CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

9

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DNA Mutations

There are two types of mutations:
Micromutations: it involves **small regions** of the DNA
Macromutations: it involves the chromosomes **as a whole**

❖ Micromutations:

- It leads to small changes in DNA
- ✓ The change can be in one or several bases
- ✓ The change can be:
  - **neutral:** it will not have an impact on the disease such as a silent mutation (ex: amino acids do not change, or change in the amino acids of the protein but the protein still functioning)
  - **negative**: the protein is damaged
  - **positive**: the proteins become better
- It can be passed to offspring if in gametes
- Types of Micromutations:

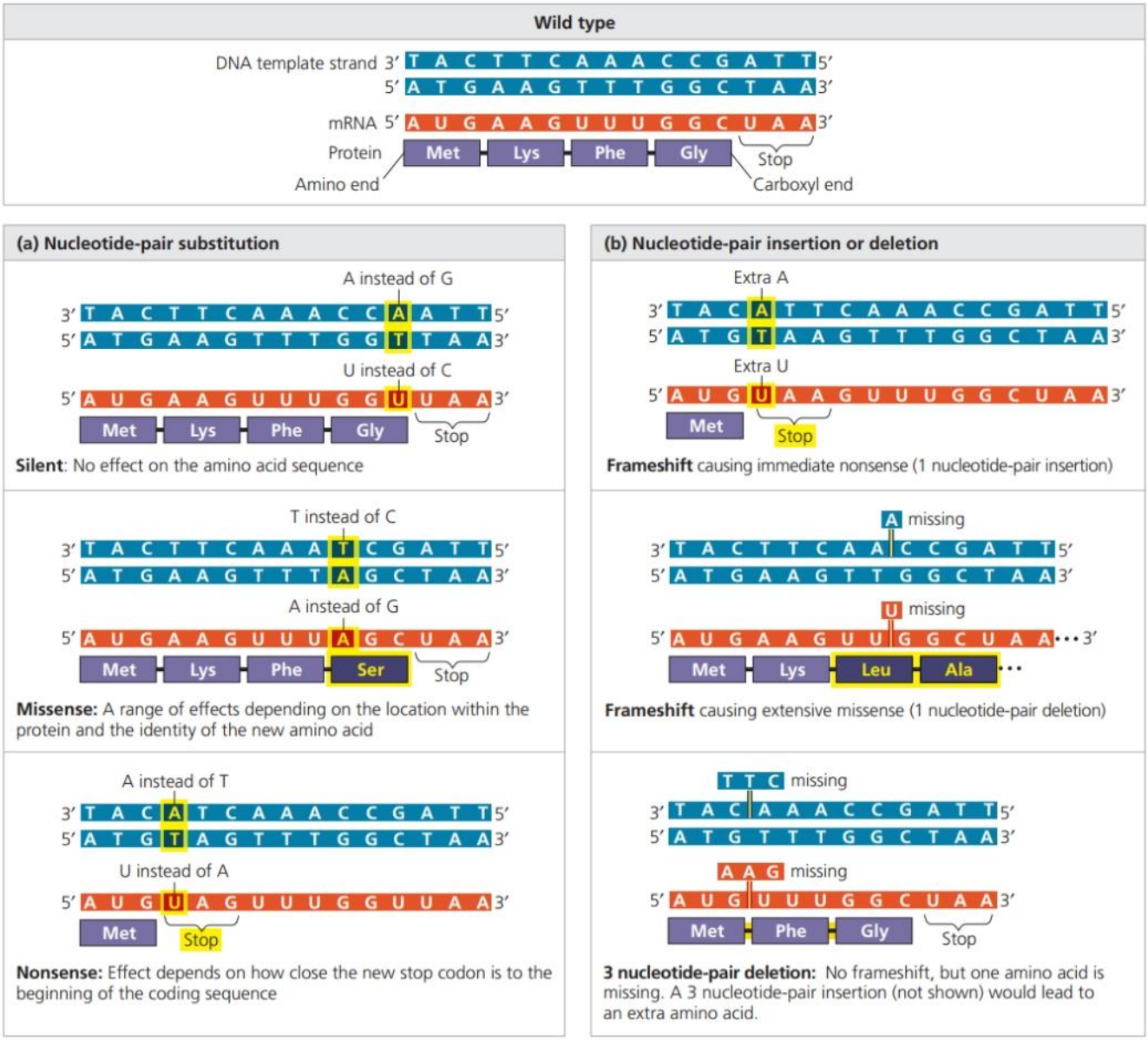
1. **Single point mutations (substitution)**:
   Mutations in a single nucleotide, which can result in multiple effects such as:
   A. **Silent**: A change in **one nucleotide** leads to the formation of a new codon that encodes for the same amino acid as the original one. It does not lead to any change at the protein level
   B. **Missense**: A change in **one nucleotide** leads to the formation of a new codon that encodes for a different amino acid
   C. **Nonsense**: A change in **one nucleotide** leads to the formation of a stop codon causing premature termination of protein synthesis

2. **Frameshift mutations (deletions and insertions):**
   A change in **one nucleotide, 2, or 3** (either by **addition** or **deletion**) leads to the change in the amino acid **sequence**, usually its effect is **great**

❖ Macromutations:

- Mutations that occur in the chromosome (millions of nucleotides)
- Types of Macromutations:

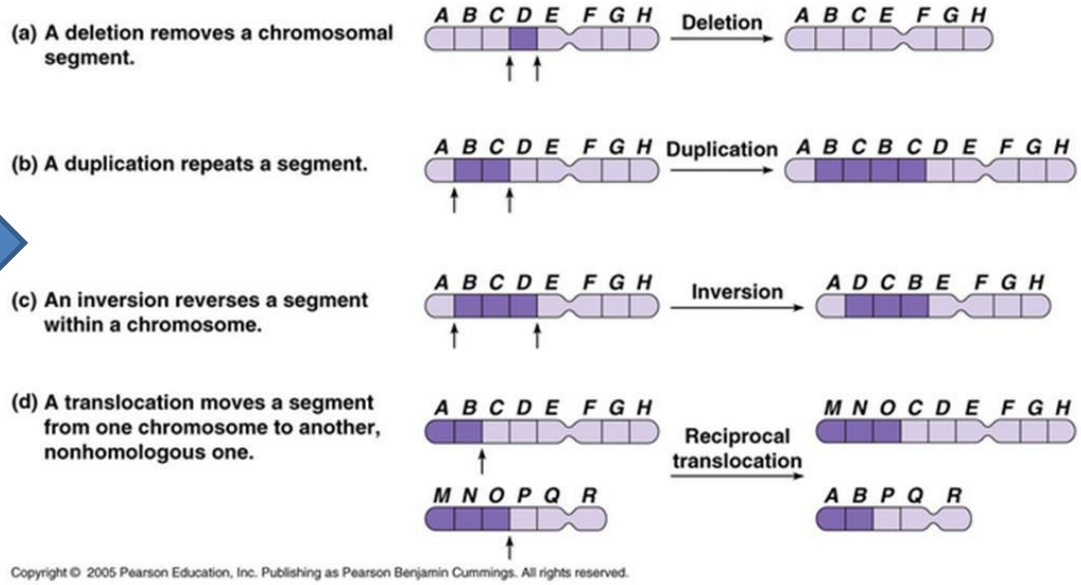
1. **Translocations**: that bring different regions of gene segments together
2. **Deletions** of a few nucleotides to long stretches of DNA
3. **Insertions** and **duplications** of nucleotides or long stretches of DNA
4. **Inversion** of DNA segments



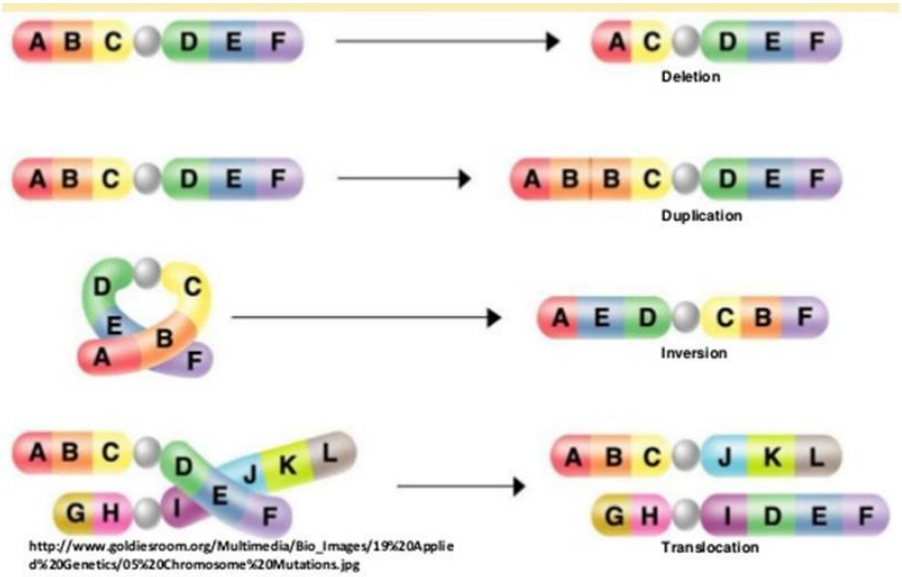
This Picture summarize the types of Micromutations

Note: the doctor didn't mention all these types (single point mutation & frameshift), so it's enough to understand them

DNA Macromutations

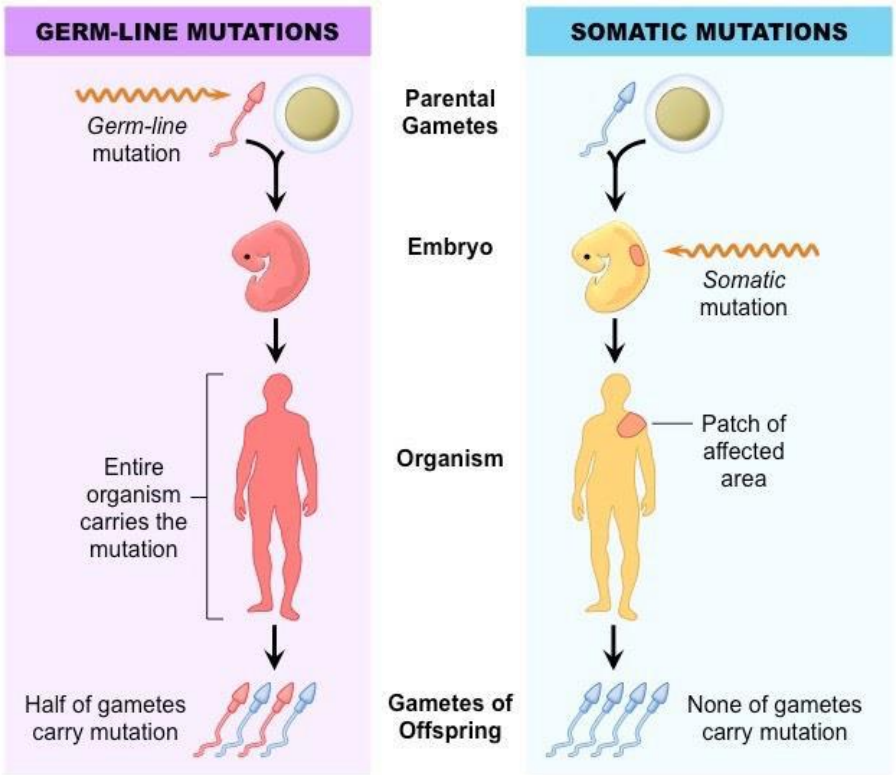


DNA Macromutations



- mutations they could exist either in the gametes (the gametes either X or sperm) or in the somatic cells.
- zygote will form and undergo mitotic divisions then rise to embryo to a fetus to a new born to a child to adult.
- the first type of mutation is **germ-line mutation** since the mutation happened in the early on original simple cell which is the zygote (maybe in the sperm or egg) all of the body cell will carry the mutation.
- On the other hand, there is another type call **somatic mutation**, here the DNA mutation happened after the zygote (maybe during the embryo or the fetus or even the child) all of the daughter cells of the mutated cell will be carrying the same mutation **not all of the body cells are having this mutation**.

Why half of gametes carry mutation?
Because if you have a mutation in one chromosome this chromosome will form 50% of gametes and the other chromosome (normal) will form the other 50%.



❖ Causes of DNA mutations

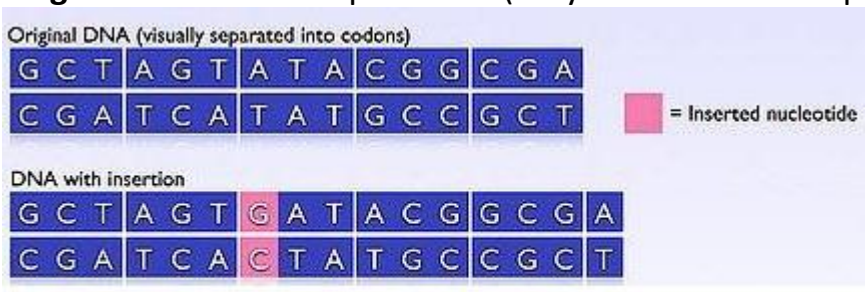
- DNA mutations can arise **Spontaneously** or **Induced**
- **Spontaneous mutations** are naturally occurring mutations and arise in all cells (spontaneous: a mutation that occur within the cell, there's no outside influence)
  - ✓ They arise from a variety of sources, including errors in DNA replication and spontaneous lesions
- **Induced mutations** are produced when an organism is exposed to a mutagenic agent or mutagen.

Numbering: **(1. &2.)** For DNA mutations
(A. & B.) For Spontaneous mutations

1. Spontaneous mutations: (the first of DNA mutation)

A. Errors of DNA replication (3 sources) (the first of spontaneous mutation)

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 (frame) of codons leading to changes in the amino acid sequence of the produced protein. These mutations often occur at repeated sequence, Frameshift mutation happens by errors of DNA polymerase.
3) **Large** deletions and duplications (they often occur at repeated sequence)

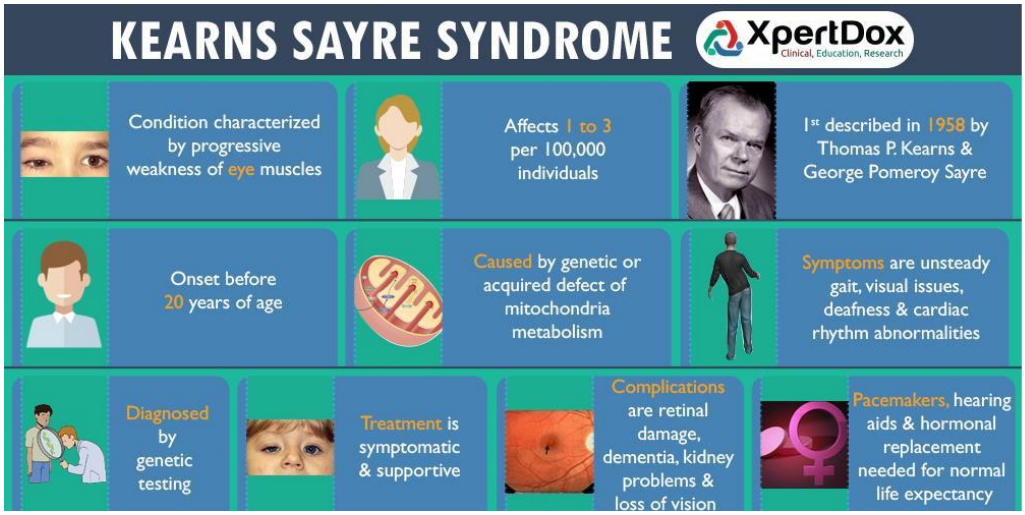


Frameshift mutations: addition or deletion of few nucleotides

➢ what's the difference between **the insertion and deletion** in Frameshift mutations and **Large deletions and duplications**?
they are the same concept, some nucleotides are added or removed in both scenarios but in **Large deletions and duplications** it's larger (maybe 100-200 nucleotides) but in Frameshift it's 3-5 nucleotides

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

1) **Deletion due to a three-base-pair repeat**
   Ex: **Kearns-Sayre syndrome** (mitochondrial encephalomyopathies)
   remember the function of mitochondria is making ATP, and there are tissues that require a large amount of ATP like muscle cells and nerve cells, so this disease a mutation happens and the protein that is involved in ATP production is defected and Kearns-Sayre syndrome occurs



2) **Expansion (duplication) of a three-base-pair repeat**

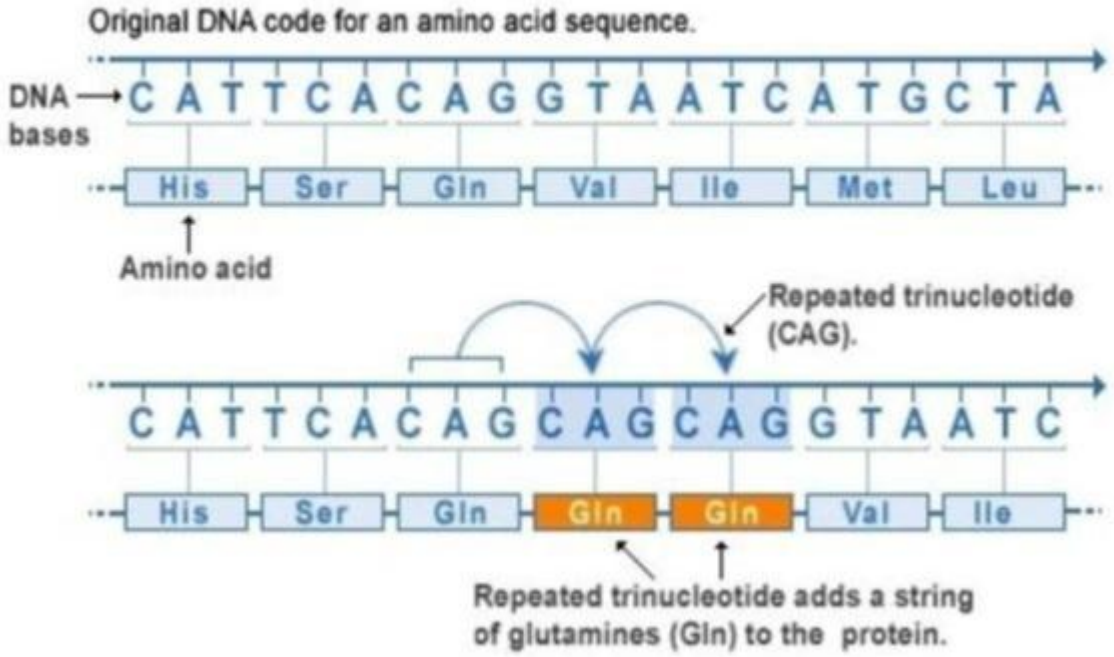
- **Fragile X syndrome** (CGG repeats in the FMR-1 gene):
  - ✓ The most common of **mental retardation** in male.
  - ✓ it's due to increase (duplicate & expanse) the no.of repeats of CGG.
  - ✓ There is a CGG that is repeated for a certain no.of times on the FMR-1 gene in the non coding region near the first exon (we all have this no.of CGG repeats less than 200 times). IF those CGG are repeated more than 200 times the disease will occur at Fragile X syndrome
  - ✓ Notice: that the sequence of those CGG are non-coding region but they influence the expression of the gene because they are close to the promoter.

- **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: We don't need to memorize the actual repeats of each disease but we need to understand that those disease are related to **repeat expansion**

Q: Are all those repeats within the non coding region close to the exon? **NO**

Repeat expansion mutation **Huntington disease** (CAG repeats)



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B. Spontaneous lesions: (the second of spontaneous mutation)

- ✓ are naturally occurring type of DNA damage that can generate mutations such as **Depurination**, **Deamination,** and **Oxidatively damaged bases**

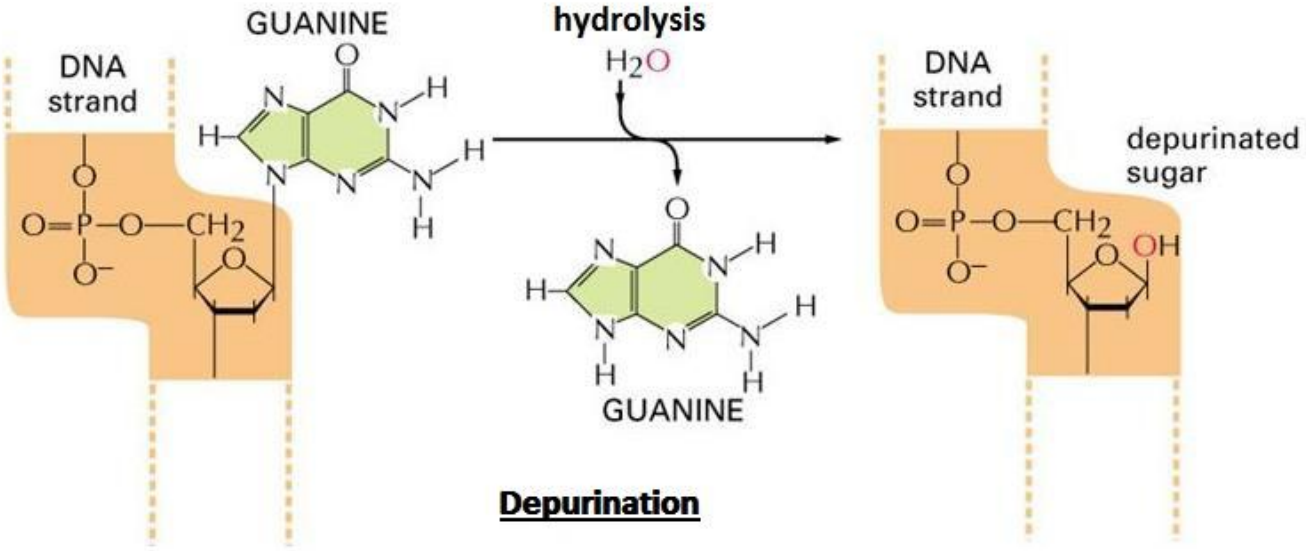
1) Depurination

- ✓ Cleavage of the glycosidic bond between the base and deoxyribose creating apurinic sites (AP sites)
- ✓ During replication, a random base can be inserted across from

- The nitrogen base is attached to the back bond through a covalent bond with **carbon one** of ribose sugar which is called the **glycosidic bond**. If this glycosidic bond is **broken** through hydrolysis, the nitrogen base will disappear creating **apurinic sites** (AP sites) this site will have only **phosphate & sugar**, this could happen for purines or pyrimidines.

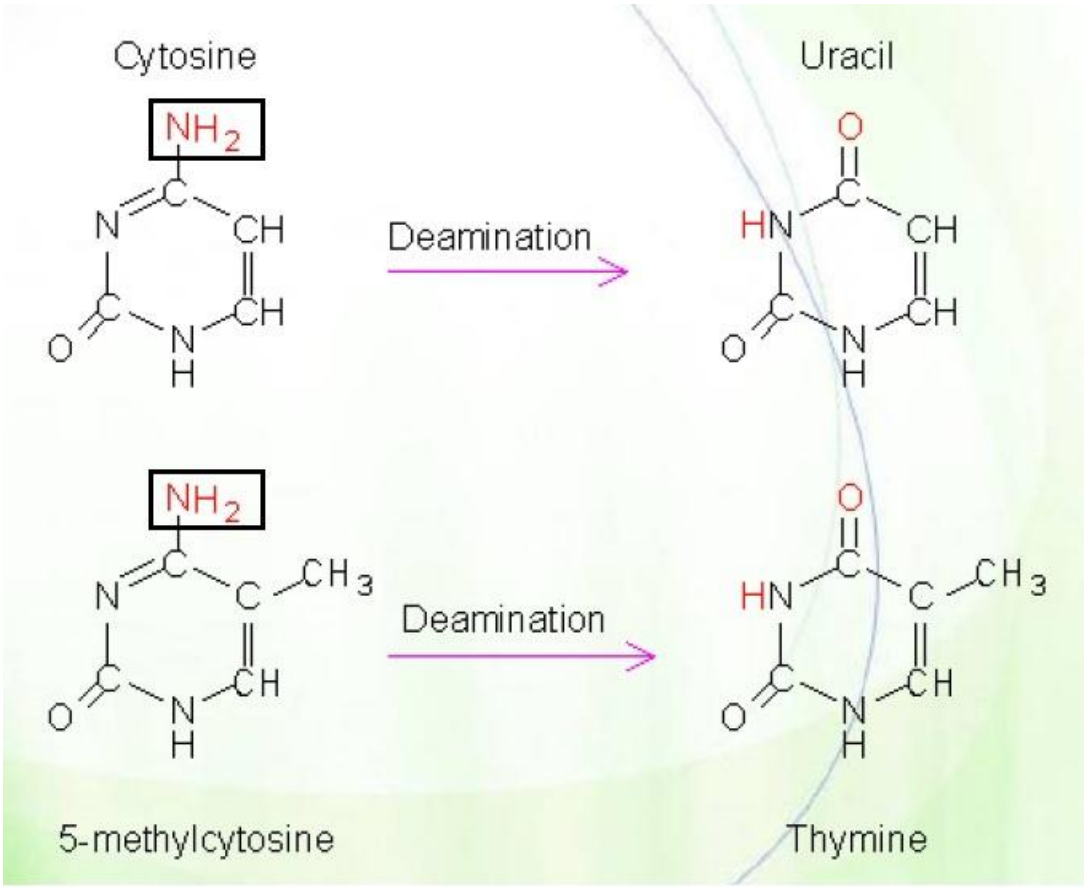
- So, when DNA replication happens the daughter strand will not find complementary to it on the apurinic sites to build, that's why the daughter strand will have **a random nucleotide** at this location

- in other words, 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 leading to a **single point mutation** and this is the most probably**.**

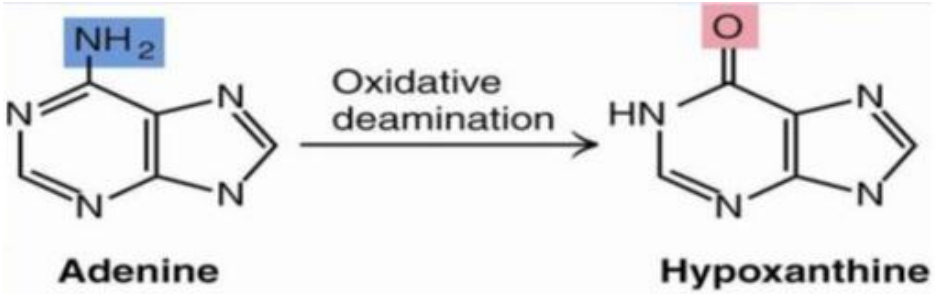


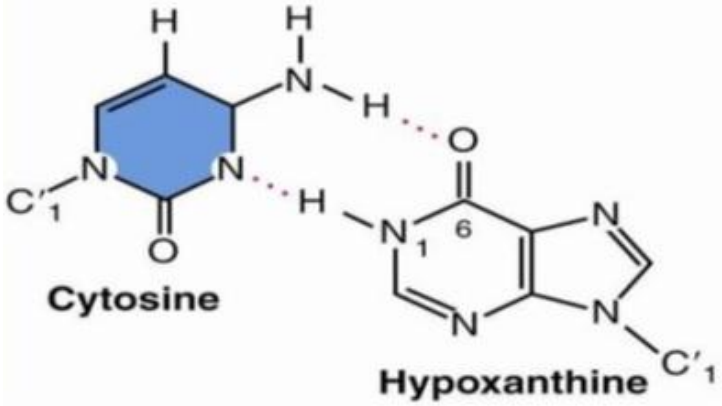
2) Deamination

- ✓ It is the removal of the amine group (NH_2) on the nitrogenous bases
- ✓ The deamination of **cytosine** yields **uracil**.
- ✓ The deamination of **methylated cytosine** yields **thymine**.
- ✓ The deamination of **adenine** yields **hypoxanthine**.



we don't need to memorize the structure of Cytosine or Thymine but we need to know that if NH_2 is removed from Cytosine it gives Uracil





Hypoxanthine should not exist in the nucleus but **if adenine deaminated it will give Hypoxanthine**. During DNA replication Cytosine pairs with Hypoxanthine and this is should not happen because **normally Adenine should add thymine**, but this is happen because Adenine became Hypoxanthine which in return pairs with **Cytosine** instead of the usual **Thymine.**

3) **Oxidatively damaged bases** (the doctor didn't mention it)

2. Induced mutation: (the second of DNA mutation)

- ✓ 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 compound (like smoke) or energy (like UV light), something you eat or drink

Mechanisms of mutagenesis:

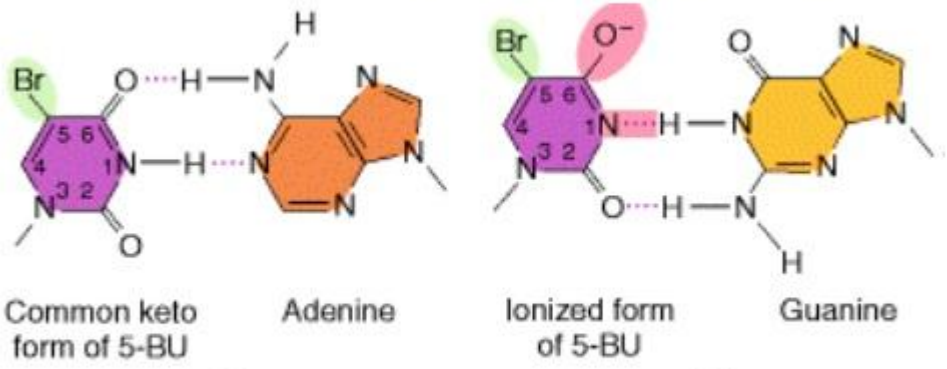
- Add a base analog during DNA replication
- Alter an existing base causing mispairing (alkylation)
- Damage a base disabling pairing with any base

1) Incorporation of base analogs

- ✓ Base analogs have similar structure to normal nucleotides and are incorporated into DNA during replication.
- ✓ 5-bromouracil (5-BU), an analog of thymine, pairs with adenine, but, when ionized, it pairs with guanine.

- Base analog: it's a nitrogen base that has similar structure to normal nucleotides and are incorporated into DNA during replication, but itself it's not normal and does not exist in the DNA

The **ionized form** of 5-BU as a complementary nucleotide to **guanine** during DNA replication (because it's an analog to Cytosine)



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

- Those two analogs (**5-BU** & **Ionized form of 5-BU**) they should not exist in the nuclease but when a mutagen creates them, they will be incorporated in newly synthesized strand during DNA replication

2) Specific mispairing

✓ some chemical reaction will happen and change the structure of the nitrogen base on the temple strand DNA molecule

✓ Bases existing in DNA can be altered causing mispairing following replication.

- Alkylating agents (it's a chemical compound) can transfer methyl group to guanine forming 6-methylguanine, which pairs with thymine.

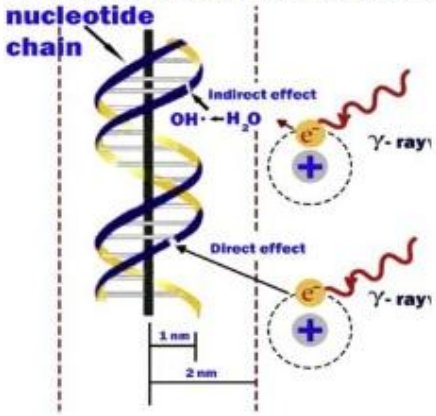
6-meG pairs with **Thymine** instead of the usual **Cytosine**

3) Base damage

✓ **Ionizing radiation (like γ ray)** results in the formation of ionized and excited molecules that can cause damage to the DNA including:

- Base damage
- the creation of **AP sites** (apurinic or apyrimidinic sites)
- strand breaks (X-rays and sunlight break the phosphodiester bond between bases)

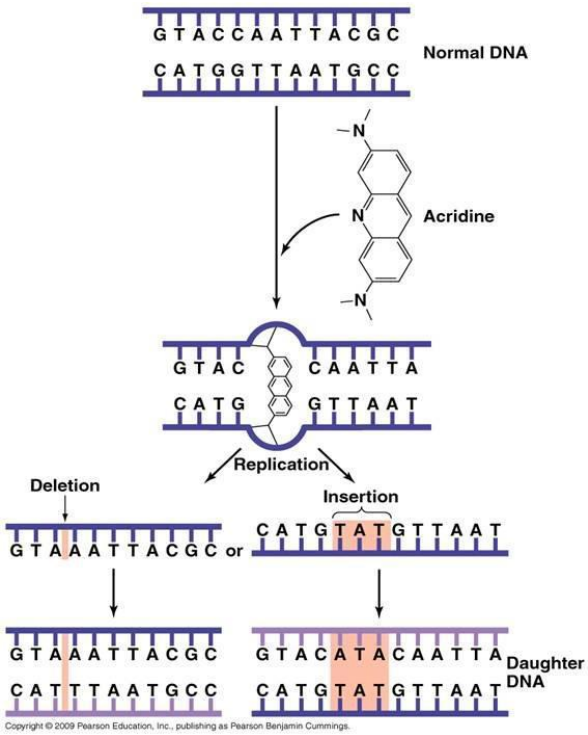
Indirect effect: when the radiation causes the formation a free radicals (which is unstable molecules) and the free radicals hits the DNA



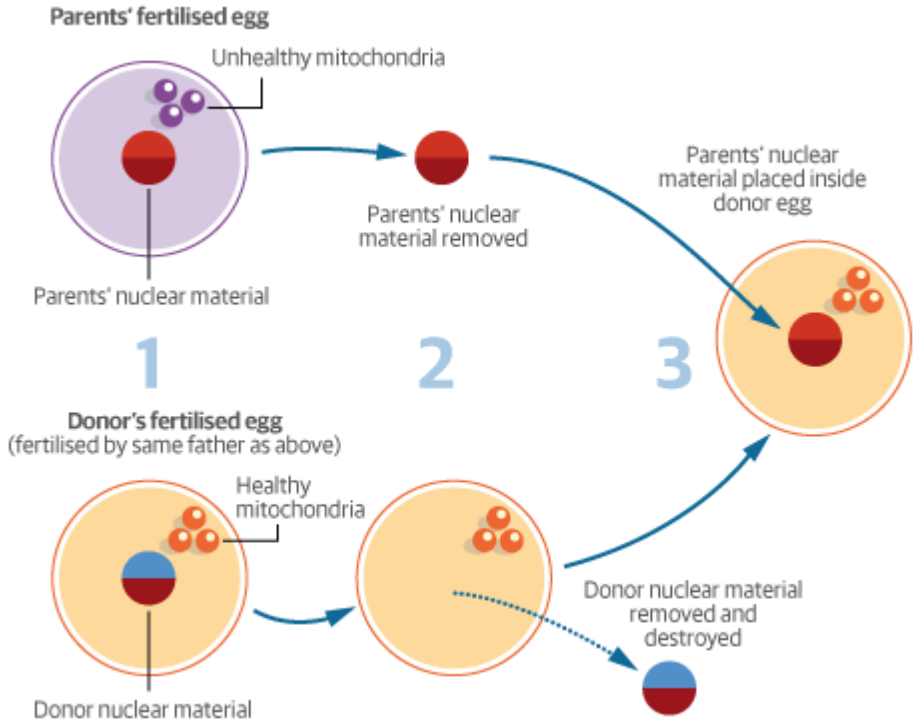
Direct effect: when the radiation hits the DNA directly breaking the phosphodiester bonds

4) Intercalating agents

The intercalating agents such as **proflavin** and **ethidium bromide** (These molecules are present naturally and are also present in labs and used for DNA staining) are planar molecules that **can insert themselves** (intercalate) between the bases and cause single nucleotide-pair insertion or deletion



❖ Controversial issue
Three-parent babies



- when the sperm fertilized the egg **only the nucleus of the sperm** goes to the egg forming the zygote, so **our mitochondrial DNA** that we have in our cells is from mother not from father.
- now, if the mother has a baby and her mitochondrial DNA was mutated, the mitochondrial DNA of baby will be mutated

- So, we 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.

- why we removed the mitochondria from the mother egg? because it's mutated and it will transfer to the baby.

- 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.