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Medical Committee
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Microbiology

◉ Sheet ○ Slides

Number : Virology 4

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Subject: Viral replication

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*Viral replication strategies:

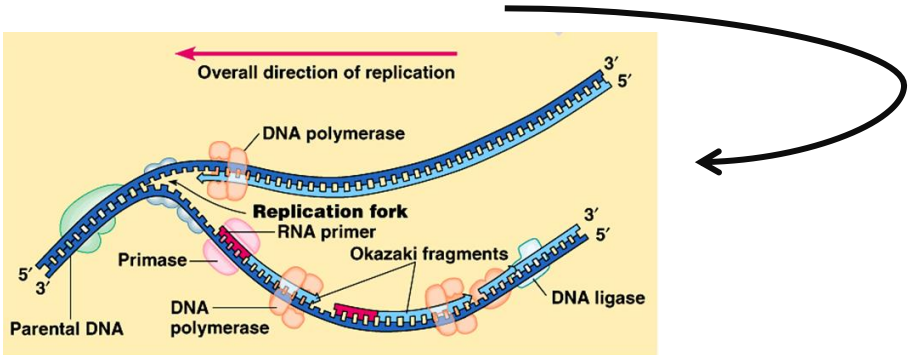
-how many replication strategies do we have?

We have **7** replication strategies:

1. ds DNA
2. ss DNA (**parvo** virus)
3. ds RNA (**rota** virus [**reoviridae** family])
4. ss RNA **(+)** sense.
5. ds RNA **(–)** sense.
6. **(+)** sense (ssRNA) with reverse transcriptase---> (**HIV**)
7. Partial dsDNA with reverse transcriptase---> (**hepatitis B** virus)

* How is double stranded DNA replicating?

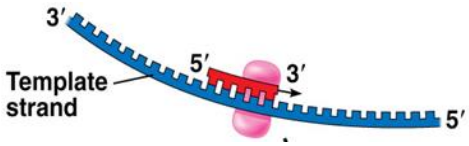
-the structure of DNA is super coiled, we need **topoisomerase** to get more linear structure of DNA. It releases the super coiled of the DNA, makes a more linear structure of the DNA. After that, the **helicase** gets in to separate the strands of DNA forming what we call " replication fork "



-then, replication of DNA occurs in two directions,

The first strand, template strand, starts at 3'

The other one starts at 5'.

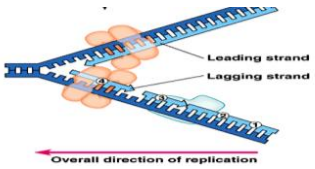


-the new strand that is going to be formed from the template ,which runs from 3', so the new strand (complementary) will be formed from 5' to 3'. It runs from the beginning to the end, there is no problem with this strand. We call this " **the leading strand** " it formed in one step.

-the other one, we call it the **lagging strand** , the template starts from 5' the problem is that DNA cannot replicate in 3' to 5' direction , the complementary strand should be formed as 5' to 3' strand ; so, what occurs?

→We need an **RNA primer** (short sequence nucleotides -from 20 to 25 nucleotides-) which is complementary to the beginning of the template strand. Then the primer binds to the strand, then DNA polymerase would give us the complementary strand for the template strand.

*in lagging strand, not all ds DNA is opened up, primer binds inside the fork and reading occurs from inside to the outside in 5'→3' direction, then the helicase is going to open up here



* In this way, replication occurs as fragments (**Okazaki Fragments**), we will have gaps between this fragments. Gaps are going to be left between Okazaki fragments, as a result of primers, so once it reads from the end of the first fragment, we have its 10-20 nucleotides are going to be left as space between the fragments. Each fragment is called Okazaki fragment.

How are these fragments joined together?

-by the enzyme **ligase**, comes and joins fragments together to form the complementary strand.

* But what about the leading strand? Do we have this problem of fragments in it?

- **Yes**, we do, just at the end of the leading strand.

*we have the enzyme **telomerase**, adds repetitive non-coding sequences, just to fill the gap, it does not encode to any gene.

* if every round of replication there is shortening of 10-20 nucleotides, with time nothing is going to be left on the genome.

Remember

**repetitive means (AAA, CCC ….ETC)

*Does this occur in the viruses?

-it does not occur exactly at this way, they have other mechanisms, we are not going to talk about them.

*so this is how the genome in the ds DNA viruses and ss DNA virus occurs, except for ss DNA. It starts by making the complementary strand into ds and replicates exactly in the same way.

*w talked about the first and second classes (ssDNA, dsDNA).

*<u>double stranded RNA</u>:

-example: **Rota** virus

-these viruses have segmented genomes, each segment is transcribed separately to produce individual monocistronic mRNAs.

*when we say ds, we have one which is (+) sense, the other is

(–) sense.

-the (+) sense serves as a template to be complemented by (–) sense and vice versa by RNA polymerase.

This is how the replication of the ds RNA occurs.

*<u>Single stranded + sense RNA</u>:

* How does the genome replicate?

-we use this (+) strand as a template to give a (-) strand intermediate which is the complementary strand to the (+) , and this (–) serves as a template to produce more (+) strands .

*<u>Single stranded - sense RNA</u>:

* How does the genome replicate?

-we use this (-) strand as a template to give an intermediate which is (+), and this (+) serves as a template to produce (-) again.

*single-stranded (+) sense RNA with a DNA intermediate:

*Example: HIV

*How does this replication strategy occur?

-we mentioned previously that HIV has 2 copies of + sense ssRNA, but it does not behave in the same way as the +sense ssRNA. So it has 2 copies (it is not ds, this means that these two copies are separated from each other), during assembly, the virus will have the two copies of the genome.

* The virus is inside the cell, and the (+) sense is released into the cytoplasm. We have the enzyme called **reverse transcriptase**, what is its function?

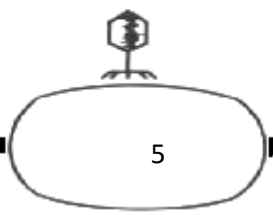
- It gives a complementary from RNA to DNA, so from its name the enzyme reversely transcribes RNA to DNA. So we get RNA intermediate and DNA intermediate, this RNA template dissociates, and the DNA strand is complemented by another strand of DNA to give us dsDNA.

The enzyme that complement ssDNA to give a dsDNA is reverse transcriptase enzyme not the DNA polymerase enzyme.

** Reverse transcriptase functions in two ways, the **first major one (mainly)** is that it reversely transcribes RNA to DNA. The second function of reverse transcriptase is complementing ssDNA to make dsDNA.

-in the next step enzyme called integrase, from its name it integrates or incorporates the dsDNA of the virus with the DNA of

The cells (host cells).



How does it do that?

-integrase makes what we call sticky ends at 5' and 3', it sticks the viral DNA to the cellular DNA, at this stage we call the viral DNA ''provirus''. Once it is incorporated within the cellular DNA,

What is the next step?

Transcription is going to occur to the cellular and viral DNA, once the cell transcribes its genome, the viral genome is going to be transcribed giving us mRNA, and it exits the nucleus into the cytoplasm.

Keep in mind ☺

**not all viruses wait the cell to start transcription, some of them once enter the cell, they control the cell to make it in continuously S phase (transcription phase).

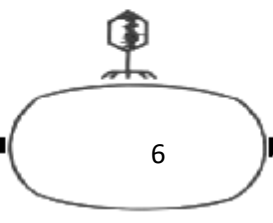
* What is the next step?

-it is translation , we consider mRNA as (+) sense , so it is going to serve two functions; the first one is as a genome (both of those mRNAs are going to be assembled and enter to the new virions) , the other mRNAs are going to ribosomes . In the ribosomes, they will give us polyprotein (chain of multiple viral proteins). How do these proteins then transport to give us individual proteins?

-we have an enzyme called **protease** which cleaves these proteins into individual proteins.

*here, we replicate the genome and synthesize the proteins, so the next step is ***assembly***.

All DNA viruses replicate in the nucleus except pox virus (replicates in the cytoplasm)



*<u>Partial dsDNA:</u>

*example: hepatitis B (**DNA virus**)

- What we start with is partial dsDNA, it enters the cytoplasm, the first step is that the partial ds DNA, we have the negative complete (outside), and the positive which is inside is incomplete (so as it is named partial).

-once the genome is within the cell, the partial ds DNA becomes complete dsDNA. Then it enters into the nucleus, BUT

Does it become integrated into the cellular genome?

- NO , in general, it enters only in the nucleus and then it benefits from the cellular machinery to transcribe this ds DNA into mRNA, mRNA exits the nucleus into the cytoplasm and once again in serves two functions which are protein synthesis and as genome.

*they start with protein synthesis because it is easier, it is going to go to the ribosomes, and proteins are going to be synthesized.

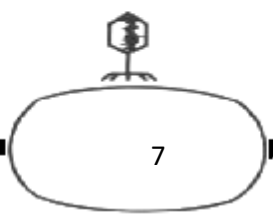
*what about the genome?

We started with ds DNA, and now we have mRNA.

This mRNA will serve as template, this template will be acted on reverse transcriptase. it is going to give us RNA DNA intermediate, then RNA is going to dissociate (in hepatitis and HIV, RNA will be destroyed by enzyme called **<u>RNase h</u>**) . Then another component of reverse transcriptase is going to give us the outer one and the complete one (it is negative),

[The dissociated RNA is (+) sense so DNA is (-)]

-**Then** another component of reverse transcriptase is going to try to make it **ds**. They found that it starts to be complemented, but it never becomes complete ds, it becomes only <u>partial</u> ds and it is released and the virion is mature.



*now, let's go back to the first two strategies, we talked about the replication but not about protein synthesis. It is easy, they replicate in the nucleus to mRNA, mRNA exits the nucleus then it enters the ribosomes to give us proteins.

*In protein synthesis, we have phases (early and late)

-**Early proteins** are non-structural proteins that are required by the virus in order for replication of the genome and transcription of it, so they are important early on during virus replication, so they are the first to be synthesized

-structural proteins are synthesized later during the replication.

NOTE

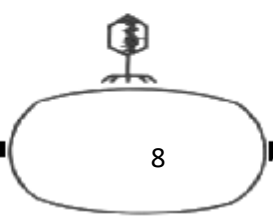
*(+) sense and HIV are made as polyproteins and then they are going to be cleaved into individual proteins.

*ds and ss DNA work the same, they do transcription forming mRNA to cytoplasm then to ribosome to synthesize proteins.

*what about dsRNA?

-in dsRNA we have (+) and we have (-), the positive goes directly to the ribosomes and synthesizes proteins, while the negative is **neglected.**

*ss (+) sense RNA, goes directly to the ribosome and synthesizes the proteins required to the viral replication.



*does this mean that the virus and its genome are enough for the formation of the viruses that are supposed to be synthesized?

-NO, it does not. It goes at the beginning, and synthesizes the early enzymes and proteins, but this is not enough. We have RNA dependent RNA polymerase, which are used for both (replication of the genome and for transcription).

It goes to the ribosomes and synthesizes the proteins (don't forget the mechanism of polyproteins).

Remember

We have mentioned that the proteins of the virus synthesized as poly protein then it will be cleaved by proteases.

* (-) sense RNA will give us the intermediate (+) that again gives us the (-) .this intermediate is going to serve in protein synthesis.

*We will talk about **monocistronic** and **polycistronic**

-what do we mean by monocistronic and polycistronic?

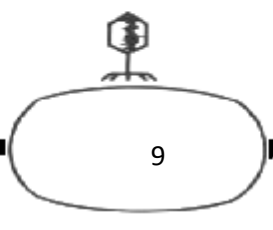
*Monocistronic

In transcription every mRNA gives one gene (encodes for one gene), so in RNA which transcripted from DNA we will have **introns** and **exons**.

* What are the introns and exons?

Introns are non-coding regions, they don't code for genes or proteins, whereas the exons are coding regions that coding for genes and proteins.

RNA splicing occur, which means taking introns out and joining exons together.



In **monocistronic**, we said that transcription occurs in which each piece of the mRNA codes for a gene.

How is this achieved in viruses?

Viruses go back and sticks in monocistronic group.

-We have **three** mechanisms by which viruses can stick to the monocistronic group:-

1. The genome is already segmented, that means every segment of the genome RNA represents one protein or one gene.

2. The last step we have poly proteins then they are cleaved by proteases to individual proteins. (Such as (+) sense and HIV viruses).

3. Transcription is sensed by promoter (at the beginning of the gene) and it stops transcription at the end of the gene .the genome can be transcribed into different three mechanisms. So transcription process in certain viruses starts by sensing the beginning of the gene and ends by sensing the end of it.

*the monocistronic mRNA problems:

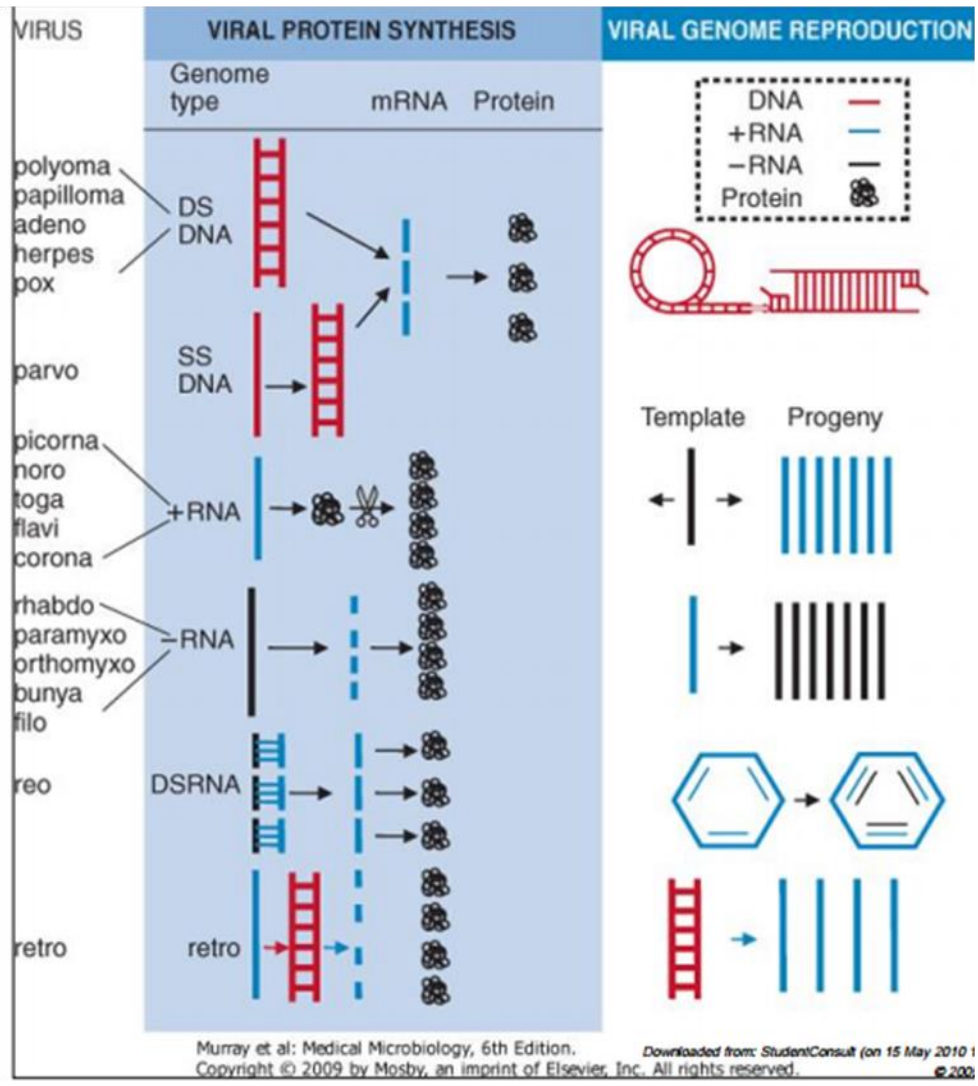
1. Make one monocistronic mRNA per protein.

2. Make a primary transcript and use alternating splicing.

3. Make a large protein and then cut it into smaller proteins. (Like HIV proteases)

4. Include special features in the mRNA which enable ribosomes to bind internally.

This picture summarize all replication strategies:



RNA-depend RNA polymerase:

there are two kinds of this enzyme,
one is associated with the genome replication only, and the other is associated with transcription and translation of the proteins.
In some cases the RNA-depend RNA polymerase which associated with the genome replication may has a mutation or something like that and then it will serve as protein translation associated enzyme.

Important notes ☺

*all the animal RNA viruses code for a polymerase. (Have polymerase enzyme)

*positive/negative/dsRNA virus genomes all encode an RNA-depend RNA polymerase.

*RNA-depend RNA polymerase is associated with negative RNA viruses.

*reverse transcriptase is associated with retroviruses and with hepatitis B as well.

- Replication challenges for DNA viruses:

→Access to the nucleus, we said that small DNA viruses (like parvo, polyoma, papilloma viruses) will uncoat in the nucleus (they enter the nucleus as nucleocapsids).

Larger DNA viruses, they uncoat in the cytoplasm and then the genome only enter to the nucleus.

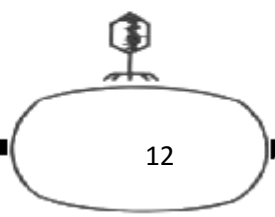
→Competing for nucleotides. We need nucleotides and enzymes to form these new strands, so the virus is competing with the cell for these nucleotides and enzymes

→Cell cycle control in eukaryotes s phase.

Why is S phase important?

Because it is the phase in cell cycle where replication occurs.

- Parvo virus is totally dependent on the cellular machinery, at most time, it waits the cell to enter the s phase.
- Other DNA viruses produce certain enzymes and proteins which applied the cell to the viral control. they put the cell in continuous s phase status, so they can use the machinery



when they want, as a general rule, we say that the virus once it enters the cell, it controls all the functions of the cell .as a result of this continuous s phase ,,

So, what do we expect?

It means uncontrollable replication, this alters in **cancer cells**, so DNA viruses are associated with transformation or cancerous transformation of the normal cells. {This is one mechanism of cancerous transformation of DNA viruses}.

Assembly:

*assembly involves the collection of the components necessary for the formation of the mature virion at a particular site in the cell.

*during assembly, the basic structure of the virus
Particle is formed.

*the site of assembly depends on the site of replication within the cell and on the mechanism
By which the virus is eventually released.

Assembly may occur in the cytoplasm or in the nucleus,
** In picornaviruses (RNA viruses), pox virus (DNA virus) and reo viruses (RNA virus) assembly occur in the **cytoplasm**.
** In adenoviruses, polyomaviruses and parvoviruses (all are DNA viruses) assembly occurs in the **nucleus.**

THE END

sorry for any mistakes ☺