VIROLOGY

Subject: Viral Replication II

Lecture no. : 2

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Viral replication cycle

In the previous lecture, we covered the first two steps of the cycle:

1- Attachment/Adsorption of the virus to specific receptors on the host cell
2- Penetration/Entry of the virus into the host cell

In this lecture, we are going to cover the two steps that follow:

3- Uncoating
4- Synthetic phase

1- Attachment/Adhesion

- This step requires receptors on target cells and glycoproteins/spikes on virus particles.

2- Penetration/Entry

- There are two mechanisms of viral penetration:
  a. Receptor-mediated endocytosis - for both, enveloped and naked viruses
  b. Fusion – for enveloped viruses only

end of recap

3- Uncoating

- This step does NOT have to do with the envelope – it only involves the *nucleocapsid.*
- The steps of uncoating take place after the entry of the virus into the host cell, and they involve:
  a. The disassembly (dismantle) of the capsid
  b. The release of the viral nucleic material (genome) and viral enzymes into the cytoplasm of the infected cell

- There isn't much information that we need to know about uncoating, except that it occurs, with *most viruses,* within the cytoplasm. An exception to this includes the smallest DNA virus (parvovirus) which undergoes uncoating in the nucleus as its nucleocapsid is very small that the whole of it can enter the nucleus through the nuclear pores and disassemble there.
- The products of uncoating and the subsequent steps of replication depend on the structure and chemistry of the nucleocapsid.

4- Synthetic phase

- This phase is divided to 3 stages:
  a. Early protein synthesis
  b. Genome replication
  c. Late protein synthesis
- The first step (early protein synthesis) involves the synthesis of viral enzymes and proteins (*non-structural* proteins) important for the process of genome replication. We know that the virus has its own proteins and enzymes, and once it enters the cell they are released into the cytoplasm to do their job in genome replication, so why does the virus need this step even though it already has these proteins? The amount of these proteins is *not sufficient* to enable the replication of the virus in huge numbers, so these proteins and enzymes are synthesized first so the virus would be able to perform genome replication and structural protein synthesis for many other viral copies.
- These three stages are not distinct stages and can overlap (occur at the same time).
- Viruses can be classified according to different bases. One of which is the **David Baltimore** classification, which arranges viruses in groups according to the mechanism of their genome replication. There were 6 classes originally, but they have been extended by the addition of a 7^{th} class (which includes hepadnavirus – the virus of hepatitis B). There are

general rules for this classification, and by knowing the class of the virus the general rules can be applied to know the replication strategy.

- The 7 classes are:
  I . Double-stranded DNA
  II . Single-stranded (+) sense DNA
  III. Double-stranded RNA
  IV. Single-stranded (+) sense RNA
  V. Single-stranded (-) sense RNA
  VI. Single-stranded (+) sense RNA with DNA intermediate (retroviruses)
  VII. Partial double-stranded (gapped) DNA with RNA intermediate

- The process of translation of viral genome into proteins has been studied in many viruses. It has been found that in certain viruses (ss RNA), the RNA genome goes itself directly to the ribosomes where translation occurs, behaving in a similar way to *messenger RNA,* and these are labelled as ***(+) sense***. In other viruses, the RNA acts as a ***template*** for the formation of a complementary strand to itself, and the complementary strand produced in this case goes to the ribosomes where the sequence is translated into a polypeptide chain, and these are labelled as ***(-) sense.***

00:00 – 11:15

CLASS I: DOUBLE-STRANDED DNA

- This class includes adenoviruses, herpesviruses, papillomaviruses, poxviruses, and T4 bacteriophages.
- Since they are DNA viruses, their replication occurs in the nucleus, **except for poxvirus** (largest DNA virus) which is the only DNA virus that replicates in the cytoplasm.

- The mechanism of replication of viral DNA is similar to cellular DNA replication:
    - DNA is ***supercoiled*** in order to fit in the small space inside the nucleus.
    - The enzyme ***topoisomerase*** acts on the supercoiled structure of the DNA, cutting and ligating it to **linearize** it.
    - The enzyme ***helicase*** opens up the double-stranded DNA, resulting in a structure called the *replication fork* (where the two strands are apart from one another).

NOTE: the two DNA strands are complementary to each other, meaning that nucleobase A on one strand pairs with nucleobase T on the other, and the same applies for nucleobases C and G.

In replication, each strand is used as a ***template*** for the formation of a complementary strand.

- The enzyme ***DNA polymerase*** adds nucleotides to the separated strands to make complementary strands. Genome replication occurs in the **5' → 3' direction**, so we're left with two strands:
    i. ***Leading strand;*** which starts at 3' and finishes at 5'. Replication starts as an *RNA primer* (a short sequence of RNA nucleotides, complementary to those of the DNA strand that is to be replicated) attaches to the template strand. DNA polymerase then starts reading, and replication occurs in a ***continuous manner*** from 5' → 3'. When the end of the strand is reached, the primer detaches, leaving behind a gap (primer size). A few nucleotides in the template strand (the ones to which the primer was attached) will not be replicated, resulting in the shortening of the strand's length. If this is the case every time the DNA replicates itself, then it would become shorter and shorter (end problem). So how does the replication mechanism compensate for this shortening? The enzyme ***telomerase*** adds repetitive non-coding sequences of nucleotides;

repetitive as in AAA, CCC… and *non-coding* as they do not code for proteins and their only purpose is to preserve the length of the DNA.

ii. ***Lagging strand;*** which starts at 5' and ends at 3'. Here, replication can neither be continuous from 3' → 5', nor can take place at 3' end as the DNA strands are not completely separated. Instead, as helicase unwinds the DNA double helix, RNA primers attach at certain points, and nucleotides are added in the 5' to 3' direction (from inside to outside). At the end, the primers detach themselves, resulting in the formation of several fragments with gaps in between. The gaps represent sites of RNA primers, and the fragments are called ***Okazaki fragments***. The enzyme ***ligase*** then ligates these fragments to form a complete complementary strand of DNA.

- This is how DNA is replicated inside our cells. Viral DNA replication is similar to it because the virus takes over (uses) the cellular machinery.

11:15 – 23:00

CLASS II: SINGLE-STRANDED DNA

- When considering single-stranded DNA, we ignore "(+) sense" and "(-) sense" because it does not matter in the case of DNA.
- The single strand of these viruses is first used as a template to produce a complementary strand (formation of a double-strand), and the steps that follow are the same as those of double-stranded DNA viruses.
- ***Protein synthesis for DNA viruses:*** The viral genome is transcribed alongside cellular genome by the enzyme ***RNA polymerase*** inside the nucleus. Transcription produces mRNA which exits the nucleus and goes to the ribosomes to start protein synthesis (early and late).

NOTE: Structural proteins – which are the proteins of the **capsid** and **spikes (glycoproteins)** – are needed in the final steps only (like assembly), that's why they are synthesized later.

CLASS III: DOUBLE-STRANDED RNA

- A member of this group is the rotavirus (which belongs to the Reoviridae family). *These are NOT two different viruses – it's a virus and the family it belongs to.* What's unique about this virus?
    - It is the only double-stranded RNA human virus
    - Its genome is *segmented*, i.e. the genome is not continuous (is found in pieces), and every one of these segments represents one gene and is therefore going to be translated into a single protein. ***Both rotavirus and influenza virus have segmented genomes.***

- In our cells, RNA is produced as a result of DNA transcription (DNA→RNA) under the action of RNA polymerase. In viruses, new RNA strands are produced from other RNA strands (RNA→RNA), and there are no enzymes in human cells capable of carrying out this process. That's why these viruses have ***RNA-dependent RNA polymerase*** enzyme (their genome contains the gene that codes for this enzyme). No replication of RNA viruses can happen unless this enzyme is present.

- General rule: *All RNA viruses replicate in the cytoplasm*, <u>except for HIV and influenza virus</u>. They are therefore less dependent on the host cell's machinery. They have more enzymes and proteins of their own to support their replication. Compare: certain DNA viruses are completely dependent on the cellular machinery, others are only partially dependent, such as the herpesvirus which uses some of its own enzymes.

- In double-stranded RNA there are two strands (after separation):
    - **(+) sense strand:** [1]serves as mRNA, and can be directly used by the ribosomes for protein synthesis. [2]It is also used as a template for genome replication.
    - **(-) sense strand:** cannot serve as mRNA, thus it is used as a template to produce complementary strand (which is (+)

sense), and this complementary strand serves as mRNA. *This strand is only used as a template for the formation of a (+) sense strand.*

- For replication, the two strands split and each strand produces its own complementary strand, (+) sense produces (-) sense and vice versa.
- For protein synthesis, only the (+) sense strand goes to ribosomes and the (-) sense strand is neglected.

NOTE: RNA strands are labelled (+) and (-) only by observation; i.e. whether the genome serves as mRNA or not

Keep in mind that no primers are required in the case of RNA replication, because the genome itself is RNA.

MONOCISTRONIC RNA

- An RNA molecule (or mRNA) is called *monocistronic* if it codes for the translation of one protein. The cell has its mRNA transcribed in this fashion. That is, each segment of the genome is transcribed separately to produce monocistronic mRNA. The same applies for viruses, and occurs in 3 mechanisms:
    - Viruses with segmented genomes, where every segment codes for one protein so mRNA that results is already monocistronic
    - Certain viruses have special enzymes which can bind internally to the genome, allowing the transcription of *certain parts of the genome* and not the whole of it (transcription can start at the middle or at the last quarter of the genome.. etc)
    - Transcription occurs for the whole genome, producing a long mRNA molecule. This mRNA goes to the ribosomes, where it is translated to a large polyprotein. A viral ***protease*** enzyme then cleaves the polyprotein into smaller, individual proteins. This mechanism represents HIV.

23:00 – 34:30

CLASS IV: SINGLE-STRANDED (+) SENSE RNA

- Examples include hepatitis A and C, common cold viruses, rhinovirus and coronavirus.
- The genome of these viruses behaves as mRNA – it goes directly to ribosomes where it is translated into proteins (early and late).
- These viruses replicate their RNA by making complementary (-) sense strands which then serve as a template for synthesizing more (+) sense copies.

CLASS V: SINGLE-STRANDED (-) SENSE RNA

- The genomes of these viruses can be divided into two types: segmented and non-segmented genomes.
- Examples include influenza virus (segmented) and Hantavirus.
- The genome of these viruses cannot go directly to the ribosomes for translation. A complementary (+) sense strand must be transcribed from the genome first, and then this is used for translation.
- Replication of these viruses' RNA occurs by making (+) sense strands which then serve as templates for synthesizing more (-) sense copies.

NOTE: Viruses classes VI and VII have ***reverse transcriptase*** enzyme. The difference is that it functions in early steps in the replication of class VI viruses, whereas for VII viruses it functions in late steps.

CLASS VI: SINGLE-STRANDED (+) SENSE RNA WITH DNA INTERMEDIATE IN LIFECYCLE (RETROVIRUSES)

- This class is unique for one family of viruses only: *retroviruses*. Examples include **HIV** and HTLV (human t-cell lymphotropic virus).

- A special characteristic of retroviruses (e.g. HIV) is that they are **diploid**, meaning that they have *two copies* of their genome (two copies of (+) sense ssRNA) within the nucleocapsid.
- Once the virus enters into the cell, it uncoats and the 2 copies are released into the cytoplasm. Then the viral enzyme ***reverse transcriptase*** comes to action. It is a multi-component enzyme.

  The first component (which is *DNA polymerase*-like) attaches to the (+) sense strand and complements it with DNA nucleotides (RNA→DNA, the opposite of transcription), producing an **RNA-DNA intermediate**. After that, the (+) sense RNA is going to dissociate from the DNA strand ((-) sense), and is destroyed by the enzyme ***RNAse H*** (which is another component of the reverse transcriptase enzyme).

- The **DNA polymerase-like component** of reverse transcriptase acts again to make the single-stranded DNA a double-stranded one. This dsDNA travels from the cytoplasm to the nucleus, where it is going to be incorporated into the cellular genome by the viral enzyme ***integrase***. This enzyme acts on both viral and cellar genomes, where it cuts a few bases from the end of each genome, producing *sticky ends* so that they can be combine together.

- Once the viral genome has been incorporated into the cellular genome, it is called a ***provirus***.

- The viral genome is then transcribed with the cellular genome by the cellular replication machinery, producing mRNA ((+) sense), which exits the nucleus to go to the cytoplasm where it can be:

    - translated into proteins (a <u>polyprotein</u> is produced first and cleaved after a while by a protease enzyme - the mechanism of monocistronic RNA)

or

- packed with the newly formed copies of the viral genome (two (+) sense copies for each virus)

NOTE: REOVIRUSES ≠ RETROVIRUSES

- Retroviruses are viruses which belong to class VI, and an example is HIV.
- Reoviridae is a family of viruses which belongs to class III, and an example is the rotavirus. The rotavirus is considered the leading cause of infant gastroenteritis during winter months.

RECALL: All RNA viruses replicate in the cytoplasm, except for HIV and influenza virus which replicate in the nucleus.

CLASS VII: PARTIAL DOUBLE-STRANDED (GAPPED DNA) WITH RNA INTERMEDIATE

- Hepatitis B (which belongs to the hepadnaviridae family) is a circular double-stranded DNA virus. It is called *partial* because one of the circular strands (the inner strand) is <u>not a complete strand</u> (it is missing a small part).
- Since it is a DNA virus, it replicates in the nucleus.
- Once this virus enters the cell, it is translocated to the nucleus where it is completed by ***DNA polymerase*** (forming a complete dsDNA circular genome). This complete dsDNA is then transcribed, so viral mRNA is produced.
- For protein synthesis, the viral mRNA goes to the ribosomes where it is translated.

- For replication, this mRNA ((+) sense) acts as a template to produce a complementary DNA strand ((-) sense), and this is done by the action of reverse transcriptase. The result is an **RNA-DNA intermediate**.

- This complementary DNA strand ((-) sense) forms the complete ring in the structure of the circular virus. DNA polymerase then works on this DNA and continues reading to produce a complementary ((+) sense) strand, until it reaches a point at which it dissociates (stops before completing) → *partial* double-stranded structure.

COMPARE the action of reverse transcriptase in the replication of viral classes VI and VII

- CLASS VI→ reverse transcriptase acts on the (+) sense RNA strand before it enters the nucleus (before replication starts), producing an RNA-DNA intermediate
- CLASS VII→ reverse transcriptase acts on the mRNA which has already been transcribed from the genome and has left the nucleus, resulting in an RNA-DNA intermediate

NOTE: To make it easier to memorize DNA viruses, remember ***HH PPPP A***:

- **H H**: hepatitis B (hepadnaviridae) and herpesvirus
- **P P P P**: poxvirus, parvovirus, papillomavirus, and polyomavirus
- **A**: adenovirus

Don't forget to go through the slides for figures and any extra information that Dr Ashraf may have missed during the lecture.

Good luck!