Microbiology

◉ Sheet ○ Slides

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Subject: Viral genetics-1

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Viral genetics and genetic changes

- ✓ In the previous lecture, we talked about viral replication, the formation of mature virion.
  Today we will start in **Genetics of viruses, mutations and recombinations**.

- ❖ Generally speaking , viruses acquire many genetic changes during their life cycle , at least during replication .For example , a **point mutation** occur every 2500 – 10000 nucleotide during replication , that's mean we have a change in nucleotide (for example A instead of C, or G instead of C …

5.1 Nature of genomes

As we said previously ,the genomes (genetic make-up) of viruses are either **DNA** or **RNA** but not both . Moreover , RNA viruses could be segmented or non-segmented , this information is very important , because we have different types of recombination accordingly.

Mutations are very common in **RNA** viruses, but they are less common in DNA viruses, because DNA have an access to ***proofreading*** in *DNA-polymerase .*

Proofreading : genetic error-correcting mechanism , usually related to DNA-polymerase .

- ❖ **Does that mean that DNA viruses don't have any mutation?**
  Not necessarily, but the rate of mutations is very low in comparison with RNA viruses.

- ❖ **Ok , is this good or bad ?**
  For a certain extent, Absence of significant changes in genome could avoid production of defective virus . However , this reduces ability to adapt and limit the evolution , so RNA viruses are subjected to evolution and adaption better than DNA viruses .

5.2 Genetic changes

Viruses generally use two mechanisms : **mutation** and **recombination** by which viral genomes change during infection and there are virologic, immunologic, and medical consequences of some of these changes .

Typically, the majority of the particles derived from a cell infected with a human virus are noninfectious in other cells.

Very very very important note :

In this lecture , we are dealing with **viral genetic changes** , not human genetic changes , so when we talk about point mutation for example , don't think about sickle-cell anemia ,it's all about **changing in viral virulence** , **antigens** , **effect** , etc …

5.3 Mutations

Note : slide 5 , 6 , 7 are not important

Types of mutations :

a) Point
b) insertion
c) deletion

5.3(a) point mutations

- **The point mutation :** is that type of mutation that Changes a single nucleotide into another one. This affects the codons that code for amino acids.

- what is the codon?
  - It's a **three letter code** which represents an amino acid, the three letter code contains three nucleotides (positions); 1st , 2nd and 3rd position.

Types of point mutation

a) **silent mutation** → If there is no change in amino acids

- **If you have a point mutation, in which one of these positions (in codon) you will not have a change in amino acid?**
  The 3rd position , but why ?

As a general role, any amino acid is coded by more than one codon (*redundancy in the genetic code)* , so any amino acid could be coded by 4 sequences theoretically . For example: CAG, CAA, CAC, CAT might represent the same amino acid .

So, some changes in the 3rd position, might not lead to change in the amino acid, and as a result is not going to affect the overall structure and function of the protein.

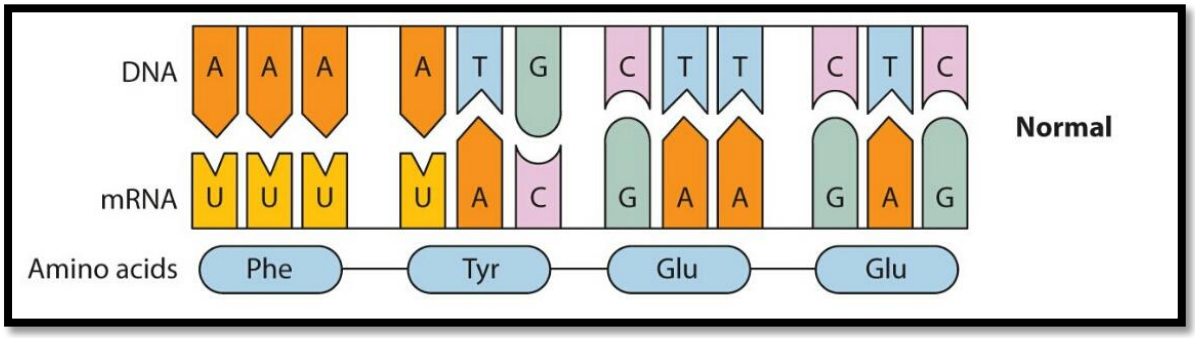
b) **Missense mutation** → any change in the 3rd , 2nd or 1st nucleotide that will lead to change in the amino acid → that may change the conformation or activity of the protein if the changed amino acid was essential .

c) **Nonsence mutation** → it will produce a **stop codon** (the codon that stops the protein synthesis)
the outcome of protein: non-functional protein and most of the time it is going to form aggregate.

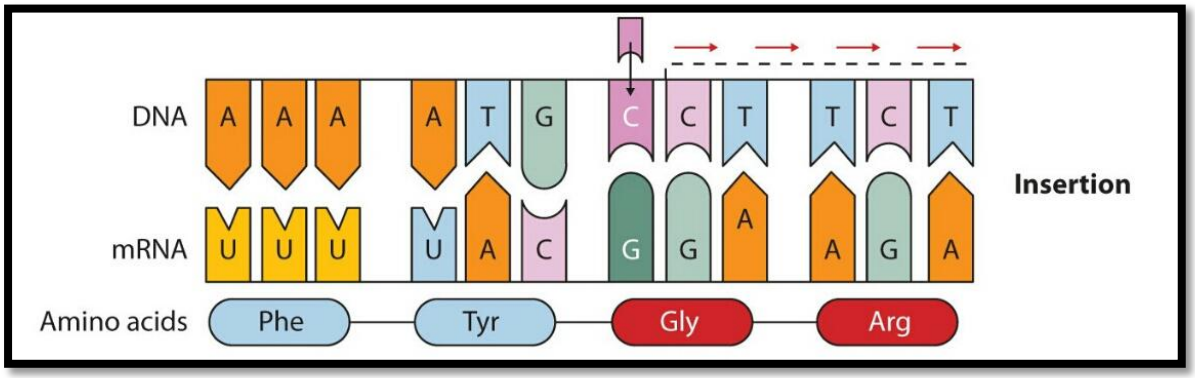
5.3(b) Insertion and deletion mutation

such mutations affect **all reading frame** (i.e. more than one codon will be affected by changing only one nucleotide)

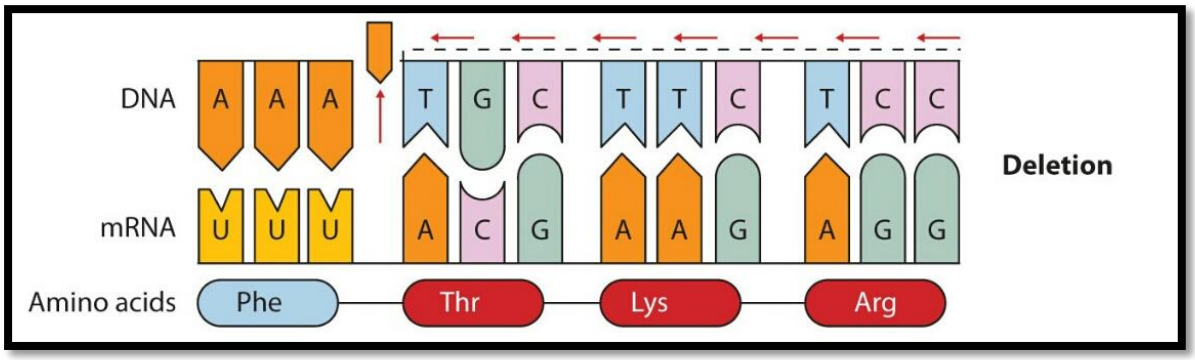
To understand this , we will talk about four codons next to each other :



1-Let's assume that you added a nucleotide to the 3rd position of the original codon, it will be the 1st position of the next codon and that will affect the whole sequence by shifting the reading of codons by one nucleotide. (Insertion) "seen below"



2-If you take\delete the nucleotide which is in the 2nd position of the codon , the 3rd position is going to move to the 2nd position, and the one in the 1st position in the next codon will be in the 3rd position of the first codon and so on …. (Deletion) “seen below”



We call these mutations **[Frame shift]**, which will lead to overall change in the order of amino acids. (Insertion / deletion)

- ❖ What is the effect of this change?
  - It might affect minimally or produce a non-functional aggregation protein depending on insertion or deletion.

Note : slide 9 is not important

5.4 recombination

Besides mutation, genetic **recombination** between related viruses is a major source of genomic variation.

Recombination requires two different genomes , after recombination we produce a new genome with characteristics from the original ones. (Mixture of both)

we have three forms of recombination:

- Classic
- Copy choice
- Reassortment

5.4(a) classic recombination

- **Classic** form is the simplest form of recombination.
- its most commonly occur in **DNA viruses.**

in this form of recombination:

We start with two different templates of genome → translocation or exchange of some segments between these two templates → new genome

we have different segments in the **new-formed genome (genome after recombination**), some of these segments acquired from one template and other segments from other template.

its an exchange process of segments have the same size or different sizes between at least two templates.

5.3 (b) Copy choice recombination:

In this form , we must have a **Viral** enzyme that depends on RNA in polymerization (i.e. **RNA-dependent-RNA-polymerase** or **RNA-dependent-DNA-polymerase**)

here , we are talking about non-segmented RNA viruses (because RNA viruses use **RdRp** or **RdDp** to replicate their genome)

The reason why copy choice recombination is restricted for RNA viruses , that RdRp & RdDp have the ability to jump from one template to another .

the mechanism of this form if recombination :

Firstly, I have two templates, let's assume that they are A and B. RdRp or RdDp starts reading from (A) in order to make a new template (which will be recombined), when it reaches a certain sequence in the middle of (A) template, it jumps to attach similar sequence in the second one which is (B) and continues reading and forming of the new template. the new template contains a combination of (A) and (B) templates.

Ok , think about it , the polymerase jumped from a sequence in a certain template , to a similar sequence in a similar template ! How can virus produce genetic variations by this process !

The answer for this question comes from the fact that **we have many similar sequences along the genome** , so to say that we have a GGCAA at the beginning of genome , after 200 nucleotide we will have another GGCAA , after 658 nucleotide we will have another GGCAA , etc **..**

So the template could be attached to the same location , earlier location , or later location .

Sooo :-

- If it started from the **same location** where it left → the new template is going to have the **same length** as one of the original templates.
- If it started from the beginning **(earlier)** → its going to be **longer**.
- If it started at a **later** point → its going to be **shorter**.
-

viral replication is not an efficient process, that's mean if we say that a virus has infected a cell and produced hundred cells, what is the percentage of infectious reproductive functional viruses?

- We are talking about 40-50-60% depending on **the virus** and **how quick replication occurs**.
  40 – 50% of newly produced viruses are non-functional viruses as a result of mutations we have mentioned previously.

In the copy choice recombination, we are talking about the same cell, how the combination occur?
In other words, one virus enters the cell, and we have more than one template (because replication is going on), how it can make recombination with itself?
- The idea here is that when virus replicates and produces new genomes **mutations will occur**, deletion, insertion, or point mutation, these mutations will produce variations.

Viruses like hepatitis C have a very quick replication, one virus might produce million viruses, this variation of the new genome as a result of replication, we call it: **[quasispecies]**

if you take a blood sample from a patient for testing, and you make sequencing to know the nucleotides on each genome, you will notice a great variations.
why?

The group of genomes that you are going to find in this same patient which was infected by hepatitis C called **[quasispecies]**
i.e we have genetic variations.

After the recombination of templates occur, the produced template is going to be used once again for another recombination.

or this template once it replicates without recombination it produces new mutations.

This is the reason for finding genetic variations within the same cell of the same virus → (mixture of mutations and recombinations).

5.3 (c) reassortment

When we talk about reassortment we are talking about **segmented** viruses only.

Remember that:

segmented viruses are (rotavirus and influenza virus).

Influenza virus has **8** segments and rotavirus has **11** segments In

segmented viruses, all of them are monocistronic, each segment represents one gene or protein.

So, what is reassortment?

Simply speaking its **mixing of segments**.

Let's take influenza virus as an example, we have avian influenza, swine influenza and human influenza.
here, recombination occur.

What does happened actually? Is there a chance to get a single cell infected by all these types of viruses?

The answer is yes, maybe in bird cell we can find swine influenza and human influenza together. in swine cell, a cell infected by human, avian and swine influenza, and so on…
and each one of these viruses are going to replicate their segments.

In which step does the reassortment occur?

At **assembly** step.

To sum up:

Reassortment occur in assembly step, segments of different species might be assembled into a single virus.

What is the effect of that on the virus and on our bodies as humans?

these new segments which are from different species (birds or pigs), they are specific form, and human body might not been exposed or sensitized against these antigens before, so the viruses are going to be more virulent, more infectious, the symptoms are going to be more sever.

The last outbreak occurred in 2009, H1N1, and this virus of this train still included in the influenza vaccine up to date.
because infections or viruses which result from reassortment are more virulent and are associated with large outbreaks.

Large **outbreaks** mean that every 10-15 years or even 7,10,12 years we have an outbreak which result from reassortment.

The outbreak means that the number of cases is more than need get during a season.

Remember:
in pathogenesis we mentioned that we have epidemic, endemic, pandemic.

Epidemic is an increase in the number of cases in a certain location and certain period of time.

Do we get something like that?

Yes , Every winter in Jordan , we have an epidemic of influenza, increase in the number of cases in winter, but for the rest of the year, the number of cases is fixed.

Pandemic involves the whole world, it's an increase in the number of cases in the whole world.

Reassortment is usually associated with a pandemic, the outbreak has been occurred in more than location.

Epidemics are in each winter (certain period of time), but every 3 to 5 years, the produced virus is going to be more virulent as well, WHY?

RdRp lacks proof-reading, it's going to introduce mutations into the segments of influenza virus. once there is introduction of mutation into the

segments of influenza virus, it might affect any gene or any protein in the virus, might be structural or non-structural protein.

What is the most affected protein? All proteins are antigens but which part? What is the most antigenic part of the virus?

Glycoproteins \ spikes.

if the change or the mutation affected glycoprotein, it might change the structure of the glycoprotein to different levels. (Spikes have an antigenic role)

- Another name for reassortment is **antigenic shift**,
  when we talk about reassortment of influenza virus we call it as another name → **antigenic shift.**

- if a change is occur in the segments as a result of mutation, we call it **antigenic drift**.

If antigenic drift occurs in glycoprotein it's going to be more pronounced, WHY?

Because glycoprotein is the **exposed part for the immune system.**

What does that mean?

You have been infected with influenza virus, but the structure of the glycoprotein this time is a little different, so the pre form antibodies are not going to be affective enough to fully neutralize the virus and prevent its entry into the target cell.

In other words:

Every year, we have been infected by influenza virus, it enters our body, recognized by our immune system to produce antibodies,
but when we have antigenic drift -every 3 to 5 years- the virus becomes more …… WHY?

Because the glycoprotein changes, and the already present antibodies become less effective.

Reassortment can occur naturally, as we said before, cell in a bird or pig can be infected by more than two viruses, and this concept is used in the production of vaccines for both, influenza and rotavirus.

5.4 vaccines

How can we use that concept in the production of vaccines?

Let's talk about influenza as an example, We have two types of influenza vaccines :

- Injectable form
- Intranasal

Intranasal is still not widely used in the whole world for vaccine, unlike **injectable form**.

Injectable influenza vaccine :

In the injectable form of the vaccine the virus is killed or inactivated and we don't usually use the reassortment concept .

How can we prepare it ?

we have 3 types of influenza, **A, B and C**.
C is not associated with illness, so we will deal with **A and B.**

A is more problematic and more virulent to humans. In influenza vaccine we have 2 **A** and 1 **B** strains. We can take the three types of influenza and grow them or culture them in eggs. They inject the virus into eggs and incubate it in the incubator at a certain degree, 33 – 34 degree, in order to replicate viruses.

Now We have the 3 strains, we can put them separated from each other, then you harvest, combine them, treat them with **formalin** to kill or inactivate them, and then put them in the syringe to inject the patient.

What is the benefits of the previous process? How can we avoid the infection of influenza?

You exposed the body to most proteins of the virus, **especially glycoprotein.**

We take information about vaccines from our knowledge in previous years, what is the strongest virus, viruses which associated with deaths,,, … And then we put these viruses in our vaccine.

So, sensitizations against the glycoprotein produce antibodies against the most problematic viruses.

What are the **benefits** and what are the **side effects** of these vaccines?

Benefits:

We have production of memory cells and antibodies , if you are infected with the same virus (which was present in the vaccine) , memory cells are going to be activated, and we get a quick response from the immune system (because its already sensitized) , produce of antibodies that can neutralize the virus.

Side effects:

Remember that we give a killed virus vaccines, so there is no chance to become active again and cause infections.

Intranasal influenza vaccine

The second type of vaccines which is intranasal is **live attenuated vaccine**.
live attenuated vaccines are capable of replication inside our bodies, without causing symptoms.

how can they make this?

By culturing the virus at **sub-optimal temperature**, the optimal temperature is 34 or 35 , they still decreasing the temperature until reaching 24 or 23 , the virus become very weak .. it can replicate, but without causing symptoms.

General role:

Live attenuated viruses should not be given to **immunosuppressant patients**, because the immune system cannot get rid of viruses, and even a weak virus can become stronger and cause infection to the patient.

How it can become stronger?

We put it in the human body, when the virus is mutated, it's in high energy state (unstable) , it still trying to reach low energy state(stable) by producing mutations.

We have segments in rota and influenza viruses, we bring segments of glycoproteins from **2 A** and **1 B** to put them in the new virus, then grow it inside the egg.

Then, glycoproteins from the three viruses will appear around the virus, then when you give this vaccine in intranasal form, the virus is still live and attenuated, it is capable to replicate but without symptoms.

Rotavirus has the same mechanism but vaccines given as **oral drops.**

Best of luck ☺