University of Jordan
Faculty of Medicine
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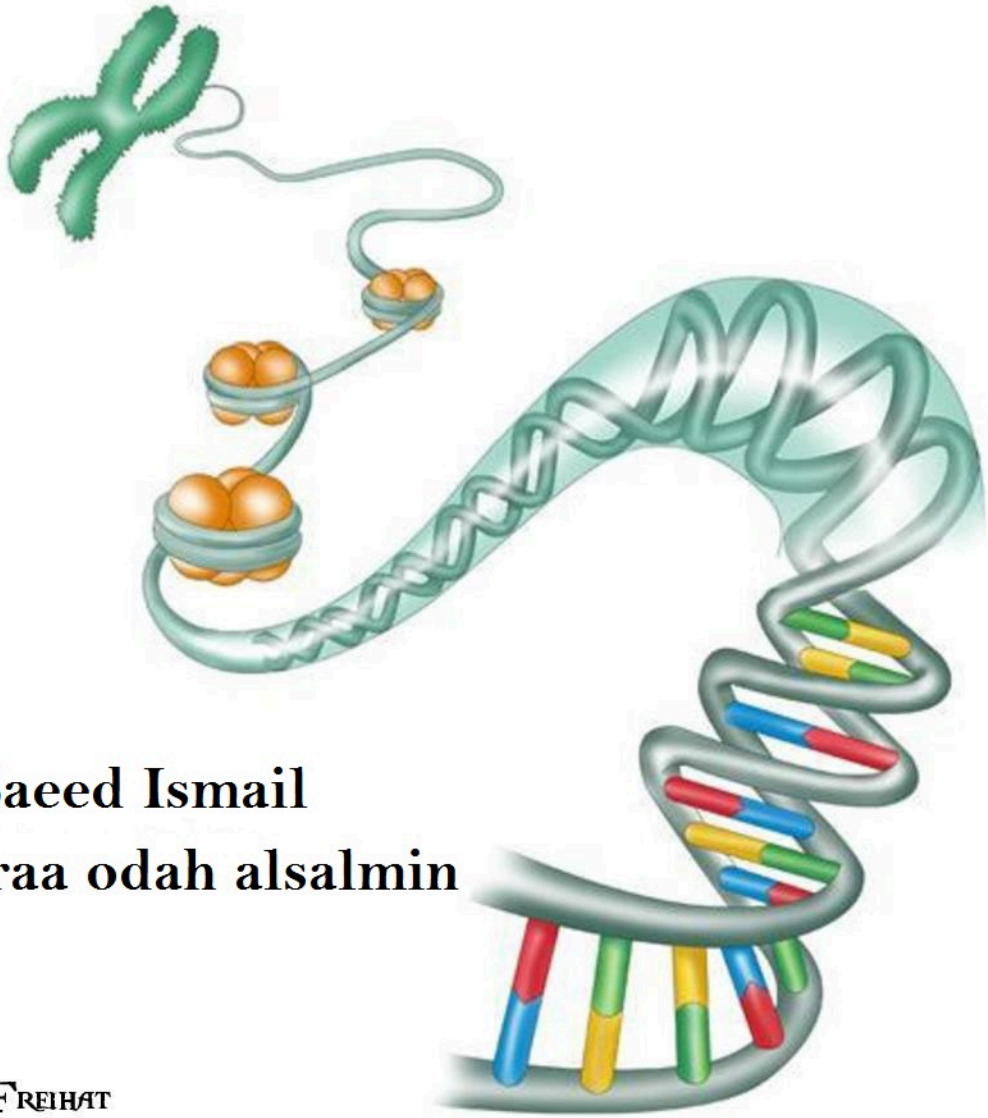
Medical Committee
The University of Jordan

Genetics & Molecular Biology

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



Sheet#: 19

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بسم الله الرحمن الرحيم

DNA Repair and Regulation of gene expression

DNA Repair:

Different damages in DNA will be fixed by different mechanisms of repair but the general scenario is:

1- Recognition; where DNA damage is recognized

2-Removal of damage region

3-Filling the gap by one of the repair DNA polymerases

4-**Ligase** in order to join the ends.

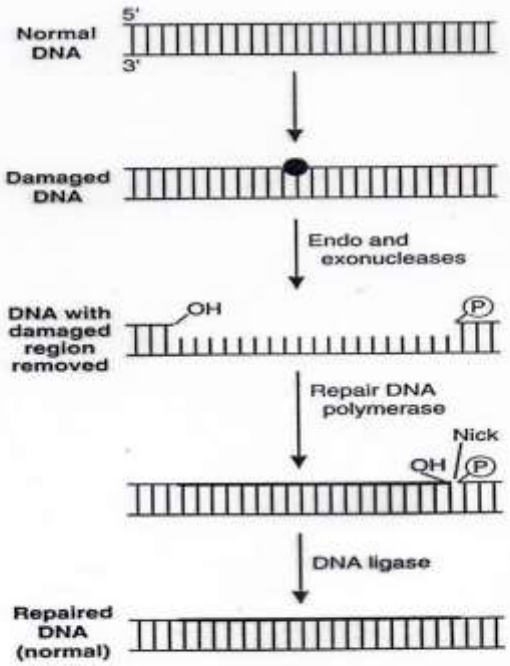


Fig. 13.14. Common steps in DNA repair mechanisms.

❖ Types of repair :

1-nucleatide excision repair (NER):

Deals with local **distortion in the DNA double helix**.►

When does distortion occur in the double helix?!

1- Mismatch repair.

2-Bulky adducts

> (From Wikipedia: *A bulky adduct is a piece of DNA covalently bonded to a (cancer-causing) chemical. This process could be the start of a cancerous cell, or carcinogenesis. Disruption of the molecules' regular helical structure occurs by introducing non-native chemical bonds or bulky adducts that do not fit in the standard double helix. When a chemical binds to DNA, the DNA becomes damaged, and proper and complete replication cannot occur to make the normal intended cell.. Without effective DNA repair, which happens naturally under normal circumstances, this can lead to carcinogenesis, the beginnings of cancer.)*

Benzopyrene separates Guanine from the coupled cytosine causing a hump in DNA structure.

3-Thymine dimer

Thymine usually binds to adenine in the *opposite* strand of the double helix by a hydrogen bond. However when we're exposed to UV light, adjacent Thymines on the *same* strand of DNA become cross-linked. These two Thymine bases will bind to each other by a strong covalent bond, and because covalent

bonds are much stronger than hydrogen bonds, the binding of the two ADJACENT Thymine bases will also cause separation of these thymines from the OPPOSITE adenines -to which they were hydrogen bonded- forming a hump. This problem is solved by special enzymes of the NER.

So any damage that would cause **damage to the double helix** will be repaired by nucleotide excision repair.

Nucleotide excision repair steps :►

1-Recognition: The damaged region is recognized by certain enzymes.

2-Removal of the damaged region "degradation" by **endonuclease**.

Pay attention that NER differs from any other repair mechanism in this step.

How?

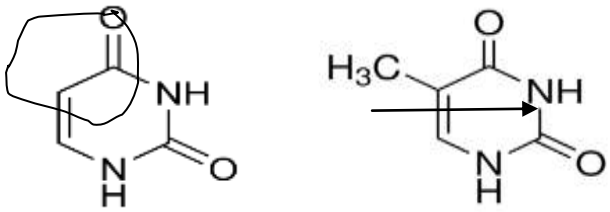
The endonuclease does not only remove the damaged nucleotide, it removes the entire region around the damaged part.

3-filling the gap by one of the **DNA polymerases**.

4-**Ligase** then joins the ends.

2- Base excision repair (BER):

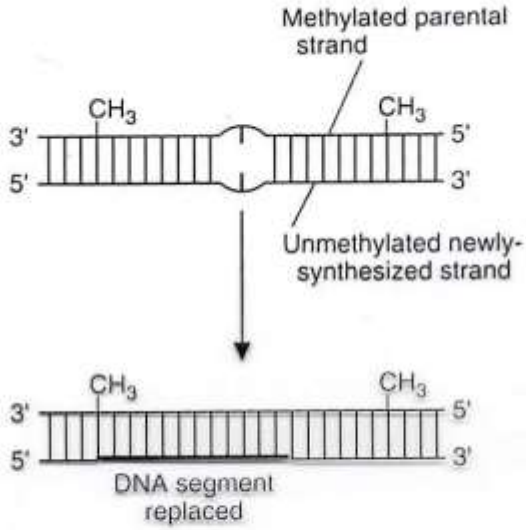
►Here the damage does not cause distortion in the double helix like damage repaired by NER. The damage is so subtle, for example it could a missing methyl group from thymine so thymine become Uracil (recall that Uracil = Thymine – methyl group), this is a mismatched base.



►How is this problem solved?

By an almost surgical method:

- First, the damaged base will be remove then the phosphate group then the sugar.



Note: <u>Only the damaged base is removed and not the whole area.</u>

- Then the DNA polymerase and ligase will complete the repair process.

<u>3-Mismatch repair:</u>

►There is no damage here; the nucleotide is normal structurally but **it is in the wrong place** (e.g.: A-C, both are nucleotides are normal but they are mismatched)

Repair steps: same scenario ►

1-Recognition

2-Removal of mismatched base

3-Filling the gap by one of the DNA polymerases

4-Ligase to join the ends

The strong question :p BUT,

If we have A-C which one will be removed?

A-remove A and put G

B-remove C and put T

C-all of the above

D-none of the above

E- A but may be B

OK! TIME IS UP :3

The answer is it's all about **<u>methylation</u>**

First you have to know where the original sequence "reference" is and where the new one is because you will assume the new one is wrong.

How can you determine the reference strand? Which one is the old one and which strand is the new one? Differentiation in prokaryotes is by **methylation**.

What is methylation? When DNA is formed in prokaryotic cells, there will be a process called DNA methylation. So any nucleotide that is not mythelated is a new nucleotide not an original one, and enzymes always remove the new nucleotide, but we do not have methylation in our cells so this repair mechanism rarely happens in eukaryotic cells. Our polymerase does not usually make mistakes but if it happens, the mismatch will be repaired by unknown mechanism.

4-Transcription-coupled repair :

►Occurs during transcription

For example, there is an A-T base pair but there must be a G-C base pair instead. RNA polymerase starts copying this gene but then it will stop and say: "Wait, this is supposed to be a G-C pair why is it an A-T pair? ((خير ان شاء)) there is a mutation here

Then RNA polymerase calls the repair enzymes "Cut ..Put the right one ..Join then I will complete the transcription."

❖ Regulation of gene expression

How do we switch on our genes? Is it possible? How do we control their expression level? But on the other hand what is the importance of this? Is it actually beneficial? Or is it a waste of time? These questions will be discussed in chapter 16 (faisal alqasem style)

Let's begin ☺

All of our cells have the same set of genes; the 25000 genes in skin cells are the same as the 25000 genes in neural cells for example. If all of our cells contain the same set of genes then what makes our cells different?! That's due to something called the **expression profile** which simply tells us which genes are expressed in different cells.

From Wikipedia: *the sequence tells us what the cell could possibly do, while the expression profile tells us what it is actually doing at a point in time*

From our first zygote cell until we become billion of cells we have had the same set of genes. What happens is that that skin cells have chosen 7000 genes out of 25000 genes to play with. These genes are necessary in skin cells while the rest 18000 genes might not be switched on by this cell at all. In order for neural cells to become specialized they need to 8000 genes, hepatocytes need 20000 genes. So each cell has chosen a set of genes according to what it needs.

How can the cells switch on a certain set of genes and switch off other group?!

Recently Scientists got the idea to activate some inhibitory genes in skin cells and thus convert skin cells into neurons. They did not stop to this extent; they were more ambitious; they converted skin cells to stem cell and to the first zygote.

Something called **"induced poly potent stem cells"** where they took a skin cell and affected its genes by 3 keys (which we will mention) allowing the cell to switch on all its genes and go back to being an embryonic stem cell or a zygote. Then scientists exposed the zygote to a specific condition and were able to make skin cells or blood cells from this zygote. Actually they can make a whole organism by this zygote.

Again how could this occur? By keys.

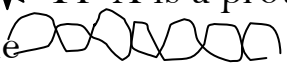
What are these keys? **Transcription factors** and the key locks are **promoters**.

So promoters control the genes and transcription factors control the promoters.

Some TF activate one gene, another TF activate several genes, and the masters of TFs control the whole genome.

This means these TFs (masters) control all other TFs subsequently these control all genes.

Imagine this story!

This gene  has a promoter  and this promoter has own TF called TF(A)  TF A is a protein encoded by a certain gene and this gene  has a promoter  and this promoter has its own TF called TF B  and TF B is a protein so it's encoded by a certain gene and this gene  has a promoter  that has own TF(C)  ....so you keep going up until reach certain TF 2 or 3 that control all other TFs which can control our genes .

"Once you have power and control over all your genes you can make cells become whatever cell you want and this called **retro-differentiation (dedifferentiation)**"

►The ability of cells to switch on/off certain genes when needed depends on:

1-<u>Developmental period</u>: embryonic cells need a different set of genes than the adult cells.

2-<u>Differentiation stage</u>: hematopoietic stem cells need a different set of genes than lymphatic stem cells.

3-<u>Enviromental changes</u>: exposure of cells to environmental conditions will induce gene expression changes e.g.: Skin cells exposed to UV light will induce the expression of the gene that is responsible for the production of melanin. So the expression profile of every cell is constantly changing.

But there are genes that are always on and others that are always off and a good number of genes that are being switched on and off.

For example:

Some genes are not expressed by liver cells. On the other hand some genes are expressed continuously and some genes are expressed under certain conditions and when exposed to certain toxins like: drugs, smoking.

The genes that are expressed all the time are called "**housekeeping genes**" and are necessary for daily activity of the cell in other words: genes that are required by so many cells just to survive. When these genes are turned on they are turned on at a volume, so there's a bit of tuning it isn't just on and off; *there's a level of expression for each gene.*

Some genes are always off like insulin gene which is only expressed in one kind of cell "beta cells of the Langerhans", 99% of our genes will never use insulin gene so it is there but switched off.

Now we will discuss the genes that switch on/off continuously in prokaryotic then eukaryotic cells.

►Prokaryotes differ from eukaryotes in regulation by having:

1-Operons:

Operon is a group of structurally/ functionally related under the control of the same promoter.

The bacterial cell is going to use their genes simultaneously, these genes give free enzymes to utilize lactose so when there's no lactose it will not express these genes, but when it has lactose it will use these genes. These genes which are called Operons give one MRNA called **polycistronic MRNA,** which is then translated to 3 different proteins.

The MRNA that gives one Protein is called monosictronic RNA. Prokaryotes have poly and mono but Eukaryotes have only monosictronic.

From the internet:
each eukaryotic mRNA contains information coding for only one protein, hence monocistronic, whereas prokaryotic mRNAs may encode more than one protein and are said to be polycistronic.

The rows of seats in Bahjat provide a useful analogy. The lights in each row (4 lights for example) could all be turned on by one switch (Polysictronic, prokaryotes). If every light of the four lights had its own switch, you'd have higher control even if you wanted to use these lights at the same time (monosictronic, eukaryotes).

2-Operator:

"A DNA sequence found in prokaryotes just downstream of the promoter, at which certain proteins called repressors can bind as an extra check point after the promoter to stop the action of RNA polymerase"

In some cases the **repressor** is always bound to the operator. If we want to transcribe the gene we need another protein called the inducer, which binds the repressor and removes it allowing RNA to transcribe.

Again:

Operator is a DNA sequence just downstream of promoter and attracts the repressor.

Repressor is a protein bind to operator to prevent transcription.

Inducer is a protein that removes the repressor allowing RNA to transcribe.

4-Co-repressor:

In some genes there is no repressor, so RNA polymerase keeps transcribing the gene but if we need to stop expressing it we put a repressor by the help of a co-repressor.

Eukaryotes do not have Operons, Operators, Repressors, Inducers or co-repressors.

5-Attenunation of transcription:

In absence of a nuclear envelope, nothing will stop translation from starting before transcription has ended.

So the ribosome working here with RNA polymerase is still there (coexistence between transcription and translation).

This happens in prokaryotes but of course does not happen in eukaryotic cells where transcription must be complete before mRNA goes to the cytoplasm to be translated.

يعطيكم العافية☺

Special dedication to my mother.

and dedication to Salam alkhreasha Ghaida khresat Tasneem abu yameen, Hana haimour , Areej abu zir and my genes Whether they expressed or not .

written by : Esraa odah alsalamin ☺