University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Introduction to

Microbiology

Title :

Replication Genomes

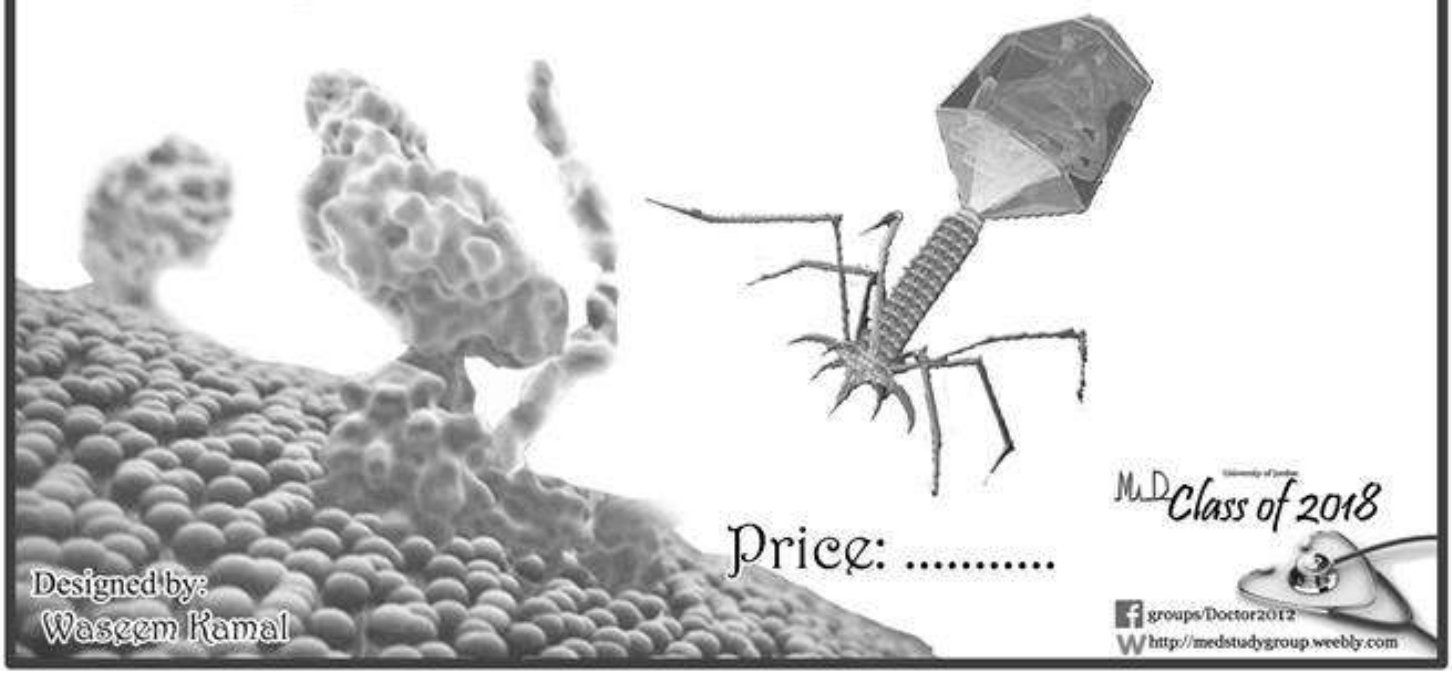
Professor:

Dr. Ashraf Al-Khasawneh

: 12

○ Slides
○ Handout
● Sheet

Done by: Zeina Al-Momani



Price: .....

Designed by:
Waseem Kamal

M.D Class of 2018

groups/Doctor2012
http://medstudygroup.weebly.com

Last time we talked about the few steps in viral replication cycle and the un-coating stage:

Un-coating: is a general term for the events which occur after penetration, we talked about adsorption or attachment, after that penetration of the virus into the cytoplasm of the target cell then we have un-coating, It has been least studied and is relatively poorly understood. Not much is known about un-coating but we will mention few steps of un-coating. (Then the dr. read the rest of slide#23 titled "un-coating")

- We talked about the entry of <u>enveloped viruses</u> into the cell via two routes:
  a) Fusion: in which part of the envelope is left as part of the cell membrane and the nucleocapsid enters into the cytoplasm of the cell.
  b) Receptor mediated endocytosis: where the whole virus with its envelope is engulfed into the cytoplasm via endocytic vesicle.
- For <u>naked viruses</u> :
  a) Receptor mediated endocytosis (viropexis): a mechanism of entry of a virus into the cytoplasm of the cell via endocytosis.

- In fusion (a type of viral entry into the cell) the nuclear capsid is released directly into the cytoplasm of the cell, but what about when the virus is endocytosed? How is the nucleocapsid released from the endocytic vesicle into the cytoplasm?

  The answer is a drop in the PH of the endocytic vesicle (both in enveloped viruses and naked viruses).

  + In <u>enveloped viruses</u> the drop in PH induces a conformational change which leads to the fusion of the endocytic vesicle membrane with the envelope of the virus, and this newly formed membrane (endocytic vesicle membrane + envelope) will eventually lyse releasing the nucleocapsid into the cytoplasm.

+ In naked viruses there are two mechanisms of release of neucleocaspid from endocytic vesicle (2 mechanisms for different viruses):

a) Lysis of the endocytic vesicle membrane itself releasing the nucleocapsid.
b) Like in the enveloped viruses, a drop in the PH of the endocytic vesicle will expose the hydrophobic domains within the nucleocapsid of the enveloped virus, these domains will stick/attach to the endocytic vesicle wall and open a small pore for the nucleocapsid to exit.

Up till now we took the following steps:

Attachment → Penetration → Un-coating. The next step is Macromolecular synthesis.

Macromolecular synthesis involves two steps:

a) Transcription and translation (as one step)
b) Replication (as another step)

In this process we have (after studying the rest of the sheet you will understand this, I hope so ya3ni):

Early mRNA→ Early protein synthesis→ Replication of the genome→ Late mRNA→ Late protein synthesis → (for some types of viruses) we have Post translational modifications; where the protein is synthesised as a polypeptide and this polypeptide is cleaved into individual proteins.

Note:

- All DNA viruses replicate within the nucleus except for Pox virus.
- RNA viruses replicate within the cytoplasm except HIV and Influenza virus.

In DNA viruses we have what's called the **early phase** and the **late phase**. (Please read through them twice to understand it is quite complicated)

The early phase:

- Involves the genome of the virus which first enters the cell.
- This genome is transcribed as mRNA and then taken to the ribosome where it is translated into proteins.
- These proteins (which are synthesized from the mRNA that was transcribed from the original viral genome) are called early proteins. They are mostly non-structural and mostly involved in enzymes. These enzymes are used and required for the replication and translation processes later on.

The late phase:

- Replication (with the help of the enzymes) then occurs forming new copies of the viral genome. These new copies are further transcribed into mRNA which goes to the ribosomes and causes the synthesis of proteins.
- These proteins are late proteins.

The difference between the early and late phase:
Early: - The origin of the template which the mRNA was synthesised from was the ORIGINAL viral genome that first entered the cell (before replication). - The proteins synthesised are enzymes that are required for the replication process (that's why we have early and late).
Late: - The origin of the template that the mRNA was synthesised from was the REPLICATED GENOME COPIES. - The proteins synthesised are structural proteins which enter in the structure of the capsid, envelope, glycoprotein etc.

*In some viruses you might find immediate early, early and late. But since we are talking about DNA viruses in general we will stick to early and late, for now.

Replication Strategies:

We said previously that viruses are classified into seven classes "Baltimore classification", these classes are:

1) Double Stranded DNA: (Adenovirus, Herpes virus)

Note: we mentioned earlier that DNA viruses replicate within the nucleus (except Pox virus). Which means the virus enters the cytoplasm of the cell needing to enter the nucleus. Depending on the size of the cell there are two ways it can enter the nucleus:

a) Small viruses: (like parvovirus) do not need to disassemble, they can enter the nucleus as a whole nucleocapsid, then in the nucleus they disassemble and start the –above mentioned- two steps.
b) Other Larger viruses: the capsid must disassemble first, and only the genome can enter the nucleus.

These viruses (DNA viruses that enter nucleus) depend mostly, and some completely, on the cellular machinery of the cells in their replication.

Examples on that:

a) Transcription: they depend on the cellular **transcriptase**, which gives the mRNA that goes to the cytoplasm and produces the early and late proteins we talked about.
b) Replication: **DNA polymerase**, which is present within the nucleus of the cell, and is used by the virus to replicate its genome producing many copies of the viral genome.

-DNA is helical and needs to be un-wound and linear for replication, this occurs by the enzyme **topoisomerase**.

-The enzyme **helicase** acts on the matching two nucleotides opening the two strands keeping them apart → this is called the replication fork.

-We don't use helicase on its own (without topoisomerase) because it will cause tangling (be3alle2) of the DNA strands. It needs to be un-wound (loosened) along the replication fork then the matching nucleotides will be separated, so it won't tangle.(مثل فك الشعر المجدّل)

In order to start this mechanism (replication) we need RNA primer. RNA primer is a short sequence of RNA which matches the beginning of the replication fork, after the primer attaches, DNA polymerase comes, taking over and continuing to replicate the DNA till the end of the strand.

- Leading strand and lagging strand: Replication goes from 5' to 3' direction(on the new strand). The strand of the DNA which is replicated from outside the fork inward is the leading strand (starts with 3'). One RNA primer is needed at the beginning.
- The lagging strand (starts with a 5') on the other hand is the other strand. It is being replicated from inside the fork outwards, when the DNA strand unwinds (helicase and topoisomerase), a RNA primer attaches (near the fork) to the unwound strand and replication continues from inwards outward till it reaches the part that was replicated before it (the okazaki fragment before it)
- The removal of the RNA primer dislodges and leaves a gap behind it which will then be removed by **DNA ligase**. By fusing the okazaki (1000 neucleotides long) fragments together.
- In eukaryotic cells the gap is replaced by an enzyme called **telomerase**, it replaces the primer a non-repetitive sequence, they code for nothing, just there to maintain the length of the DNA. Mafe menno bel virus.

2)Single stranded (+) sense DNA:

- Replication occurs in the nucleus, involving the formation of a (-) sense strand, which serves as a template for (+) sense strand RNA and DNA synthesis.
  - Please refer to the picture it's much easier to understand
  - In DNA synthesis the virus enters to the nucleus. In the nucleus there is an intermediate that forms; this intermediate is composed of a double stranded DNA. Those double stranded DNA intermediates perform two things:
    a) They are transcribed into mRNA which then leaves to the cytoplasm, attaches to ribosomes, and synthesizes early and late proteins. (Produce proteins)
    b) They are used for the replication of the genome, giving single stranded DNA molecules which are going to be used for assembly of the new virions. (Replication)

3) Double stranded RNA: (Rotavirus, Reovirus)

- These viruses are segmented, each genome segment is transcribed separately to produce monocistronic mRNA's.

> ***Note:***
>
> What is monocistronic and polysistronic?
>
> These are terms we use when we talk about transcribed mRNA. When we say monocistroinic it means that that mRNA codes for only one protein or one gene.
>
> Polycistronic on the other hand refers to an mRNA that codes for the entire genome (multiple proteins).

- Since these viruses are segmented, each segment of mRNA codes for a certain protein, meaning that they are monocistronic.
- This is exactly what the doctor said ana ma fhemto:
  DNA viruses use the cellular machinery, and the eukaryotic cells, or our cells, stick with the monocistronic rule. **How?** By splicing mechanism; there are exons and introns, introns are taken out and exons are fused together, and each segment of mRNA that

was transcribed using this splicing method will be monocistronic, encoding for one protein. So, DNA viruses stick to this rule.

- RNA viruses vary (could be polycistronic or monocistronic), and there are three cases in which they must revert to (go back and use) the monocistronic rule. These cases are:

  a) Being segmented: each segment encodes for one protein or one gene. So, by being segmented, it sticks to the monocistronic rule.
  b) Production of polyprotein or polypeptide that is cleaved (discussed later in the sheet in ssRNA (+) sense).
  c) In Non-segmented single stranded (-) sense RNA: (transcriptase fixed at the beginning of the gene and stops at the end of the gene)

  ***Note**: In books there is a lot more said about this but the dr. only wants us to know that transcriptase can bind at the beginning of the gene or the protein and end its transcription at the end of the protein.)

- This is double stranded RNA, so it has (+) sense strand and (-) sense strand. The (+) sense strand will behave like the single stranded (+) sense RNA strand where it goes directly to the ribosomes and synthesizes proteins. The (-) sense strand, using **RNA dependant RNA polymerase**, gives the (+) complementary strand which then goes to the ribosome and synthesizes proteins.
- For transcription and replication, all RNA viruses must pack (pair) with them RNA dependant RNA polymerase which helps them in these two steps (replication and transcription).
- For certain types of RNA viruses like picornavirus those two steps overlap, for other viruses, transcription and replication are separate steps. How can viruses –knowing that they use RNA dependent RNA polymerase for both steps- maintain separate

lines (they may happen at the same time, but what is imp. here is that they are separated)? Ya3ni how does the cell know that this enzyme is for transcription or replication?
The answer is, is that there are some functionally distinct RNA dependent RNA polymerases that can be used ONLY for transcription.

4)Single stranded (+) sense RNA: (Hepatitis A & C)

- We mentioned before, for the transcription and translation, that the (+) sense RNA strand enters the cytoplasm, goes to the ribosomes and synthesizes proteins.
- For the translation step, translation results in the formation of a polyprotein product which is subsequently cleaved for mature protein, so its polycistronic. The whole genome is transcribed into an mRNA then translated into a polypeptide or a polyprotein chain. After that, cleavage occurs to this polypeptide at certain sites to give us the individual proteins for this virus. This is how it reverses back to the monocistronic rule we talked about earlier.
- For replication, we have an intermediate. RNA dependent RNA polymerase gives us a (-) sense RNA intermediate from which new copies of (+) sense RNA strand are produced.

5)Single stranded (-) sense RNA: influenza virus (segmented), Hantavirus (non-segmented).

- We have segmented and non-segmented.
- **Segmented**: first step in the replication is transcription of the (-) sense RNA genome by viral RNA dependant RNA polymerase to produce monocistronic mRNA which serves as a template for genome replication. This mRNA is (+) sense because it was transcribed from a (-) strand of RNA. Part of the mRNA will go to the cytoplasm then ribosomes and produce proteins. The other

part will be used as a template to produce more (-) sense strands (the complementary for (+) sense is (-) sense).

- **Non-segmented**: replication occurs as above and monocistronic mRNA are produced (the 3rd case in which RNA viruses may return to monocistronic mechanism).

6)Single stranded (+) sense RNA with DNA intermediate: (Retroviruses-HIV-)

- It should behave like the other (+) sense RNA but since it's in a different class it behaves differently.
- HIV is unique in the replication mechanism, and is unique in that is has two separate copies of the single stranded (+) sense RNA strand in its capsid. It is a DIPLOID. It does not serve directly as mRNA but as a template for reverse transcription into DNA; once the virus enters into the cytoplasm there's an enzyme called **reverse transcriptase** which reversely transcribes RNA into DNA. DNA single stranded then becomes double stranded, then moves from cytoplasm into nucleus (Remember we said that exception of RNA viruses -that replicate in the nucleus- HIV and influenza virus) so it enters the nucleus as dsDNA and becomes part of the cellular genome by facilitation of an enzyme called **integrase** (integrates the viral genome into the cellular genome) . At this stage it is called **provirus**. Then the cellular machinery generates mRNA then new proteins (structural and non-structural). And when it replicates the cellular DNA it replicates the viral genome.

Note:

- We said that the DNA viruses are dependent totally or partially on the cellular machinery to perform replication and transcription. But are the enzymes required for these viruses' genome replication always present in the cell?
  The answer is no, it depends on the phases of the life cycle of the cell (G-phase, S-phase) the cell replicates in the S-phase. So certain types of viruses (DNA viruses), like Parvovirus, which is totally dependent on the cellular machinery, wait for the cell to

enter the S-phase then uses its machinery and enzymes to replicate itself.

Other viruses don't behave the same way, bring certain proteins or enzymes with them. These proteins and enzymes, once inside the cell, induce the cell to enter the S-phase -becomes in S-phase all the time-, and that's how they get the enzymes needed for their replication.

- We talked about HIV and how it produces its proteins, now, how does it replicate its genome?
  ANS: the transcribed mRNA (a (+) sense RNA) part of it goes to the ribosomes producing proteins, and the other part as a new replicated genome ((+) sense), goes to new progeny viruses.

7) Partial double stranded (gapped) DNA with RNA intermediate:

- The sixth class -we talked about- uses reverse transcription on entrance of the virus. In this class reverse transcription occurs at the last step. How?
- Hepatitis B is a partial double stranded DNA, both of the DNA strands are incomplete, they complement each other at certain steps and there are gaps in between.

  Once the virus enters the cell, the first thing the cell offers is the DNA polymerase which completes these gaps in the partially double stranded DNA and makes it fully double stranded.

  After that it enters into the nucleus. In the nucleus it's transcribed into mRNA which goes to the cytoplasm to the ribosomes for synthesis of protein. At the same time this mRNA / (+) sense RNA is used as a template on which reverse transcriptase acts and transforms those from RNA to DNA. Which becomes partial dsDNA, once these newly formed/ progeny viruses infect other cells, the cycle starts again.