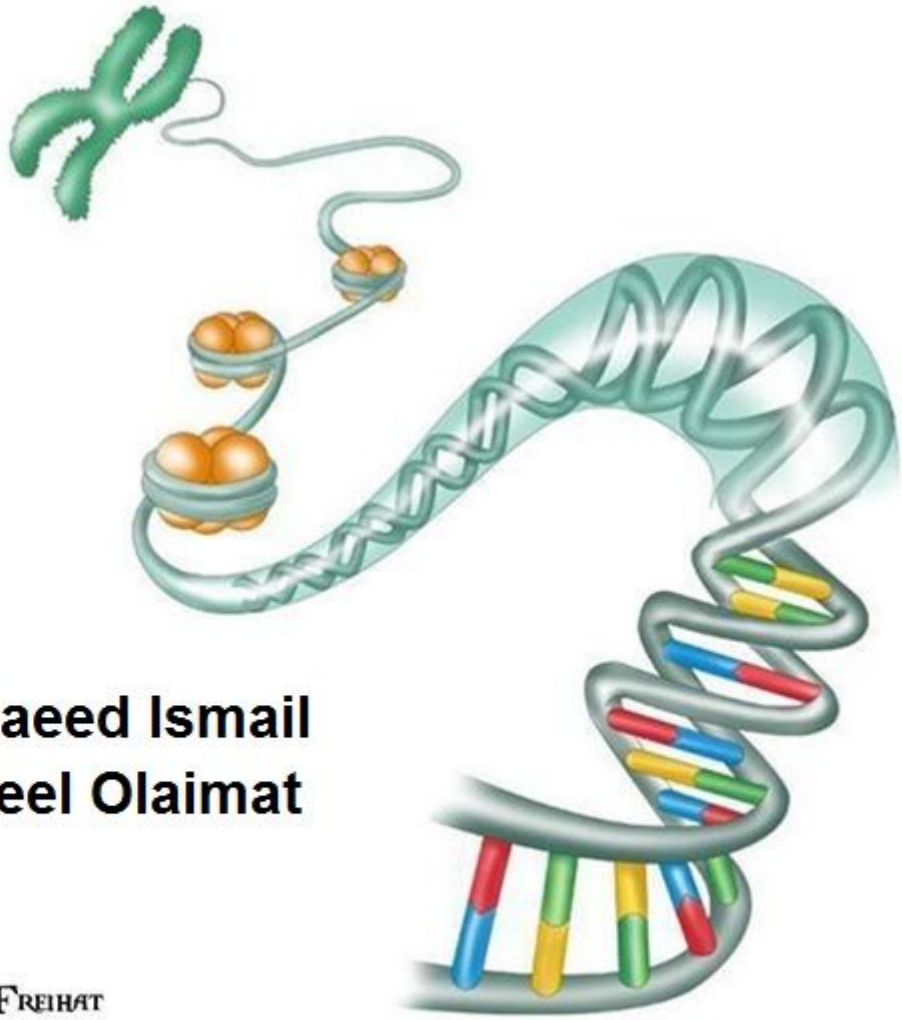
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ○ Sheet ○ Handout ○ other.....



Sheet#: 18

Dr. Name: Saeed Ismail

Done By: Aseel Olaimat

Designed by Nadeen Al-Freihat

DNA Mutations, Rearrangements, and Repair

Assalmu alaikum ♡

Dr.Saeed started by correcting that amino-acyl tRNA synthetases are 20 in number not 50 (1 for each amino acid).

Generally, mutations are:

1- Small scale mutation (Substitution, Deletion and Insertion).
2- Large chromosomal Rearrangement (DNA arrangement).

>Small Scale<

Point mutation = Single base pair substitution or SNP (single nucleotide polymorphism): one nucleotide which affected by the mutation.
Eg. AT pair ➔ GC pair.

*Polymorphism: a change in the sequence that involves nucleotide and this is the most common variation and mutation happens in Human genome.

*We as Human beings might differ in one nucleotide for every thousand nucleotides, so we are 99.9% identical.

Mutations are mostly point mutations and more common than insertion and deletion (which are more serious).

Q: why the point mutation that are less serious are more common than mutations that are more serious (insertion/deletion)?

The more serious the mutation, the more serious the disease that result, the less likely is the person carrying that mutation to live long to pass it to next generation (he will die at 12 age, he will not married and give his mutation to his children – so no transmission to next generation).Whereas who has less serious mutation will live enough to pass his mutation to the next generation (cuz it's must less affect his life production).

*Pay attention that we care about protein level in mutation.

Point mutation types:

* **Silent mutation**: Does not result in a change in amino acid sequence of protein (change in the nucleotide level but there is no change in the protein level).

And what I concerned with is the protein level, so if there is no change in the protein level (same protein), in this cause we have silent mutation.

e.g. CGA ➔ CGG : both code for Arg.

Affect the 3rd position of nucleotide, why? cuz redundant nucleotides in genetic code differ in the 3rd one means that : A.A which has more than 1 codon , it's codons differ in the 3rd position.

***Missense mutation**: Results in a change of amino acid.

e.g. CGA ➔ CCA : Arg ➔ Pro

Will this cause a disease for the cell that requires this gene? It depends on position of A.A which determine the importance of this A.A and if the new A.A similar to old one in its properties (like non polar, small and hydrophobic) and can fill the same gap, so the overall structural of protein will not change and function will not too.

New A.A could be completely different from the original one which causes structural change in the protein and as a result, Completely Loss of function will happen because the position of the old A.A is very critical to maintain the structure of the protein. (Remember that there is relationship between the function and the structure of the protein).

And if the old one was potent gene ? Disease may result as a mutation in Gb gene (Thalassemia and sickle cell anemia).

*__Nonsense mutation__: The base change results in a stop codon.
e.g. CGA ➔ UGA.

The resulting codon after point mutation will be stop codon and of course will affect the function of the protein cuz it will produce a much shorter protein and may not function at all.

***__insertion and deletion__ ➔ causing frame shift.**

When we insert or delete one nucleotide following the site of insertion the overall sequence changed.

There is exception where frame shift less happening and the causes less damaging: when we add or delete 3 or multiple codon in frame.

Even though the addition of 1 extra A.A will cause less damage than frame shift, will it have an effect in the protein level?

It depends, if it was added at a place within polypeptide which طرفيis not important to main structure motif, it will not affect.

* If it was added in a place which affect protein structure ➔ protein will lose its function.

But most likely addition of 1 A.A doesn't affect.

SICKLE CELL ANEMIA

Switch from TA to AT, SNP.

Glutamic acid (-ve , polar , acidic and hydrophilic) to Valine (non-polar and hydrophobic)

hydrophilic A.A located in the surface of the protein facing water , when valine (hydrophobic) substituted glutamic acid, Valine will fall down to escape the water and make a groove in one side which will meet a protrusion in another side of globin chain ➔ so they now fit each other as a Lego and starting to elongate and making fibril of hemoglobin which will extend within the RBC (changing in its shape from globular or circle to sickle shape) , and instead of freely moving of Hb within the RBC , There will be limited movement.

But why is that bad?

1- Thrombosis == accumulation of Hb fiber will block small capillaries.
2- Oxygen transferring ability will decrease cuz concavity shape will decrease. Concavity shape of RBC maximizes the surface area to increase transferring of Oxygen ... So it's an effect in structural and functional level.

Q: How these mutations Happen? It's due to external factors, not internal, these mutations happen during DNA replication, when DNA polymerase works it may make a fault; for eg. Transcript A instead of G , but this occur in bacterial cell not Human , why ? Cuz we have fidelity which is due to 3' == 5' exonucleases which let only 1 error per 100 billion.

* So according to evolution thinking: 🎗

If we say Human being is still evolving or they are supposed to evolve, why their DNA polymerase that accurate?

Bacteria polymerase error rate is 1 every 10,000 so in 3,000,000 nucleotide will have so many 100s of mutations (Whether these mutations are advantages or disadvantages ... It's How bacteria evolve)

But in human, when we say fidelity 1/100 billion, it means our polymerases tend to preserve its code as it is.

لقد خلقنا الانسان في احسن تقويم We've the best code; there is no need to change" it. :)

So Dr. Saeed believes that our mutations are due to external factors as Radiation - chemicals - pollution rather than an error in DNA polymerases.

<Genetic Rearrangement (Large chromosomal scale)>

A- Homologous Recombination(normal process) : sort of chromosomal level rearrangement that happens naturally , during meiosis where homologous chromosomes aliens next to each other - because they have similar sequences - and they will catch each other and exchange their pieces together.

Homologous recombination = crossing over; exchange of equal parts of homologous chromosomes during meiosis ... It's useful because it's a type of biodiversity.

Notice that we aren't 50%paternal and 50% maternal origins ➔ due to cross over.

For example : chromosome 9 , you can't claim paternal line is 100% paternal and maternal line is 100% maternal == but may paternal line is 30% paternal and 70% maternal and maternal line is 70% maternal and 30% paternal.

So father gives to his offspring his mixed chromosomes and also these mixed chromosomes will be mixed again with the mother chromosomes during meiosis ➔ ↑ Biodiversity. Don't care about "Holiday structure" which mentioned in the slide.

B- Translocation

Same mechanism, its exchange in large chromosome pieces but between non homologous chromosomes.
eg. chr(9-22)/chr (8-14)

- not equivalent portions and doesn't happen in meiosis and embryogenesis, it does occur in adult cells as a result to radiation, chemicals and smoking.

- So it's unequal and non-homologous chromosome exchange in short or long arms of the chromosome and it's associated with Diseases most likely leukemia & lymphomas.

C-Rearrangement of stem cells - esp. Precursor of B-cells which produce Abs (antibodies).

There is huge portion of chromosome 14(immunoglobulin region) containing so many different exons (where immune cells use to make Ab against different Ags)

Whenever immune cells counter different Ag. , they use different exons , different combination and different cocktail to make different 3aseer aah I meant different Ab. :P Now when these precursor B-cell (which make many types of antibodies) decide to dedicate one of their daughter B-cells to make one Ab against certain antigen , she doesn't give all its DNA , she gives just a part !

this mother (B-cells) which make 20,000 Ab type with her 20,000 exons , when her daughter decides to make Ab against Ag , will not give all her DNA , she will give it only 3 exons to A.A ➔ and this is a type of arrangement.

This is very specific example, and In other words : Stem cells(Immature precursor of B-cell in long arm of chr. 14) contains many exons to enable cells to make different Abs , and the DNA of immature mother will not pass all DNA to daughter cell ... And we consider that as a genetic rearrangement.

D- Transposons:

Firstly, it's a general event and happened on all cells.

So they are Jumping genes, jump from one place to other, we've 10s of them in our genome, they code an enzyme transposase, once it produced at a protein Level it goes back to same sequence (gene) which produce it and transfer this gene to another place and so on, and How it identifies it ? By border sequence called direct repeats.. So it will cut at this Direct repeats and join it randomly into completely different base on another chromosome, and it keep jumping in non-coding region, but may jump in coding region too which will causes problems.

Transposones:

1-**Classical** (transfer or jump as DNA from one place to another).

2- **Retrotransposons**, they don't migrate physically from place to another, they copy themselves through mRNA intermediate and send that copy to somewhere else.

Then it makes RNA copy and reverse transcript RNA to DNA . Then this DNA copy will ligated to another place. So it did actually replicate itself through RNA intermediate which is very similar to Retro viruses (RNA viruses) ➔ reverse transcriptase

* Remember:

Reverse transcriptase enters our cell and reverse transcript RNA to DNA then integrate randomly.

Hence, suggestion that there is association between retro viruses and retrotransposons, they replicate from RNA intermediate and both have sequence (Direct repeats in retrotransposon) and (Low terminal repeats in retro viruses).

Almost identical and both they are almost same behavior and sequence and numbers.

* There are 2 theories:

1- Vast majority of retrotransposons were their origin is retrovirus that infected our ancestors then started to pass them.
If this side of the theory is right, retro virus to be passed to next generation must enter germ cells which fertilize other germ cells and give new spring which has the virus.

2- Other tells that retrovirus came from retrotransposons.

Once upon time, retrotransposons were jumping and one time it got emerged and jump out of the cell, it's cell hydrolyzed and it's RNA surrounded by an envelope.

Ω 3rd evidence that they are related is: most of our oncogene is formed in retro virus (C-mech and v-mech?)

How oncogenes come to the virus?

When transposons (I think here dr. Meant retrotransposon) transcript itself as a sudden he saw an oncogene and transcript it as well as and also

when he exit the cell he took this oncogene with him , Which gave it a survival advantage.

And once it's in another cell, it can switch it on.

<Simple introduction to Repair>

We've very efficient repair mechanism, to imagine this, being under the UV light(mutagenic factor) for 30-60 mints will lead to accumulation of many mutations (around 70,000 mutation in each cell in our body) which efficiently repaired by repair mechanism.

So any one born with one mutation affecting its repair pathway 90% will have cancer, cuz there is balance between mutagenic stress (factors that cause cancer) and DNA repair mechanism.

There is specialization in repair mechanism, for eg. : this set of repair enzymes just specialized in repair thiamin dimer which happen due to UV light and that group of repair enzymes just to repair damage which caused by Benzo[a]pyrene in smoking.

Regardless to all of this, there are 4 main steps in all repair mechanism:

1- **Recognition**, DNA damage is recognized, which is not easy in the molecular level.

2- **Removing** the damage by nucleases whether endo or exo.

3- **Filling** the gap with new nucleotides by DNA by polymerase (minor ones).

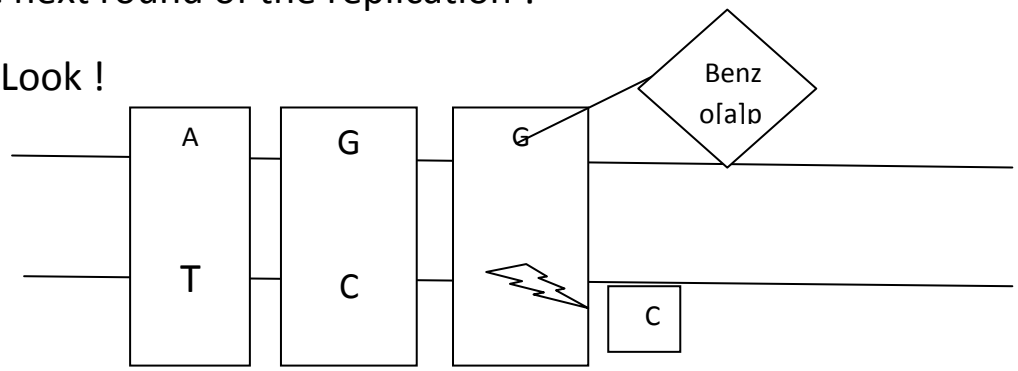
4- **Joining** Last bits by ligase.

***mutagen** can be chemical or radiation.

Eg. On How external factor (chemical) cause mutation , Benzo[a]pyrene(can be found in cigarettes) , chemical compound that cause carcinogens , it interacts with DNA components , no one should interact with DNA at all , DNA should be preserve and intact , anything will react with it will cause damage. .

Benzo[a]pyrene has high affinity (chemical affinity) with guanine that is higher than affinity of G to C , so when Benzo[a]pyrene come will remove C and replace it , causing Hump in the double strand, but till now , there is no mutation ,Cs there and Gs there ! ok there is distortion in the double helix but there is no change in the sequence till now , but the problem will arise in the next round of the replication !

Look !



Look at the strand which has G which still helped be Benzo[a]pyrene , DNA polymerase appeared , he saw A so he put T , and he saw G he put C till he reached G- Benzo[a]pyrene , He said what is this ? Is it A ? Is it G ?

If he identifies it as a G and put C ➔ no problem .

If he said it looks like A and put T ➔ it will be G-T base pair .

In next round of replication this T will incorporated with A , so it is 2 cell divisions :

G-T and T-A

What about Benzo[a]pyrene ? will it be there ?

It depends if the person is still smoking or not

So please stop smoking, please. Yes you, do you hear me? stoppp it .

There is another scenario, if the DNA polymerase does not recognize it as A or G, the DNA polymerase may skip it which will lead to deletion (frame shift) and as a result there will be big problems.

The End ..

I' m totally mrash7a so Pls forgive me .

This sheet dedicated to Aseel Al-Khateeb ♡

قال الإمام محمد الغزالي :

" إننا لو وصلنا الليل بالنهار دأبا ، ثم حرمنا عنياة السماء فلن نحصل من تعبنا إلا بوارا"

Aseel Olaimat