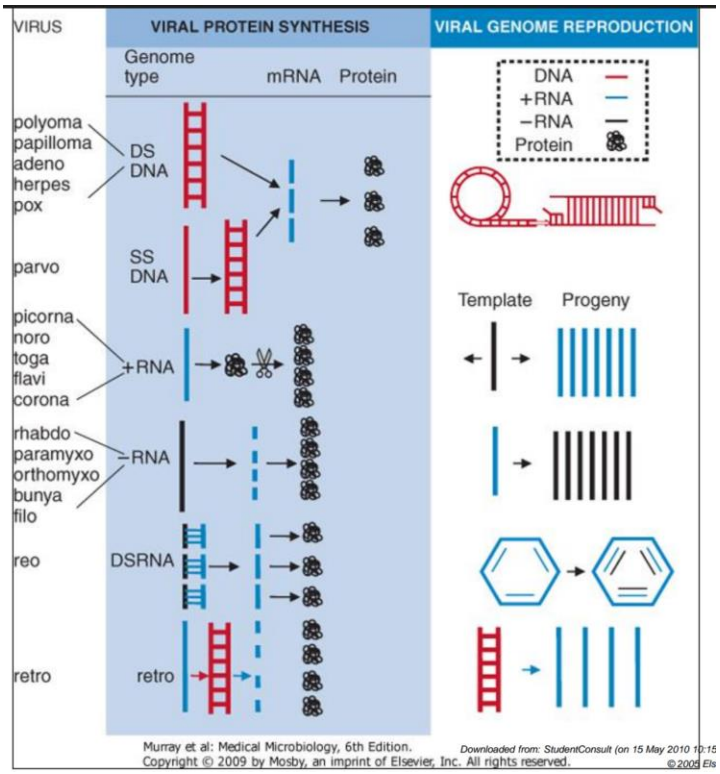
In this sheet we will continue talking about genome replication (briefly), last three steps of viral replication then some definitions in viral pathogenesis.
Drink some water, take a deep breath, let's go!



Explanation of the figure:

- **DSDNA** is transcribed to mRNA which will go to the ribosome to synthesize proteins.
- **SSDNA** becomes DSDNA which will be transcribed then it goes to ribosomes for protein synthesis (and its replication is similar to DSDNA)

- **Positive sense SSRNA** (blue lines in the figure above) will go directly to ribosomes where it is going to be translated producing a **polyprotein**, then this polyprotein is further cleaved into individual proteins (as in retrovirus or HIV), this mechanism is considered a general rule for protein synthesis of +ve sense RNA viruses.

Genome replication method : it makes a template (negative sense, black in the figure above) which will give new copies of +ve sense (blue) and so on.

- **Negative sense SSRNA** will make complementary strand (+ve sense, blue) then this +ve strand will go to ribosomes for protein synthesis.
  For replication, it makes a template +ve sense strand which will give new copies of -ve sense and so on (opposite to +ve sense replication method).

General rule for viral protein synthesis:

Positive sense strands go directly to ribosomes and produce proteins
Negative sense strands are neglected (they only serve in genome replication).

NOTE: Classes VI and VII include an enzyme called **reverse transcriptase**, that's why they produce **RNA-DNA intermediates** in their life cycle.

- **Retroviruses (class VI)**
  Replication starts with +ve sense SSRNA (blue) > RNA-DNA intermediate is produced by reverse transcriptase > RNA dissociates > DNA strand is complemented producing DSDNA > DSDNA is **integrated** into the cellular genome > Transcribed when the cell transcribes its genome > mRNA is produced > Goes to ribosomes > Synthesizes protein as a polyprotein which is later cleaved into many smaller proteins.
  Some of the +ve sense mRNA copies will be **packed** as a genome (instead of going to ribosomes) when the assembly of new viral copies occurs.

- **Partial DSDNA (class VII)** (Hepatitis b/ Hepadnaviridae)
  Partial DSDNA > The incomplete strand is completed > **Complete DSDNA** is transcribed in nucleus > mRNA is produced > Goes to the cytoplasm > (Some of the mRNA copies will go to ribosome for protein synthesis) whereas some copies will serve as a template for reverse transcriptase producing RNA-DNA intermediate (mRNA strand and a complementary complete DNA strand) > RNA dissociates > **DNA polymerase <u>like</u>** will make a complementary DNA strand > Since the first DNA strand is **circular**, DNA polymerase like won't fully complete the production of the complementary

DNA strand, instead it will dissociate leaving the complementary strand **gapped** (that's why it's called partial DSDNA).

"واضح ؟ لازم تكون واضح لأنو عليها أسئلة" - دكتور أشرف

All animal RNA viruses code for a Polymerase

(DNA viruses don't code for them since they replicate in the host cell's nucleus therefore they depend on the usage of the **cellular machinery**)

RNA-dependent RNA polymerase plays an important role in the production of complementary RNA strands for both +ve and –ve sense RNA strands (-ve sense viruses use this enzyme for protein synthesis and for genome replication, that's why they need it more than +ve sense viruses, which only use it in replication).

RNA-dependent DNA polymerase (another name *for reverse transcriptase*) is associated with retroviruses like HIV (where it serves **early** in replication) and with Hepatitis B virus (where it serves **late** in replication)

Replication challenges for DNA viruses

DNA viruses face some challenges in their replication because they replicate in the **nucleus** and use the **cellular machinery**. Which means they will *compete* with the host cell on replication (who will replicate its genome first), unlike RNA viruses which replicate in the cytoplasm and bring their own enzymes for replication.

The cell replicates its genome during the *S phase* , and any DNA viruses in the cell must replicate in this phase as well. For example, Parvovirus is completely dependent on the cellular machinery, It *waits* until the host cells enters the S phase and then it'll start replicating itself.

Other DNA viruses might *induce* the cell to enter the S phase and actually *remain* there as long as the resources are available, and replication continues.
What does this imply ??
The cell will continue to replicate uncontrollably = **cancer**, hence you can see the link between DNA viruses and cancer.
Example:

- Cervical cancer - in females - is linked to human papilloma virus.
- Herpesviridae - Human Herpesvirus 8 which is associated with Kaposi's sarcoma and AIDS
- Adenovirus is linked with cancerous transformation in *animal tissues only*, but not in humans - although humans are the natural host for this virus.

NOTE: keep in mind that the amount of nucleotides and other sources in the cell are limited, and once they are depleted the ultimate fate of the host cell is death)

This is -of course- beneficial for the virus more than the cell at this stage (DNA viruses replicate at much higher pace than the cell).
DNA viruses face the challenge of **accessing the nucleus**, some DNA viruses can enter and uncoat in the nucleus, others can enter as nucleocapsid or even as genomes only.

After we finished talking the synthetic phase, we will continue talking about the **viral replication cycle** :assembly, release and maturation.

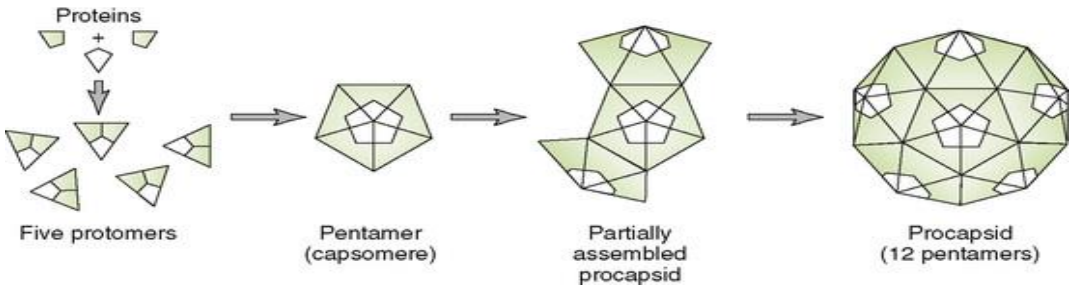
Recall
steps of viral replication are : adsorption, penetration, uncoating, synthetic phase (early nonstructural proteins synthesis, genome replication, late structural protein synthesis), assembly, maturation and release.

Assembly :

Assembly involves the *collection* of all the components necessary for the formation of the mature virion at a particular site in the cell, that site is usually where uncoating had occurred.
During assembly, the basic structure of the virus (*the nucleocapsid*) is formed.

Recall that viral capsids are of three kinds: Icosahedral (most of DNA viruses), Helical (for RNA viruses), Complex (specific for Poxvirus)**.**
Let's take a step back and remember the **building units** of each capsid:

- Helical capsid : **Capsomers**, which are rod shaped structures.
  Capsomers arrange together to form a disk which is hollow in the center. Once this disk is formed, RNA is going to wind up with the disk. Then these disks will continue to form and stack on top of each other in both directions until we get a nucleocapsid with the RNA inside.
- Icosahedral capsid : **Protomers** (the circle inside the triangle).
  These Protomers are arranged to form capsomers (If the formed capsomer has 5 protomers it is a *pentomer*, if it has 6 protomers it is a *hexomer*).
  Icosahedral is composed of 20 triangles that meet in 12 angles. This goes for all viruses regardless of their size. However, the difference in size is due to the difference in the number of protomers (small one would have 10 protomers inside the triangle while a big one would have 30-40).
  The icosahedral capsid looks *spherical* if we look at it under the microscope.
  NOTE: During assembly, Icosahedral capsid is formed **except the top** of it , which is left open so that the genome would be able to get inside the capsid, when the genome is assembled the capsid will close.



The genome of certain DNA viruses looks like a chain. To be more specific, they are multiple copies of a complete DNA genome linked together forming what is called a **concatemer**. Once the virus is being assembled, one of these genomes is going to enter into the icosahedral capsid, the virus will sense the end of the genome and make a cut there, so as only one genome from the concatemer is going to be assembled within the capsid. (However, chances of error are still there, like the entry of more than one genome in a capsid or improper cutting)

NOTE: Other errors occur during RNA viruses' assembly, that's why we may find a virus that contains mRNA molecule that belongs to the **host cell**!

The site of assembly depends on the site of replication within the cell and on the mechanism by which the virus is eventually released:

- Picornaviruses (RNA), Poxviruses (DNA), and Reoviruses (RNA) all replicate in the cytoplasm therefore their assembly occurs in the cytoplasm as well
- Adenoviruses, Polyomaviruses, and parvoviruses assemble in the nucleus. (*remember that parvovirus and other small DNA viruses uncoat in nuclei*).

Maturation :

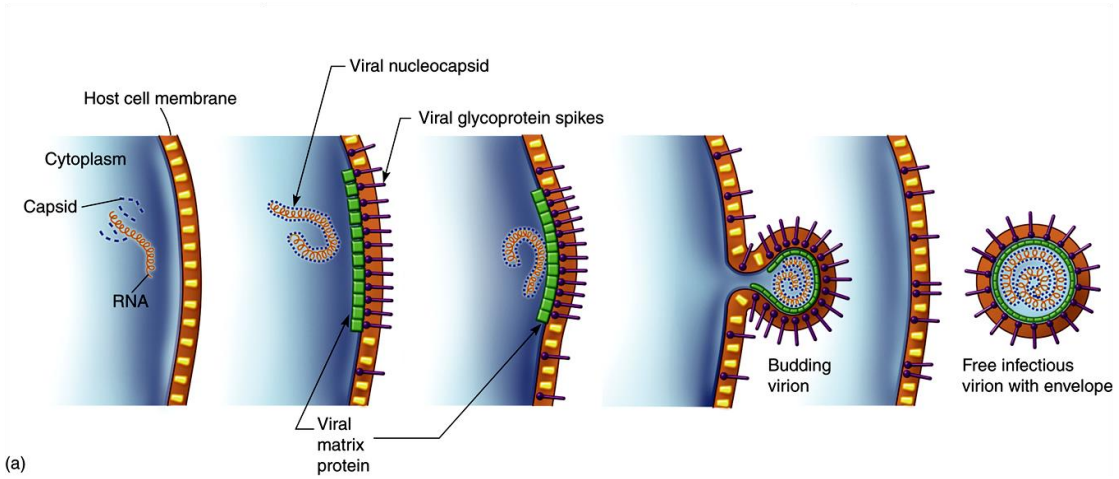
- A mature virus is the one that's able to cause infection. Its proteins are taking their last conformational change and are perfectly functional
- Maturation is a matter of time.
- Both viral and cellular enzymes can play a role in maturation. Meaning that the virus may depend on its own enzymes in maturation process or it may need assistance from the cellular enzymes to complete maturation.

Release :

Since there are two types of viruses (based on presence of envelop) then the releasing method will differ:

- When **naked** viruses are released
  - They won't take a part of the cellular membrane
  - They will be released when the cell **lyses**.
- When **Enveloped** viruses are released
  - Where do they acquire their envelope from ?
    Generally, from the **cellular membrane**.
    Exception: some viruses acquire their envelopes from nuclear membrane and Golgi apparatus

How does the virus know that it should leave from this area specifically ?
There's a **viral matrix protein** that will attach **internally** to the cellular membrane, As a result, the virus knows that the budding will be there, so the nucleocapsid will go to that area. At the same time, the viral matrix proteins will bind the spikes and fix them in their transmembrane positions (opposing the viral matrix protein). When the virus exits it'll take the nucleocapsid and the membrane (envelop) and the glycoproteins. (In the case of RNA viruses, they will take proteins and enzymes with them as well).



What's the effect of this on the host cell ??
It's losing a part of the cellular membrane. So it will try to adapt/compensate by **regeneration** until it reaches a stage where it can no longer cope with the membrane loss, then it will lyse and die.

Possible consequences to a cell that is infected by a virus:

- **Acute infection**
    - lasts for a short period of time 5-7 days e.g. influenza and other viruses that cause upper respiratory tract infections
    - Symptoms (sneezing, coughing, fatigue etc..) disappear after 7 days *but coughing remains* for several weeks in order to clean the area. The cleaning was originally the done by ciliated cells, but these cells have died as a result of the lytic infection.
      It takes 4 to 8 weeks for cilia to get back to their normal shape.
    - ***Causes lysis of the cell quickly*** (Immune system has a role here)

- **Persistent infection**
    - Lasts for a long period of time
    - Lysis doesn't occur in short period of time
    - Two types: 1. Chronic (hepatitis b and c)
      2. Latent (They'll be discussed them in viral pathogenesis)

- **Transformation**
    - A cell becomes cancerous
    - Prolongation of infection, the virus "*immortalizes*" the cell (prolongs the life span of the cell and doesn't kill it in a short period of time) in order to use its resources as long as possible.

Viral pathogenesis

Here are some terms you must be familiar with :

• **Endemic**: (مستوطن) Disease present at fairly low but **constant** level.
(e.g. malaria is present in Nigeria), number of new cases and mortality together keep the occurrence rate of the infection constant.
Here we usually describe **nations** (a village might have 70% of its population infected but when we major for the whole country the percentage decreases)

• **Epidemic**: Infection greater than usually found in a population.
(associated with a certain geographic area at a certain period of time with an *increased* number of cases like *influenza*, influenza cases in Jordan in winter are higher than the rest of the year)

• **Pandemic**: Infections that are spread worldwide
(Global level : H1N1, Corona, MERS -middle east respiratory syndrome)

• **Infectivity**: The frequency with which an infection is transmitted when contact between a virus and host occurs
(e.g. a person has a flu and he's sitting in a class where 100 people are present, depending on the infectivity of the virus 45-50% are going to be infected).
SO, viruses vary with their infectivity rates, some have high infectivity rates (like measles, 95%, that's why infected individuals shouldn't mix with people) while others have low infectivity rates.

Note: infection doesn't equal illness

In other words if you got infected that doesn't necessarily mean that you're going to get sick.

• **Disease index**: number of persons who developed disease/ total infected
(Total of diseased people/total infected but didn't necessarily develop a disease)

How do we know if someone is infected or not?
By a method called *seroconversion*. Upon exposure to foreign bodies(antigen) the body will form antibodies. If we detected the presence of these antibodies then you are infected (again not necessarily going to be sick).

• **Virulence:** number of fatal cases/ total number of cases
(those who have the disease not only infection)

• **Incidence**: the number of new cases within a specific period of time in a certain geographic area.
(e.g. number of *new* HIV cases in Jordan in 2016)

• **Prevalence**: the total number of cases of a disease that are present in a particular population at a given time.
(e.g. *total* HIV cases in Jordan in 2016 and *before*)

Note: incidence and prevalence are two terms that usually come together, make sure you don't mix them up.

"Have some fire, be unstoppable, be a force of nature, be better than anyone here, and don't give a damn what anyone thinks. There are no teams in here, no *buddies,* you're on your own. *Be* on your own."

- Dr.Cristina yang/ Grey's anatomy