Genetics
& Cell biology

◉ Sheet ○ Slides

Number

5

Done by:

Yaman Jarrar

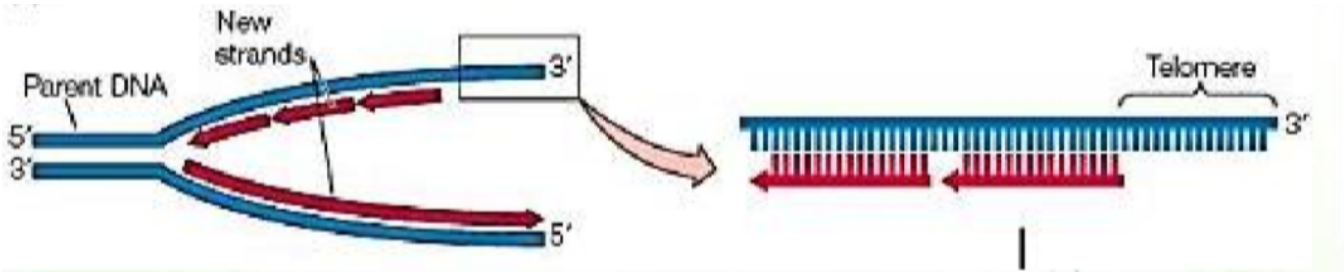
Corrected by:

Enas M.Ajarma

Doctor

Mamoun Ahram

Unfortunately we all are going to die because there is a problem in the lagging strand, why? Because what could happen is that we have a leading strand that is going to continue until it reaches the end of the linear chromosome and then the lagging strand template is not completely replicated because there is no space for the primase to put a primer, so at the end we are going to have a gap. This gap is going to be in the telomere region which is at the end of the chromosome and its function is to stabilize the DNA, so the shorter they get, the more unstable DNA becomes leading to more mutations ,fragmentations .. etc , then the cell commit suicide and die, that's why organs failure occur. However, we have an enzyme called **telomerase** which keeps the chromosomes long(prevents the progressive shortening of the lagging strand) , so telomerase elongates the telomeres to keep them stable, its activity and protein level are high in younger people. Its expression and activity becomes less as we age.



How it works? the telomerase has an oligonucleotide associated with it, and the oligonucleotide functions as a template/primer.

So, what happens exactly, the telomerase elongates the template where the lagging strand is, creating enough space for the primase to add the primer and the DNA polymerase to extend the final **Okazaki fragments** so they can bind to each other, thus telomerase makes the DNA more stable.

[The telomere itself is composed of repetitive nucleotides such as GGTTA extending about 10,000 nucleotides].

Dolly (the sheep) when she was born she was 1 day old , but in reality she was 5 years old because her DNA was taken from a 5 years old sheep , so dolly lived 5 years and died because aging appeared on her early.

So, people thinks that if we can increase the activity of telomerase we can be immortal and this just a theory.

Some cells are immortal such as cancer cells because it has a very high activity of telomerase enzyme.

Now we are going to talk about a new topic which is DNA Mutations.

-We have different type of mutations which is divided into two different types:

1-Micromutation which is on the level of DNA.

2-Macromutation(large) which is at the level of chromosomes(ex. Extra chromosome in down syndrome)

-DNA micromutations can be divided into:

1-**Single point mutation** such as sickle cell anemia and it can be :

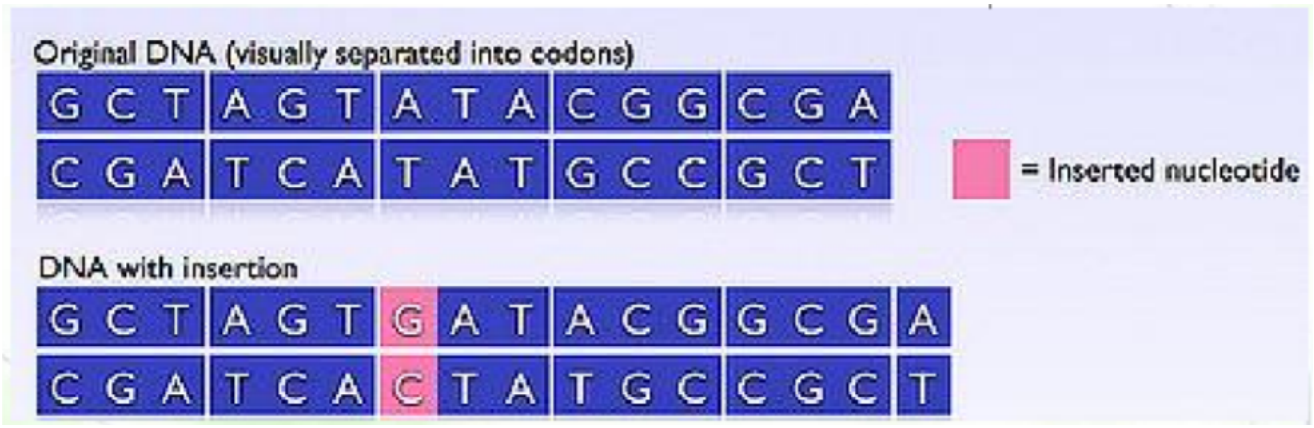
a-**Missense** leads to change of an amino acid in the protein

b-**Non-sense** leads to change of an amino acid and leads to stop of the protein synthesis in early stages

c-**Silent mutation**:does not lead to any change at the protein level ,it may change the rate of the protein synthesis or protein interaction with DNA, it also contributes to genetic variability among individuals.

d-Some single point mutation can be **Frame shift mutation** :basically we have a codon which creates an amino acid and each codon is composed of 3 nucleotides , so if I inserted a base in the middle of these 3 nucleotides , a shift is going to happen to all the codons , so frameshift mutation affects the amino acid sequence of the protein and can lead to production of a defective protein.

Frame shift mutations often occur at repeated sequences. So at these repeats RNA polymerase may insert or delete one or few bases that result in changing the reading of codons and can lead to changes in the amino acid sequence of the produced protein (**large deletions and duplications**)



2-**Translocations**: that bring different regions of gene segments together

3-**Deletions** of a few nucleotides to long stretches of DNA,

4-**Insertions and duplications** of nucleotides or long stretches of DNA

5-**Inversion** of DNA segments

-We have two types of DNAmutations:

1-**spontaneous mutations**: naturally occurring mutations and arise in all cells

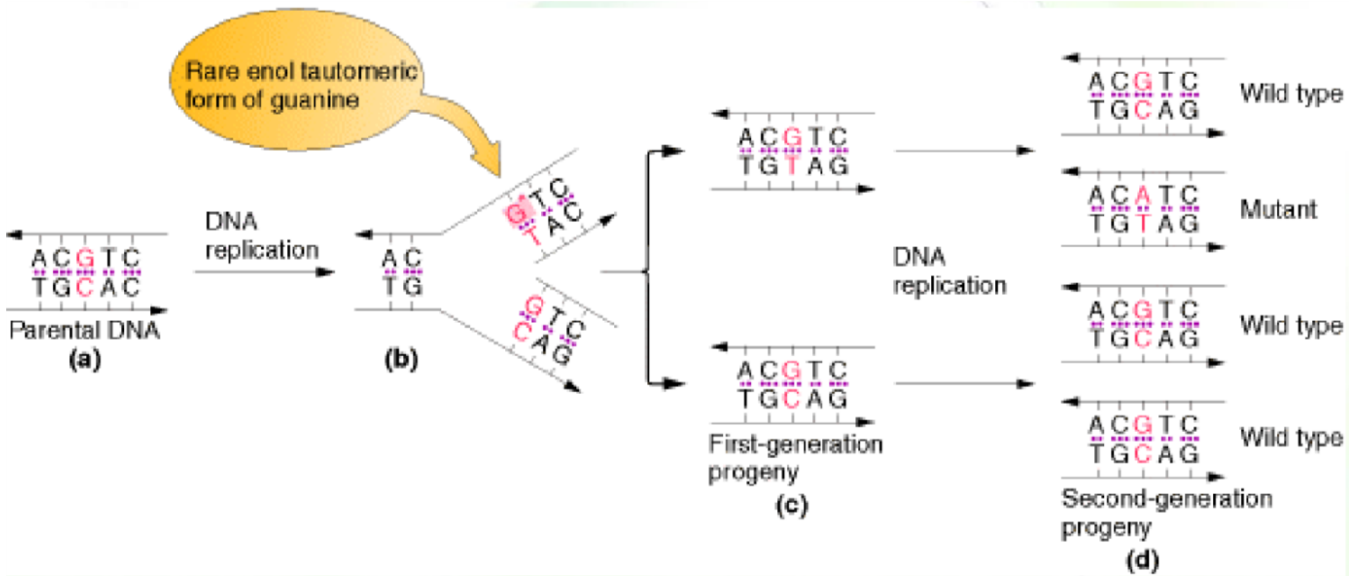
2-**induced mutation**: produced when an organism is exposed to a mutagenic agent, or mutagen(external source)

-Sources of errors in DNA replication: mispairing between the nucleotides and what we called **tautomers** which are several forms(isomers) for the same base. (interconverting constitutional isomers of organic compounds).

Normally , we have C with G and A with T , but we may get an isomer of the nucleotide that result in change in the structure leading to wrong hydrogen pattern (A-C, G-T).

Tautomers lead to either:

1-**transition mutations** which is the mutation when we change purine into purine or pyrmidine into pyrmidine.



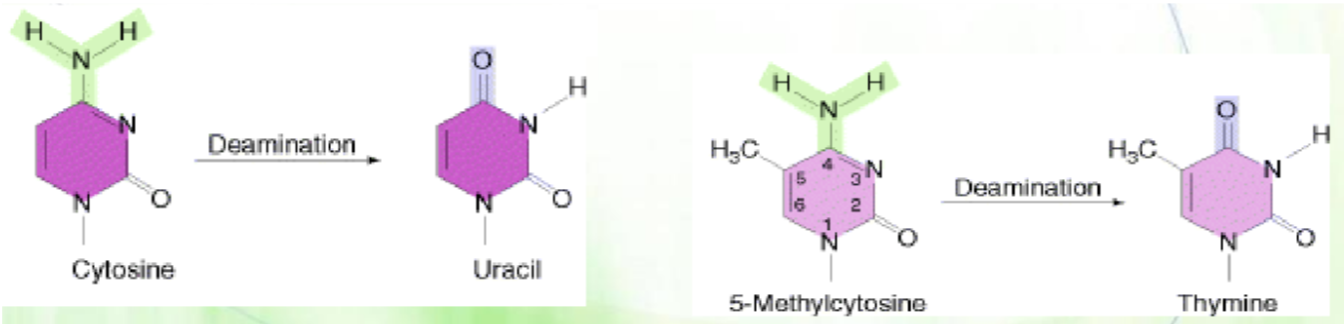
2-**transversion mutation** which is the mutation when we change purine into pyrmidine or the opposite.

-Spontaneous lesions are Injuries that occur to the DNA. We have three types, depurination, deamination and oxidatively damaged bases.

1-depurination is removing the purine base from the nucleotide (nucleotide missing a base)

So, when the DNA polymerase recognize that there is a nucleotide that doesn't has a base it either continues and puts a random base leading to single base mutation or it stops (suicide).

2-deamination is the removal of the amino group and putting a ketone group, for example is changing cytosine into uracil, Uracil residues will pair with adenine during replication, resulting in the conversion of a G-C pair into an A-T pair (a GC→AT transition). if cytosine is methylated, and we deaminated the methylated cytosine it is going to be transferred into thymine and leading to a transition mutation.



3-Oxidatively damaged bases which happens in tissues with high metabolism because it has large number of mitochondria so we will have more reactive oxygen species [free radicals] such as hydrogen peroxide so they oxidize DNA. if hydrogen peroxide took an electron from the guanine for example it is going to be transformed into 8-oxo-7-hydrodeoxyguanosine [Oxo DG]. If this compound was in the DNA, it is going to pair with adenosine not with cytosine and this is a transversion mutation.

Note: There is a mistake in slide 16, the first point should be deletion of mitochondrial DNA as a result of the presence of repeats not expansion of three-base -pair repeat

So in the mitochondria we are going to have repeats in the DNA and DNA polymerase makes a mistake so deletion occur to a large piece of DNA resulting in Kearns-Sayre syndrome.

-Expansion of a three-base-pair repeat: (you should know the gene associated with each disease)

1-Fragile X syndrome (CGG repeats in the FMR-1 gene)

2-Kennedy disease (X-linked spinal and bulbar muscular atrophy (CAG repeats in the androgen receptor)

3-Myotonic dystrophy (CTG repeat in the non-coding region of a kinase gene)

4-Huntington disease (CAG repeats in HTT gene)

Induced mutations

Now we are going to talk about Induced mutations which could happen because of:

1-replacing a base with something looks like a base and this is called **base analogue**.

2-altering a base so that it mispairs with a wrong one.

3-damage a base so that it can no longer pair with any base under normal conditions.

*__Base analogues__ basically are compounds the look like nucleotide but they are not nucleotides so the DNA polymerase looks at these bases and thinks that they are nucleotides and incorporate them with the DNA.

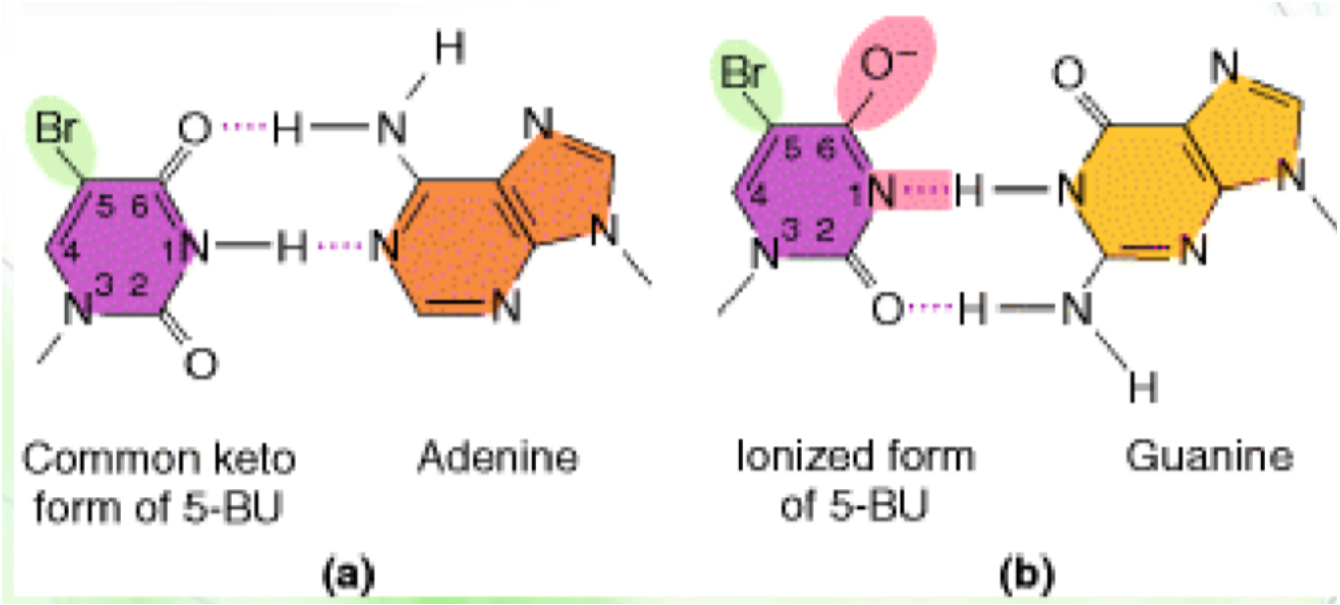
Examples

5-bromouracil:

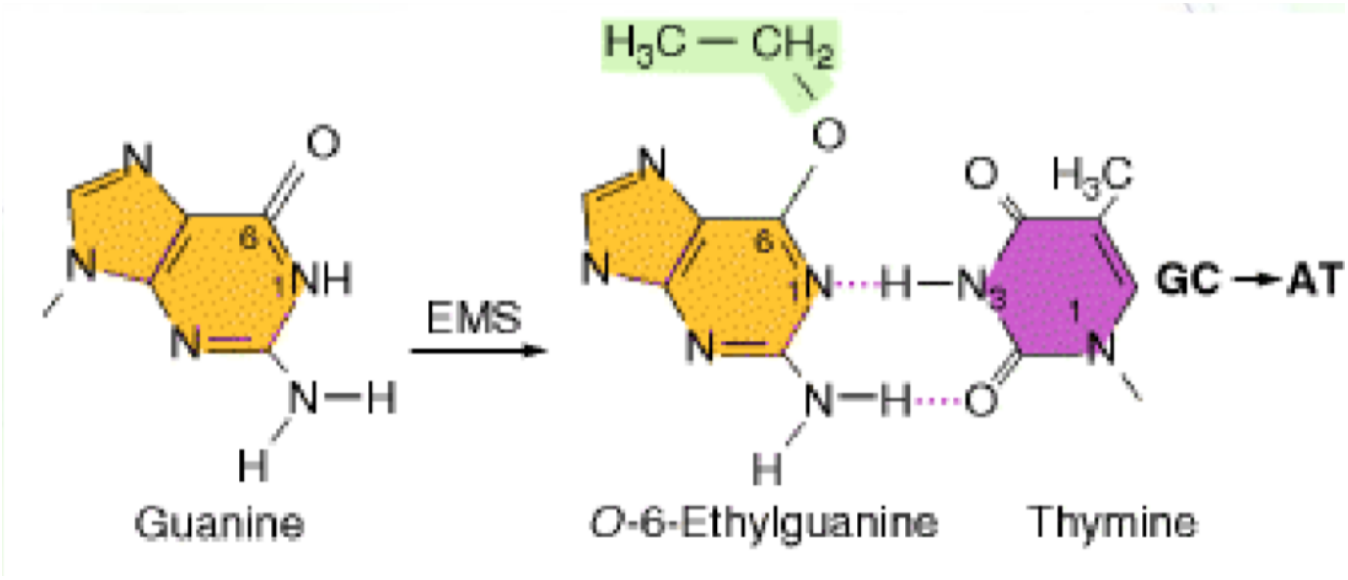
5-bromouracil (5-BU) is an analogue of thymine

It pairs with adenine because it is an analogue of thymine.

5-bromouracil can be ionized (happens spontaneously in the DNA) and the ionized form pairs with G leading to a transition mutation.



Some Mutagens are not incorporated in the DNA but a chemical reaction could happen to a base that is already incorporated in the DNA such as alkylation of G leading to pairing with T rather than C in the second round of replication leading to transition mutation.



-Damage to the bases could also occur because of ionizing radiation such as X rays leading to DNA mutations.

-Many different types of reactive oxygen species are produced that:

1-can damage bases

2-can cause breakage of the N-glycosidic bond (AP sites),

3-can cause strand breakage.

***The intercalating agents** such as proflavine and ethidium bromide are planar molecules that can insert themselves (intercalate) between the stacked nitrogen bases imitating base pairs.

So when the DNA polymerase reads the DNA and recognize the intercalating agent as a foreign base it is going to pair the intercalating agent with a random base which result in single-nucleotide-pair insertions or removes this base (decision making) leading to frameshift mutations.

An example is ethidium bromide which is carcinogen and is used in DNA staining

Mutagens can be carcinogens so all carcinogens are mutagens but not all mutagens are carcinogens.

if anybody wants to know if this chemical is carcinogen or not, he must see if this chemical is mutagenic or not. However, in the past it wasn't easy to know until Bruce Ames developed a bacterial system to identify if a chemical is carcinogen or not.

What is this system? He brought a bacterial culture (salmonella) and he:

1- mutated the gene that makes histidine so these bacteria can't survive without adding histidine to the culture.

2-mutated their DNA repair system.

3-They also carry a mutation that eliminates the protective lipopolysaccharide coating of wild-type Salmonella to enable the entry of many different chemicals into the cell.

His system is based on a concept called reversion mutation so he took a normal cell and transferred it into and abnormal cell, the mutation he was studying is the mutation in the gene related to the biosynthesis of histidine, so if he put a chemical and this chemical was mutagenic, there is a high probability to get a mutation in the mutated gene (which he mutated at the first) to return to normal, and that's why he called it reversion mutation.

This reversion can happen spontaneously or as the result of a mutagen (induced mutation)

So, if we took these bacteria and put it on petri dish, the second day we will see that we have 10 colonies, how did they live without histidine? Because they have high level of spontaneous mutation resulting in a spontaneous reversion mutation and if we put the mutagen(chemical) we are going to have a spontaneous reverse mutation and induced reverse mutation and we will have more than 10 colonies.

So, **his point is that the number of colonies must be doubled when we put the mutagen(chemical)**

let's take the example from the slide

Water	Motor oil	Alcohol	Drug X	شيبس أبو 5 قروش
10	50	43	9	200

When we add water we got 10 colonies(control), when we added motor oil we got 50 colonies which means that motor oil is mutagenic.

When we added alcohol, we got 43 which means that alcohol is also mutagenic.

When we added drug x we got 9 colonies which means that this drug is not mutagenic.

Normally when we ingest anything, it is going to be processed in the liver by liver enzymes so Bruce Ames mixed the liver enzymes with the mutagens (chemicals) to study the role of liver enzymes in metabolizing these chemicals.

Let'slook at the same example but with the liver enzymes:

Condition	Water	Motor oil	Alcohol	Drug X	شيبس أبو 5 قروش
-liver enzymes	10	50	43	9	200
+liver enzymes	12	22	50	35	500

In water, we got 12[not mutagenic , this increase come from spontaneous mutations]

With motor oil we got 22 colonies which mean it is not mutagenic [liver enzymes metabolized motor oil into a safer compound which is not mutagenic].

With alcohol we got 50 colonies which means that it is mutagenic and liver enzymes don't have any role.

With drug X we got 35 which means that liver enzymes converted drug x into a mutagenic molecule.

Liver enzymes also can convert a mutagenic molecule to be more and more mutagenic.

" Believing is half the cure " ,,,,,, **GOOD LUCK CURE**