Genetics & molecular biology

◉ Sheet ○ Slide

Number:	7
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In this lecture, we will be discussing DNA mutations and DNA repair mechanisms.

DNA Mutations

There are 2 types of mutations:

1- **Micromutation**: It involves **small regions** of the DNA that **can't** be seen by the **naked** eye, consequently; we will have to do **PCR** or **DNA sequencing**.

"The type of mutations we will be discussing in this sheet"

2- **Macromutation**: Is a mutation of a **large phenotypic** effect that involves the chromosomes **as a whole**.

"Will be discussed with Dr. Belal"

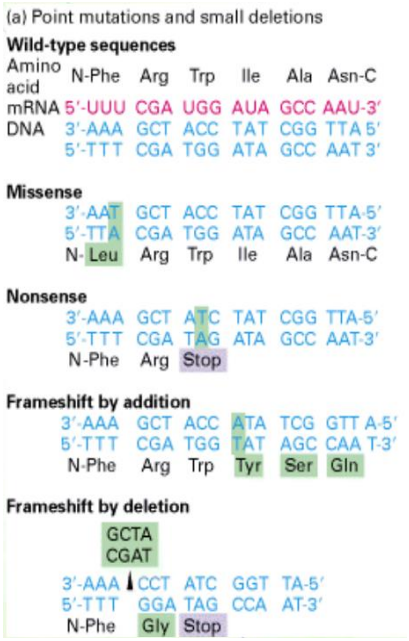
Micromutations can be:

A- **Single point mutations**: Mutations in a **single nucleotide**, which can result in multiple effects such as:

1- **Silent:** A change in **one** nucleotide leads to the formation of a **new codon** that encodes for the **same** amino acid as the **original one**.

➔ **Net Result**: It does **not** lead to any change at the **protein level** but contributes to **genetic variability** among individuals.

> ***Note:*** *A codon is a sequence of **3 nucleotides** that codes for either an **amino acid** or a **stop codon**.*



2- **Missense:** A change in **one** nucleotide leads to the formation of a **new codon** that encodes for a **different amino acid**.

Example: *Sickle cell anemia; a change in one nucleotide changed the amino acid from **Glu** (its codon: GAG) to **Val** (its codon: GTG)*

3- **Nonsense:** A change in **one** nucleotide lead to the formation of a **stop codon** causing **premature termination** of protein synthesis.

4- **Frameshift:** A change in **one** nucleotide *(either by **addition** or **deletion**)* leads to the change in the amino acid **sequence**, usually its effect is **great**.

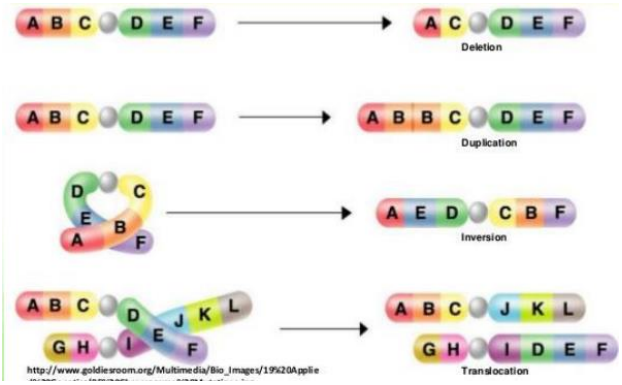
> ***Note:*** *In the case of **Nonsense** and **Frameshift** mutations, the effect depends on **where** the mutation occurs. If it's at the **end** of the gene, the effect won't be as great as if it occurred at the **beginning** of the gene.*

B- **Deletions** of a **few** nucleotides to **long** stretches of DNA.

C- **Insertions** and **duplications** of nucleotides or long stretches of DNA.

D- **Inversion** of DNA segments.

E- **Translocations**, that bring **different** regions of gene segments **together.**



***Note 1**: Please note that mutations (B-E) involve mutations in **more** than **one** nucleotide unlike the single point mutations.*

***Note 2**: **Macromutations** include the **same** above-mentioned mutations **except** for the single-point mutations.*

Causes of DNA Mutations

The causes of DNA mutations can be either **Spontaneous mutations** or **Induced mutations**.

1- **Spontaneous mutations**: They are naturally occurring mutations that arise in all cells from a variety of sources, including **errors of DNA replication** and **spontaneous lesions**.

A- Errors of DNA replication. DNA polymerase can cause mutations in 3 ways:

1- Formation of **inaccurate** nucleotide pairs (A-C or G-T) leading to **base substitution**.

2- **Frameshift mutations**: Insertion and deletion of one or a few bases can change the reading of codons leading to changes in the amino acid sequence of the produced protein.

The DNA polymerase can also cause frameshift mutations in regions of **repeated sequences**.

***Explanation:** Imagine you have a sequence of (CCC) repeated for **8 times**, so that's **24** nucleotides in total. Now, when the polymerase reaches nucleotide number 21 it might **lose** count. So, the Polymerase here has 2 choices, either **stopping** the sequence and adding a nucleotide **other** than C leading to a frameshift mutation, **OR** continuing the sequence with an **additional** number of nucleotides, so instead of adding **4** more nucleotides, it adds **7** nucleotides (just in case) leading also to a frameshift mutation.*

3- **Large deletions and duplications** also often occur at repeated sequences.

There are many diseases related to mutations altering the number of repeats:

1- **Deletion due to a three-base-pair repeat**.

Example: Kearns-Sayre syndrome: mitochondrial encephalomyopathies.

2- **Expansion of a three-base-pair repeat** *"instead of 10 repeats we have 20 or 50 repeats".*

Examples:

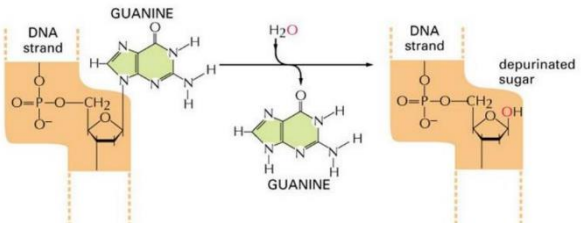
- Fragile X syndrome (CGG repeats in the FMR-1 gene).
- Kennedy disease (X-linked spinal and bulbar muscular atrophy "CAG repeats in the androgen receptor").
- Myotonic dystrophy (CTG repeat in the non-coding region of a kinase gene).
- Huntington disease (CAG repeats in HTT gene).

> ***Note:*** *The doctor didn't focus on this part much, memorize it just in case.*

B- Spontaneous lesions *"2nd spontaneous mutation"*: They are a naturally occurring type of DNA damage that can generate mutations such as **Depurination**, **Deamination,** and **Oxidatively damaged bases**.

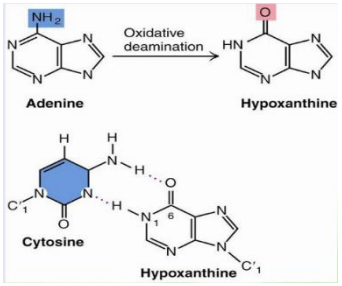
1- **Depurination**: It is the cleavage of the **glycosidic bond** between the base and deoxyribose creating **apurinic sites** (AP sites). This will end up with a site that has sugar + phosphate **only** without a base.

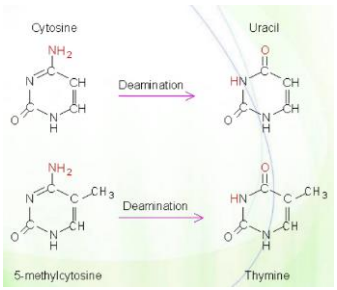
Now, when the DNA polymerase arrives at this site it either **stops** the synthesis which will lead to **cell death** or it adds a **random** base out of the 4 bases *(the chance of adding a wrong base is 75%)* leading to a **single point mutation**.



2- **Deamination** is the removal of the amine group on the nitrogenous bases, examples:

- **Adenine** usually pairs with **Thymine**. However, **oxidative deamination** of **Adenine** yields **Hypoxanthine** which in return pairs with **Cytosine** instead of the usual **Thymine**.
- Deamination of **Cytosine** yields **Uracil** which shouldn't be in the DNA. So, if the cell is in a rush and didn't repair the **Uracil,** the DNA polymerase would recognize the **Uracil** as **Thymine** and pairs it with **Adenine** which should have been **Guanine** in the normal state *(U-A instead of C-G).*
- Deamination of **methylated Cytosine** yields **Thymine** *(which is normally present in the DNA),* so the pairing becomes T-A instead of C-G.





> ***Note:*** *All the previous examples on* ***deamination*** *lead to a* ***single point mutation****.*

3- **Oxidatively damaged bases**: In cells with a **high** metabolism, the production of **ROS** and free radicals increases, in which they attack anything (e.g. DNA) in order to gain an electron to become stable causing **DNA damage**.

----------*End of the 1st causes of DNA mutations: Spontaneous mutations*----------

2- **Induced mutations:** Produced when an organism is **exposed** to a **mutagenic agent**, or a **mutagen**, so it does not occur naturally.

A **mutagen** is an agent that **changes the genetic material**, could be a chemical, smoke, UV light, something you eat or drink, etc.

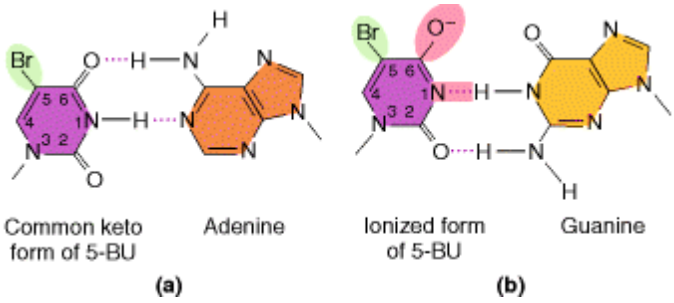
Mechanisms of action:

A- Incorporation of base analogs:

Base analogs have a similar structure to normal nucleotides and are incorporated into DNA during replication.

Example: **5-bromouracil** (5-BU), it is an analog of **Thymine**, so normally it pairs with **Adenine.**

However, when it's in the **ionized** form it pairs with **Guanine** instead. Therefore, in the 2nd round of replication G-C pairs form instead of A-T pairs *(single point mutation).*



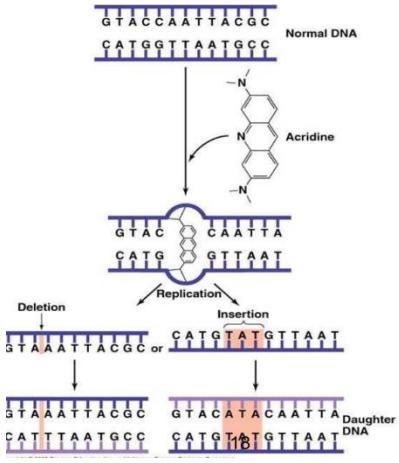
B- Specific mispairing: Altering an existing base chemically causing mispairing.

Example: **Alkylating agents** can transfer a methyl group to **Guanine** forming **6-methylguanine** (6-meG), which pairs with **Thymine** instead of the usual **Cytosine**. In the 2nd round of replication, T-A pairs form instead of G-C pairs *(single point mutation).*

C- Base damage: Ionizing radiation results in the formation of ionized and excited molecules that can cause damage to the DNA through Base damage, the creation of AP sites *(apurinic or apyrimidinic sites)* or strand breaks *(X-rays and sunlight break the phosphodiester bond between bases).*

D- Intercalating agents: They are planar chemicals such as **proflavine** and **ethidium bromide** that can insert themselves (intercalate) **between** the bases and cause single nucleotide pair insertions or deletions. These molecules are present **naturally** and are also present in **labs** *(used for DNA staining).*

Intercalating agents' mechanism of action: The DNA polymerase senses something abnormal when it reaches the area of the **intercalating** agent, this causes:

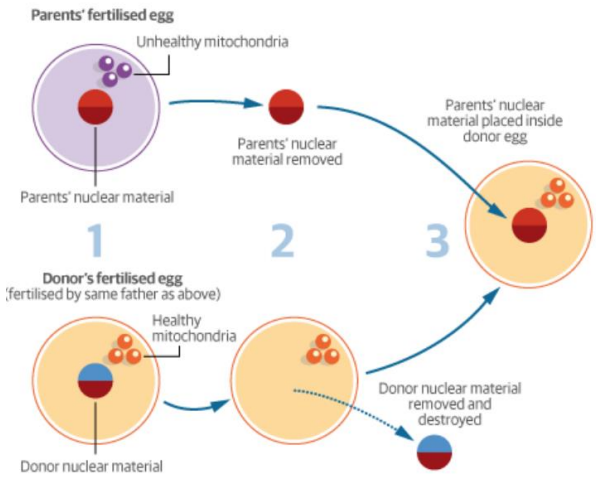


a- **Frameshift insertion**, if the DNA polymerase added a **random** base.

b- **Frameshift deletion,** if the DNA polymerase **skipped** the agent and the sequence **around** it.

Controversial issue: Three-parent babies.

A British woman had a mutation in her mitochondrial DNA, and she didn't want to transmit this mutation to her children. So, they got someone who donated her egg to the couple, and then we removed the donor's nucleus from the egg and kept the cytoplasm with the mitochondria as it is. Then we added the mother's nucleus to the egg then fertilized the egg with the father's sperm. We ended up having a healthy baby.



Note that sometimes we resort to using mitochondrial DNA to confirm paternity and that would be a problem in this case because the baby has 3 DNA molecules from the mother, father, and donor.

https://www.theguardian.com/science/2015/feb/02/three-parent-babies-explained

> ***Recall:*** *We took in the last semester that the mitochondria of the sperm gets destroyed by the egg cell after fertilization. That's why mitochondrial DNA is inherited only from the mother.*

A quick recap of all DNA mutations

SPM → Single point mutation.

Causes of mutations			Original Bases	Changes/Errors	Pairing Change	Type of mutation
Spontaneous Mutations	Errors of DNA Replication		-	Inaccurate pairing.	-	SPM
			-	Issues at repeated sequences/insertions/ deletions	-	Frameshift
	Spontaneous Lesions	Depurination	-	AP sites → Random insertion.	-	SPM
		Deamination	A-T *2nd round: T-A*	A deaminated into HX	HX-C *2nd round: C-G*	SPM
			C-G *2nd round: G-C*	C deaminated into U	U-A *2nd round: A-T*	SPM
			C-G *2nd round: G-C*	Methylated C deaminated into T	T-A *2nd round: A-T*	SPM
Induced Mutations	Base analogs		5-BU *(T analog)* **pairs** with A *2nd round: A-T*	Ionization of 5, BU	Ionized 5-BU **pairs** with G *2nd round: G-C*	SPM
	Specific mispairing		G-C *2nd round: C-G*	Methylation of guanine into 6-meG **pairs** with T	6- meG -T *2nd round: T-A*	SPM
	Base Damage		-	Chemically induced AP sites/ strand breaks	-	SPM
	Intercalating agents *(e.g. proflavine and ethidium bromide)*		-	Random insertion.	-	Frameshift Insertion
			-	Skipped sequence.	-	Frameshift Deletion

DNA Repair Mechanisms

Fortunately, our cells have repair mechanisms to **detect** and **correct** many types of **DNA damage**, such as:

1- **Prevention of errors before they happen**
2- **Direct reversal of damage**
3- **Excision-repair pathways**:
- Base excision repair (Specific excision repair).
- Nucleotide excision (General excision repair).
- Transcription-coupled repair.
- Mismatch repair.

4- **Trans-lesion DNA synthesis.**
5- **Repair of double-strand breaks.**
6- **Recombinational repair.**

Throughout this lecture, the mechanisms **1-3** will be discussed.

1- Prevention of errors before they happen

In our body, some enzymatic systems neutralize potentially damaging compounds **before** they even **react with the DNA**, for example:

Superoxide dismutase and Catalase.

- Reactive oxygen species are **unstable molecules** "free radicles" that **damage** the DNA and other parts of the cell upon **interacting**.
- **Detoxification** of reactive oxygen species and oxygen radicals happens through **Superoxide dismutase** (SOD) and **catalase**.
- **SOD** converts $O_2^{\bullet -}$ into $H_2O_2 + O_2$, while **catalase** converts H_2O_2 into H_2O.

$$O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

$$H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

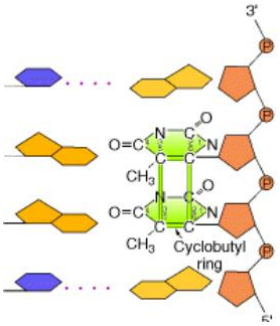
2- Direct reversal of damage

Some damages that affect our DNA can be **reversibly** repaired, two of such damages are:

a- Cyclobutene pyrimidine

- **UV light** that hits DNA results in the formation of a **covalent** interaction between two **adjacent** pyrimidine bases forming structures known as **"Cyclobutene Pyrimidine dimers"**. This most frequently occurs between two **Thymines**.

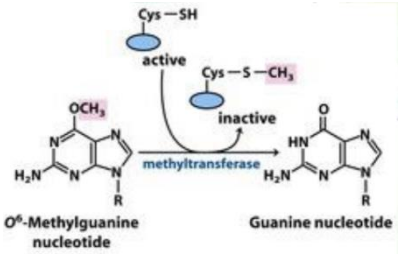
- If this damage **isn't repaired**, DNA polymerase would fall off the strand during the replication *"replication ceases"* and eventually the cell may **die**.
- This **mutagenic photodimer** caused by UV light "Cyclobutene Pyrimidine" can be repaired by a **photolyase** that has been found in **bacteria** but **not** in humans.



The enzyme **binds** to the photodimer and **splits** it, generating the **original bases**.

b- O6-methylguanine

- When a **methyl group** is attached to the **oxygen** atom in **guanine**, this produces 6-O-Methylguanine.
  It base-pairs to thymine rather than cytosine, causing DNA damage.
- This can be reversed through a **dealkylating enzyme**, called **O6-methylguanine methyltransferase.**



3- Excision Repair Pathways

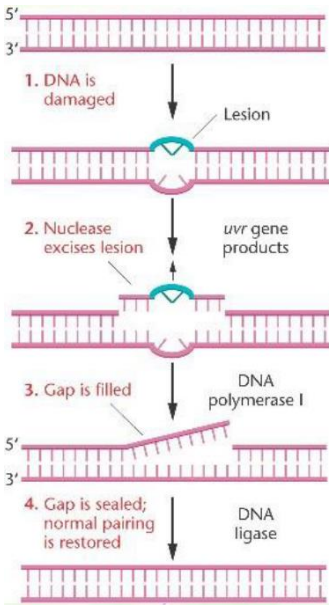
Excision repair pathway **types**:

First type: General excision repair *(Nucleotide Excision Repair "NER"):*

In Bacteria:

UvrABC protein complex is a system that includes:

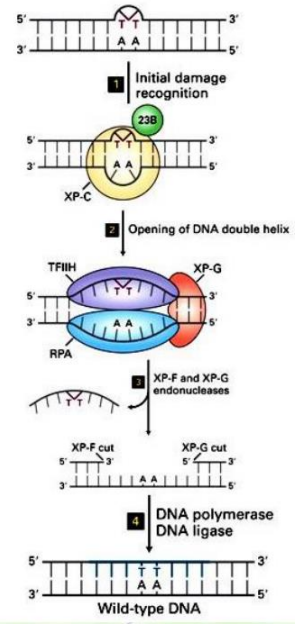
- ➔ Breaking of a **phosphodiester bond** on either side of the **lesion** (e.g. Thymidine dimer), on the **same** strand, resulting in the **excision** of an oligonucleotide.
- ➔ A **helicase** then comes in and **removes** the **excised segment**.
- ➔ The **gap** is filled by **DNA polymerase**.
- ➔ Finally, a **ligase seals** the breaks.



Recall: *A **pyrimidine dimer** can be **repaired** through **2** pathways, **General excision repair** (UvrABC complex) and **Direct reversal of damage** (photolyase).*

In Humans:

- In human cells, the process is **more complex** than how it is in bacteria. However, the basic steps are the same as those in **E. coli**.
- **XP proteins** play a key role in the nucleotide excision repair pathway **in humans**.
- The XP proteins are **7 in number** (XPA, XPB..., XPG). These proteins have **different functions** including **damage recognition** and **enzyme activities** (endonuclease, helicase) throughout the nucleotide excision repair (NER) mechanism.
- A transcription factor, **TFIIH**, plays a role in NER as a **helicase** that **unwinds** the DNA double helix after the damage is initially recognized.
- A single-stranded DNA binding protein called **replication protein A** (RPA) **protects** the **undamaged** DNA strand from the **nucleases** recruited throughout this process.



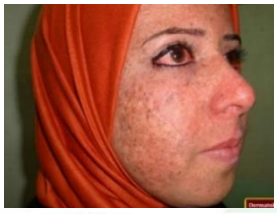
Second type: Transcription-coupled repair.

- Transcription-coupled repair (TCR) is a **subpathway of NER** dedicated to the repair of lesions located on the **transcribed** strands of active genes.
- In both eukaryotes and prokaryotes, there is a **favored repair mechanism** of the **transcribed** strand of DNA. When encountering a **lesion**, RNA polymerase **pauses:**

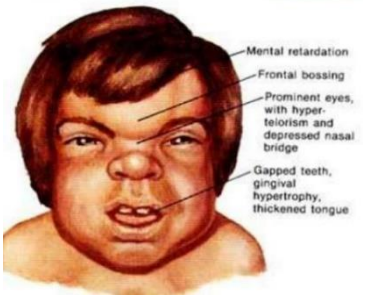
In prokaryotes, a transcription-repair coupling factor "*Mfd"* displaces the RNA polymerase. This recruits **UvrABC complex** to repair the lesion. Thus, Transcription can continue **normally**.

In Eukaryotes, the **CSB proteins** recognize the arrested RNA polymerase due to the lesion. This recruits **XP proteins**, **TFIIH** and other factors to carry out the incision, excision, and repair reactions. After that, transcription can continue **normally**.

Defects/mutations in the **NER** proteins results in **severe human diseases**, such as:



1- Xeroderma pigmentosum, which may lead to skin cancer. This occurs mainly due to a mutation in the **XP proteins**.

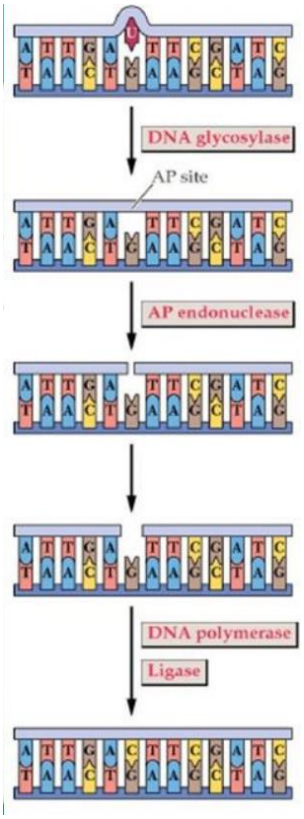


1- Cockayne's syndrome.

If the **CSB protein** is **mutated**, there would be **no** recognition and thus the NER pathway cannot function. This causes the **Cockayne's syndrome**.

Third type: Specific/Base excision pathways "BER".

- BER is initiated by **DNA glycosylases**, which is an enzyme that cleaves the **N-glycosidic** (base-sugar) bonds of damaged bases "specific", **liberating** the altered **base** and generating an **apurinic** or an **apyrimidinic** site, both are called **AP sites**.
  - ➔ Then an **AP endonuclease** cleaves the **phosphodiester** bonds at the **AP sites**.
  - ➔ The deoxyribose is **removed**.
  - ➔ **DNA polymerase** fills in the gap, and **DNA ligase** and re-forms the bond.



Recall: *In **BER**, **DNA glycosylases** cleaved the **N-glycosidic** bonds, then the **AP endonuclease** cleaved the **phosphodiester bonds**. This is unlike **NER** where, initially, the **phosphodiester bonds** were cleaved.*

- Numerous **DNA glycosylases** exist, example: **uracil-DNA glycosylase**, which removes uracil from DNA.

  Uracil residues, which result from the spontaneous deamination of cytosine, can lead to a **C →T transition** if unrepaired *"recall from DNA mutations"*.

Fourth type: Mismatch Repair *(Post replication system).*

In Prokaryotes:

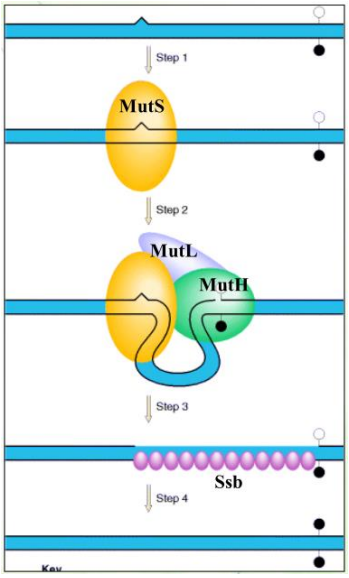
- DNA is **methylated** following **replication** by the enzyme **adenine methylase**. However, the methylation of the **newly synthesized** DNA "daughter strand" is **delayed**.

  Keep in mind that methylation can change the activity of a DNA segment, that's why we want everything to be perfect before methylation occurs.

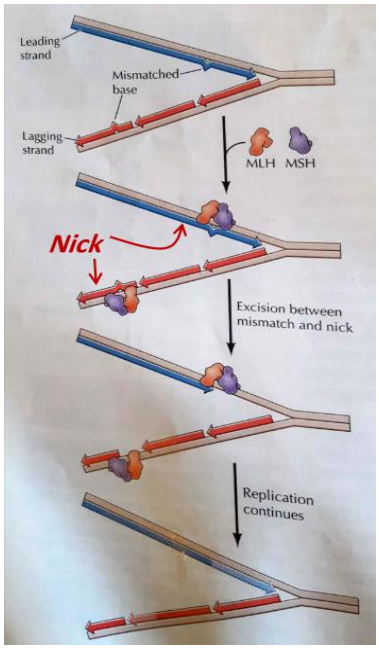
- The mismatch repair system in bacteria takes advantage of this delay to **repair** mismatches in the newly synthesized strand.
  1- This system **recognizes** mismatched base pairs. Then, it **determines** which base is the **incorrect one**, which is on the new **unmethylated** -yet- strand.
  2- Finally, it **excises** the incorrect base and carries out **repair synthesis**

- The past steps are mediated through the **"Mut" protein system.**

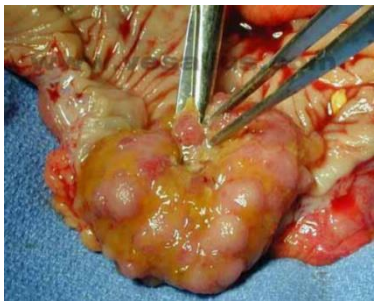
"The doctor didn't explain the steps in details so don't worry much about the figure".



In humans:



- The **mismatch repair system** has also been characterized in **humans**. It is **similar** to the one in prokaryotes but it's **not** the same.
- Two of the proteins, **hMSH2** and **hMLH1**, are very similar to their bacterial counterparts, **MutS** and **MutL**, respectively.
- A **mutation** in those proteins (hMSH2 and hMLH1) can cause **cancer**.
- Hereditary nonpolyposis colon cancer (**HNPCC**) resembles 15% of colon cancer cases.



- HNPCC is caused by mutations in the proteins of the mismatch repair system, where **50%** of them is due to a mutation in **MSH** and most of the **remaining** are due to a mutated **MLH**.

Good Luck ♥