University of Jordan
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# GENETICS & Molecular Biology

Number: 6

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DNA mutation

We can classify DNA mutation into two types :

1) Micromutation
2) Macromutation

Macromutations are large mutations involve insertion or deletion of big chunk of chromosome ( we will learn about it later )

Micromutations are little small mutations happen at DNA level

There are different types of such: the main one is single point mutation which happen at a single base, which is divided into many types:

1) Missense mutation : is a case where the change in a base will result in the change of amino acid of the synthesised protein
2) Nonsense mutation : is a case when replace amino acid or codon with stop codon , and the synthesis of the protein is terminated prematurely and the resulted protein become shorter
3) Silent mutation : change in one base that not resulting in change of amino acid- due to the flexibility of the last letter of the codon
4) Frameshift mutation : ( remember : the RNA is read as a codon and the codon contain three bases , you can imagine that if each codon is placed in a frame , so we have frame 1,2,3, and so on ) if we insert one base between base 1 and base 2 of the first codon it become{ base 1, the inserted base, base 2} and base 3 become the first base in the second codon and so on , that mean there are shift of the bases downstream of the insertion, changing every single codon read after the insertion, that result in synthesis of completely different protein

Now ,let's say that you have codon 1 , codon 2 and you insert three bases between them , what will happen to the protein ?

The answer :Insertion extra amino acid to the original sequence

In the past example , if you insert three bases within a codon , what will happen ? (Note: not between the codons, but rather between the bases of a codon)

The answer : Frameshift mutation

There are other types of mutation that can happen such as :

1) Translocation : where small part of the DNA is translocated from one part to other part of the DNA
2) Inversion : invert the bases ( base 1,2,3 become 3,2,1 )-refer to diagram in slides
3) Deletion and insertion which can cause frameshift mutation or addition of codon or may be nothing

Now, what does cause DNA mutation ?

DNA mutation can occur spontaneously or induced

Spontaneously by means in the life of the cell, during DNA replication mainly, where errors may occur and the DNA can be damaged as an example

Induced mean that something is added to the cell and induces the mutation

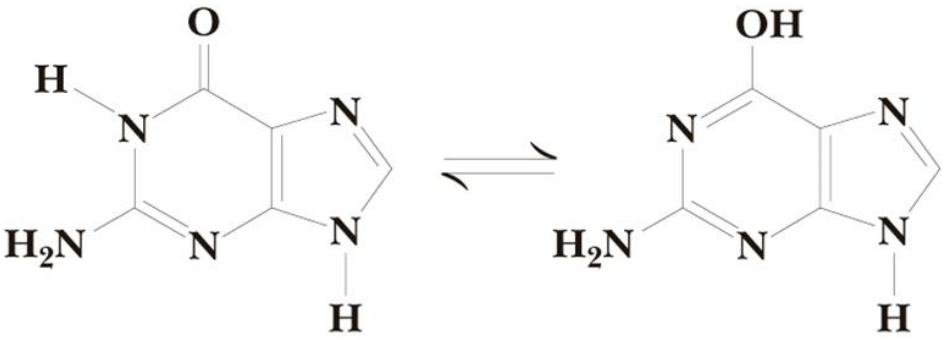
Let's talk about spontaneous mutation :

There are different types , one of them is error in DNA replication :

Basically , the DNA polymerase would insert the wrong base ,A-C instead of G-C , they can be corrected but if it isn’t, it cause mutation

Now, sometimes these mutations occur because there are certain features in these bases , they have “tautomers”

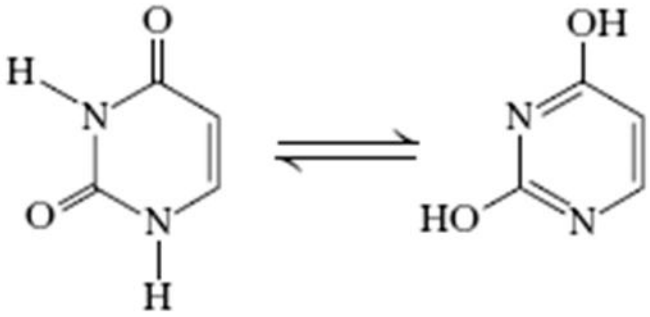
Tautomers are isomers of the bases



Keto form Enol form

Example : in the left structure above we have double bond attach to O this double bond can be interconverted to become as in the right structure above , that will completely change the hydrogen bonds pattern. In this case, the G will form 2 hydrogen bonds instead of 3 so the polymerase will think it is A ( which form 2 hydrogen bonds ) instead of G

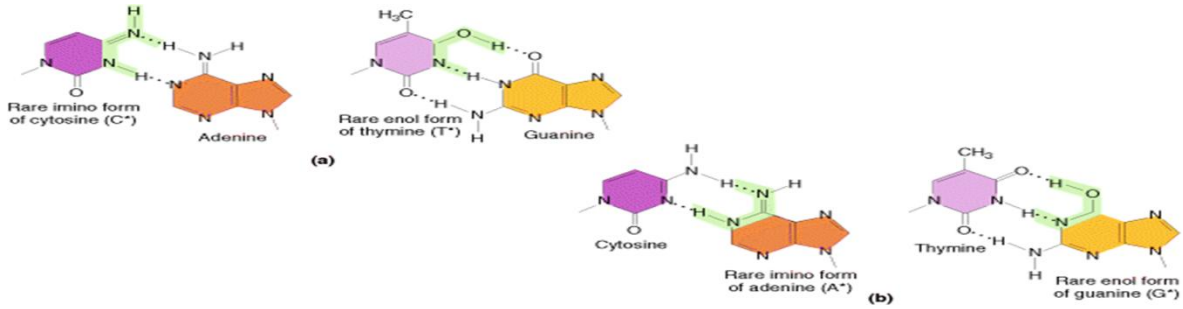
Same thing about pyrimidine :



Lactam Lactim

Normally , we have( A with T) and( C with G)


But if we have the other form of the base ( as we mentioned above ) C will pair with A and T pair with G



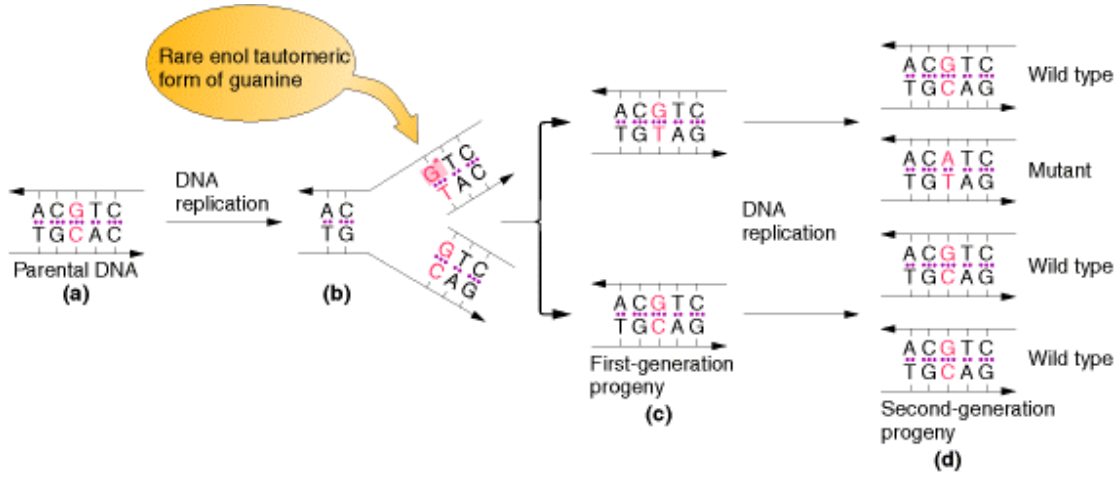

Or if we have the tautomiric form of guanine and adenine, then there will be a mispair again (C with A ) and ( T with G ) and that normally happen in the cell sometimes

We have two types of mutation ( as definition ) : transition and transversion

Transition mutation : when you change a purine for a purine and a pyrimidine for a pyrimidine

Transversion mutation : when you change a purine for a pyrimidine and a pyrimidine for a purine

So let's take this example :




In the middle of parental we have G&C , after replication we have normal G&C pair but there are abnormal G&T pair , let's say it is not fixed and enter another round of DNA replication so we have in this case G with its complementary C and T with its complementary A ( nothing wrong happen in the second round )

Look at the second generation , in the middle we have A&T pair instead of G&C pair in the parental DNA

This mutation is transition because G is replaced by A in the same strand and C is replaced by T in the same strand

There are other error in DNA replication such as deletion , insertion and duplication , they happen in sequence repeats

So for an example , sequence contain CAG repeat (CAGCAGCAG…..) some individuals have 10 repeat ,other have up to 35 repeats so there a variation among people . These repeat normally occur in our genome, so let's say we have 10 repeats of CAG, the polymerase will get confused, it may add extra repeats or less, that can lead to a frameshift mutation or to a deletion or duplication or insertion

Spontaneous lesion: it cause injury to the DNA, and it normally happen, there are 3 types of such lesion :

- Depurination
- Deamination
- Oxidatively damaged bases

Depurination:

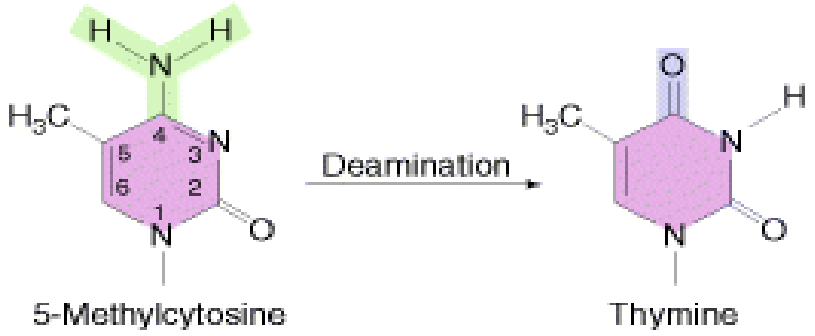
- We have certain DNA strand and the purine is removed (cleavage of the glycosidic bond hydrolytically ) so we have a base less nucleotide. When DNA polymerase replicate the DNA and reads that the base is released it get confused. There are two decision that can be made : continue replication (which creates a mutation) or kill the cell.

At decision time if polymerase reads a base less nucleotide, it will usually add whatever base that depurination normally happen in the cell

Deamination :

-Normal process where the cytosine ,more specifically , is deaminated so the amine group is removed and replaced by a ketone and converted to a uracil. Usually when the repair system fixes the error. Let us say that it's not fixed so when the DNA replicates, the polymerase insert A because U look like T resulting in transition mutation.

Sometimes the cytosine can be methylated then deaminated so resulting in a convertion to a thiamine




5-Methylcytosine Thymine

Oxidativly damaged bases :

-During cell metabolism, reactive oxygen species are normally produced. These molecules is oxidized need electrons and they are reactive , so free radical steal electron from membranes or proteins or DNA which will result into mutation . One type of the products can be resulted from these molecules is 8-oxo-7-hydrodeoxyguanosine (8-oxodG, or GO). When we have it inserted in the DNA, it is read as T and the polymerase adds A resulting in transversion because the changing of G to T ( more explain : G is paired to C , G is converted to GO which is paired with A so we replaced C by A so it transversion )

## Induced Mutation

Here,there are something external cause damage to the DNA

Things that cause DNA mutation are called mutagens. Some mutagens can cause cancer and are called carcinogens , so all carcinogens are mutagens but not all mutagens are carcinogens

There are three types of induced mutation can be happened :

1) Insertion of a base analogue : insertion of something look like a base but is not a base that will cause a mutation
2) Normal base is damaged as a result of something external when the base is part of DNA strand or damage to the phosphodiester bond so the whole DNA is broken in different pieces

3) Alter a base so that it specifically mispairs with another base (alkylation)

One of the most important analogue is 5-bromouracil , the polymerase think it as a thiamine and adds Adenine which is normal , but what happens is that 5-bromouracil is interconverted to an ionized form which is read as cytosine so it place G on the other strand that cause transition mutation. (A to G)

Sometimes you can alter the base when it part of the DNA , so if we have G it can be alkylated , once alkylated it form ethylguanine that will be paired with thiamine instead of cytosine and this is transition mutation (C to T)

The base itself might be damaged in a different way. One way is radiation (X-ray as an example ) which break the phosphodiester bond within the DNA strand.

Intercalating agents :

They are molecules that insert themselves within the bases. When DNA polymerase comes in and replicate the DNA it sees the free space and then the intercalating agents.

((Refer to slides for the diagram for further understanding))

Decision time: the polymerase see something is unknown to it , it has two options :

1. Insert bases
2. Jump , when it jump over the intercalating agent sometimes jump just over this agent or jump over other several bases resulting in deletion

So, the intercalating agents are highly mutagenic and highly carcinogenic because it cause insertion or deletion resulting in frameshift mutation.

The agents can be beneficial and used to stain DNA in lab.

There must be a test to see if a certain chemical is mutagen or not and must be cheap which is the Ames test :

This test uses genetically modified bacteria calledSalmonella typhimurium which has three features :

1) the LPS around it is gone , meaning any chemical I added to this bacteria it will go to the cell –No barrier
2) there is a defective DNA repair system so whatever mutations happen it cannot be fixed –No repair system
3) this bacteria normally grow in the absence of histidine because it have a gene code for enzyme produce histidine , there are a mutation in this gene so this bacteria cannot grow in the absence of the histidine

The test based on something we called a "**reversion mutation**"

The normal bacteria can grow in absence of histidine, but there are mutation that make it unable to grow in the absence of histidine, and the reversion mutation is a mutation happen in the gene that synthesizes the enzyme for producing histidine that *reverse* the cell to the normal type

If we have a group of cells ( million cells as an example ) and grow them in the absence of histidine , how many colonies these cells form ?

Let's say 10 or 20 but where did they come from ?

- Come from spontaneous mutation because it have high rate of mutation resulted from defective repair system

To test chemicals and how mutagenic they are;

Chemical is addedto the cells, and these chemical not mutagenic, how many colonies formed ?

The same number 10 or 20

Mutagenic chemical? There are two types of mutation will occur, spontaneous and induced, as a result of this chemical. How many colonies do you predict to see? A lot more ( more than 10 or 20 )

In order to determine that a chemical is mutagenic or not , the number of colonies must be duplicated

Example :

| Water | Motor oil | Alcohol | Drug X | شيبس أبو ٥ قروش |
|---|---|---|---|---|
| 10 | 50 | 43 | 9 | 200 |

Water is the control

Motor oil and alcohol and " شبس ابو 5 قروش" are mutagenic but drug X is not mutagenic

We have another factor in our body which are liver enzymes. They detoxify chemical and metabolize substances , they can change chemical from one form to another. Sometimes it change harmful chemical into harmless and vice versa. Liver enzymes are used to determine how mutagenic a chemical is after being metabolized in the body.

| Condition | Water | Motor oil | Alcohol | Drug X | شيبس أبو ٥ قروش |
|---|---|---|---|---|---|
| -liver enzymes | 10 | 50 | 43 | 9 | 200 |
| +liver enzymes | 12 | 22 | 50 | 35 | 500 |

There no effect of liver enzymes in the mutagenicity in water

Motor oil in presence of liver reduced from 50 to 22 and become not mutagenic ( less than double of 12 ) so liver enzymes reduce the mutagenicity of the oil

Alcohol still mutagenic and there are no effect of lever enzymes

Drug X become mutagenic in presence of liver enzymes , so liver enzymes increase mutagenicity

" شبس ابو 5 قروش" is become more mutagenic in presence of liver enzymes

Last slide is not included.

## Test yourself!!

Imagine yourself in the laboratory with two ***ds-DNA*** samples (A and B), the sample A which has a concentration X microgram per ml exposed to heat reaching the **melting temperature(71.3 $C^o$ ) ,** at that point you noticed that the absorbance of 260 nm of UV from sample A increased 2 units in comparison with the original

sample(before heating) . On the other hand, the sample B needed a temperature 90 $C^{o}$ to make equaled concentrations of both ds DNA and ss DNA .

According to the previous case, answer the questions 1 and 2:

1.the concentration(microgram per ml) of the sample A (X value) and the absorbance of UV from the original sample A (before heating) respectively are:

a. 150 , 8
b. 300, 8
c. 150, 6
d. 300, 6
e. 200, 5

2. all of the following are NOT true except:

a. sample A is less resistant to denaturation than sample B.
b. sample B has higher content of G.C pairs than sample A.
c. the increase in the absorbance of UV due to the increase in ss DNA concentration .
d. adding sodium ions to samples increases the Tm due to neutralizing the negative charges found in DNA.
e. all of the above

A restriction endonuclease cuts at 5'ACGT 3'(between G and T) which is palindromic in this strand of an allele
5' GACCTACGTGCCAGTTCCACGTAAGTC 3' with its complementary strand , but this sequence may be mutated at nucleotide number 7- near to 5 prime- to G.
Depending on the previous case, answer the questions 3 and 4:

3. suppose that a person is heterozygous for this allele ( has the normal and the mutated allele) and you want to detect this using ***southern blotting*** with a probe carrying this sequence

3’ GGATGCACGGTC 5’ , so to ensure that he is heterozygous you must see how many bands in the blotting ( assuming that the probe must match at least **6 bases** in each strand of the allele to give a signal):

a. 1 b.2 c. 3 d. 4 e.5 f. 6

4. using ***only*** gel electrophoresis , how many bands do you expect to see in the gel for a homozygous person (normal alleles):

a.1 b.2 c.3 d. 4 e.5 f.6

5. About DNA replication in prokaryotes , what is (are) the wrong:

a. both DNA polymerase III and( DNA polymerase I) have the ability to add deoxyribonucleotides and remove deoxyribonucleotides but polymerase I removes from 5 prime to 3 prime and polymerase III from 3 prime to 5 prime.

b. both DNA helicase and DNA gyrase are ATP dependent .

c. DNA ligase makes the phosphodiester bond between the two primers of two okazaki fragments.

d. the two replication forks proceed in opposite directions until they meet up roughly halfway around chromosome.

e. more than one of the above.

Answers:

1.d 2.e 3. c 4. c 5. e

(Thanks to Abdullah Qaswal for writing the previous multiple-choice questions)