6 – ambisense RNA viruses :-

- **single stranded RNA viruses**, which has a (+) sense segment at one end of the genome and (-) sense segment at the other end. The replication (make a copy) of the (+) sense strand can act as mRNA. The genome can be huge as it contain +ve and –ve RNA and the polarity of this genome make a relationship with mRNA. The first step in the replication of these viruses is the transcription of the 5'end of the genome, so that part of sequence of the genome which transcribed into mRNA should be –ve sense.

- The transcription has two phases :-

  1. **Early phase** :- The genomic RNA (-ve sense ) segment yield sub-genomic (smaller sections of the original transcribed template strand) mRNA (+ve sense). This mRNA synthesis the structural components of the virus. Thus viruses must carry with them an RNA polymerase and transcriptase, otherwise can't replicate so these enzyme must be brought to the cell.

  2. **The late phase** :- Although the other segment of the genome is +ve sense but we can't use it as mRNA directly. The replication occur to the genomic RNA to yield what we called replicative intermediate. This replicative intermediate (antigenomic in the figure (1) below ) transcribe the sequences that are not transcribed in the early phase. So these sequences which are mRNA (positive sense) have the same sequences of the genome itself, same polarity. This mRNA synthesis the structural components of the virus. As if the genome contains sequences which are positive in sense and sequences which are negative in sense so we call them ambisense (the same strand act as +ve and –ve sense).

- The mRNA then translated to proteins that are essential in the replication. Thus viruses must carry with them an RNA polymerase and transcriptase, otherwise can't replicate so these enzyme must be brought to the cell.

- We have to families that have ambisense genome :-

  1. **Bunya viruses** :- (multipartite) have 3 single-stranded RNA (ssRNA) fragments

  2. **Arenaviruses** :- (multipartite) have 2 single-stranded RNA (ssRNA) fragments
Figure (2): Replication scheme of ambisense RNA viruses.

step 1) After entry and partial uncoating.
Encapsidated viral RNAs are partially transcribed by the "nucleocapsid-associated RNA-dependent RNA polymerases" (RdRp's) produce mRNAs.

step 3). The nucleocapsid protein and RdRp is produced by translation.

step 4) These enzymes produced catalyze RNA replication through the synthesis of replicative intermediate (antigenomic nucleocapsid) which serve both:

a- As templates for transcription of mRNAs (step 5) for the other viral proteins (step 6).

b- For the production of more genomic nucleocapsid.

**Replicated genomic nucleocapsid serve as:-**

A- Templates for transcription (step 7) and then translation as in the (step 8).

B- And for assembly with the structural proteins to produce progeny infectious virus particles (step 9).

7- **double stranded RNA viruses :-**

- One family of viruses that has a double stranded RNA genome which is segmented, these are the reoviruses, other example is rota viruses.

The genome is segmented into 10-12 segments that are double stranded, as these viruses enter the infected cell, they undergo uncoating (partial uncoating) the vertices of virus (icosahedra) are opened and the transcriptase and RNA-dependent RNA-polymerase enzymes are present within them, so in these partially opened viruses these enzymes transcribe the mRNA utilizes one of the strands of the dsRNA which is the negative in this regard as a template to produce mRNA, so they produce a +ve sense mRNA from each of the segments, and they extrude from the opened vertices, mRNA molecules are released in large quantities from the partially opened vertices. **These mRNA +ve strands will be used for two purposes :-**

1- Some of them will be translated into proteins.

2- The others will wait for These proteins to assemble as empty structures to enter to the newly synthesized particles where they act as a template for synthesis of complementary strand, which result in the availability of double stranded RNA virus.

Note :- the +ve strand which used to synthesis protein will be degraded.
II-DNA viruses replication:

- There are four strategies for transcription and replication:

  1- Double-stranded DNA viruses that replicate in the nucleus:

  - There are three families that belong to this category:

    1- Papovaviruses include: papillomavirus and polyomavirus

    1- Adenoviruses

    2- Herbesviruses

    - It is convenient to gather these families to one strategy as they all contain ds DNA viruses and they all utilize the transcriptase of the cell.
There is a difference between these families in relation to the capability to synthesize the protein and enzymes necessary for the transcription:

1. Papovaviruses encode a single protein that binds in close proximity to the origin of the viral DNA synthesis to stimulate the cellular polymerase complex of the host cell to replicate the viral DNA and it also acts as helicase.
2. Adenoviruses encode their own DNA-polymerase but they depend on the host cell for providing protein necessary for transcription and replication of the genome.
3. Herpesviruses (HSV) encode all the necessary proteins and enzymes for replication (it said that herpes viruses are capable of replication in the environment extracellular to the cell if they are provided with ribosomes and precursors of replication. Even they synthesis proteins that can maintain their genome in an episomal state.

Note: A genetic element that originates outside the host, in a virus or another bacterium. It can replicate free in the cytoplasm or when integrated, a new copy of the episome will be made as the host chromosome undergoes replication.

The mechanism of cellular DNA replication is similar to the eukaryotic DNA replication as the viral polymerase are similar but they are faster and less accurate than cellular polymerase. On the other hand, they all utilize standard Watson-Crick base pairing and rolling circle mechanism to replicate their genome.

* Rolling circle replication describes a process of unidirectional nucleic acid replication that can rapidly synthesize multiple copies of circular molecules of DNA or RNA, such as plasmids, the genomes of bacteriophages, and the circular RNA genome of viroids. Some eukaryotic viruses also replicate their DNA via a rolling circle mechanism. (wiki)

Flow of events during the replication of dsDNA viruses in the nucleus:
For Papovaviruses, there are 2 phases of transcription:
1. Early phase
2. Late phase
The regulatory proteins and enzymes are produced in the early phase. And the structural components are produced in the late phase.

While in Adeno & Herpes viruses there are 3 phases of transcription:

1) Immediate early (pre-early) phase
2) Early phase
3) Late phase

The products of each phase are necessary for the initiation of the second phase.

- Pre-early phase codes for proteins that are necessary for activation of the early phase. And the early phase is responsible for the activation of the late phase of transcription resulting in the synthesis of the structural components of the virus.

After completing all the phases (production of proteins [ regulatory and structural ] & transcribing as well as replicating the viral genome ) they assemble together into viral particles. Then if the virus is enveloped they will get the envelope and if the virus is naked like adenoviruses and papovaviruses they will kill the cell and leave it.

To fulfill your understanding you can return to these animations about herpes virus

http://darwin.bio.uci.edu/~faculty/wagner/movieindex.html
2- double stranded DNA viruses that replicate in the cytoplasm:

- Although this category includes only Poxviruses/Poxviridi family, this family includes large numbers of viruses and Most phases of the poxviruses replication occurs in the cytoplasm.

- Those viruses must carry their own necessary enzymes & proteins for replication and transcription because host cells cannot provide any help because they replicate in the cytoplasm, so this replication and transcription mechanism has diverged from the cellular mechanism.

- Although poxviruses' DNA has been detected in the nucleus but most phases of replication occur in the cytoplasm of the infected cell.

- The first phase of transcription of the genome of the virus takes place utilizing viral transcriptase and DNA–dependant RNA polymerase in the partially uncoated virus. So, after the entry of the virus to the infected cell, partial uncoating takes place, followed by early limited transcription of the genome, then synthesis of proteins that complete the uncoating process. After completion of the uncoating, further transcription takes place in which more than 100 genes or More are transcribed by this mechanism (large genome "complex virus") and this transcripts will produce regulatory protein and enzymes as well as structural components.

- Then viral DNA replication takes place by virus encoated DNA polymerase, and the assembly of the viral particles is achieved in the cytoplasm of the infected cell with the acquisition of an envelope from the membrane of the cell. Finally, the release of the virus either directly from the infected cell to the environment or by transfer of the virus from one to the other through inter-cytoplasmic bridges between cytoplasmic membranes.

3- single stranded DNA viruses (parvoviruses)

- Represented by a single family known as Parvovirus's/Parvoviridi. That contain single stranded DNA

- They are divided into 2 types:

1) Dependo viruses
2) Autonosm viruses (independent)
Autonomous viruses.

- Those viruses can replicate on their own.
- There are 2 human pathogens that represent them:

1- Parvovirus B19

- They infect immature erythrocytes (nucleated blood cells) because they replicate in the nucleus of this immature erythrocyte.
- They can infect other cells than erythrocytes but the primary targets are erythrocytes.
- B-19 Paroviruses infect immature erythrocytes, which are actively dividing. They usually infect the cell when it is in the S phase of growth cycle where enzymes, nucleotides & precursors are available. So, the virus makes use of this growth phase of the infected cell to replicate its own genome.

2- Boca viruses

- They infect the respiratory cells in which they cause respiratory tract infection.

Dependoviruses:

- Are those that require help. This help is provided by other viruses; most commonly Adeno-viruses that provide them with necessary structures & enzymes to enable them to replicate in the infected cell. thus, they cannot replicate on their own. In this case, these viruses are known as Adeno Associated Viruses (AAV). In addition to Adenoviruses, Herpesviruses can provide help as well. They can't replicate in the absence of the adenoviruses or herpesviruses so they integrated in the genome of the host cell without any harm.
- They replicate in the nucleus. And they utilize nuclear enzymes of the infected cell for both replication (polymerases) and transcription (transcriptase). In addition, they require help in the form of other proteins from another virus (Adeno or Herpes virus). They usually infect mitochondria active cells and usually obtain precursors such as enzymes necessary for replication from cell that are in the s phase.
- The major problem in the replication of this viruses is the lack of double stranded DNA because transcription and replication can't be achieved on single stranded DNA as the enzymes cannot work on single stranded DNA, they can only transcribe and replicate double stranded DNA.
- So the first step in the replication of these viruses is to create double stranded sequences, without this, no replication can proceed.
To overcome the problem of single strandedness, these viruses convert themselves from ssDNA viruses to dsDNA viruses. And this is made possible by the presence of identical inverted repeat sequences that are present at both termini of the genome to create dsDNA.

By folding back on itself, the genome forms double stranded sequences, and this is the critical step in the replication of these viruses, which takes place in mitotically active cells. And now, after folding of the palindromic sequence on itself, Gab-fill synthesis takes place and this is continued with displacement of the strand followed by Gab filling of sequences with the formation of hairpin Y or T shapes for these sequences until the viral genome is amplified several times. So what result in this process are multiples of the viral genome because of copying each sequence. The resulted genome composed of 9-10 segments of the genome of the virus means that the original genome of the virus copy itself 9-10 times.

A palindromic sequence is a nucleic acid sequence (DNA or RNA) that is the same whether read 5' (five-prime) to 3' (three prime) on one strand or 5' to 3' on the complementary strand with which it forms a double helix.
Actually, there are only 3 genes in the genome as this genome is too small. So this ds sequence is transcribed into 3 proteins. One of these proteins is the structural proteins serve as the protein coat of the virus.

These structural components will assemble together forming empty pro-capsid that associate themselves with the sequences of the genome at certain exact sites and then the genome is cut at sites to produce 9-10 segments of the original genome of the virus by deoxyribonucleases.

So the empty-capsid will associated with a segment of the genome of the virus and this will create new viruses that will lyses the cell because they are naked viruses. Finally, viruses are released from the infected cell.

Each virus contains a DNA sequence that is not necessarily the same; it is complementary to another sequence. But when the virus starts new cycle of replication, the complementary strands will be produced, so it does not influence the protein that’s coded for by the virus. And therefore, whether it is the primary strand or the complementary strand, after infection of a new cell, these strands serve as templates for transcription and a complementary strand for each of the previous ones will be generated and the ds DNA of the virus will be received.

4-Hepandaviruses :-

- **circular partially dsDNA genome** The only human pathogen virus in this category is *Hepatitis B virus*. Hepatitis B virus has closed circular partially dsDNA virus, partially double stranded, meaning that there are sequences that are double stranded "complete" and others that are single stranded "uncompleted".

- The virus (hepatitis virus) enters the cell (hepatocyte) and the first step after entry to the infected cell is to complete the double strandedness. A DNA polymerase of the virus completes the double strandedness and as a consequence, the virus becomes a **Complete Circular Closed Double stranded DNA (CCC dsDNA)** virus.

- Host cell transcriptase transcribes the genome into 2 types of RNA:

  1) Multiple (sub-genomic) mRNA that code for the structural components & enzymes of the virus.

  2) Genome long RNA which acts as the **genomic RNA**. It is the genome of the virus. And this genomic RNA will be the template to produce a ss DNA molecule by a reverse transcriptase carried by the virus.
So now, instead of the ss RNA (degraded) the virus has a ss DNA. And because the reverse transcriptase carries the ribonuclease activity, it will degrade the RNA molecule that acted as a template for the synthesis of DNA. Then the DNA-polymerase starts copying the genome of the virus (DNA) into a complementary strand to produce dsDNA. But before completion of the function of the enzyme (completion of double strandedness), the virus is released from the cell. So it’s released as partially double stranded DNA. And that’s because the enzyme was not given sufficient time for completion of double strandedness, so it stops (pauses) at this stage waiting for another cycle to enter the cell and the first step after entry into the cell is the completion of double strandedness and the virus inside the cell becomes a double stranded DNA virus.

**Note:** there are similarities between the replication of these viruses and that of Retroviruses.

They are similar to retroviruses in exactly the same events but retroviruses start replication by production of ss DNA from and RNA molecule, while in hepadnaviruses, it is the end of replication in which an RNA single strand is utilized to synthesize ss DNA which then acts as a template to produce the double stranded DNA. But the virus is not given the sufficient time to complete this process and as a result, the virus is released as partially double stranded DNA virus.

*Please refer to the slide "replication of hepadnaviruses "flow of events.*

**Assembly ,Maturation , and Egress of viruses from infected cells**

- Once the virus has completed the processes of transcription, replication and of course translation of the transcripts into proteins, all necessary components are available for the virus. And now, assembly, maturation and egress of viruses from infected cells take place.

- Assembly of DNA viruses, except pox viruses occurs in the nucleus (where replication occurs) and they require transport of the viral proteins (structural components) from the cytoplasm (the site of their synthesis<<ribosomes on the RER>>) into the nucleus.
And this involves "right leading sequences / proteins" that lead the viral proteins to the nucleus where assembly takes place. Assembly of poxviruses and RNA viruses takes place in the cytoplasm. Assembly process starts when the structural components (protein capsid of the virus) are made in sufficient concentration and the assembly occurs in a process much like the crystallization reaction.

- **There are two different mechanisms of assembly depends on the architecture of the virus weather icosahedral or helical :-**

A- **Icosahedral viruses** :- assemble spontaneously into empty procapsids (structural components of the virus) even if they are mixed in vitro (test tube). Then the genome of the viruses weather DNA or RNA and weather in the cytoplasm or nucleus will go inside this empty capsid utilizing packing sequence nucleotides that are transcribed from the genome of the virus then farther modification of the virion takes place. After that the virus is released even by the killing the cell like naked capsid viruses or acquire an envelope from the infected host cell membrane. Usually the genome is inserted through the vertices that remain open and then theses vertices close.

B- **Helical viruses** :- These viruses assemble while the genome is coiling. So, while the genome is assuming its shape, building blocks are added, and that’s why there are no empty structures. These processes take place simultaneously; coiling of the viral nucleic acid with the addition of the structural components of the virus. There are no helical DNA viruses, so all helical viruses are RNA in nature. They don't kill the host cell because they are all enveloped so they must acquire an envelope from the infected host cell before release.

- Acquisition of an envelope occurs after association of the nucleocapsid with regions of host cell membrane modified by matrix protein and glycoproteins.
- For **enveloped proteins**, modification of the sites of maturation by matrix proteins happens. These matrix proteins line and promote the adhesion of the nucleocapsid with the modified membrane. And that also promotes the release of the virus from the infected cell, as interactions take place between matrix proteins & the nucleocapsids. Where-
as in the case of naked viruses, there is no such requirement. But the cell in this case is destroyed and the virus is released after assembly.

- Acquisition of an envelope occurs at sites that are either:
  1) Cytoplasmic structures: Golgi apparatus, endoplasmic reticulum, nucleus.
  2) Cytoplasmic membrane

**strategies for maturation :-**

- Three fundamental strategies for maturation have been described:-

1- **Intracellular assembly and maturation for naked viruses :**
   - Naked viruses weather DNA or RNA kill the infected cell .

2- **Enveloped viruses :-**
   - The last step in assembly of (-) strand RNA viruses is linked with their egress from infected cells by budding from the cytoplasmic membrane or other membranes. Viruses that mature and egress by budding vary considerably in their effects on host cell metabolism and integrity.
   - They range from highly cytolytic (toga, paramyxoviruses ) which kill the cell to viruses which are frequently non-cytolytic (retroviruses).
   - All enveloped viruses carry on their envelope a spike (glycoprotein).
   - Viruses modify the structure of the infected cell with the inclusion of a full protein that will make the cell foreign and stimulate the immune response to attack the infected cell and as a consequence the cell is killed.
   - So killing may be immune mediated not necessarily induced by the virus because enveloped viruses when they enter the cell, they leave their envelop proteins at the surface of the cell, so the cell is modified. And that’s why the immune response is stimulated in the case of viral infections, resulting in the killing of the cell.
3- Strategy for herpesviruses :-

- Herpes viruses are enveloped viruses. They acquire the envelope from the nuclear membrane. The nucleocapsid are assembled in the nucleus. Matrix proteins are produced and they associate with the nuclear membrane, and so, the virus acquire an envelope from the nuclear membrane.

- Now, there are some speculations. Some authors believe that the viruses acquire the envelope from the inner lamella of the nuclear membrane and then it lose it in the inter-laminal space and then it acquire another envelope from the outer lamella and then after this, it they are released from the outer lamella of the nuclear membrane coated within a vesicle and they follow certain cytoplasmic paths that open at the cytoplasmic membrane and they are released from the infected cell.

- The productive infection by herpes viruses is followed by killing of the cell. After the release of Herpes viral particles from the infected cell, the cell dies either by the productive infection or by importing new antigens on the infected cell which make the cell foreign for the body and so the immune system target it.
**glycosylation and budding**: 

- Viral proteins are glycosylated. The envelope of the viruses has glycosylated proteins. Glycosylation of viral proteins is important in processes of budding from the infected cell.

- The glycosylation of viral proteins takes place utilizing cellular pathways which is used to glycosylate host cell's hormones, cytoskeleton pathways or proteins that will be recycled outside the cell.

- After synthesis of proteins, they are sent to the endoplasmic reticulum with a signal sequence of about 15-30 hydrophobic amino acids which facilitate the binding of the protein to a receptor on the cytoplasmic side of the RER and will lead the viral proteins from the cytoplasmic side of the RER to its luminal side where the signal peptide is first removed by signal peptidase, so the viral protein now is inside the RER allowing the addition of manose-rich sugar. After that the glucose is removed by glycosidase and further trimming (cutting) is achieved by modification of this structure then the glycoprotein is transferred to the Golgi apparatus for the addition of fatty acids (acetylation).

- Transfer of glycoproteins to specific sites/structure in where budding will take place, if it is the cytoplasmic membrane, it is transferred to the cytoplasmic membrane or if it the membrane of some other organelles in the cell like Golgi bodies. For example, corona viruses acquire the envelope from internal structures. Probably, this is done by the help of leading sequence known as the “postal address or zip code” sequence to enable the proteins of finding their address. After reaching their destination, the glycoproteins wait for the virus that associates with matrix proteins and start budding.

- During this process, glycoproteins are subjected to cleavage by proteases or other enzymes. By that, the glycoproteins are split into 2 domains; hydrophobic which will be exposed to the extracellular environment & hydrophilic which will anchored to the membrane. And they are covalently bound by S-S bonds, and this is very essential for the virus; without it, the virus will face a dead end, in which it will not be able to infect...
new cells. So creation of infective viral particles requires this cleavage, because this cleavage will expose the viral attachment peptide.

- If cleavage doesn’t take place, the viral attachment peptide will remain buried inside the structure of the glycoprotein, so the exposure of the virus attachment peptide requires this cleavage. Without it no infection can take place. So all glycoproteins of enveloped viruses must be cleaved by enzymes that are necessary to keep these two subunits covalently bound by disulfide bonds.

- After that the virus is released from the cell by exocytosis which mediated by the help of the matrix protein (the reversed form of endocytosis). It enters by endocytosis and leaves by exocytosis at which large numbers of viruses are released by consecutive waves and they are ready to infect new cells.

- All the processes that we are talking about from penetration to uncoveting, replication, transcription, assembly and release each of them has a rate of failure that is why only 5-10% of the viruses are infections and the remaining viral particles that are produced are faulty because of false process during viral replication. So during viral replication large number of defective viral particles are produced, especially when we have a segmented genome for example. The association/assortment of the right exact segments may not take place in the process of replication, and that’s why many defective viruses are released from the cell. And these defective viruses that are released have consequences, they may influence infection.

- **It is convenient to classify defective viruses into two groups.**

1- Viruses that lack one or more essential genes (genetic defect) and therefore they can’t replicate independently. Some of this viruses can integrate their genome to the cell genome and transform the infected cell into malignant cell.

2- Viruses that are defective in their replication (can’t replicate efficiently) due to mutations and deletions in their genome. Some of this viruses are associating with diseases (here the doctor say many diseases). Because they can’t complete their replicative cy-
cle like another viruses the may cause very significant diseases. They can cause tumors (cancer).

- Such defective viruses, whether from first or second category may be the best gift of viruses to humans. They can be utilized as vectors for gene therapy. We may insert the required defective gene (lacking gene of an individual in these viruses) by molecular technology (genetic engineering) and then infect these cells, which will deliver the gene required. So it acts as vehicle.

- Viruses are the only organisms that can integrate their genome with the genome of the infected cell.

I know you are waiting for this word "The end"

Done by: Hamza Al-NIMIR

"تسامح في حق نفسك وتشدد في حق أمتك، تكن عند الله عبدا كريما، وفي أمتك مواطنا مستقيما" 
مصطفى سباعي