Genetics & molecular biology

◉ Sheet ○ Slide

Number:		12
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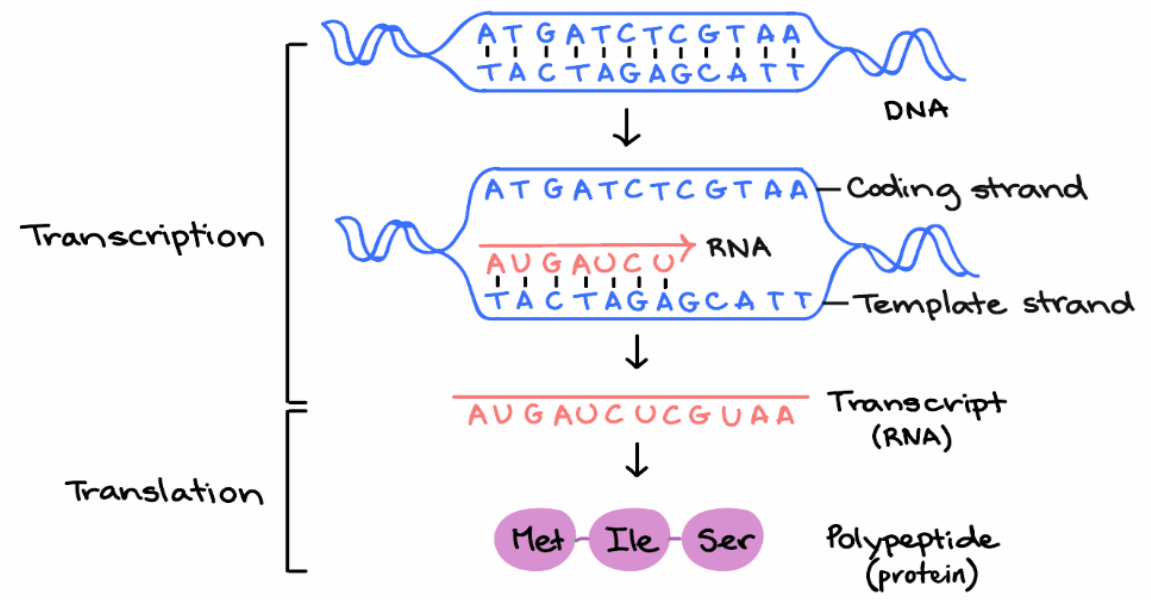
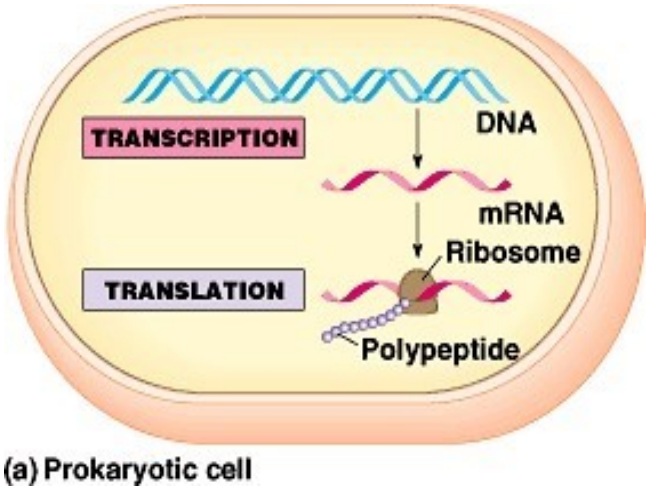
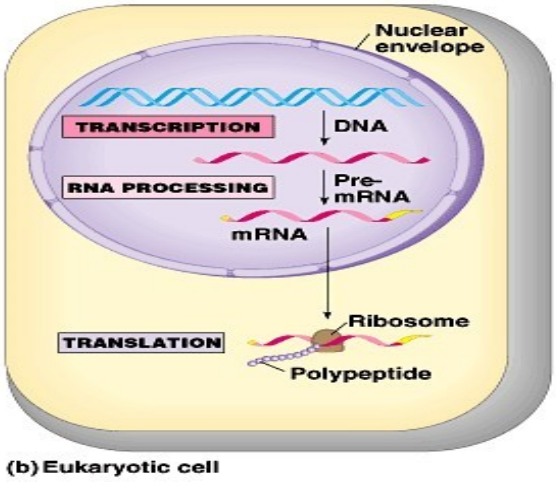
Protein Translation



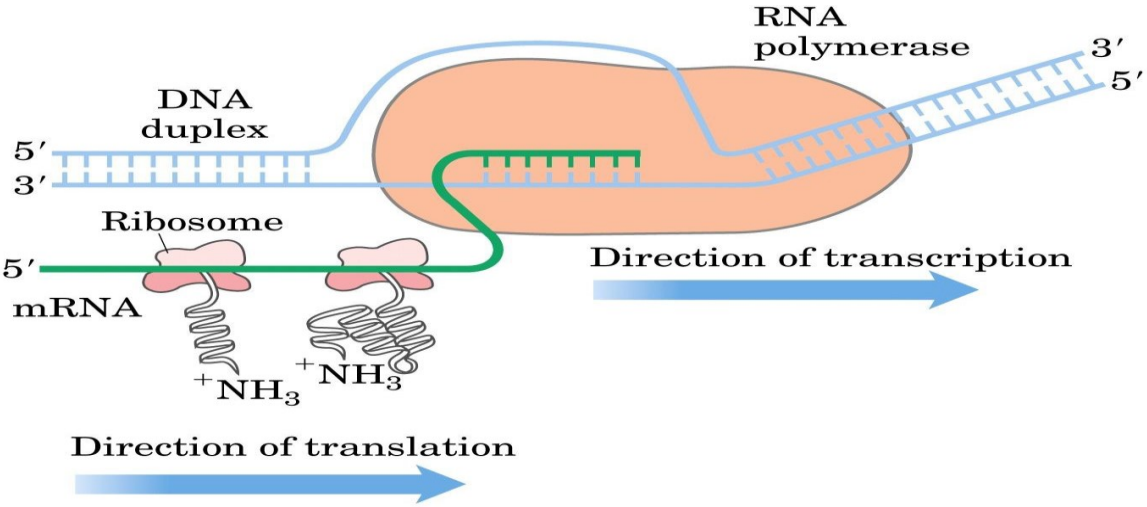
At first, We will talk about the differences between the transcription and the translation in the prokaryotes and the eukaryotes:



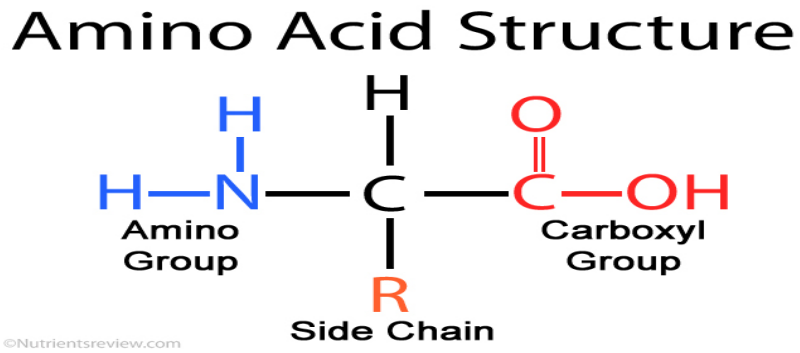


*** The translation and the transcription in prokaryotes take place in cytoplasm. In eukaryotes, the transcription (pre-mRNA) and the processing for the RNA occurs in the nucleus while the translation occurs in the cytosol.

Translation in prokaryotes:

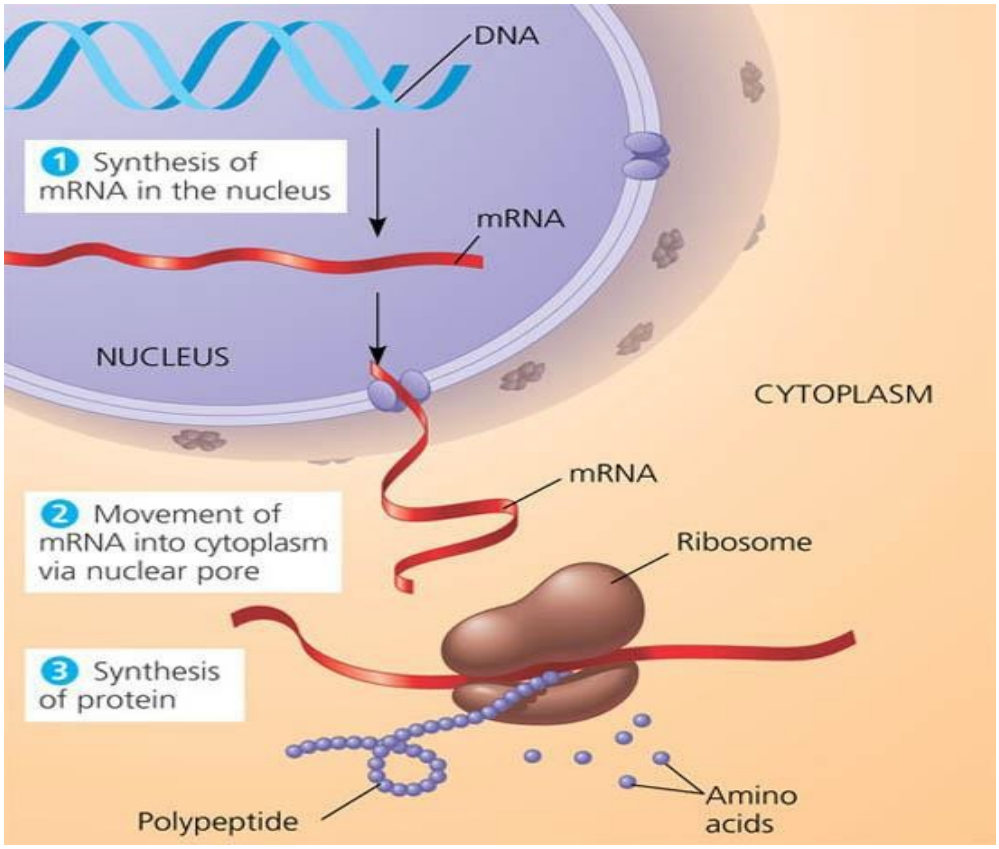


We have RNA polymerase, and the transcripts that came down. In prokaryotes' translation, it's possible that more than one ribosome holds the transcripts and do the translation.
in prokaryotes, the direction of the transcription is from 5' to 3', also the direction of translation is from 5' to 3' (the direction of the ribosome movement). In proteins, the first thing that becomes synthesized and appears is the "N" because in the protein there is an "N terminal and a C terminal" and the end of the protein is an amino acid (amino acid = carboxyl group + amino group that are connected to each other by a peptide bond) .



At the end, we will have a protein having an N terminal group (amino group) on one side and on the other side we will have a C terminal (hydroxyl group).

-- let's say it again: the direction of RNA polymerase while doing transcription is from 5' to 3'. The direction of the translation (movement of ribosome) is also from 5' to 3'.

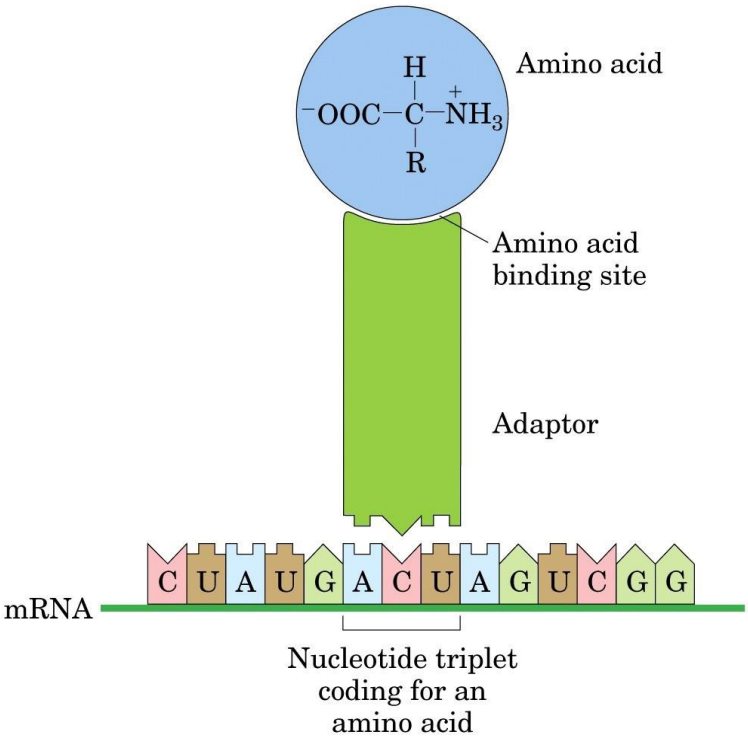


In eukaryotes, it is similar to prokaryotes (movement of the transcripts then translation).

** Genetic Code:
We have 4 letters of DNA (A, C, G, and T), 20 amino acids, this means that the 4 letters encode the 20 amino acids, But we want to know how much of nucleotides are making amino acid !!? every 3 nucleotides is a codon to the amino acid.

I don't have 64 amino acids! Although I have a 20 amino acids and 64 combinations (يعني احتمالية تشكيل هاد الكودون) that are responsible of giving 20 amino acids.

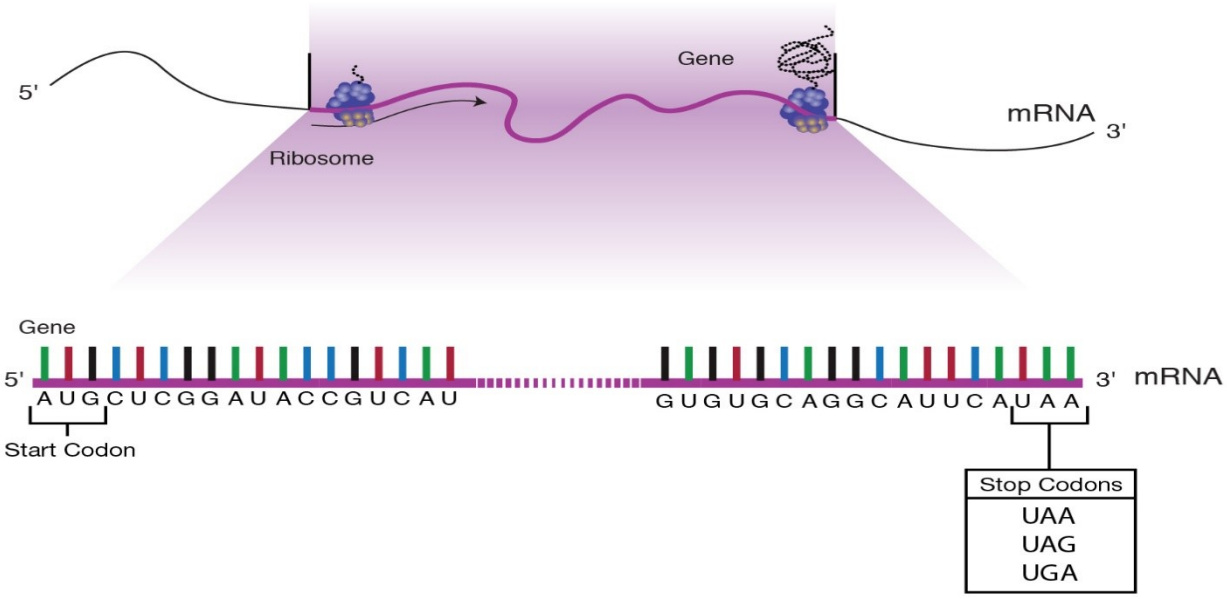
So the **genetic code** is a triplet of nucleotides, the feature of the genetic code is that there is no punctuation (no stop, بيقرأ بس ورا بعض ما بوقف بمكان). Ribosomes read the transcripts 3 nucleotides after 3 nucleotides and doesn't stop! Here I'm talking about the DNA and amino acids (تختلف عن لغة الامينو اسيدDNA لغة ال) so I need an adaptor that understands both (DNA and amino acids) . This adaptor is the TRNA.



This is the anti-codon region

The codon is found in the mRNA, the anti-codon region must be complement to the codon. There is a side that is called (CCA) which is added during the processing of the tRNA (this side will bind to the amino acid which is called the **amino acid binding site**).
"A" residue in specific will bind the amino acid on it.

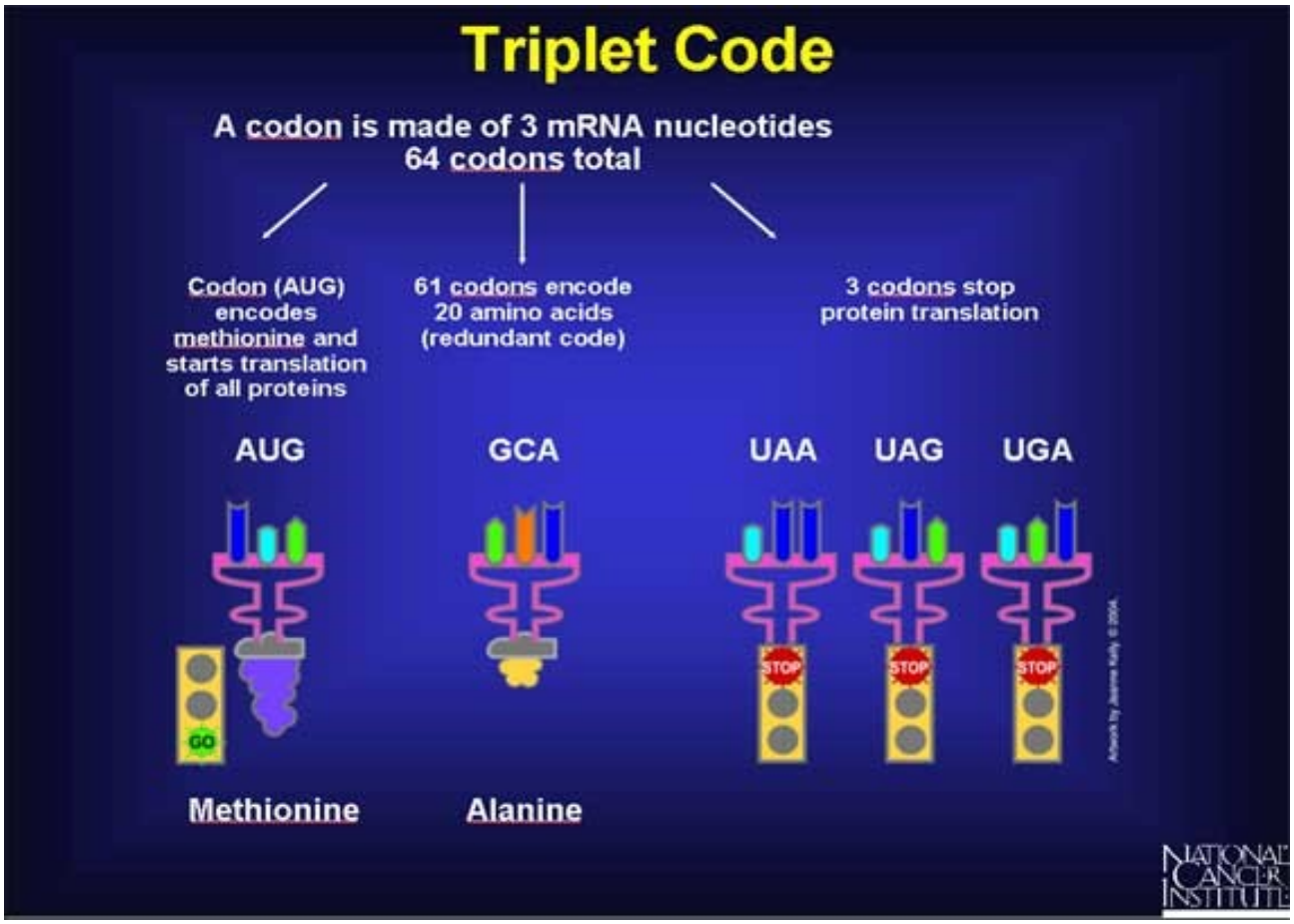
** If I have 64 combinations, will we have 64 tRNA!?
No, because I have only 32 tRNA, 20 amino acids, 64 combinations (that are not responsible for giving me all the 20 amino acids) because I have 3 that are stop codons.
Therefore, I have 61 codons that are responsible to give the 20 amino acids.



BE CARFUL *: when asked, we must know that the 61 codons are responsible to give us the 20 amino acids (all of them) But I have 64 combinations.
stop هم منهم 3 و بس 61 عندي انا لانو غلط فهو كلهم 20 ال بيعطونا اللي هم combinations64 ال انو حكولنا اذا codons .

I have 32 tRNAs and just 20 amino acids. This means that it's possible to have more than 1 tRNA for the single amino acid.

the 32 tRNAs: (31 of them give the amino acid and there is 1 tRNA for the initiation (AUG codon = methionine))
** the methionine in the initiation codon have a tRNA that differs from the tRNA that puts the methionine within the protein sequence.



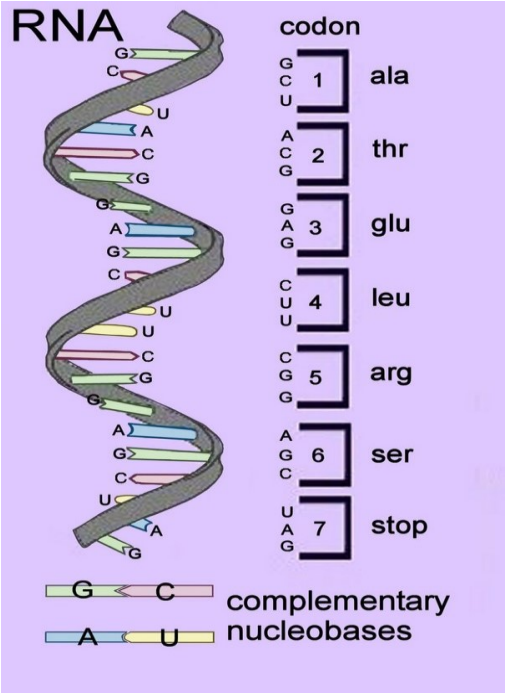
NOT ALL THE PROTEINS BEGIN WITH METHIONINE. (JUST THE FUNCTINAL PROTEINS).

BUT ALL THE TRANSLATION BEGINS FROM AUG because it's the initiation codon.

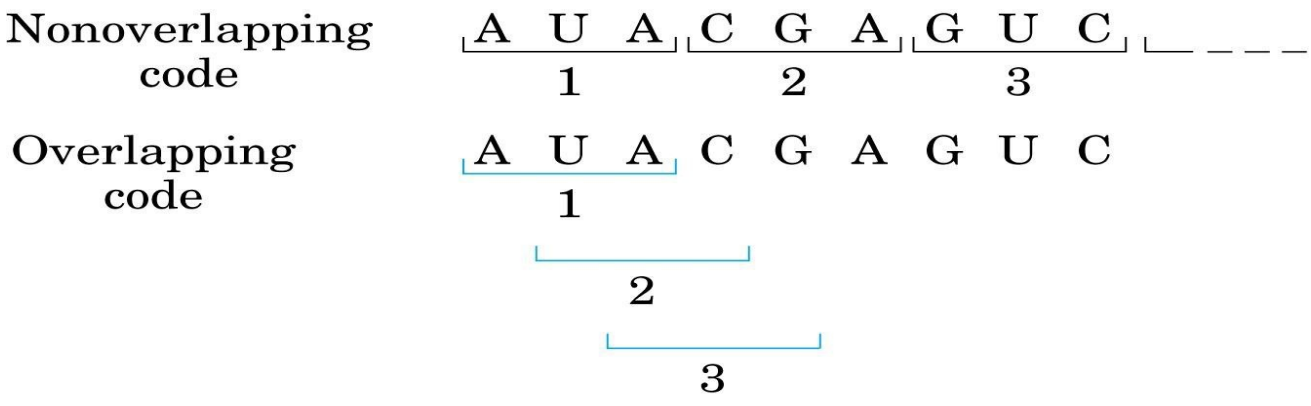
** processing to the protein must occur to get its native structure and to become a functional protein.

Characteristics of the genetic code:

1) The Genetic code consist of 3 nucleotides (triplet).



2) There is no Overlapping between the codes



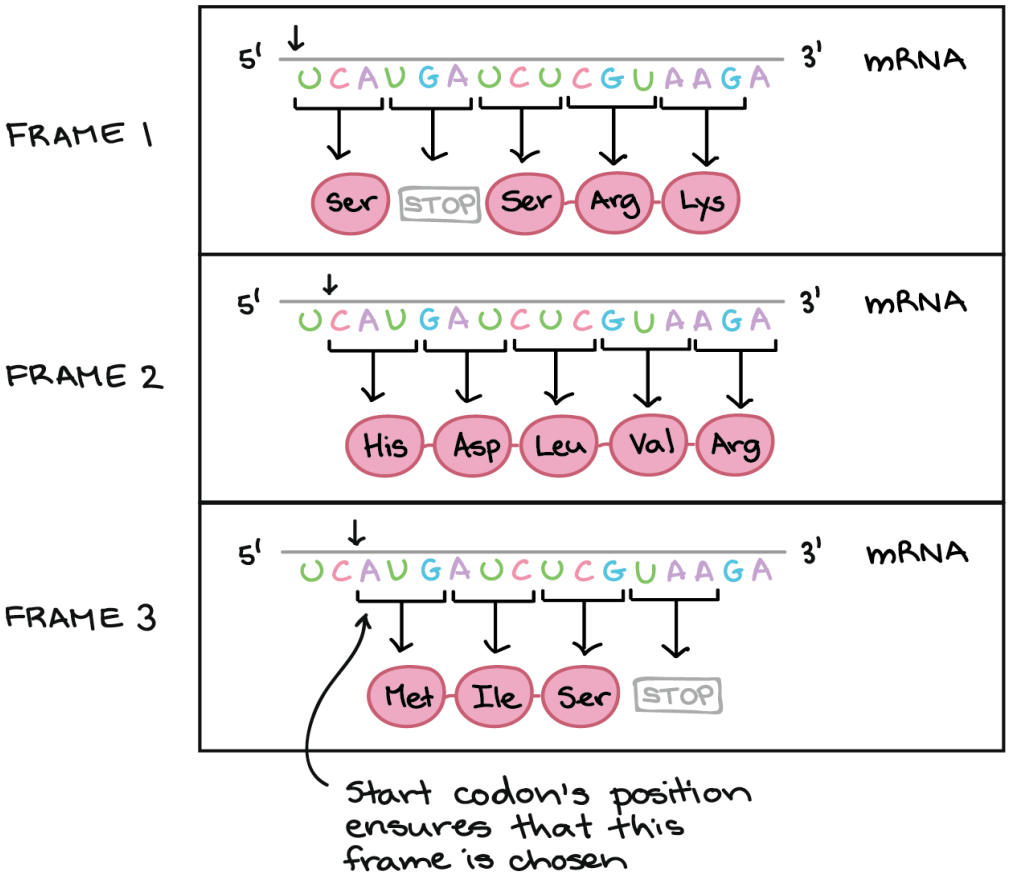
يعني بوخد كل 3 ورا بعض من دون ما اعيد نيوكليوتايد من اللي قبل، كل 3 نيوكليوتايد بيعطوني 1 امينو اسيد

Reading Frame:

It indicates the site at which the ribosome will start (the tRNA will bind).

*Each transcript has 3 reading frames and JUST one of them is functional (correct) and it is the real transcript that will give the protein.

- if I have any INSERTION\ DELETION the reading frame will be different.



Open reading frame (ORF): means that in the end I must reach something that is called Termination codon among 50 or more codons.

** NOT all the mRNA will be translated. We have sites that are called UTR (untranslated regions).

3) Genetic code has no punctuation.

Cracking the genetic code by Marshall Nierenberg experiment
The base sequences of the codons were deduced from experiments using synthetic mRNAs of known composition and sequence.

He did 61 combinations, and used an enzyme that is called “polynucleotide phosphorylase”.

$$(NMP)_n + NDP \leftrightarrow (NMP)_{n+1} + Pi$$

This means that he took a nucleotide without a template.
linking to بعمل فهيك. بنربط النيوكليوتايد في بعض فهيك بتزيد بواحد inorganic phosphate بس بطلّع ال RNA.

*** UUU = Phe (this codon is almost universal) because in the mitochondrial DNA there are codons that don’t give what the nuclear DNA gives.

4) Genetic code is almost universal

** Some amino acids have more than one codon. ONLY Met and Trp have 1 codon.

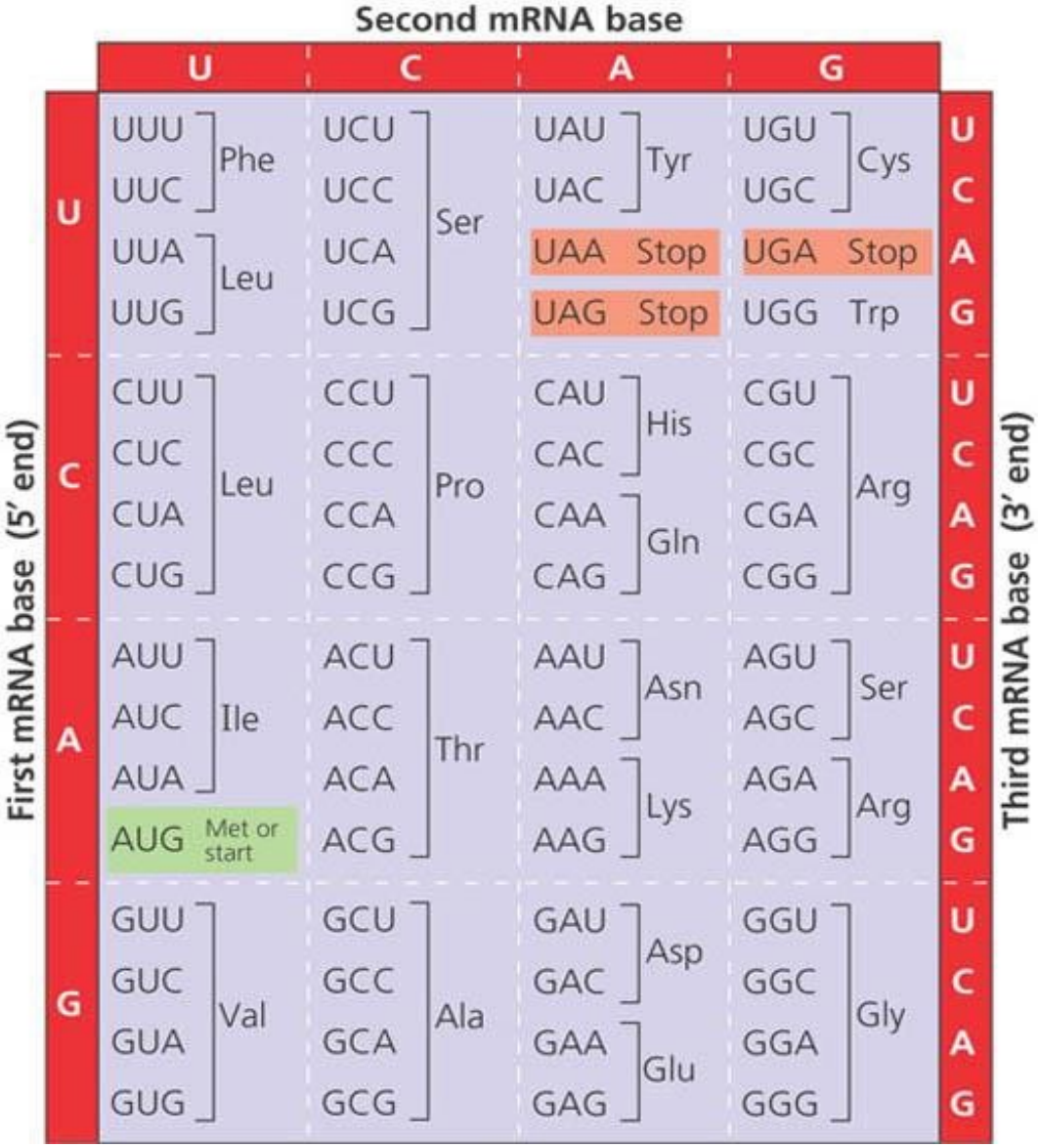
All other amino acids have **2-6** triplets.

EX:

UCU Ser UCA Ser
UCG Ser UCC Ser

Note that when just the last letter is changed, the same amino acid is given and this is called “**wobble base**”.

Dictionary of amino acid code words in mRNAs



Each cell transcribes specific genes that are regulated in the regulatory sequence (in the promoter region) according to the type of the cell and its products (genes turn off /on).
Housekeeping genes: genes that are always expressed in transcription. However, **inducible genes** are only expressed when activated by proteins called activators that bind to the enhancer upstream the **+1,** or they are not expressed by proteins called repressors that bind to the silencer region upstream the **+1**.
Note: enhancers and silencers are DNA sequences that are regulated. Activators and repressors are proteins that bind to the DNA to regulate expression.
One of the proteins that participate in the expression and activating of the genes is the co-activator.
The difference between the activator and the co-activator is that the activator binds directly to the DNA but the co-activator binds to the activator and not the DNA sequence directly. (The same is for the repressor and the co-repressor).

When the transcription process happens, the promoter region participates in the regulation, but if we want to enhance or suppress the process, we use activators or repressors.

Concentration of RNA depends on:
1- Rate of synthesis which indicates the presence or absence of activators or repressors.
2-Rate of degradation of mRNA which is related to the half-life of the mRNA. The longer the period the mRNA remains, the more translated. If the he synthesis and degradation of an mRNA are balanced, the concentration of the mRNA remains in a steady state. A change in either rate will lead to net accumulation or depletion of the mRNA. Degradative pathways ensure that mRNAs do not build up in the cell and direct the synthesis of unnecessary proteins.
How does the degradation happen?
In eukaryotes, it starts with exoribonuclease activity of the poly A tail - shortening, by a protein complex consisting of 10 types called exosomes

that degrade poly A tail from 3' to 5', while for lower eukaryotes degrade from 5' to 3'. Then, a decapping enzymes removes the cap at the 5'.
In prokaryotes, an endoribonuclease cuts the mRNA into fragments, then exoribonuclease degrades these fragments.

tRNA (transfer ribonucleic acid) in prokaryotes is very similar to that in eukaryotes, ⚠️except that some tRNA in eukaryotes may contain an intron that must be spliced.
tRNA is a single stranded in the primary transcript that folds to form the secondary structure (🍀 clover leaf)
<u>Processing of tRNA</u>:
1- Base modification: some types of nucleosides must be modified (methylation, psuedouridine, dihydrouridine...) This happens by changing the base binding using an enzyme called (psuedouridine synthase). The purpose of this process isn't clear, but it's believed that it helps in gaining the full function of the tRNA in transferring amino acids in the translation process.
2- 5' cleavage by Rnase P enzyme, which is a ribozyme- RNA with catalytic activity, cleavage of (100-200 nucleotide)
3- 3' cleavage by Rnase D enzyme, which is a protein enzyme at the 3' end.
4- CCA addition on 3', forming an intermediate. These nucleotides are very important for the binding of the amino acids to the A residue.
5-To reach a mature tRNA, the intron sequence must be removed by splicing.
⚠️***<u>Reminder</u>***: some tRNA contain introns in the eukaryotes, but not prokaryotes.

rRNA (ribosomal ribonucleic acid)
What is the ribosome?
It is a two-subunit organelle consisting of proteins and rRNA whether it's a prokaryotic or eukaryotic. However, the prokaryotic ribosome (two subunits) is 70S, while the eukaryotic ribosome is 80S. **(S)** is the sedimentation coefficient and it is not related to the molecular weight.
The prokaryotic ribosome subunits are:

30S - small subunit, and 50S - bigger subunit.
30S precipitates faster than 50S.
Both have many proteins and a certain type of rRNA that differs between big and small subunits. They are different among eukaryotes and prokaryotes and the only similarity is the 5S rRNA in both eukaryotic and prokaryotic big subunit.
In prokaryotes, the precursor rRNA is 30S rRNA.

Processing:

1- Base modification, mainly methylation- other than methylation we have uridine, psuedouridine, etc.. But the main one is methylation.
2- Cleavage: there are sites on the precursor rRNA that are processed by certain types of enzymes- cut them: RNase iii, RNase P, RNase F. That's why it's called **processing** and **not SPLICING,** because there's no rejoining after cutting.
After cutting, sometimes one of the mature RNA is a tRNA, which means that the precursor can be a 30S rRNA and not only pre-tRNA.
The mature rRNA is then surrounded by proteins to form the big and small subunits.

True or False: RNase P is a ribozyme and is involved in both tRNA and rRNA processing : true✔

In eukaryotic ribosome, subunits are:
40S- small subunit, and 60S- bigger subunit.
The pre-assembly happens in the nucleolus, while the assembly happens in the nucleus.
The precursor is 45S, which binds to a 90S preribosome

The processing:

1- Base modification by methylation, psuedouridination..etc
2- Special proteins called ***snoRNPs*** enters the complex in the nucleolus to make the cleavage in order to make the pre-subunits. The 5S is part of the 60S and it doesn't come from the precursor, but from outside - another gene-

3- Additional cleavage happens to prepare the mature ribosomal subunits.
The complete assembly happens in the cytoplasm.

Types of ribozymes:

Can be natural: in mitochondria, prokaryotes. It is an RNA enzyme that have a catalytic activity, which requires a substrate - RNA. Therefore, it's an RNA that catalyzes another RNA.

Types: RNase P

group 1 & 2 introns: self-cleavage through transesterification.

Peptidyl transferase: mRNA enzyme that makes the peptide bond between amino acids.

Hammerhead: plant virus RNAs, needs Mg+2 as a cofactor , it will make a cleavage in RNA, and high temperature causes degradation.

Synthetic: in virus, injection, laboratory... Etc.

How to get rid of the transcripts?

An accumulation of certain type of proteins called processing bodies, or P-bodies that surround the transcript and let the exosomes enter and make the shortening of the poly A tail and decapping to degrade the mRNA.

It also acts as a storage for the mRNA, when it is needed to be translated again, the P-bodies disassemble. If the poly A tail and the cap are removed, this means that mRNA will be degraded, otherwise it's kept surrounded by the P-Bodies.

Central dogma of molecular biology:

DNA TO DNA :DNA Replication

DNA TO RNA :Transcription

RNA TO PROTRIN :Translation

RNA TO DNA :Reverse transcription (retrovirus, telomerase)

RNA TO RNA :RNA Replication, has an enzyme called replicase (RNA dependent RNA polymerase) that enters the host cell but not the genome of it.

Applications for restriction enzymes:

- Restriction Fragment Length Polymorphism (RFLP): screening for some mutations

اذا كان عندي sequence محدد وصار فيه substitution mutation ممكن يصير عند بالصدفة restriction site وبتحفز انزيم القص EcoR1. هاي العملية بتساعد للبحث عن الطفرات وبتميز اذا كان عندي heterozygote or homozygote

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Applications for PCR:

- Reverse Transcription PCR: They study the activity of genes and they have many applications in disease which depend on Gene Activity.

How do they know if the Gene is active or not?

1. Checking where the proteins is active and where it is not.
2. Checking the mRNA because it is the bridge between RNA & DNA.!

ولكنPCR بتعامل مع DNA template not RNA وهون بيجي دور ال reverse transcription وبعرف اذا كان DNA الي mRNA الخاصة بالفايروسات اللي بتحول ال enzymes عن active طريق تصميمprimer الله.

ملاحظة: ال DNA الناتج من عملية ال Reverse Transcription بكون اصغر من ال DNA الأصلي وبميزه بالرمز cDNA واصله بكون RNA .

Processing for tRNA:

tRNA is very important in translation

ولكن بعض المناطق بتعمل base وبصير عليهprocessing مثل mRNA بطلع ال tRNA ك single srand مع بعض وبصير شكل معين وهاد بكون pairing primer transcript .

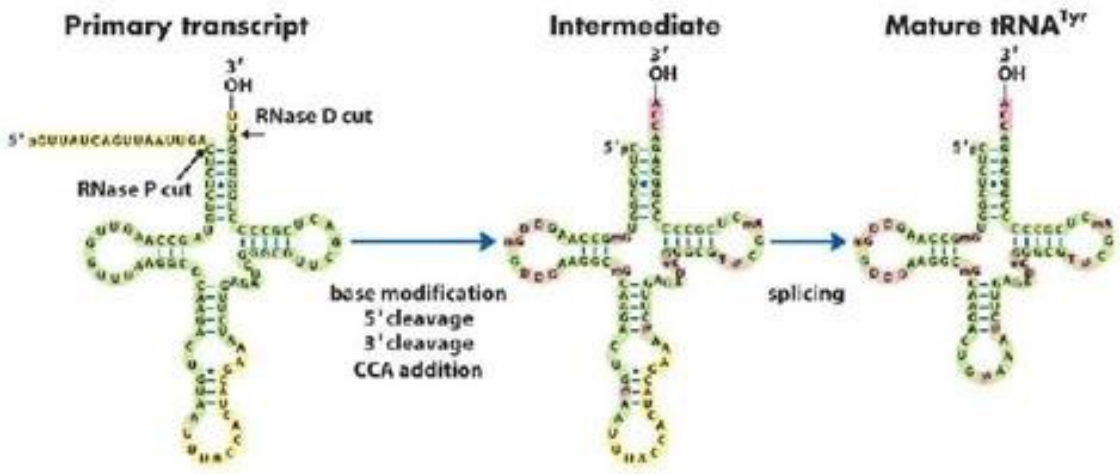
Types of processing:

1. Base modification: methylation, reduction and deamination

عشان تقوم بوظيفتها modified basesرح يطلع عندي بعض ال

2. Cut: For 3' end & 5' end by RNases
3. Splicing

وبعض منها بكون عبارة عن RNAهي بروتينات بتقص ال RNase Riboenzyme .



transferRNAnucleotidal عبر انزيم CCA وبنحط مكانه '3 في جهة UAA nucleotide لل cutبصير

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rRNA& tRNA بتطلع rRNA gene and tRNA genes عندي جينات معينة تسمى
وجين واحد ممك يطلع 16sRNA, 5sRNA & 23sRNA ؟ عندي عدة انواع منه : rRNA genes كيف يتم انتاج بعدة اشكال. cut بوساطة عملية ال tRNA هدول ال 3 جينات بالاضافة الى

لل splicing (مرت في عملية ال cleavage in vertebrate بتدخل في عملية ال snRNA وعندي كمان mRNA(

تتراوح مدته من half time وكل واحد اله degredation بعد ما تمت له عملية الترجمة مصيره ال mRNA ثواني قليلة الى عدة ساعات.
اللي خلصت شغلها وبتكون في مراحل RNA ال بتم تكسير P bodies عملية التخلص منه تحدث في منطقة تسمى والتكسير بكون بوساطة turn over ولما اشيلهم ببطل محمي وبصيرله tail & cap. اولها اني اشيل ال decapping& detailing enzymes .
بطلع على شيئين زي كفتين الميزان همي: RNA وعملية التكسير مهمة زي عملية التكوين ولما اطلع على نتائج ال rate of degradation & rate of product .

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template:. يكون RNA بعض الاحيان ممكن ال

1. Reverse transcription
2. بعض الفايروسات عندهم انزيم يعرف باسم RNA dependant RNA polymerase لانه بعض الفايروسات كل مادتهم الوراثية بتكون RNA ما في عندهم DNA وبتسخدمه ك template

بتسبب سرطان وايدز retrovirus بنسميها RNA عندهم بتكون genetic materialالفايروسات اللي ال

For transduction, they enter their genetic material into the DNA of the host cells and they make integrations in the genome and become part of it and sometimes make mutation in important sites. (they cause cancer and AIDS)

Scr-viral: (v-scr): in viruses. Oncogen (gene that causes cancer)

الفيروسات ممكن تسبب سرطان بطريقتين:

1. Integration in our genes
2. V-Scr بيعطينا الفيروس DNA وخلايانا بتنتجه كبروتينات وبتصير عملية ال transformation وبتتحول من خلية سليمة لخلية سرطان وهيك بتبلش عملية التكاثر للخلايا السرطانية.

AZT a drug used against retroviruses and EIDS :

لما يدخل الفايروس على الخلايا واحط هاد الدوا بوقف عمل deoxythymidine dialog هو عبارة عن reverse transcriptaseال لانه بتفاعل مع ال active site بمنع replicationال تبعه وبالتالي

DNA recombination: The rearrangement (reshuffling) of genetic information within and among DNA molecules (e.g. chromosomes).

وتحدث في 3 انواع: مهم في عملية هندسة الجينات وتحدث في خلايانا بشكل طبيعي

1. **Homologous / general recombination:** crossing over in non-sister chromatin
   اعادة تشكيل اشياء ما كانت موجودة. وهاي العملية بتلزم وجود homology كبير بين المقاطع.
2. **Site-specific recombination:** the exchanges occur only at a particular DNA sequence. - occurs between sequences with a limited stretch of similarity.
   بقدر استخدمها في تدخيل مقاطع من ال DNA في مناطق معينة
3. **Transpositional recombination:** like transposomes. They don't need homology

Functions of genetic recombination systems

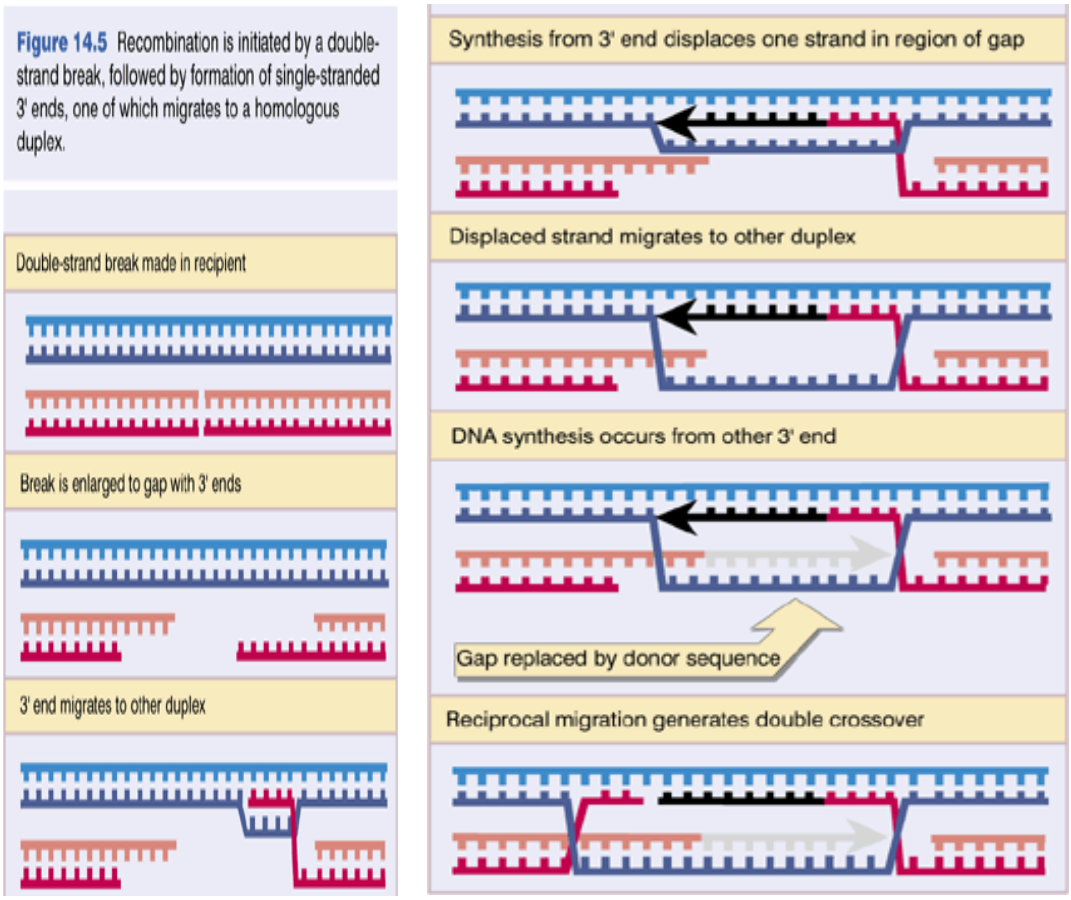
- **Biodiversity:** we will have a new alleles that weren't in parents
- **DNA repair system:** repair DNA double strand
- Rearrangement for some genes in developing phases like immune-globulin genes
- **Regulation of the expression of certain genes.**
- **Facilitate the segregation process in meiosis (in tetrad phase)**

Crossing over:

Occurs on tetrad phase in a specific area (chiasma) between non-sister chromatins. If the crossing over occurs, the result will be 4 alleles (2 new) and if not, there will be 2 only.

ملاحظة: كل ماكنت الجينات بعيدة عن بعض بتزيد احتمالية حدوث ال cross over.

Recombination pathways:



1. Double strand break then enlargement of the break by enzymes (degradation). Here, we must have two double strands (homologous pair)

واحد من ال pairs رح يصيرله كسر بوساطة انزيمات ويصير degredation للاطراف كما في الصورة لحد ما ينتج نهاية تكون حرة حتى يتحرك, وبعد ما صار التكسير رح يطلع الجزء الحر ويعمل displacement ويصير migration (بطول) والجزء اللي فوق بنزل لتحت وبصيرله انبعاج معين وهيك شكلوا اجزاء على شكل X تسمى هاي المناطق holliday model

الفجوات اللي صارت بيعبيها ال DNA polymerase (DNA synthesis)

بنفصلوا عن بعض بوساطة انزيمات resolvase والناتج بعتمد على طريقة الفصل او القص