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Faculty of Medicine
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جامعة جيلالي ليابس
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قسم الصيدلة

Course Handout

Intiteled

Molecular Genetics Course

Presented by

Dr. Benchiha Nawel Nassima

Intended for students specializing in Pharmacy , General Medicine , and Dental Surgery,

&

Bachelor's and Master's degrees in Biological Sciences.

April 2025

وفوق كل ذي علم عليم

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FOREWORD

Molecular biology is a scientific discipline at the crossroads of genetics, biochemistry and physics, whose object is the understanding of the mechanisms of cell function at the molecular level.

Molecular biology emerged in the 20th century, following the development of the laws of genetics, the discovery of chromosomes and the identification of DNA as the chemical carrier of genetic information.

Following the discovery of the double helix structure of DNA in 1953 by James Watson (1928-), Francis Crick (1916-2004), Maurice Wilkins (1916-2004) and Rosalind Franklin (1920-1958) saw significant developments in molecular biology, making it an indispensable tool in biology. modern from the 1970s onwards.

Since the late 1950s and early 1960s, molecular biologists have learned to to characterize, isolate, and manipulate the molecular components of cells and organisms. These components include DNA, the carrier of genetic information, RNA, and proteins, the most important structural and enzymatic molecules of cells.

This course handout is a support for the genetics module intended for science students. Medical and molecular biology subjects; intended for undergraduate and graduate students in science biological.

The handout comprises seven chapters: the first six chapters concern molecular biology in all its forms, which will allow students to understand the structure and organization of DNA, with all its complexity of replication, transcription, translation and post-modification transcriptional. As well as mutations, repair, and protein synthesis. While the last chapter presents the entire organization of chromatin, thus providing students with a clear understanding of the unit of chromatin structure with its histones and histone variants. The latter have been detailed from the point of view genomic distributions, functions and chaperone proteins or remodeling complexes. Their Implications in cancers and other genetic diseases have also been explored.

1. Nucleic acids

Nucleic acids result from the condensation of very long chains of basic structures called **nucleotides.**

Nucleotides result from the sharing of a base and a phosphoric acid . and a sugar. However, there can be one to three phosphoric acid groups.

We will study each of these three structures in turn, and then see how These come together to form more complex entities.

1.1 Phosphates

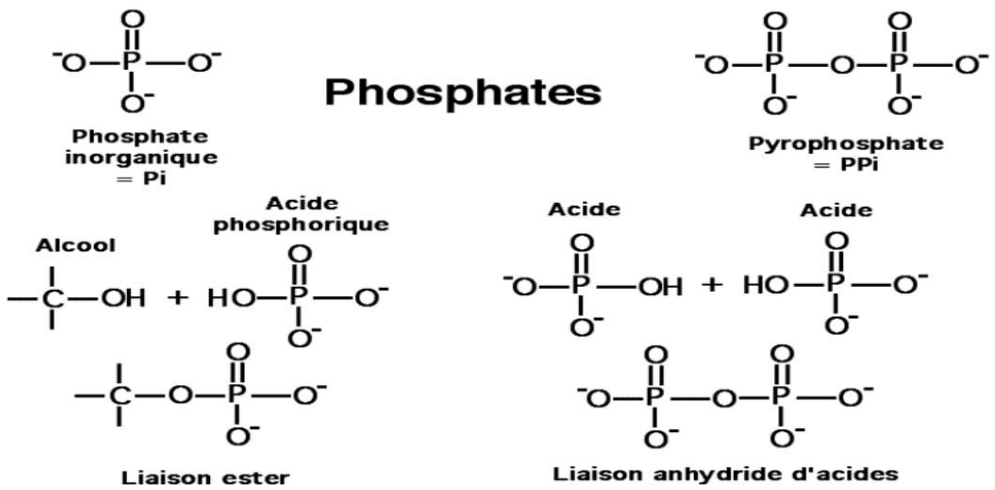


Figure 1: Phosphates

Nucleic acids are composed of simple molecules such as acid phosphoric

(PO4H3), 5-carbon sugars (pentoses) and nitrogenous bases (purines or pyrimidines).

Inorganic phosphate is a stable ion formed from phosphoric acid. PO4H3. It is often written Pi.

Phosphate esters can form between a phosphate and a group hydroxyl

free (alcohol, enol, phenol...).

The condensation of a phosphate (phosphoric acid) and another acid, for example Another phosphate gives an anhydride. There are also mixed anhydrides with acids carboxylic acids, for example.

Pyrophosphate is an ion derived from pyrophosphoric acid (P2O7H4), which is itself a phosphoric acid anhydride.

1.2 Ribose, deoxyribose

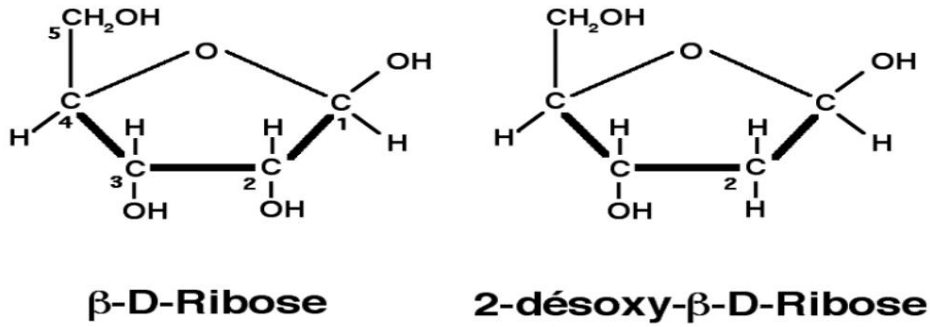


Figure2: Ribose, deoxyribose

The two sugars encountered are **ribose** in RNA and **deoxyribose** in DNA.

Ribose is a D-series pentose, in which all hydroxyl groups are right-handed. (Fisher representation). More precisely, for ribose, it is bD-ribofuranose, and for the deoxyribose of bD-deoxyribofuranose (it is derived from the previous one by reduction of the alcohol function carried by carbon 2).

Deoxyribose is much more stable than ribose, which is suitable for the conservation of genetic information. Indeed, it is considered that the elimination of The hydroxyl group at the 2' end of ribose reduces the possibility of DNA hydrolysis by a factor of 100. compared to RNA.

Noticed :

According to legend, the name ribose comes from the initials of the institute where it was discovered, the "Rockefeller Institute of Biochemistry" in New York.

1.3 Nitrogenous bases: Purine, Pyrimidine

The nitrogenous bases of nucleic acids belong to two classes of molecules according to the aromatic core that forms its skeleton.

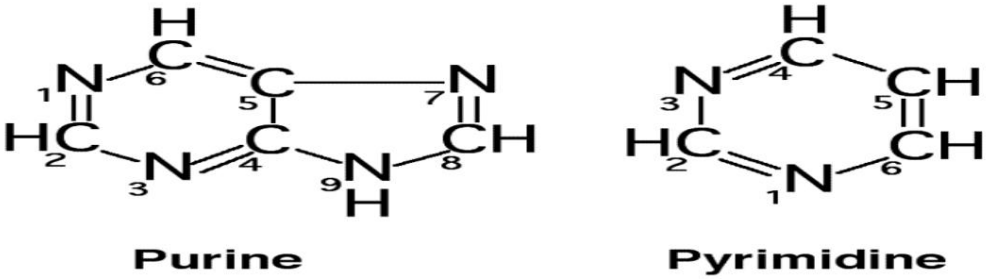


Figure 3: Nitrogenous bases: Purine, Pyrimidine

The pyrimidine ring is the simplest: it is an aromatic ring with six atoms, four carbons and two nitrogens; the two nitrogens in meta position (no. 1 and 3).

The purine nucleus consists of two joined heterocyclic nuclei, one of which is six atoms and the other of five atoms, having two carbons in common in the middle. Compared to these common carbons, the nitrogens occupy symmetrical positions (numbers 1 and 3 on the left, number 7 and 9 on the right).

The different bases found in nucleic acids are derived from them according to the substituents that the atoms of these nuclei carry.

1.4 Purine bases:

There are two purine bases, **adenine** and **guanine.**

Purines have a double aromatic ring with a hexagonal ring on the left. of 4 carbons and 2 nitrogens and on the right a pentagonal ring of 3 carbons (2 of which are shared with the previous one) and 2 nitrogens.

Adenine consists of a purine ring in which carbon 6 is substituted by a amine function. It is the only nucleic base whose formula does not contain an atom of oxygen.

Guanine consists of a purine ring in which carbon 2 is substituted by a amine function and carbon 6 by a ketone function (Housset and Raisonnier, 2010).

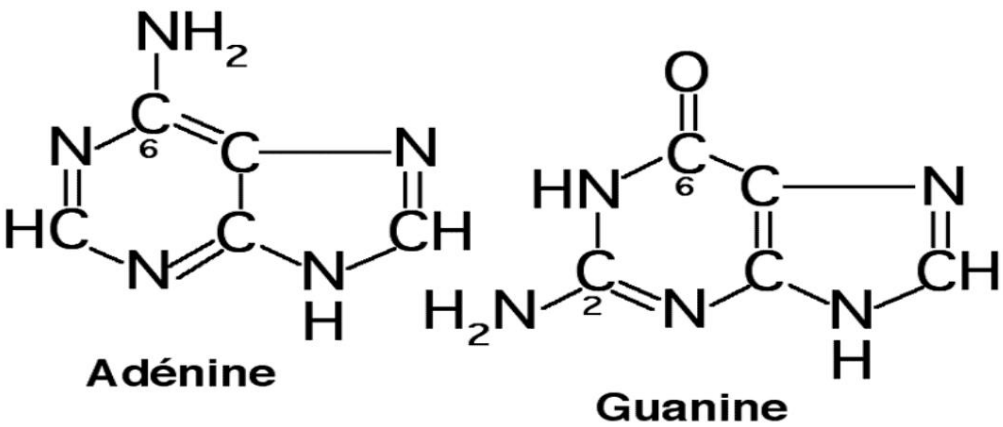
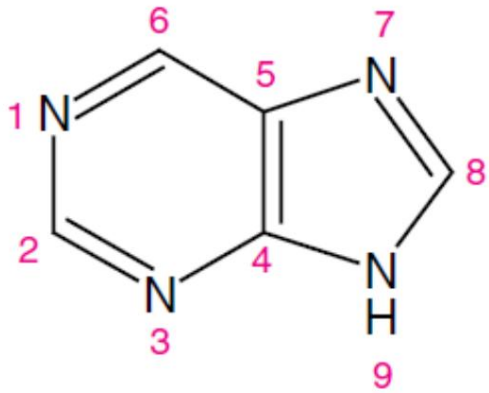


Figure 4: Purine bases

The numbering rules require that the pyrimidine nucleus be numbered first, then the imidazole nucleus.



1.5. Pyrimidine bases:

There are three pyrimidine bases: cytosine, uracil, and thymine. Pyrimidines have a hexagonal aromatic ring of 4 carbons and 2 nitrogens.

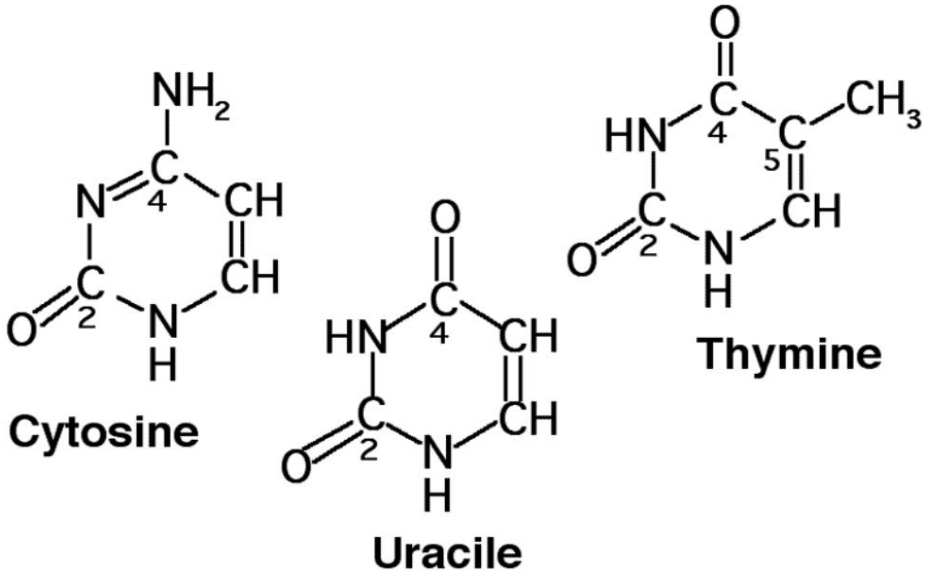


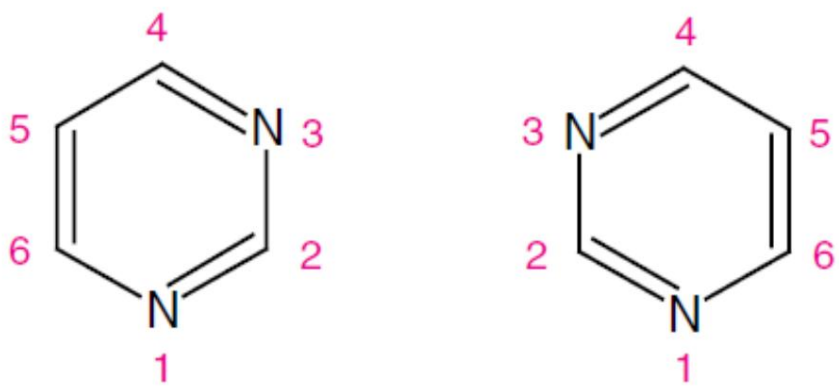
Figure 5: Pyrimidine bases

Cytosine consists of a pyrimidine ring in which carbon 4 is substituted by an amine function and carbon 2 by a ketone function.

Uracil consists of a pyrimidine ring whose carbons 2 and 4 bear ketone functions.

Thymine also consists of a pyrimidine ring whose carbons 2 and 4 carry ketone functions, but with carbon 5 substituted by a methyl group.

The numbering rules imply that one of the nitrogen atoms is assigned the index 1 and the other the index 3 regardless of how the molecule is represented.



1.6 Important properties of nitrogenous bases:

The nitrogenous bases are:

-slightly soluble in water

-basic

-absorb in the UV range at approximately 260 nm.

Each base has a characteristic spectrum. All bases generally have 2 absorption maxima (190-230 nm and 240-290 nm). This property allows for the quantification of nucleic acids in a solution.

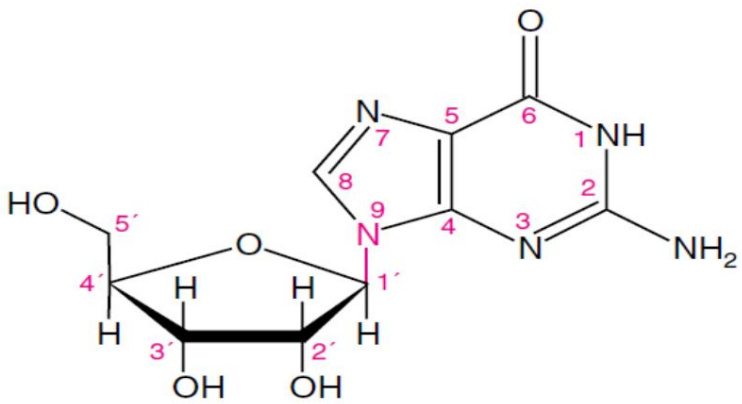
-tautomerism. Bases can exist in 2 forms Tautomers according to pH: enol form and keto form

1.7. Nucleosides :

Sugars (ribose or deoxyribose) bind to nitrogenous bases through bonds involving one of the nitrogen atoms in the base (nitrogen #1 of pyrimidines or nitrogen #9 of purines) and the carbon number 1 of the sugar (reducing carbon or semi-acetal function). These are N- bonds carbohydrates.

The bonding of a nitrogenous base with one of the sugars forms a nucleoside. A nucleoside It is therefore composed of a base and a sugar linked by an N-glycosidic bond. In a nucleoside, The atoms of the base are numbered with digits: 1, 2, 3, etc... and to distinguish them, the The sugar carbons are numbered 1', 2', 3', etc…

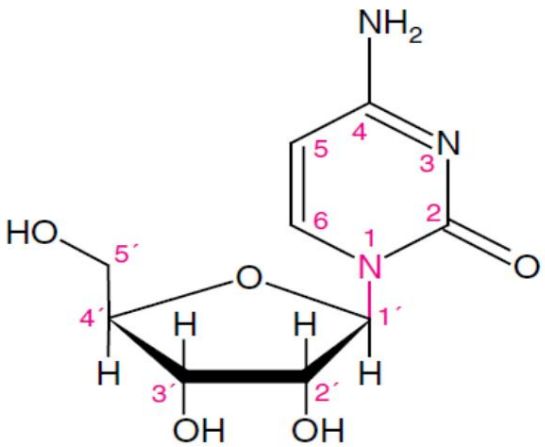
With a purine base, the bond is formed between carbon 1 of the sugar and nitrogen 9 of the base (Housset and Raisonnier, 2010).



Guanosine

Figure 6: Structure de la guanosine.

With a pyrimidine base, the bond is formed between carbon 1' of the sugar and nitrogen 1 of the base.



Cytidine

Figure 7: Structure de la cytidine.

Table 1. Nucleoside nomenclature.

	Désoxyribonucléosides	Ribonucléosides
Bases	**Nucléosides**	
Thymine	Désoxythymidine ou thymidine[1]	—
Cytosine	Désoxycytidine	Cytidine
Adénine	Désoxyadénosine	Adénosine
Guanine	Désoxyguanosine	Guanosine
Uracile	—	Uridine
Hypoxanthine	—	Inosine

1 Deoxythymidine can simply be called thymidine since initially, It was only present in DNA; but we now know that thymines are found in tRNAs, originating from doubly methylated uracils. It is therefore better to specify the name in deoxythymidine.

1.8. Nucleotides:

They result from the bonding of a base, a sugar, and one to three phosphoric acids. following the rules and procedures outlined above.

The bonding of a nucleoside with a phosphate occurs through esterification of the primary alcohol function (carbon #5') of sugar and one of the three acid functions of phosphate.

• The resulting ester is a nucleotide. A nucleotide is therefore composed of a nitrogenous base, linked by a glycosidic bond to a sugar, itself linked by an ester bond to a phosphate.

• Nucleic acid metabolism involves substrates rich in energy, nucleoside triphosphates. A nucleoside triphosphate is a nucleotide whose phosphate is itself linked to one or two other phosphates by anhydride bonds of acids.

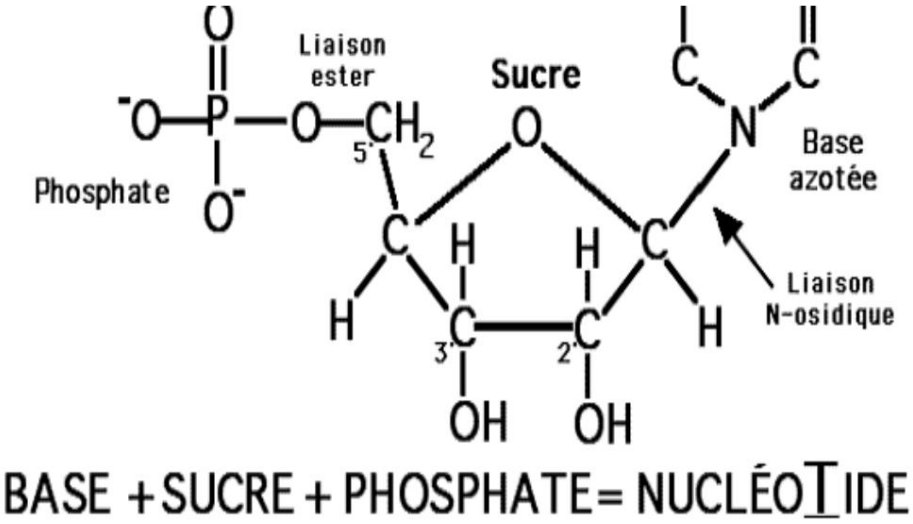


Figure 8: Structure of a nucleotide

The nucleotide ATP (Fig. 9) has very specific roles: substrate of RNA polymerases, energy-carrying coenzyme, energy reserve…

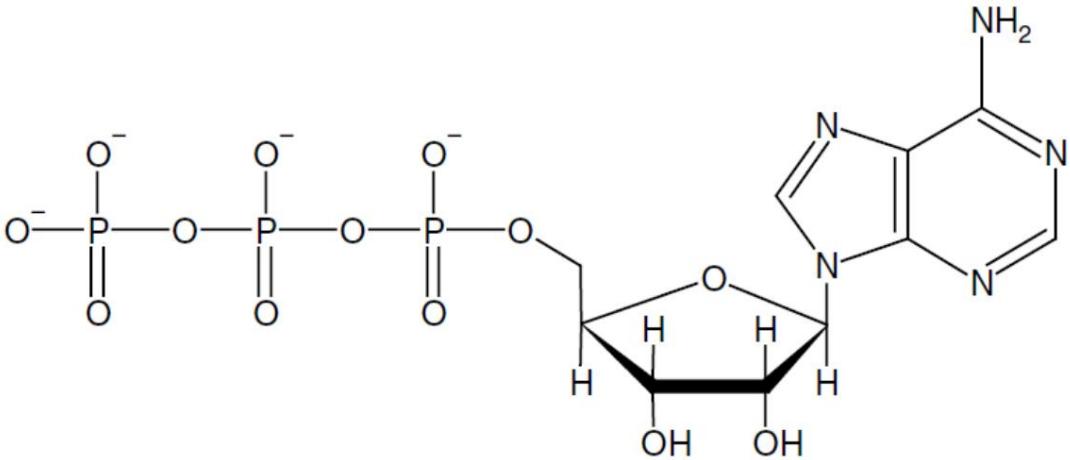


Figure 9: Structure de l'ATP.

Figure 9: Structure of ATP

Table 2 lists the nomenclature of monophosphate nucleotides.

Table 2 Nomenclature of monophosphate nucleotides.

	Désoxyribonucléosides monophosphatés	Ribonucléosides monophosphatés
Bases	**Nucléotides**	
Thymine	Acide désoxythymidilique dTMP	
Cytosine	Acide désoxycytidylique dCMP	Acide cytidylique CMP
Adénine	Acide désoxyadénylique dAMP	Acide adénylique AMP
Guanine	Acide désoxyguanylique dGMP	Acide guanylique GMP
Uracile	—	Acide uridylique UMP

Each nucleoside can be linked to one, two, or three phosphates. These are designated by... Conventional abbreviations: GMP for guanosine monophosphate, CDP for cytidine diphosphate, ATP for adenosine triphosphate, etc...

Nucleotides are defined as nucleosides monophosphates: AMP or acid adenylyl, dTMP or deoxythymidylic acid, etc...

Nucleosides polyphosphates are diphosphates: ADP or GDP... or even... triphosphates, the richest in energy: ATP or GTP; etc...

Nucleic acids are formed by a polycondensation of AMP nucleotides, CMP,

GMP and UMP for ribonucleic acids, dAMP, dCMP, dGMP and dTMP for deoxyribonucleic acids.

1.8.1. The bond between nucleotides:

When a nucleic acid is in solution, the molecules form bonds hydrogens link the nucleotides in pairs, so that one nucleotide binds to adenine with a thymine nucleotide (or a uracil nucleotide in an RNA) and a guanine nucleotide with a cytosine nucleotide.

• This bond is referred to as **hybridization.**

• Adenine-thymine hybridization is less stable (2 hydrogen bonds, -21 kJ) than that between guanine and cytosine.

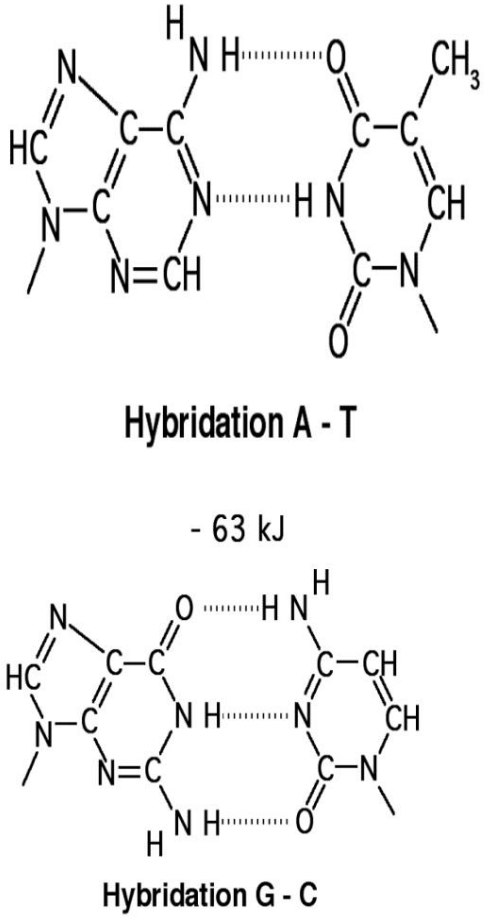


Figure 10: The bond between nucleotides

Guanine-cytosine hybridization is more stable (3 hydrogen bonds, -63 kJ) than that between adenine and thymine.

Nucleotides are linked together on each strand by covalent bonds.
(phosphodiester bonds between a phosphate group and two deoxyribose molecules)
adjacent nucleotides)

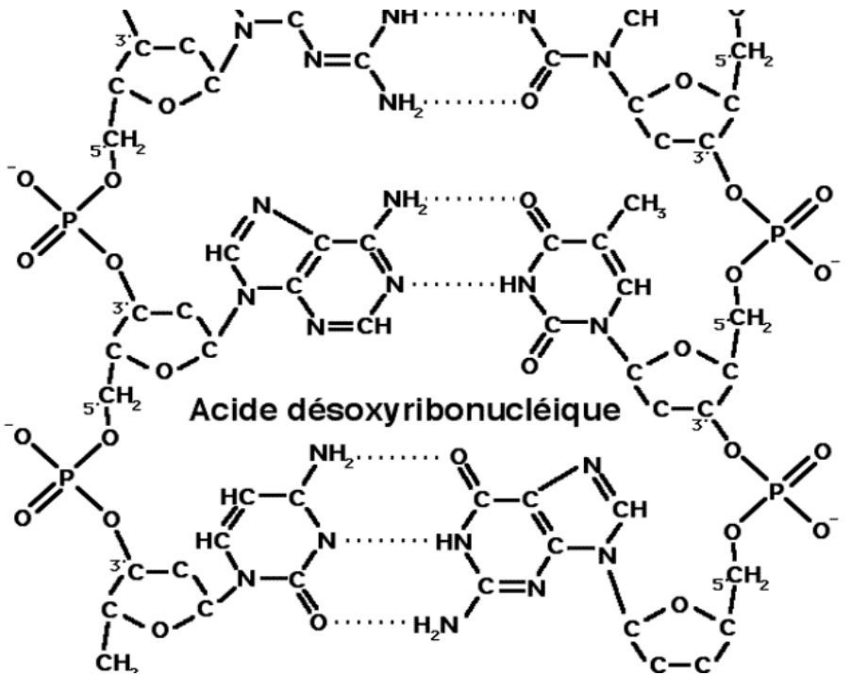


Figure 11: Hydrogen bonds of nucleotides

1.8.2. Hydrogen bonds of nucleotides

A hydrogen bond is a weak energy bond between two atoms attracted to each other. towards the other for electrostatic reasons, one being rich in electrons and therefore nucleophilic and the other having only the protons of its nucleus, therefore electrophilic.

Thus the hydrogen atom, whose single electron is necessarily in the orbital which unites this atom to the rest of the molecule, can only be electrophilic and as such attracted to the atoms having lone pairs of electrons.

Nitrogen and especially oxygen atoms have two and four atoms respectively electrons in their outermost shell that do not participate in bond orbitals covalents of the molecule. This gives them a nucleophilic character which allows them to exert an attraction on neighboring electrophilic atoms, in particular atoms of hydrogen: this attraction constitutes a hydrogen bond.

When the orbitals that link the hydrogen atom to the molecule on one side On the left and on the other side the lone pairs of electrons with the nucleophile atom are in the On the same axis, the hydrogen bond is stronger.

2. Properties of DNA revealed by X-ray diffraction (Works of Watson and Crick):

2.1. Context of revelation

The double helix structure of DNA was elucidated by Watson and Crick in 1953. Two researchers then have the following information:

(a) the chemical composition of DNA (deoxyribose, nitrogenous bases, and groups phosphate);

(b) X-ray diffraction patterns of crystallized DNA, patterns due primarily to Rosalind Franklin and Maurice Wilkins of King's College. These photographs show a cross-shaped figure, characteristic of helical structures;

(c) the work of Erwin Chargaff, which had shown that for any molecule of DNA, the number of adenine molecules is equal to the number of thymine molecules, and that of cytosine is equal to that of guanine;

(d) electron microscopy analyses, which had shown that the diameter of the DNA molecule is 20 Å, suggesting that this molecule comprised two strands of deoxyribose-phosphate.

It was by developing several molecular models in succession that Watson and Crick succeed in propose a structure that satisfies all the data crystallographic and biochemical data then available (Housset and Raisonnier, 2010).

This structure is now known to everyone; it has become the emblem of the Molecular biology: two strands made up of phosphate groups and sugars form a double helix where the orientations of each of the strands are opposite.

The nitrogenous bases are linked to the sugars on each of the two strands, each base of a One strand is held opposite a base of the other strand by hydrogen bonds. Cytosine always faces guanine, and adenine faces thymine. The two strands of a DNA molecules are said to be complementary.

Crick, Watson, and Wilkins received the Nobel Prize in 1962 for this work, which was described by Peter Medawar as "the greatest scientific achievement of our century". Rosalind Franklin would likely have been associated with this prize if illness hadn't intervened. taken from us prematurely.

2.2. Spatial structure of DNA:

X-ray analysis of DNA molecule crystals, performed in 1953 by Watson and Crick, indicates a structure with the following configuration:

- a double helix formed from two DNA strands whose base pairs are complementary

- the two chains are antiparallel

- the sugar-phosphate chains form parallel helical skeletons external, the planes of the bones being almost perpendicular to the plane of the bases

- the planes of the bases are perpendicular to the axis of the helix and each of the bases One chain is paired with the other chain by hydrogen bonds, the two bases being located in the same plane

- The paired bases are complementary (A/T) and (G/C) and are within the central cylinder of the propeller.

• Heat can separate the two strands: this is DNA fusion. This fusion is reversible: the two chains can hybridize again.

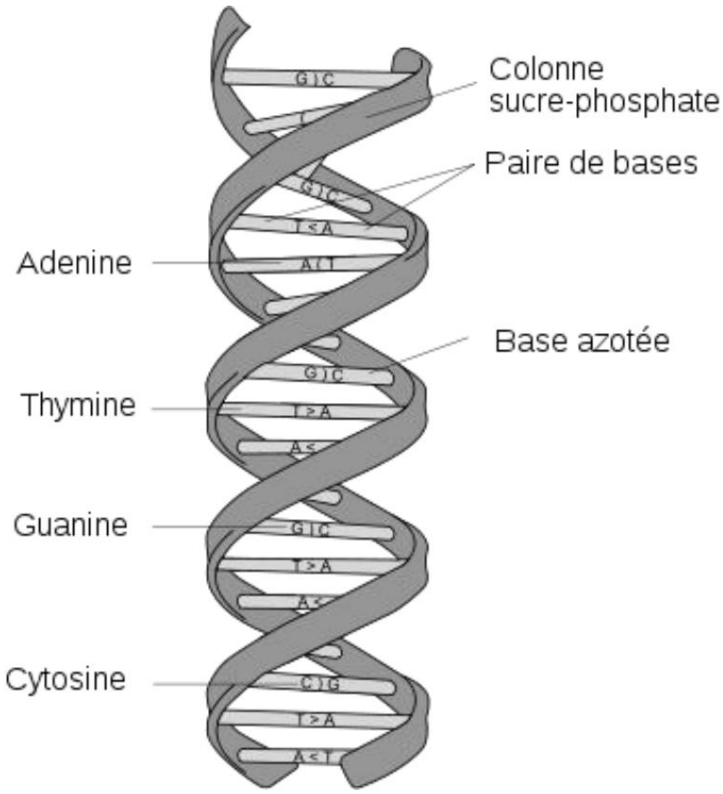


Figure 12: Model of DNA structure

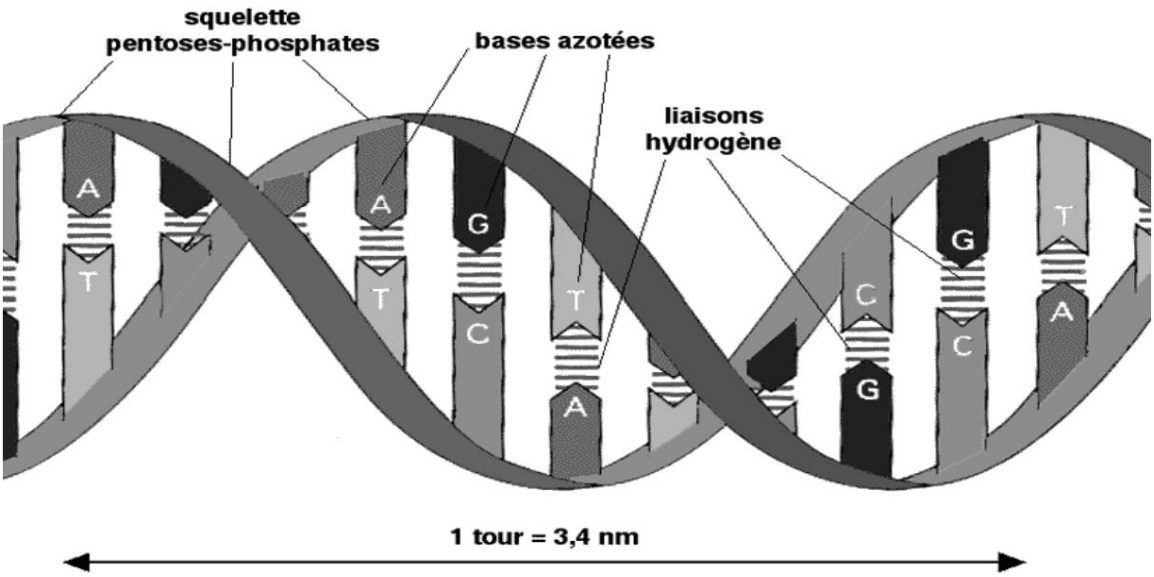


Figure 13: The double helix (ribbon model)

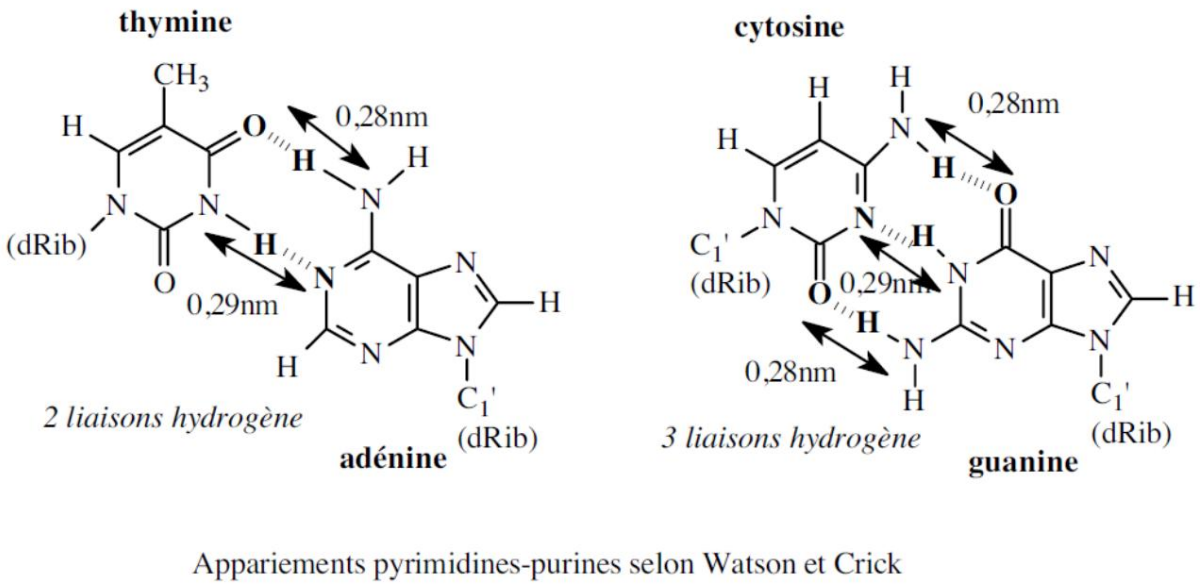


Figure 14: Watson and Crick pyrimidine-purine pairing

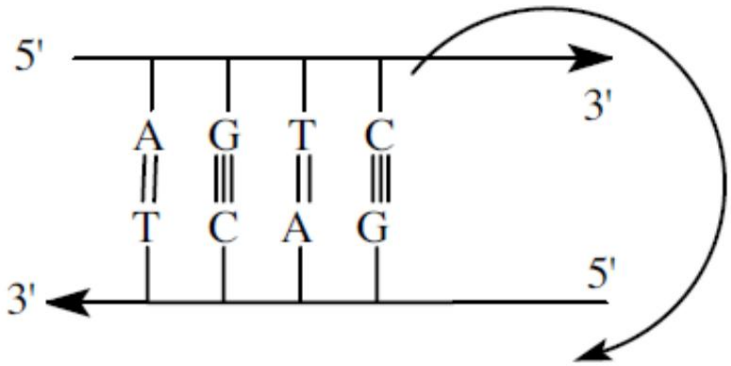
The secondary structure of DNA is such that the two strands are wound around each other. on the other. Each of the two strands is oriented (5'ÿ3') in the opposite direction to the other strand (3'ÿ5'). They are said to be antiparallel.

• The nitrogenous bases are oriented towards the inside of the double helix so that Each one hybridizes with a base on the other strand (A with T, C with G, etc.). We say that the The successive bases of each of the strands are complementary.

• The double helix has a "pitch" of 3.4 nm, meaning there are approximately 10 pairs of nucleotides for each turn of the helix.

2.3. The isoforms of the DNA double helix (A, B, and Z forms):

A double helix is formed from two antiparallel strands of DNA that coil around each other. either to the right (in the direction of a corkscrew or clockwise) or to the left

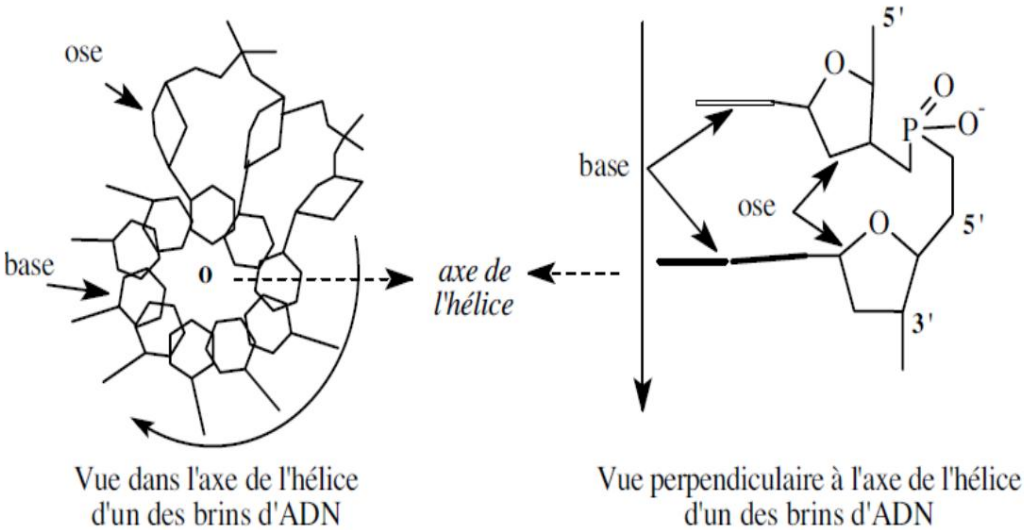


This double helix is characterized by:

- its main axis,

- the winding direction,

- the step.



The stability of the secondary structure of these different DNA double helices is primarily due to:

- hydrogen bonds between the complementary bases of each strand

- hydrophobic and electrostatic interactions of successive bases stacked in the helix structure, where the distance between the planes varies from 0.26 to 0.37 nm

This stability does not lead to rigidity of the DNA molecule, and it can adopt different conformations depending on the region.

Several conformations corresponding to different winding directions or pitches Several different ones were found, the main ones being:

Conformation B:

This is the model described by Watson and Crick, which is found to be the form principal native under physiological conditions.

It is a right-hand propeller with a pitch of 3.4 nm (10 bps per revolution).

- The inclination of the bases relative to their axis of rotation perpendicular to the axis the main angle of the helix is 1°.

- The distance between two successive basis planes is 0.34 nm.

Conformation A:

When the water content of a solution containing a DNA molecule is decreased, For example, during crystallization, the molecule changes conformation and adopts a conformation noted A. This change is reversible.

Conformation A has the following specific characteristics:

- right helix (same as conformation B)

- more compact propeller

- pitch of 2.8 nm (11 bp per revolution)

- distance between successive baseline planes of 0.26 nm

- the planes of the bases are rotated 180° relative to conformation B

- the inclination of the bases relative to their axis of rotation perpendicular to the axis The main angle of the helix is 20°.

This conformation is found *in vivo* in:

- the DNA of certain bacterial spores, formed in response to the desiccation of the medium

- DNA-RNA hybrids that form transiently at the start of replication, and during transcription.

Z-conformation:

This conformation is present in short regions of DNA in a general conformation of type B (native). These specific regions are generally Pur/Pyr alternating sequence segments (GCGC).

The Z conformation has the following specific characteristics:

- **left helix**

- 4.5 nm pitch (12 bp per turn): the molecule is more elongated in this conformation

- distance between successive baseline planes of 0.37 nm

- the inclination of the bases relative to their axis of rotation perpendicular to the axis The main angle of the helix is 9°.

This conformation is found *in vivo* for segments of the DNA molecule, with alternating Pur/Pyr chains, the bases of which are often methylated. This would have a role in gene expression.

These different conformations describe the secondary structure of DNA molecules Double-stranded. These conformations are very stable, but possess considerable flexibility. which can define tertiary structures (structure in space of a double helix) different.

DNA replication is the synthesis of two identical DNA molecules from of a DNA molecule

Since Watson and Crick (1953), it has been known that DNA is a molecule made up of two antiparallel strands, forming a double helix. From their original publication on the structure From DNA, Watson and Crick proposed that this double helix could open, allowing thus the synthesis of new strands, complementary to the original strands.

1. Watson and Crick Postulate

"It has not escaped our attention that the specific matching of the bases that we have proposed immediately suggests a possible replication mechanism for the genetic material

DNA can thus serve as a template for its own replication, an essential step in the cycle cellular. This duplication of DNA (and therefore of chromatids) allows the transition from chromosomes with one chromatid to chromosomes possessing two identical chromatids, carrying the same genetic information. During mitosis, these two chromatids are distributed, each daughter cell inheriting one chromatid from each chromosome. The result is thus two cells possessing the same genetic information as the parent cell.

The problem that then arose was to understand how this was achieved Replication: **by what mechanisms does a DNA molecule made up of two strands with two identical double-stranded DNA molecules?**

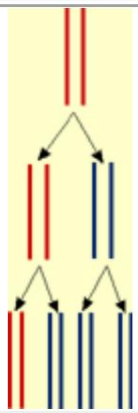
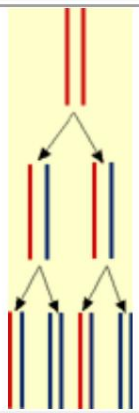
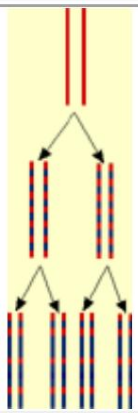
1.1. Proposed Models of DNA Replication

In the 1950s, three different mechanisms were proposed for replication of DNA.

Conservative theory : Both parental strands remain intact after replication DNA

Semi-conservative theory : Each of the daughter DNA molecules consists of a parent strand and a new strand.

Dispersive Theory : Parent and daughter DNA molecules are broken down into fragments

"Mother" DNA molecules and their fate newly formed DNA	**Fate of DNA in three successive generations of cells**	
		
Hypothesis 1: model conservative Starting of a double-stranded DNA molecule strands "mother", we form a biactenary new DNA molecule double-cabled. We therefore retain a complementary element here, molecule "mother", No modified (it is therefore double-catenary). Each new double-catenary "daughter" conserved), while "creating" the "daughter" molecule does not conserve a new molecule, therefore, that half of the ("girl").	**Hypothesis 2: model semi-preservative** We separate the two of the DNA molecule strand function. Each strand therefore serves as fragments matrix for the synthesis of a strand the whole the assembly reforming a DNA molecule molecules "mother" molecule.	**Hypothesis 3: model dispersive** We do not keep any intact. The copy is made via the "mother" scattered in of DNA, allowing the two DNA system.

The now classic experiments of Meselson and Stahl proved that the DNA replication is **semi-conservative,** in that each of the daughter molecules inherits

from a strand of parental DNA. There are advantages to semi-conservative replication, since it allows for the *post-facto* correction of errors made during replication, in the to the extent to which the parental (and therefore correct) strand can be identified.

2. Meselson and Stahl experiment:

This experiment dates from **1958.** It demonstrates the semi-conservative nature of the multiplication of the DNA molecule in bacteria.

2.1. Description of the experiment: comparison with the models

The Meselson and Stahl experiment therefore demonstrates the presence of hybrid DNA at the end of a cellular generation.

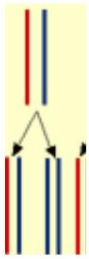
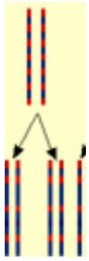
Nitrogen is a major component of DNA. Nitrogen-14 (14N) is by far the most abundant isotope on Earth, unlike nitrogen-15 (15N), which is considered heavy. The latter is not not radioactive, just heavier than the common isotope, because it contains a neutron additional.

Escherichia coli bacteria are grown in a medium containing 15N.
When DNA is extracted from these cells and then centrifuged on a saline density gradient, DNA migrates until it reaches the point where its density is equal to that of the saline solution.
After that, the E. coli strains previously cultured in a medium containing 15N are returned to a normal medium containing 14N for a single cell division.
The DNA from these bacteria is then extracted and centrifuged to compare the results. density turns out to be intermediate. If replication had been conservative, a equivalent amounts of heavy and light DNA, but no density hybrid DNA intermediate, excluding Thus there replication conservative.
The result could, however, correspond to either dispersive or semi-dispersive replication. conservative. In both models, DNA is made up of an equivalent amount heavy nitrogen and light nitrogen, responsible for an intermediate density.

In the next part of the experiment, cells cultured in a medium containing 15N were then cultured in a normal 14N medium for two cell cycles. The result obtained is an equivalent amount of DNA of two densities. One corresponds to the density DNA the intermediate found during the previous part of the experiment, the other corresponding to

cells cultured exclusively in a medium containing 14N. This result allows us to exclude the dispersive model that would have given an intermediate density, lower than that of the cells having divided only once, but greater than that of cells cultured exclusively in medium containing 14N, the quantity 15N being distributed over more and more DNA strands.

The results of these experiments are consistent with the semi-conservative model of the DNA replication, with the proportion of lightweight DNA increasing with the number of generations having divided within the contained medium of 14N.

hybrid DNA	**DNA heavy (15N)** And **lightweight DNA (14N)**	**DNA hybrid** (molecules) formed from a heavy strand and of a strand light)	**DNA hybrid**
result observed	expected result For the **model** for the **conservative**	expected result **semi-model conservative**	expected result for the **model dispersive**
hybrid DNA And lightweight DNA	 **DNA hybrid** And lightweight DNA	 **DNA hybrid**	
result observed	expected result for the **semi-conservative model**	expected result for the **dispersive model**	

The experiment by Meselson and Stahl thus highlights the fact that **the Replication occurs in a semi-conservative manner.**

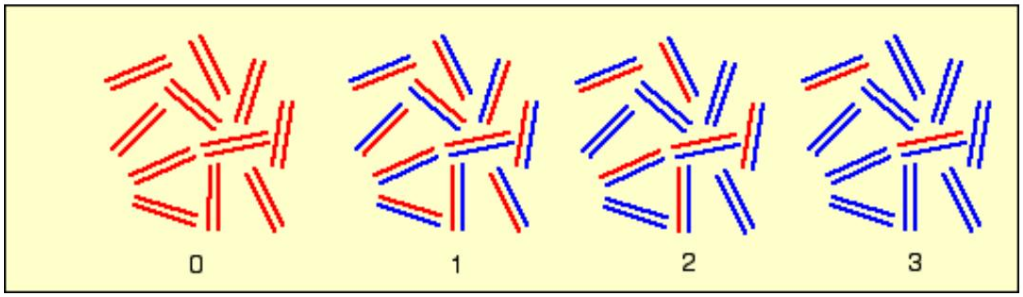


Figure 15 : Schematic representation of the DNA fragment population at over generations.

After 1 generation, all DNA is "hybrid" and consists of a "heavy" strand (15N) and of a "light" bit (14N).

This conclusion has since been confirmed by more precise studies, leading to the current operating model of replication.

3. General information on replication

- The synthesis is **bidirectional and asymmetric. It takes place on both DNA strands after the DNA molecule is opened.**
- The synthesis is polarized and can only occur in the 5'->3' direction.
- Since the two parental strands are antiparallel, the growth of the strands

Newly synthesized molecules are produced continuously on one strand and discontinuously on the other strand. the strand that forms discontinuously, a small fragment (called an Okazaki fragment) is synthesized and then we go back to synthesize the rest.

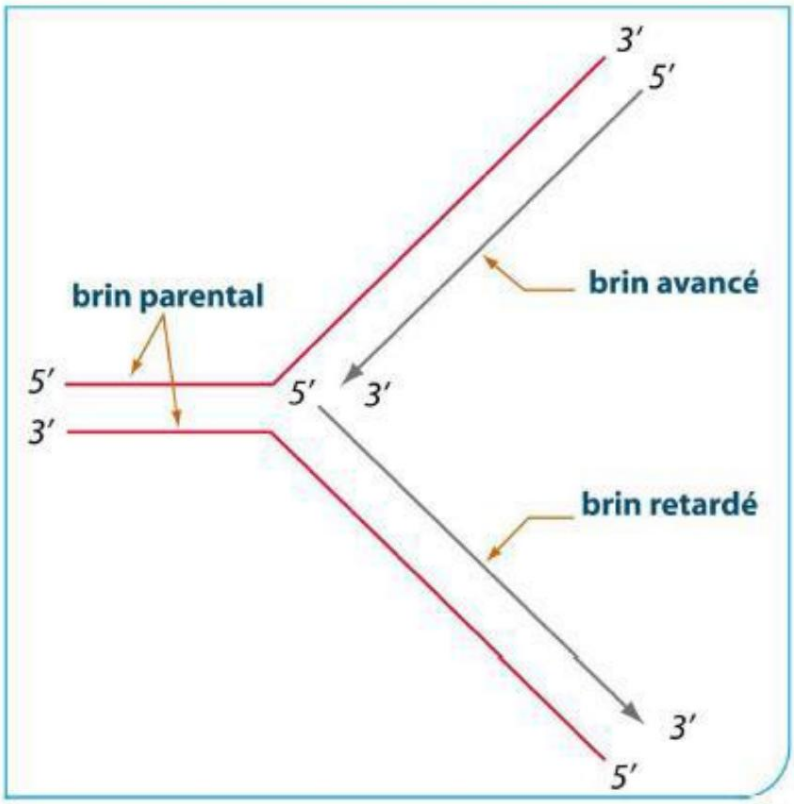


Figure 16: Progression of replication on the 2 DNA strands

4. Replication in prokaryotes:

4.1 Introduction

- First, there is the synthesis of RNA primers. These are short complementary sequences of template DNA synthesized by primases which are DNA-dependent RNA polymerases. (That is, they produce RNA) (using DNA as a model).
- Molecules called helicases gradually open the replication fork.
- The DNA regions that have become single-stranded are stabilized by SSB (single strand binding) proteins.

ÿ There is recruitment of enzymes necessary for replication and formation of the replisome. **A replisome contains:**

-DNA gyrase removes supercoils

-Primosome = helicase/primase unwinds and primes DNA by RNA primer synthesis

Replication first involves the separation of the two strands of the DNA molecule. (DNA denaturation), by breaking the hydrogen bonds between complementary bases. In prokaryotes, this process is initiated at a single site consisting of a sequence of 250 base pairs called origin of replication *(ori).*

The breaking of hydrogen bonds, and therefore the untwisting of the double helix, is catalyzed. by helicase and is an energy consumer in the form of ATP, which is hydrolyzed by ATPases. DNA renaturation upstream of helicase is counteracted by SSB proteins. Each strand can then serve as a template for the synthesis of a complementary strand, catalyzed by DNA polymerase III.

This creates a replication eye consisting of two replication forks. Replication is bidirectional: it progresses simultaneously and in opposite directions on each forks

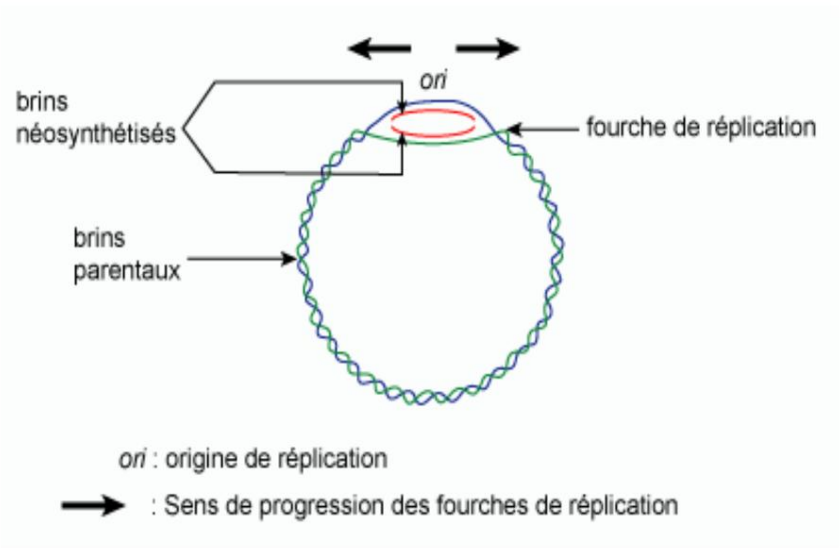


Figure 18: Formation of the replication eye in a prokaryote

4.2 Replication Progression: Replication Elongation:

Polymerases have two roles:

Synthesis of the two daughter DNA molecules by a complex of several proteins

1- Incorporation of the basics while respecting the rules of complementarity >>>> summary continuous from a new early strand (DNA pol III)

2- Degradation of RNA primers through its 3'->5' exonuclease activity and resynthesis of the missing part to ensure fidelity of replication (DNA pol I).

The properties of DNA polymerases have two important consequences:

- Every synthesis of a strand of DNA will be preceded by the synthesis of a RNA primer, catalyzed by primase, which is an RNA polymerase.
- The synthesis mechanism will be different for the two strands. The strand whose polymerization can occur in the direction of progression of the fork of Replication will be the subject of continuous synthesis. It is called the master strand or strand advance.

However, the strand whose polymerization (which is only possible, let us remember, in the sense $5' \rightarrow 3'$) is done in the opposite direction to the direction of travel of the fork will be the subject of a Discontinuous synthesis, by segments of 1000 to 2000 nucleotides: the Okazaki fragments. Its synthesis occurs with a time delay relative to that of the master strand; it is called follower strand or lagging strand.

The synthesis of each Okazaki fragment stops when DNA polymerase III encounters the 5' end of the RNA primer from the previous Okazaki fragment. It is then DNA polymerase I comes into play, which hydrolyzes the primer (it therefore acts as a nuclease) then catalyzes the synthesis of a polydeoxyribonucleotide that replaces the primer.

The splicing of the two Okasaki fragments, to form a continuous strand, is ensured by the ligase.

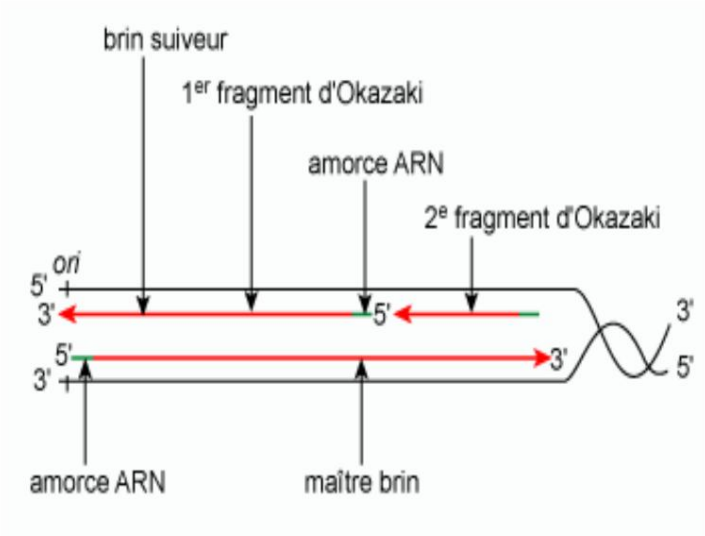


Figure 17: Schematic representation of a replication fork

4.3 Termination

It takes place at the 5' end of the template DNA molecule, in the presence of proteins specific.

At the level of a TER sequence located opposite the recognized origin of replication by the Tus protein which terminates replication.

The " ***Tus*** " protein , "Substance Use Termination," recognizes the sequences stops replication and blocks DNA b.

Meeting of 2 replication forks (Type IV topoisomerase ensures ligation) at level of a TER sequence located opposite the replication origin recognized by the Tus protein that terminates replication

When the replication of a circular chromosome is complete, the 2 molecules the obtained are linked together, like the links of a chain.

>>> Separation is achieved by a topoisomerase

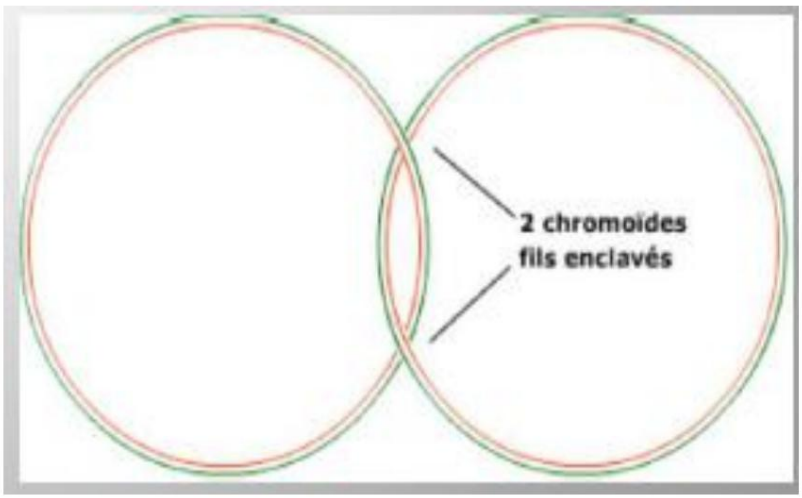


Figure 19 : End of replication; enclosed daughter chromoids

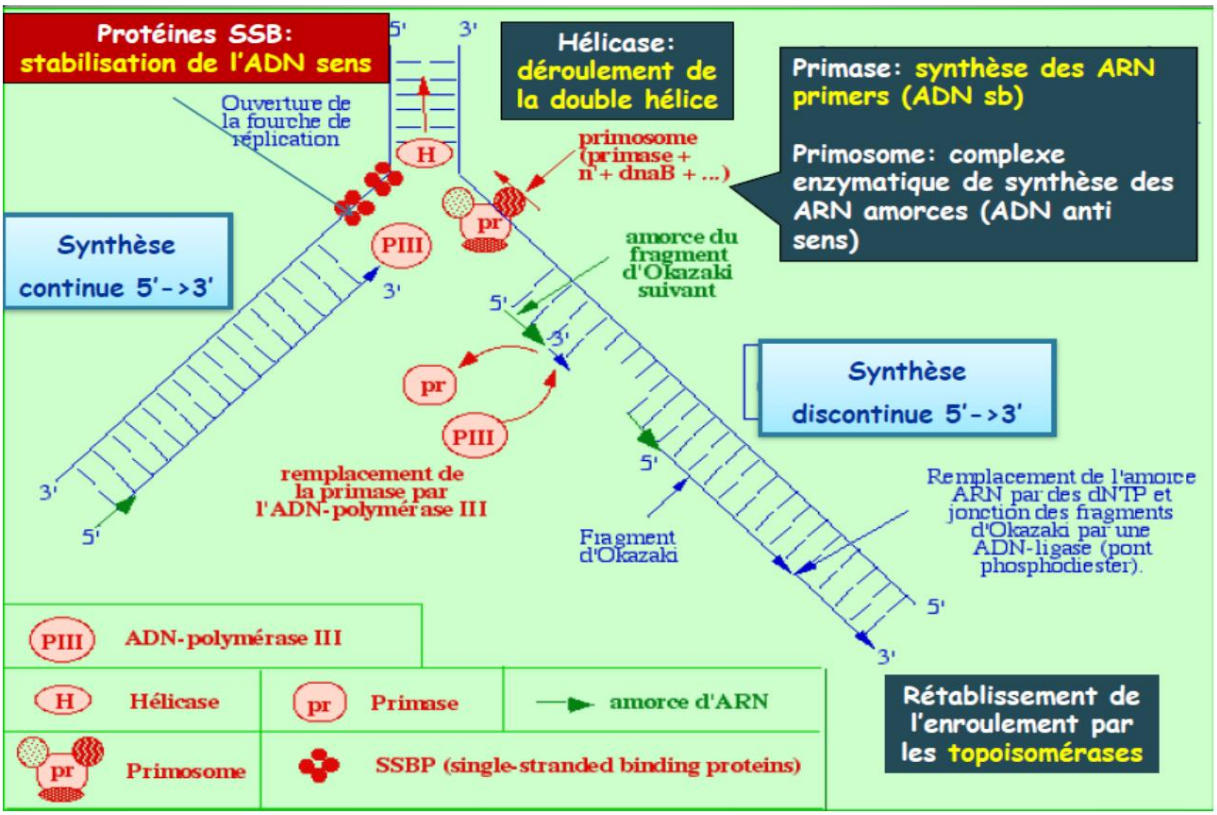


Figure 20: General diagram of replication

5. DNA polymerases

5.1. General Information

DNA polymerases (or deoxynucleotidyltransferases) are the enzymes responsible for the polymerization of nucleotides during DNA replication. They are DNA-dependent, meaning they need a DNA template to produce the strand newly synthesized, and to do so they read the 3' to 5' matrix strand to synthesize DNA in the 5' to 3' direction.

Prokaryotic DNA polymerases are of 3 types (I, II and III) and DNA Eukaryotic polymerases of 5 types (ÿ, ÿ, ÿ, ÿ, and ÿ). DNA polymerases require a a number of activity conditions:

- The 4 deoxyribonucleotides 5' triphosphate (dATP, dTTP, dCTP and dGTP) in equimolar quantity.
- Magnesium ions (Mg2+) that stabilize DNA and proteins.
- A DNA matrix (single or double stranded).
- A DNA or RNA primer having a free 3'OH end.

5.2. Activities of DNA polymerases

During the formation of the phosphodiester bond between a 5' deoxyribonucleotide tri-phosphate and the elongating strand, there is hydrolysis of the triphosphate function and formation of pyrophosphate (PPi).

DNA polymerases have very specific activities:

- A **5' to 3' polymerase activity** which is their main activity, This is the basic activity for elongation.
- An **exonuclease activity** that corresponds to the degradation of a the ends of the newly synthesized DNA strand during replication and which can be of 2 types:
    - From 3' to 5', which corresponds to the degradation starting from the 3'OH end. The 3' to 5' exonuclease activity allows what we

This is called **proofreading.** Polymerase, also known as proofreading or self-correction. It is

in the opposite direction to the others to go back in case of a problem to allow the excision of the mismatched nucleotide and to start again.

- From 5' to 3', which corresponds to the degradation starting from the 5' phosphate end, during the joining of the segments of DNA synthesized on the delayed strand.

5.3. Prokaryotic DNA polymerases in replication

As mentioned previously, there are 3 types of prokaryotic DNA polymerases, and for Regarding replication, we will primarily study the function of DNA polymerases I and III.

- DNA **polymerase I** (or **Kornberg enzyme)** are the most numerous (400 molecules/cell). They exhibit 5' polymerase activity towards 3' as well as the 5' to 3' and 3' to 5' exonuclease activities. The rate of DNA polymerase I synthesis is low (20 nt/s) and these are enzymes with low activity. processive (10-20 nt/event); these characteristics do not allow them to do the majority of prokaryotic DNA replication. They are used in the DNA repair as well as filling gaps left by DNA polymerase III.
- **DNA polymerase III** (or **core enzyme)** molecules are multimers. heterogeneous large molecular weight molecules that are responsible for the synthesis of long fragments of DNA, with a rapid synthesis rate (approximately 1000 nt) incorporated) as well as high processivity (10ÿ nt/event). They exhibit the 5' to 3' polymerase activity and 3' to 5' exonuclease activity, but not exo-nuclease from 5' to 3'.

6. The different proteins involved in prokaryotic DNA replication:

- **Recognition proteins** (DNA A) recognize the sites of initiation and termination.
- The **helicases** (or DNA B) unwind the double helix by breaking the hydrogen bonds present between the two strands of DNA, with consumption **of ATP.**

- **SSB proteins** (for *single stranded binding protein)* have a strong affinity for single-stranded DNA and thus prevents it from rewinding during the migration of replicative forks.
- Primase is a DNA **-** dependent RNA polymerase that synthesizes the primer.
- Topoisomerases release the torsional stresses **of** DNA,

They are of two types, and only type II topoisomerase consumes ATP .
E-Coli type II topoisomerase is called DNA gyrase.

- DNA **ligases** (or DNA G) catalyze the formation of the bond phosphodiester, but is unable to place nucleotides. During repair of

The DNA nucleotides in place are no longer triphosphated, so DNA ligase needs of an **ATP supply.**

1. Definition of mutation:

The term mutation is used to refer to an irreversible change in information.
genetic and hereditary factors in the sequence of a genome. In other words, it is the modification **sudden** and **irreversible** alteration of genetic material resulting in a variation in the sequence of nitrogenous bases of DNA. When a mutation occurs in a sex cell, or gamete,
It is transmissible to offspring and is, most often, stable.

It is a consequence of:

- An error occurred during faithful reproduction during replication of DNA;
- Or an instability of the nucleic bases or of the N-glycosidic bonds;
- Or various injuries caused by chemical or physical agents.

Mutations are very rare events. A mutation can randomly affect any
which gene, from any cell, at any time. It's a random event.

For an organism, the consequences of a mutation will differ depending on the cell type.
which is affected. If a mutation occurs in a somatic cell, this can sometimes
trigger the development of a cancerous tumor. If it is a germ cell that is
If infected, the individual themselves will not be affected, but some of their descendants may be.
The being. In humans, such a mutation can thus be the cause of a genetic disease,
hereditary, the defective copy of the gene is then passed on from generation to generation.

2. Natural origins of DNA mutations:

The DNA molecule possesses the remarkable property **of self-replication: from** a
DNA polymerase produces two identical daughter DNA molecules from the parent DNA molecule .
the original molecule.

In a human cell, at each replication, there are 6.4 billion nucleotide pairs
which are thus replicated.

• Since no copying system is infallible, it is easy to understand that errors can occur
Occasional **errors occur:**

- incorporation of a non-complementary nucleotide

- an "oversight"

- the addition of an extra nucleotide

Even though the **reliability of DNA polymerase can be considered** excellent, we estimates that it "makes a mistake" about once per 100,000 nucleotides inserted.

Furthermore, even outside of replication periods, DNA can be damaged and its the sequence will be modified.

3. Mutagenic agents

DNA modifications are **spontaneous and** their frequency is low. However, some These factors have the property of increasing this frequency. They are classified as mutagenic agents .

3.1. Chemical mutagens

They are distinguished by their mode of action. Some act through similar mechanisms. In addition to spontaneous mechanisms, others act more like radiation.

3.1.1. Analogues of the bases:

Their chemical structure is reminiscent of purines and pyrimidines. They can be incorporated into DNA during replication. Bromouracil (BU), similar to T (Br replaces CH3), **It pairs** with A. It has a strong tendency to tautomerize into "enol". It then pairs with G. Aminopurine, an analogue of A , **pairs** with T and causes A-T transitions in GC or The opposite.

3.1.2. Chemical substances altering the structure and pairing of bases

Nitrous acid is produced by the digestion of nitrites (food preservatives). It is at the origin of **deaminations(=** loss of an NH3 group (e.g., C -> U; meC -> T; A -> hypoxanthine).
Nitrosoguanidine, methylmethanesulfonate, and ethylmethanesulfonate react with the bases by **adding methyl or ethyl groups.** Degradation can go as far as the production of sites without databases which, upon replication, generates mutations.

3.1.3. Intercalating agents:

Acridine, proflavin, and ethidium bromide are molecules that **insert themselves between the bases** of the DNA. This causes the DNA to stretch. The polymerase then inserts a base supernumerary in the face of the foreign molecule.

3.1.4. Agents that alter the structure of DNA:

Some large molecules **bind** to bases and thus become " **non-coding** ".
Other agents cause **intra- and inter-strand crosslinking** (e.g., psoralen found in... plants And used In THE treatments of there skin).
Chemicals cause breaks in DNA (e.g., peroxides).
These agents probably do not directly induce mutations but induce processes repair mechanisms that are mutagenic.

3.2. Physical agents

Radiation is the main mutagenic agent.

3.2.1. The electromagnetic spectrum:

Visible light and other forms of radiation are electromagnetic radiation.
Their wavelength varies and is inversely proportional to their energy.
Among short wavelengths, energy increases in this order: FM waves, TV waves, Microwaves, infrared, visible light, UV, X-rays, and gamma rays. The **biologically active** fraction is made up of UV, X-rays and gamma rays.

3.2.2. Ionizing radiation:

X-rays and gamma rays are energetic enough to produce reactive ions (atoms charged or molecular molecules) when they interact with biological molecules. This is referred to as of **ionizing radiation.** This term also includes corpuscular radiation,
A stream of atomic and subatomic particles emitted by radioactive elements. These are of two types: alpha particles (helium nucleus 2H+ + 2 neutrons) and beta particles (electrons).

UV rays are not ionizing but can react with DNA or other molecules biological.

The unit used to measure ionizing radiation is the **rem** (roentgen equivalent man): 1 REM of any ionizing radiation produces the same biological effect.

3.2.3. Sources of radiation:

Natural sources produce "background" radiation. These are the rays cosmic (including solar radiation), radioactive elements from the soil or products from the ground (wood, stone) and from the atmosphere (radon).

The other sources are **artificial :** X-rays for diagnosis, nuclear tests, TV, etc. Overall, the average exposure rate is **350 mrem/year,** the majority of which is due to radon.

3.2.3.1. Biological effects of radiation:

The damage caused to cells by radiation is the result of the production of **free radicals** **free** radicals originating from water (the OH or hydroxyl radical). Free radicals possess electrons They are unpaired and chemically very reactive. They interact with **DNA,** proteins, and the lipids of the membranes. The resulting damage leads to the involvement of organelles, the Blockage of cell division or cell death. **Short cell cycle** cells (bone marrow stem cells, gastrointestinal tract lineage) are the most affected.

The severity of the effects depends on the dose received:

• Lethal dose: (100 - 250 rems): nausea and vomiting, latency period of 1 to 2 weeks followed by malaise, anorexia, diarrhea, hair loss, then recovery.

• Lethal dose (350-450 rems): nausea and vomiting, latency of 1 week followed by severe symptoms with internal bleeding; 50% risk of death due to alteration blood and gastrointestinal cells

• Supra-lethal dose (>650 rems): nausea and vomiting, shock, pain abdominal pain, diarrhea, fever, and death within hours due to system damage nervous system and heart.

3.2.3.2. The genetic effects of ionizing radiation

Ionizing radiation has numerous effects on DNA, both through **free radicals and** other means. that they create and by **direct action :**

• breaks in one or both strands (which can lead to rearrangements, deletions, loss of chromosome fragments, or cell death in the absence of repair).
• Alteration or loss of bases (mutations)
• Entanglement of DNA with itself or with proteins

There is a relationship between radiation dose and mutation rate, the effect of radiation. being cumulative.

3.2.3.3. The effects of UV

They are not among the most energetic and are not ionizing, but their wavelengths are **preferentially absorbed** by DNA bases and by acids aromatic amino acids of proteins.

Among UV radiation , we can distinguish :

• UV-C (180-290 nm): the most energetic. They are lethal but absorbed by the layer of ozone.
• UV-B (290-320 nm): These can be lethal. They constitute the radiation mutagenic to sunlight.
• UV-A (320 - visible): they have harmful effects because they create free radicals oxygen but they produce few pyrimidine dimers.

Tanning lamps produce UV-A and UV-B rays.

Most lethal lesions are dimers **between pyrimidine bases (TT or TC)** in DNA, resulting from the establishment of a covalent bond between pyrimidines adjacent on one strand.

These dimers, like most chemically induced lesions, block the transcription and replication. They are lethal if not repaired. They generate also chromosomal mutations and rearrangements.

4. The different types of mutations

4.1. One-off changes

We speak of **point mutations when they affect only one base of the DNA.**

There are 3 types:

4.1.1. Substitution mutation

Replacement of one nucleotide (of a base) by another nucleotide.

Purine-to-purine or pyrimidine-to-pyrimidine substitutions (transitions) are the most frequent:
— A ÿ G, C ÿ T, G ÿ A and T ÿ C

The other substitutions are transversions:

— A ÿ C, A ÿ T, C ÿ A, C ÿ G, G ÿ C, G ÿ T, T ÿ A and T ÿ G

A substitution can lead to very different results after translation. This depends on its position relative to the reading framework. Transversions cause more mutations than the transitions.

• A substitution in a codon can result in the same amino acid: it is said to be synonym. There will be no change to the translated amino acid.

• A substitution in a codon can result in a different amino acid: we say that it is nonsense. It will be all the more detrimental to the protein's functions the new The amino acid will be different from the one that should have been translated.

• A substitution in a codon can result in a termination codon: we say that it That's nonsense. The translated protein will be truncated at that point.

4.1.2. Mutation by addition (insertion)

Insertion of an additional nucleotide.

The insertions vary in importance depending on their length:

— by 1 or 2 nucleotides, they shift the reading frame (codons)

— of 3 nucleotides, they result in the addition of an amino acid in the expressed protein

— because of their great length, they can completely alter the translation of exons

— in introns or in untranslated exons, long insertions are often found of variable structure which have no apparent effect on gene expression.

4.1.3. Mutation by deletion : Loss of one or more nucleotides.

The deletions vary in importance depending on their length:

— by 1 or 2 nucleotides, they shift the reading frame (codons)

— of 3 nucleotides, they result in the deletion of an amino acid in the protein expressed

— of great length, they can suppress the expression of one or more exons, or even of an entire gene.

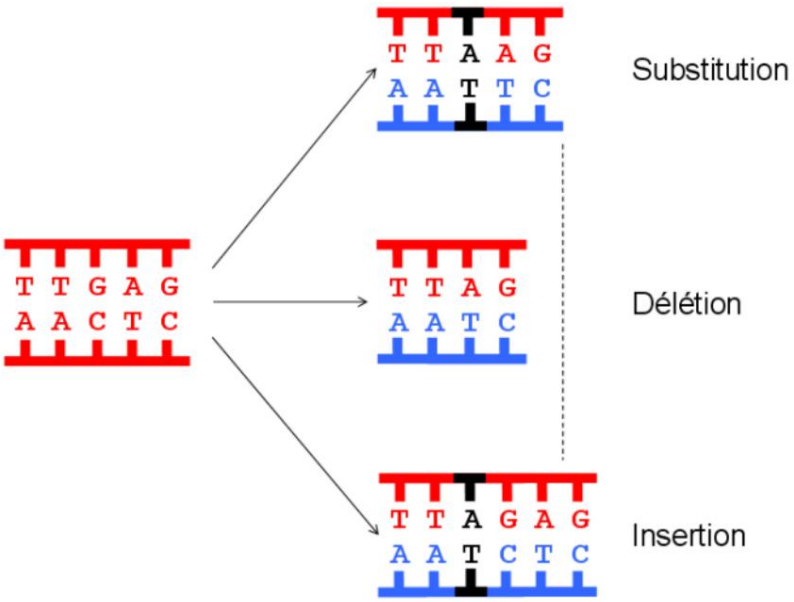


Figure 21 : Different types of point mutations

4.2. Consequences of point mutations

May lead to the modification of an amino acid in the protein.

- Sometimes little or no effect.
- Sometimes it decreases or eliminates the effectiveness of the protein.
- Rarely does it confer a new property (more efficient enzyme or one possessing new catalytic properties for example).
- In some cases, a codon becomes a STOP codon ==> stopping synthesis before the end = nonsensical mutation

4.3. Chromosomal mutations

These are quantitative modifications of hereditary information. These are changes deep within the structure of one or more chromosomes.

Most chromosomal abnormalities occur **accidentally** during **development gametes** due to **non-disjunction** or **chromosome breakage.**

Since the regulation of a gene's activity depends in part on its location in The genome, chromosomal mutations generally have extremely significant effects. dramatic, because they alter the amount of gene product, or modify the timing of a a given gene is activated or deactivated.

4.3.1. Inversions (inv):

An inversion results from two breaks on the same chromosome followed by rejoining. after inversion of the intermediate segment (Figure 2).

The inversion is **paracentric** if the break points are located on the same arm chromosomal.

The inversion is **pericentric** if the break points are located on either side of the centromere.

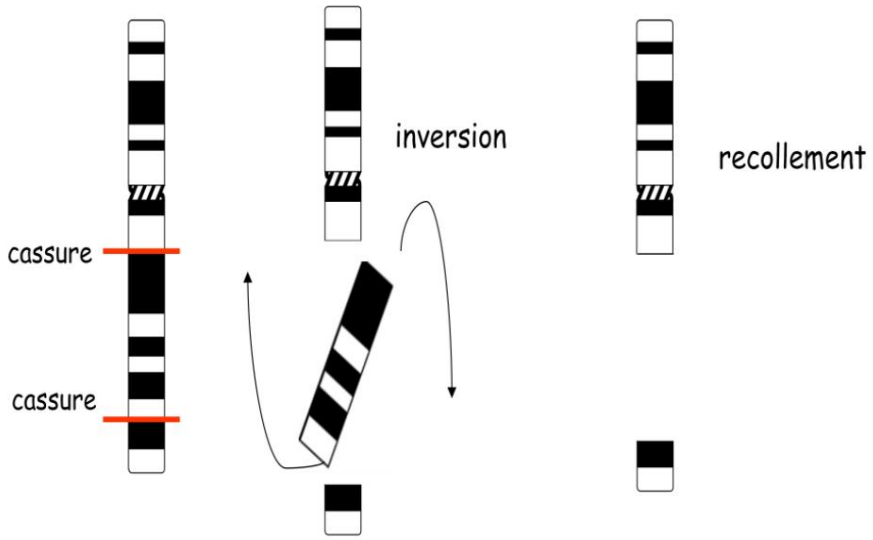


Figure 22: Mechanism of occurrence of a reversal

4.3.2. Deletions:

A deletion results from a chromosomal break with loss of the distal segment (deletion terminal) or two breaks on the same arm with loss of the intercalated segment (deletion) interstitial or intercalary).

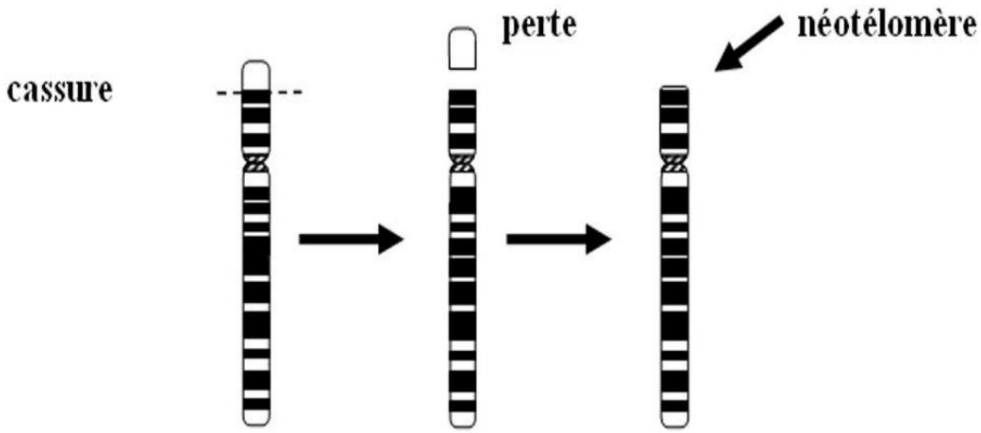


Figure 23: Mechanism of occurrence of a deletion

4.3.3. Duplications (dup):

A duplication is defined as the repetition, one or more times, of a segment of chromosome.

The duplicate segment can be:

- in the same orientation as the original segment, it is a direct (tandem) duplication.

- reversed at 180° with respect to the original segment, it is an indirect (mirror) duplication.

A duplication is an unbalanced chromosomal abnormality.

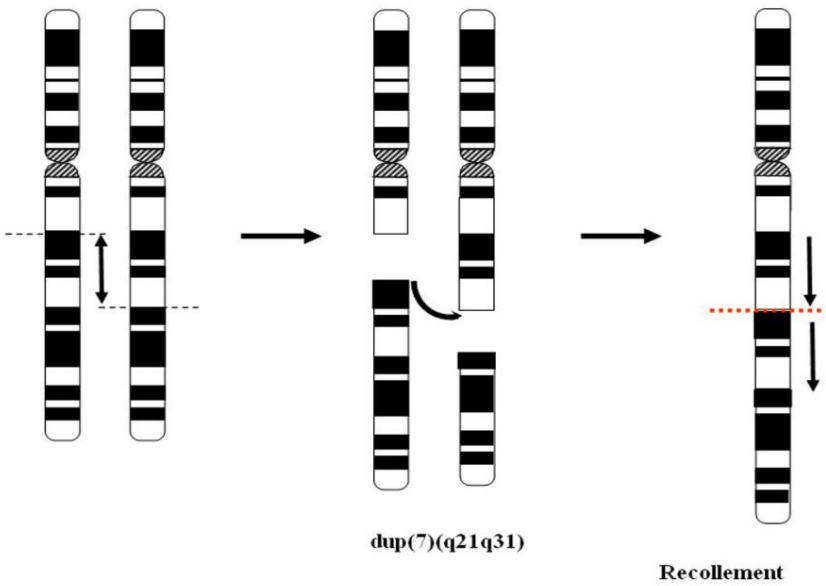


Figure 24: Mechanism of duplication

4.3.4. Isochromosomes (i):

An isochromosome is an abnormal chromosome consisting of two long arms or two arms short chromosomes with loss of the other arm.

An isochromosome can be monocentric or dicentric depending on the mechanism of formation. The most commonly encountered isochromosome is the isochromosome for the long arm of the X chromosome which constitutes a cytogenetic variant of Turner syndrome.

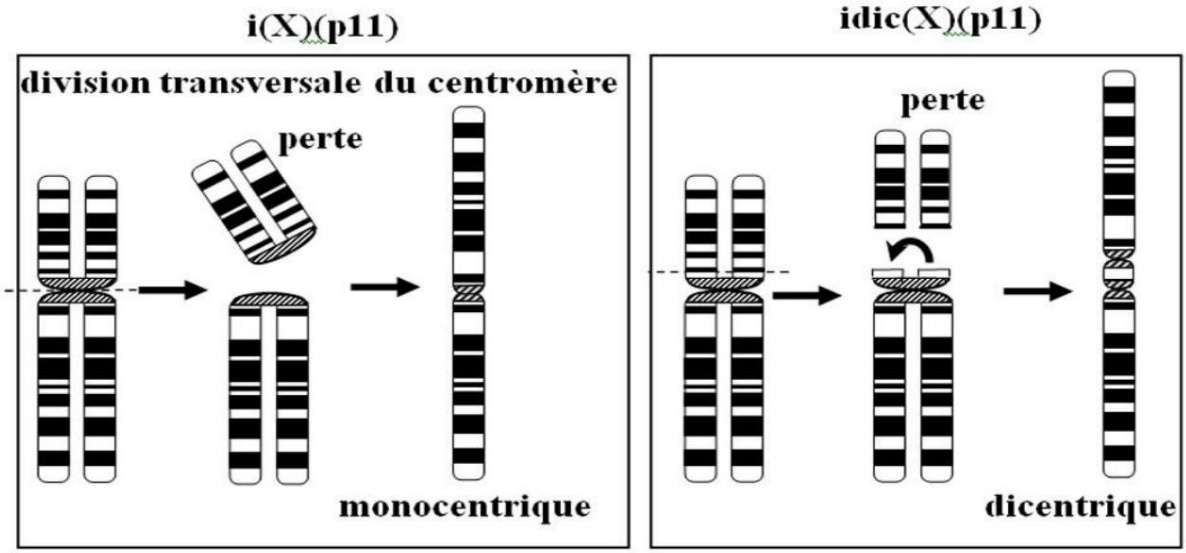


Figure 25: Mechanism of isochromosome occurrence

4.3.5. Ring chromosomes (r)

A ring chromosome results from a break at each end of a chromosome. followed by reattachment with loss of distal segments.

A ring is an unbalanced anomaly, most often *de novo;* it is a structure unstable during mitosis and gametogenesis.

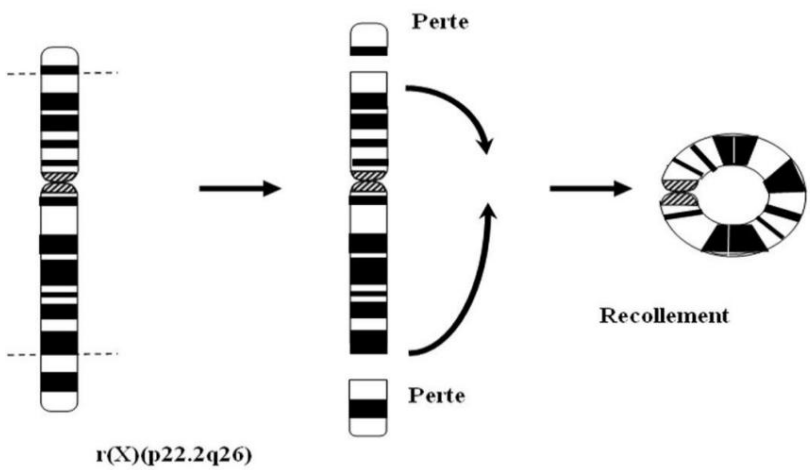


Figure 26: Ring chromosome

4.4. Abnormalities involving two or more chromosomes

These are primarily balanced translocations, their overall frequency is ~1/500-1/600 i.e. that it affects one couple out of 250-300.

They most often involve two chromosomes but can sometimes be more complex (3 or more chromosomes).

They result in varying risks of imbalance for offspring.
Screening is recommended in children with associated intellectual disability and syndrome recognizable or unrecognizable polymalformative (e.g., T21), in cases of repeated abortions or for male hypofertility or sterility.

4.4.1. Reciprocal translocations:

Reciprocal translocations are characterized by two breaks on two chromosomes. different and reattachment after exchange of distal segments.

They can involve any chromosome. In most cases, a reciprocal translocation is apparently balanced and the phenotype of the carrier individual is That's normal. They are transmitted *de novo.*

They sometimes lead to an abnormal phenotype even though the translocation is apparently balanced: in this case, there is a possibility of deletion or duplication at the point of break, break in a structural gene, position effect, etc.

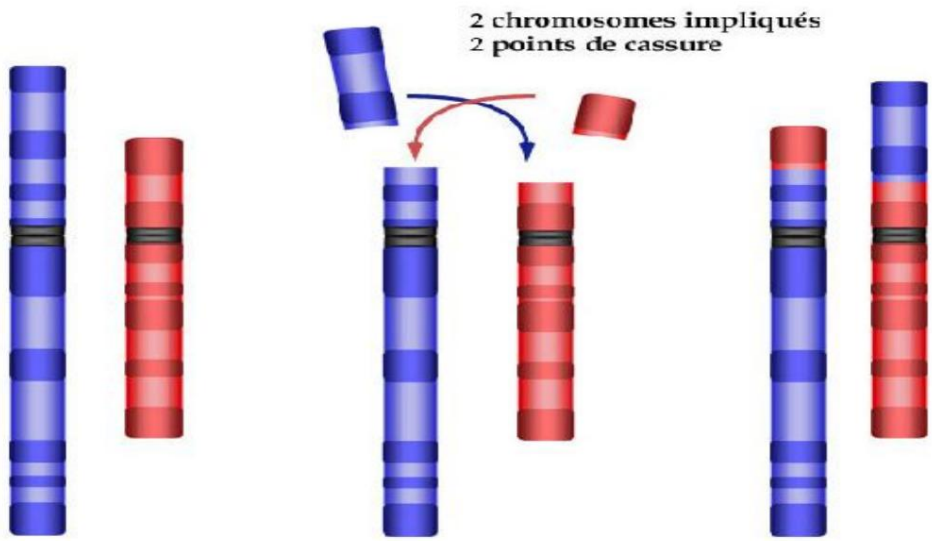


Figure 27: Reciprocal translocation

Reciprocal translocations are responsible for reproductive abnormalities because the Translocations prevent the normal progression of meiosis. They can be responsible of infertility, repeated spontaneous miscarriages, or the birth of a child polymalformed.

4.4.2. Robertsonian translocations:

A Robertsonian translocation is defined as a centric fusion (near the centromere) of 2 acrocentric chromosomes (13, 14, 15, 21, 22).

The translocation involves both long arms of the chromosomes involved (sequences coding) with the loss of part of the short arms (heterochromatin, satellite DNA).
The individual carrying the translocation is phenotypically normal because there is no loss of genetic material.

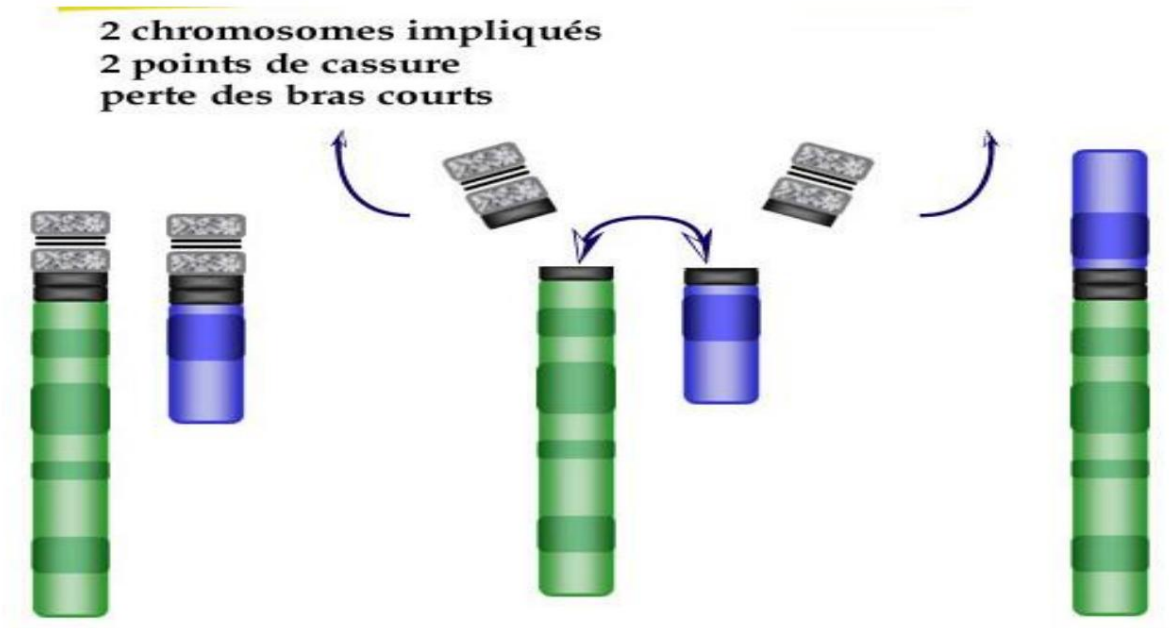


Figure 28: Mechanism of formation of a Robertsonian translocation

4.4.3. Insertions:

Insertions are less common. They result from two breaks on a chromosome arm. with insertion of the intercalated segment at a third break point any part of the genome.

They result from a three-break mechanism, two on the donor chromosome and one on the recipient chromosome. The chromosomes: donor and recipient, can be a single one and same chromosome (intrachromosomal insertion). The inserted segment can retain its orientation relative to the centromere or take an inverse orientation.

During meiosis, the formation of monosomic or trisomic gametes can be observed. for the inserted segment.

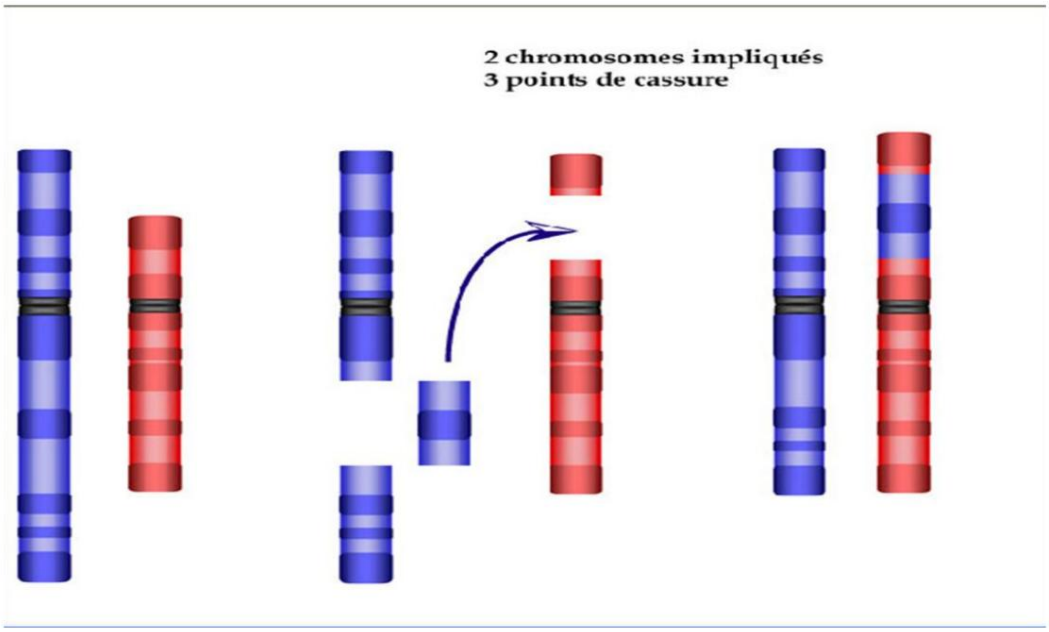


Figure 29: Mechanism of insertion formation

4.5. Genomic mutations:

Genomic mutations occur when an entire chromosomal configuration is replicated, or when the chromosomes of two different genomes merge into the same cell. In nature, these phenomena, which occur rarely, are summarized under The term "polyploidy". A polyploid individual can be born from the fusion of gametes from two

different species, or the failure to reduce the number of chromosomes during meiosis. The Genomic mutations are generally disastrous for an organism, because they disrupt the delicate balance of functions of thousands of genes. Despite this, it is common that New plant species emerge through polyploidy, while the phenomenon is well rarer in animals.

The first major type of genomic mutation is a change in the number of chromosomes. Changes in chromosome number are generally listed in two groups:

4.5.1. Changes in complete sets of chromosomes (aberrant euploidy)

4.5.1.1. Aberrant euploidy:

Organisms possessing a number of chromosomes that is a multiple of a haploid number of chromosomes are said to be euploid.

Euploid organisms possessing more than two sets of chromosomes are called polyploid. organizations that own a number of games that is higher or lower than the normal number of games chromosomes are aberrant polyploids. We speak of triploids (3n), tetraploids (4n), pentaploids (5n), hexaploids (6n), etc. An individual belonging to a species normally Diploid, which has only one set of chromosomes (n), is said to be monoploid.

4.5.1.2. Changes in parts of sets of chromosomes (aneuploidy).

An aneuploid is an organism whose number of chromosomes differs from the type wild by part of the chromosome set. Generally, the chromosome set aneuploid differs from the wild type by only one chromosome or a small number of chromosomes. Aneuploids may have a greater or lower than that of the wild type. The nomenclature of aneuploids is based on the number of copies of the chromosome in question in the aneuploid state.

-affecting autosomes

- Trisomy 21 (Down syndrome): 47,XX or XY, +21, it represents 1 birth alive on 800;

- Trisomy 18 (Edwards syndrome): 47, XX or XY, +18, affecting 1 birth alive out of 9000
- Trisomy 13 (Patau syndrome) 47,XX or XY, +13, affecting 1 live birth out of 20,000

-affecting the sex chromosomes

- Extra X chromosome (triple X syndrome) affecting 1 in 1000 girls: 47,XXX
- Supernumerary X chromosome (Klinefelter syndrome) affecting 1 in 1000 births, mosaicism including: 47,XXY
- Extra Y chromosome (double Y syndrome) affecting 1 in 1000 boys: 47,XYY

However, other viable trisomies, or tetrasomies (2n+2), exist, but they are very rare. exceptional.

1. Fidelity of DNA replication

Replication fidelity is very high and largely due to DNA polymerase, which incorporates nucleic bases according to the complementarity of Watson-Crick (A-T, CG) pairings. Indeed, this very low error rate of DNA polymerase during replication is due to the fact that replicative DNA polymerases have a proofreading activity which allows them to verify that the last incorporated nucleotide is indeed the Good.

However, it is estimated that the DNA of an average human cell undergoes several tens of thousands of lesions per cell per day, under normal conditions of metabolic activity and exposure to environmental factors. Exposure to Ionizing radiation is just one additional risk factor among others.

These lesions are generally harmful (cancer, aging, etc.) but mutagenesis is also a driver of evolution.

A highly efficient set of biochemical processes **identifies, signals ,** and **corrects** the DNA damage and thus helps maintain the integrity of the cellular genome.

This "DNA repair" machinery is always active, reactive, and finely tuned. controlled by the cell and is linked to several related mechanisms.

But this machinery is not infallible ... ***failure increases the risk carcinogenic***

2. Prevention: cell protection systems (superoxide dismutase, acid-base balance:

2.1. Superoxide dimutase:

A free radical is an atom or molecule that has gained or lost an electron. An example of biological interest is the dioxygen molecule, which gains an electron during the Cellular respiration leads to the superoxide radical. This is due to the odd number of electrons. which makes the molecule unstable. It will then constantly capture or donate an electron to another molecule from its surroundings, thus propagating the phenomenon.

When it occurs in the body, this chain reaction is commonly called **oxidative stress.** It causes extensive damage to tissues, organs, and It can alter certain genes. It is involved in many diseases such as cataracts, arthritis, cardiovascular diseases or cancers.

To neutralize its harmful effects, the cell developed defense systems antioxidants. One of these mechanisms is, in fact, detoxification by metalloproteins of the **superoxide dismutase** (SOD) family.

The transformation of the superoxide radical into H2O2 can occur spontaneously but SOD accelerates it approximately 10,000 times. It attacks the superoxide anion to stop the base the chain reactions. There are three different classes of SOD, all catalyzing the Same reaction.

Copper and zinc SOD (Cu, Zn SOD) is found in the cytosol and at the level extracellular fluids, iron SOD and manganese SOD, in the mitochondria.

2.2. Acid-base balance

Acid-base balance, or pH homeostasis, is a major function of the body. human, which aims to regulate its pH . Plasma pH normally varies from 7.38 to 7.42. We speak of acidosis in the event of a decrease in pH and alkalosis in the event of an increase in this one.

A plasma pH below 7 or above 7.8 is incompatible with life. pH regulation involves numerous systems (respiratory function, the kidneys, the proteins, etc).

The accumulation of acids in tissues can lead to various consequences: increased inflammation, slowed metabolism, demineralization, a general weakening of the body, increased sensitivity to stress, a decrease in pain threshold or an acceleration of the aging process.

Here are some pathologies that may be linked to an acid overload in the body: Rheumatism, diabetes, kidney and bladder problems, Graves' disease (overactive thyroid gland), cancer in general, the leukemia, osteoporosis.

3. DNA repair mechanisms

When DNA is damaged or replicated incorrectly, the result is that The nucleotides located on the two strands are no longer complementary (A-T or CG). We say that it There is a **mismatch** between the nucleotides.

• Repair aims to replace a nitrogenous base, or a nucleotide or a fragment of nucleic acid by complementary nucleotides of the sequence of the other strand.

• Since replication is semi-conservative, one of the two strands is identified as matrix and it is the other strand that will be repaired in case of mismatch.

• Several repair mechanisms allow for the replacement of a base, a nucleotide or a more or less long fragment of a strand of DNA.

3.1. The excision repair system.

3.1.1. Base excision repair (BER):

In the case of a damaged base, repair can be done by a simple excision of base followed by the replacement of the base with a normal nucleotide.

• Excision is performed by a **DNA glycosylase** that opens the double helix, then hydrolyzes the N-glycosidic bond of the damaged base and leaves an apurinic nucleotide (without a base).

The repair will be carried out by one of the DNA repair polymerases: the **DNA polymerase ÿ** which hydrolyzes the apurinic nucleotide, then replaces it with the nucleotide expected on the free 3'OH end and the breach will be closed by the DNA ligase which reconstitutes the phosphodiester bond at 3' of the added nucleotide.

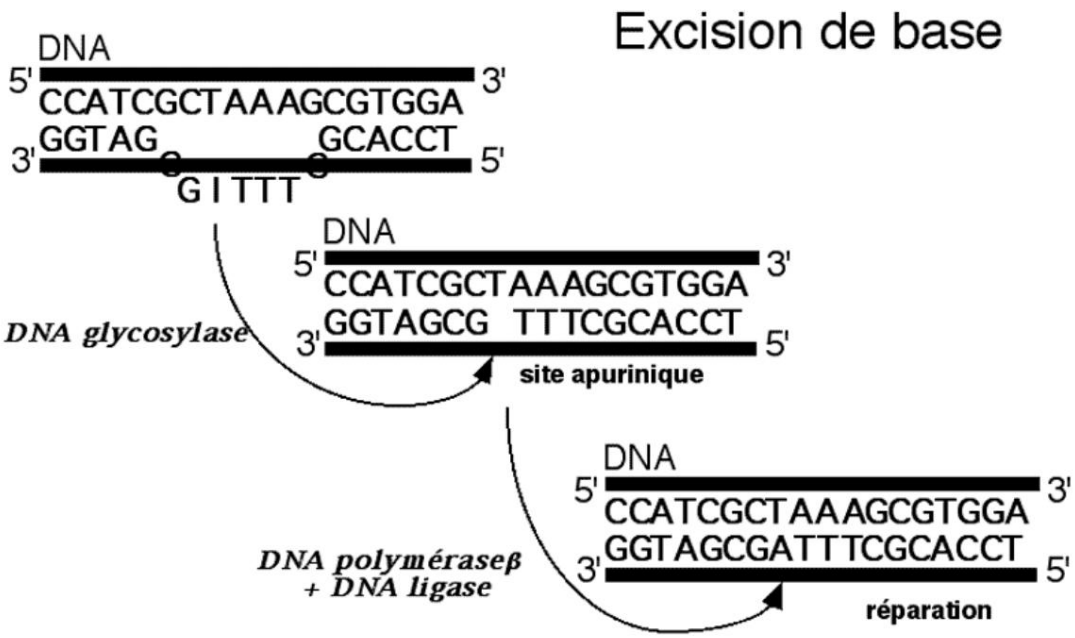


Figure 30 : Base excision repair

3.1.2. Nucleotide excision repair (NRE):

This mechanism intervenes on significant DNA damage that creates blocks of replication and transcription (such as UV-induced dimers and chemical products). They are probably not recognized as structural defects but by the distortion of the double helix they generate.

It consists of a **cleavage of the DNA strand** on each side of the connection by Endonucleases. The polymerase then completes the missing region. Mutants that do not Those who can implement this type of repair have been isolated: they are sensitive to UV and to chemical products that act like UV rays.

3.2. Recombination repair

This repair system is used in case of DNA breakage or if the two strands of the molecule is altered.

The system will then recover the damaged information from the uncopyable copy altered on the homologous chromosome.

Note. *When the nucleotide sequence is too altered, or if the breaks are too numerous, a last-ditch repair system will be added randomly selects complementary nucleotide sequences. This system is used in last resort because it introduces numerous mutations leading either to the death of the cell either to processes of carcinogenesis.*

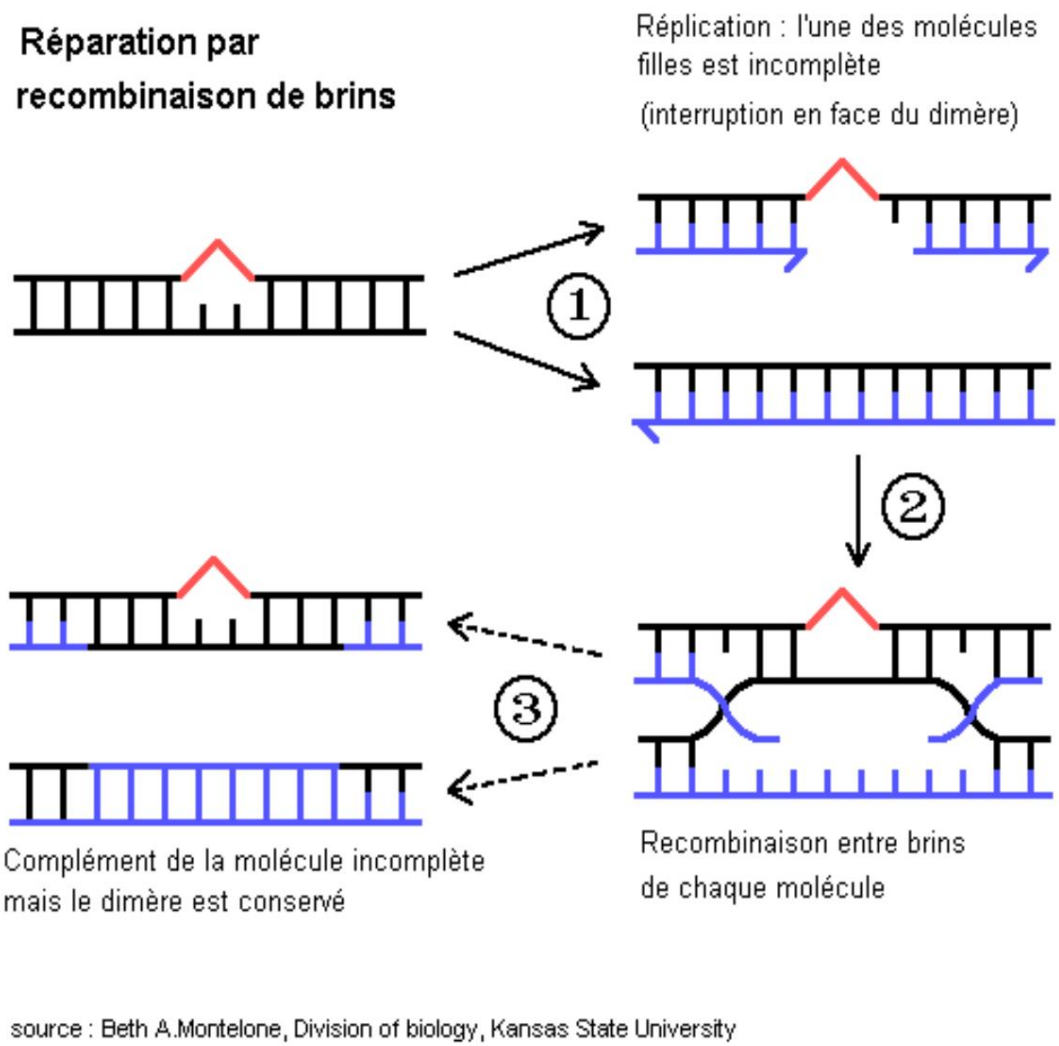


Figure 31 : DNA repair by recombination

3.3. Direct repair – Photorectification-

Repair by reversal of lesions:

This type of repair uses very few proteins and immediately restores the connections.

Photo-reactivation: photolyases are enzymes activated by energy luminous and which participate in DNA repair by breaking covalent bonds at level of thymine dimers.

Reversal of single-strand breaks: by a DNA ligase when there are no losses basics.

Reversal of depurination by a purine insertase: restores glycosidic bonding. enzyme specific to a base.

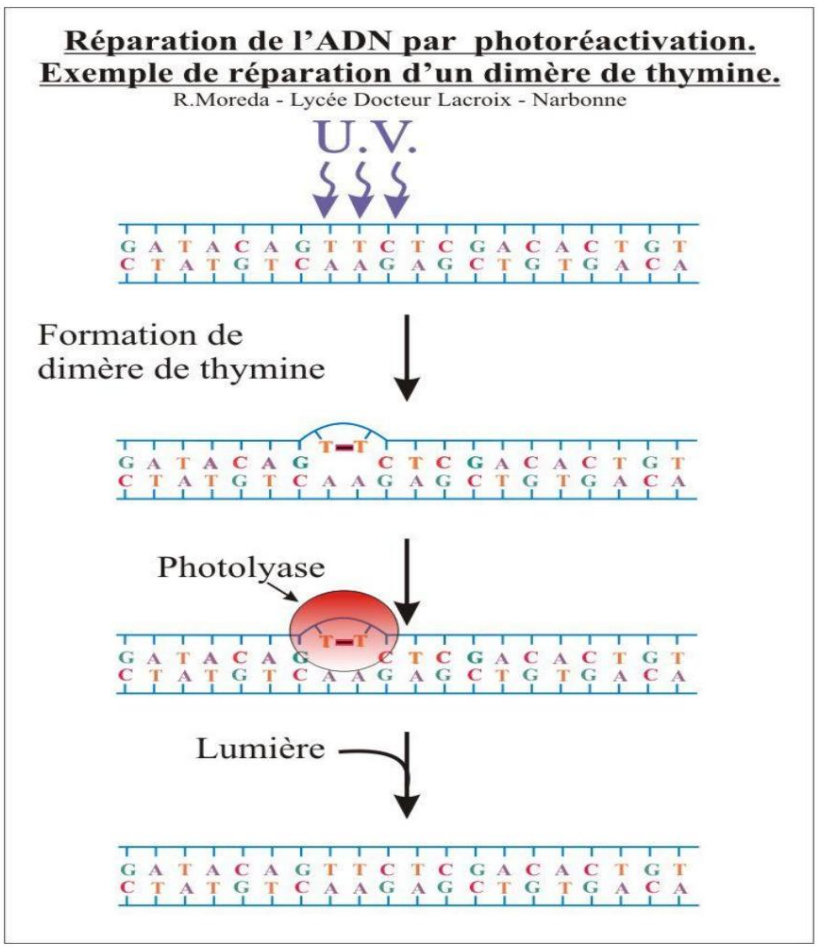


Figure 32: DNA repair by photoreactivation (direct repair)

4. The limits of repair:

When nucleases and other repair systems fail to correct
Due to the error, the placement of mismatched nucleotides is permanent.

During the next replication, the nucleotide mistakenly incorporated becomes part of the strand matrix and will direct the incorporation of its complementary nucleotide, so there will no longer be mismatched nucleotides, but a definitive change in the DNA sequence, that is to say
a mutation.

1. General characteristics:

RNAs are characterized by:

1.1. The sugar: ribose replaces the 2'-deoxyribose present in DNA

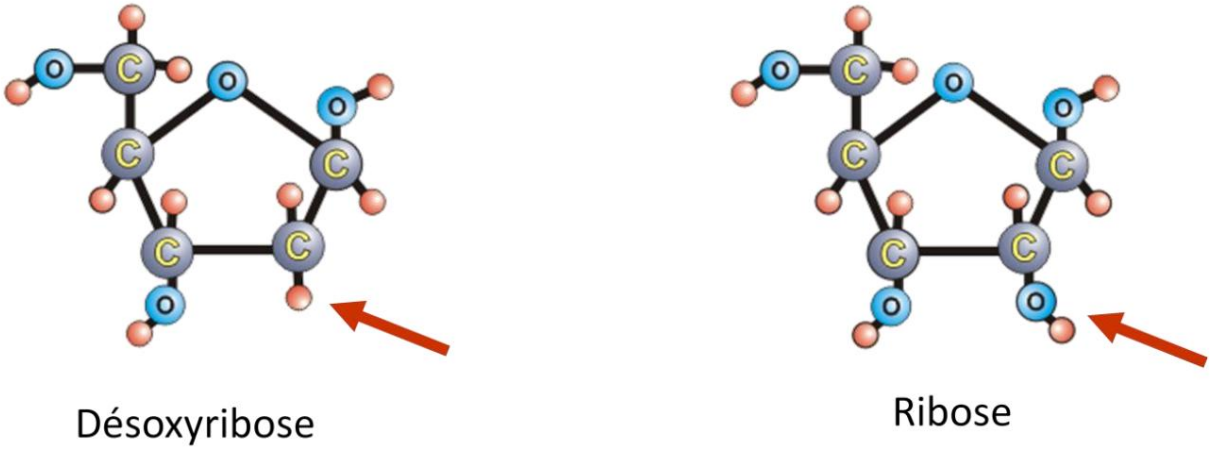


Figure 33 : RNA Ribose

1.2. The purine and pyrimidine bases encountered are: A, C, G, U (the latter is replaced by T in DNA.

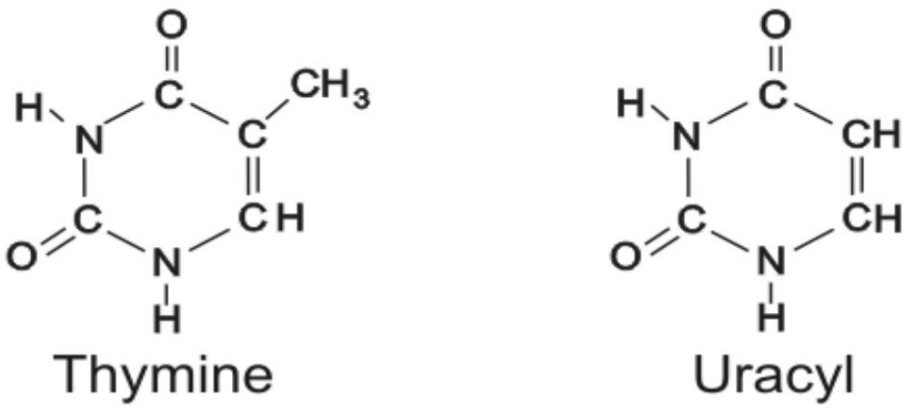


Figure 34: Structure of Uracil

1.3. A single nucleotide chain (instead of two in DNA). This single chain is shorter than DNA strands.

Note: within the same RNA chain, portions may be in double-stranded form

(A is linked to U, and C is linked to G)

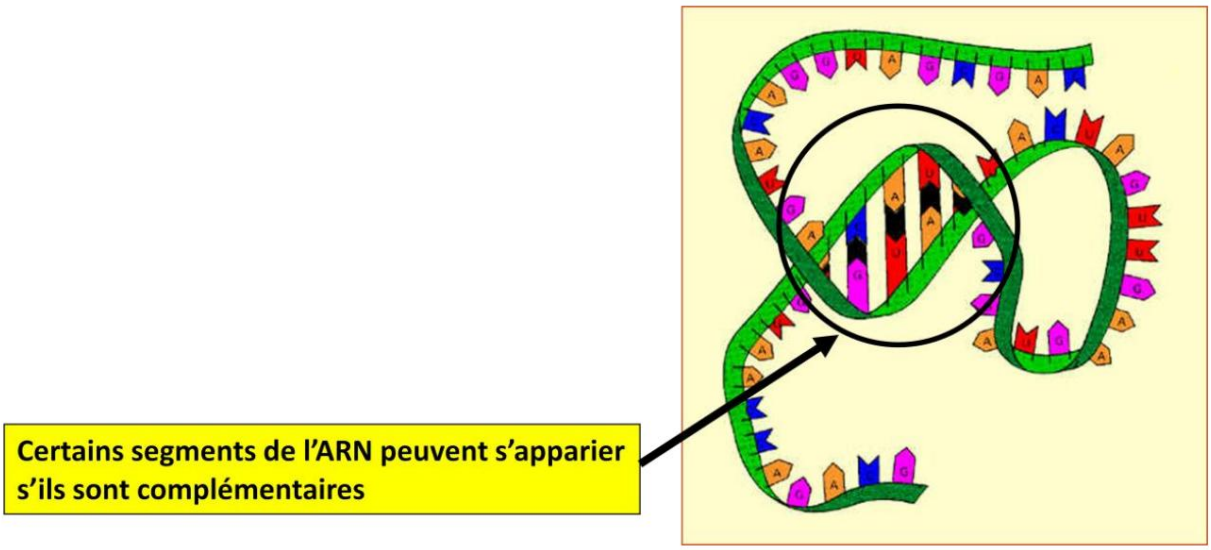


Figure 35 : Double-stranded structure of RNA

2. The different types of RNA:

Cells essentially contain four types of RNA:

Ribosomal RNAs (rRNA)

Transfer RNAs (tRNAs)

Messenger RNAs (mRNAs)

Small nuclear RNAs (snRNAs) and small cytoplasmic RNAs
(scRNA)

Within the cell, rRNAs are the most abundant (at least 80% of all RNAs).
the cell), and the least abundant snRNAs.

2.1. Ribosomal RNAs:

Ribosomes are intracellular organelles located in the cytoplasm (but we also have
(also found in mitochondria) and which are " *the protein manufacturing plant* "
of the cell. We can distinguish between the rRNAs of prokaryotes (E. coli) and the rRNAs of
eukaryotes.

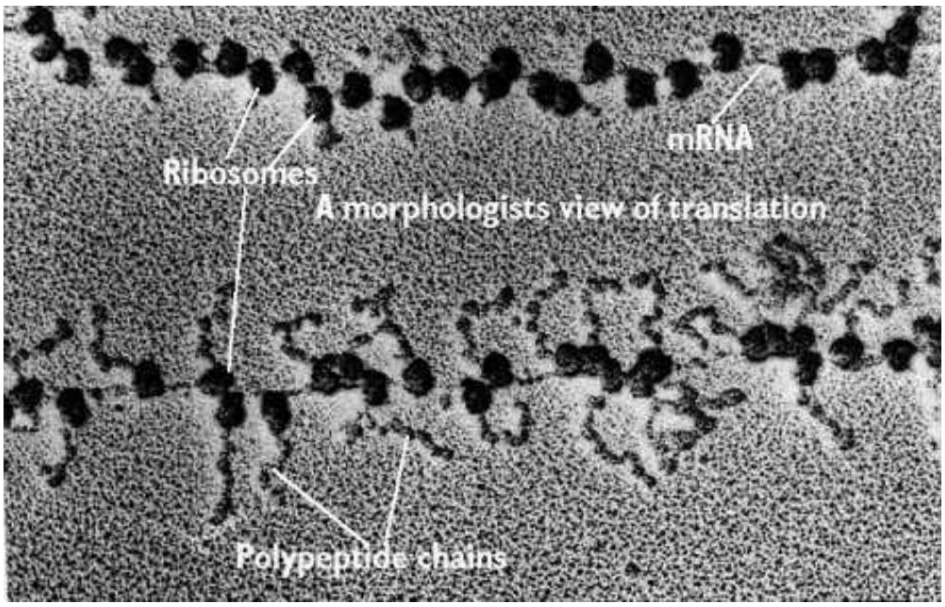


Figure 36: Micrograph of ribosomes

2.1.1. Prokaryotic rRNAs:

The so-called 70S ribosomes are composed of two subunits:

- A large subunit (50S)
- A small subunit (30S)

Each subunit comprises proteins called ribosomal proteins (or r-proteins) and RNA too.

The large 50S ribosomal subunit of prokaryotes contains the following rRNAs:

- 23S rRNA (2904 nucleotides in *E. coli) ;*
- 5S rRNA ; it is not bound to 23S rRNA.

The small 30S ribosomal subunit contains the following rRNA:

- 16S rRNA.

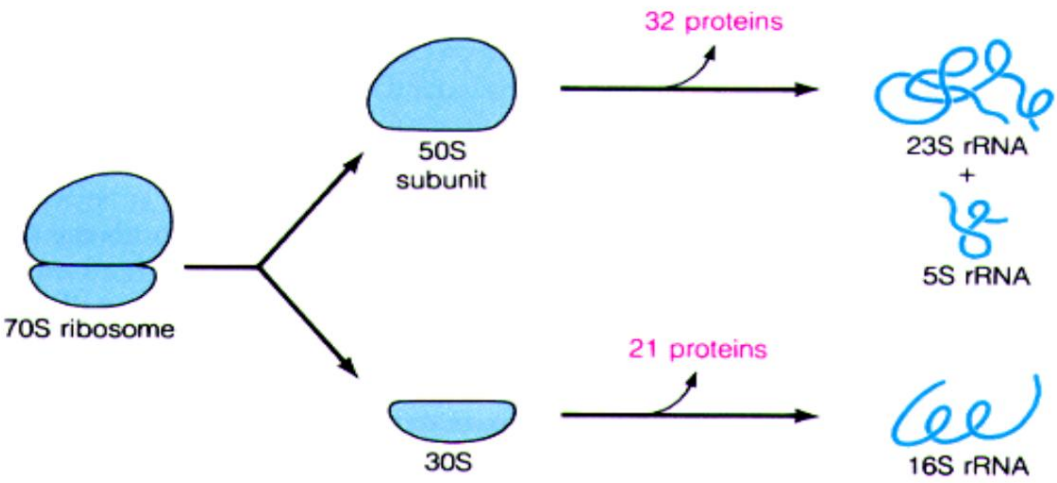


Figure 37: Structure of the E.coli ribosome

2.1.2. Eukaryotic rRNAs:

In eukaryotes, ribosomes are larger (80S) and also have a two-strand structure subunits (40S and 60S).

The 60S subunit contains three different RNAs (5S, 5.8S and 28S)

The small 40S subunit contains only one rRNA (18S).

The number of ribosomal proteins within each subunit is greater than in prokaryotes.

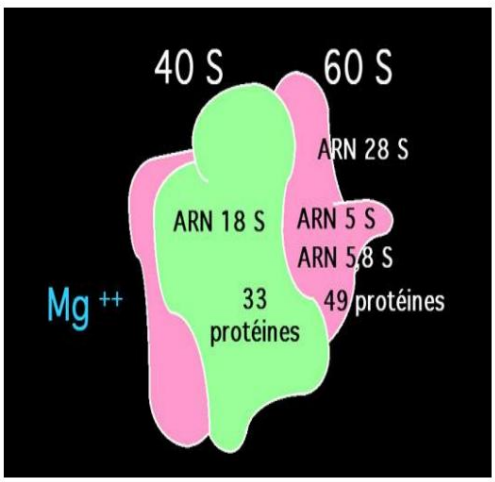


Figure 38 : Structure of a eukaryotic rRNA

2.1.3. Functions of the different rRNAs

rRNAs play an essential role in the structure and maintenance of the integrity of ribosomes. in association with ribosomal proteins.

- rRNA of the small subunit (16S or 18S)

The RNA of the small subunit is involved in reading messenger RNA. It is the one that verifies that the interaction between the codon located in the A site of the ribosome and the anticodon of The tRNA is correct. The RNA of the small subunit is therefore the fidelity controller of the translation of the genetic message into protein .

- rRNA of the large subunit (23S or 28S)

The large RNA of the large ribosomal subunit is involved in the formation of peptide bonds. It is the direct catalyst for protein biosynthesis.
The active center of the ribosome, called peptidyltransferase, is composed exclusively of RNA. ribosomal. The crystallographic structure of the ribosome showed that there was no protein within 50 to 60 Å of this active site. The rRNA of the large subunit is therefore a ribozyme.

2.2. Transfer RNAs

tRNAs are the vectors that will transfer amino acids to the ribosome factory or protein synthesis takes place.
Indeed, there is no affinity between messenger RNAs and amino acids, neither in vivo nor In vitro, the link between the code and what it specifies is made via these molecules adaptors are transfer RNAs. These small molecules (approximately 70 nucleotides) possess two essential functions: the possibility, for each of them, to link to a a specific amino acid and, on the other hand, to recognize a precise codon thanks to an anticodon that is, a complementary triplet of the codon. Anticodon-codon recognition relies on on the complementarity of bases and involves the primary structure of transfer RNAs, the Specific recognition of an amino acid is much more complex and involves the three-dimensional architecture of these particular molecules.

2.2.1. Structure of tRNAs

The tRNA chains are made up of approximately one hundred nucleotides; we present tRNAs are often clover-shaped. Important tRNA sites include:

- At the 3'-OH end there are three characteristic nucleotides: CCA-3'-OH,
  It is at this end that the amino acid will be attached and transported by the tRNA.

- The anticodon, which corresponds to a group of 3 nucleotides (or triplet) located on a loop of the tRNA. This triplet pairs with the corresponding codon present on a RNA, messenger through antiparallel hydrogen bonds.
- Finally, the 5' end of tRNAs contains a phosphate group.

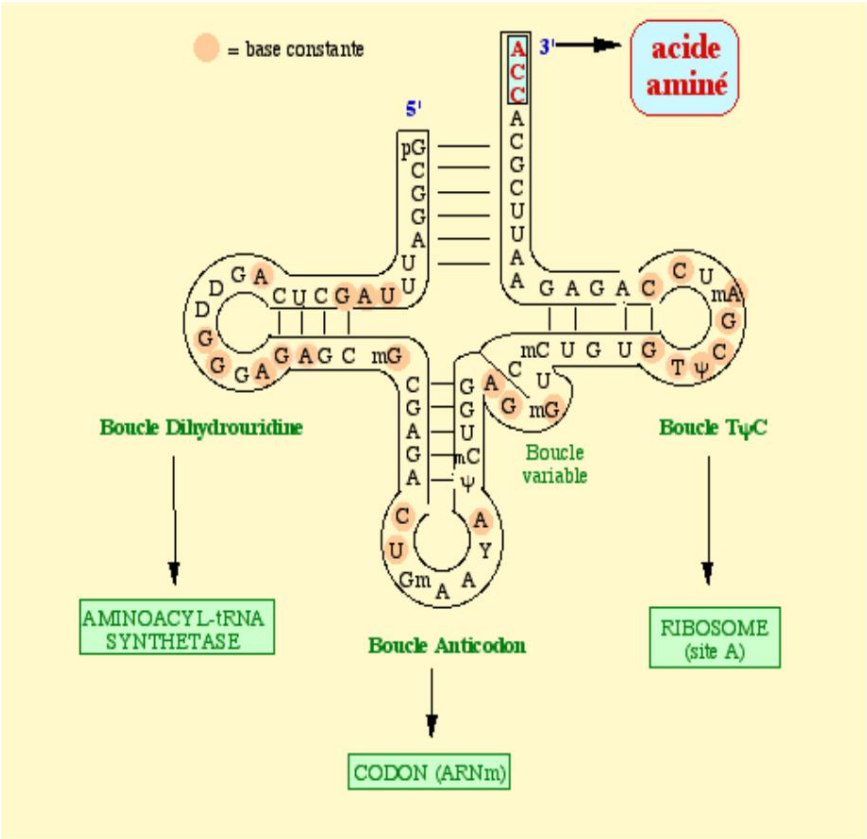


Figure 39 : Structure of a transfer RN

Note: Transfer RNAs contain so-called "rare" bases such as pseudouridine, dihydrouridine, inosine... which are actually modified bases after the transcription. These bases contribute significantly to the establishment of the structure three-dimensional by unusual hydrogen bonds.

2.3. Messenger RNA

Messenger RNA (mRNA) is formed in contact with DNA and its role is to transcribe a DNA sequence and then to transport the genetic information gathered from the nucleus to the cytoplasm. The messenger RNA will then bind to a protein assembly unit, the

ribosome, where it will be translated to build a sequence of amino acids necessary for the protein synthesis.

The genetic information of a cell, which governs all aspects of its life, is contained in its DNA. This information must be translated into proteins, which are the effector molecules of the living organism. Rather than directly using the matrix Instead of using DNA to synthesize proteins, evolution established an intermediate molecule, RNA. messenger, which is a replica of a part of DNA called a gene. Indeed, to a gene This generally corresponds to a protein. Passage through an mRNA intermediate also allows for regulate gene expression. mRNAs are labile molecules, whose lifespan is limited, varying from a few minutes to a few hours. Their production can be adapted by the cell to the specific conditions it faces. When a protein is necessary, the cell transcribes the corresponding mRNA. Conversely, when it no longer has any When necessary, gene transcription stops and the mRNA is progressively degraded by ribonucleases (or RNases). Thus, protein production can be stimulated or repressed by depending on the needs.

Like all RNAs, mRNA is a nucleic acid resulting from the polymerization of ribonucleotides linked by phosphodiester bonds.

2.4. Small nuclear RNAs

Small nuclear RNAs (or snRNAs) are present in the cell nucleus and are involved in certain stages of transcription, that is, the copying of DNA into ARN messenger.

These snRNAs are present in the form of ribonucleoprotein particles which are called snRNP: small nuclear ribonucleoparticles or snurps.

In the cytoplasm, one can find small RNAs (or scRNAs) which also exist under form of ribonucleoprotein particles (called small cytoplasmic scRNPs) ribonucleoprotein particles or scyrp)

1. When DNA becomes RNA

The information contained in genes will be used to manufacture thousands of proteins which play a role in the functioning of the cell. The first step in the expression of a
Gene production involves copying its information in the form of a molecule very similar to DNA. ribonucleic acid or RNA.

Definition: Transcription is the copying of a DNA molecule into an RNA molecule.
The enzyme responsible for this transcription is RNA polymerase.

RNA polymerase is an enzyme complex responsible for the synthesis of RNA.
ribonucleic acid, or RNA, is produced from a DNA template. In eukaryotes, there are essentially three RNA polymerases

— RNA polymerase I, RNA polymerase II and RNA polymerase III

— ensuring the synthesis of the four types of RNA : ribosomal RNA , messenger RNA, RNA transfer RNAs, and small RNAs: small nuclear RNAs

2. Transcription in Eukaryotes:

Their transcription takes place in two stages: formation of a pre-messenger RNA and then maturation of this pre-messenger RNA to form one or more messenger RNAs.

2.1. The formation of a pre-messenger RNA

2.1.1. Initiation of transcription

Not all DNA is transcribed; only the regions corresponding to genes are. And even then,
This expression can be regulated according to the stage of development and cell type.
the environment, etc... Therefore, an actor must intervene to determine where a
The DNA region must begin to be transcribed: this is the role of the **promoter.**

- **The promoter**

The promoter corresponds to a non-transcribed region of DNA, usually just upstream
from the beginning of the transcribed region, whose sequence allows the recruitment of RNApol II.

The developer states:

- The beginning of the gene to be transcribed into mRNA (where the RNA polymerase must bind to DNA)
- Which strand of DNA needs to be transcribed?

Certain sequences of the promoter (nicknamed "boxes") have particular importance in this process, essentially because these sequences are specifically recognized by different proteins belonging to the initiation complex:

The promoter in eukaryotes contains a nucleotide sequence made up solely of bases A and T. TATA(A or T)A(A or T)A

Most often it's the sequence TATAAATA. This sequence is called the TATA box (it is located on the 5'-3' strand approximately 25 nucleotides before the start of the gene).

- **The initiation complex**

Unlike what happens in prokaryotes, eukaryotic RNA polymerase II does not recognize not alone the proximal promoter. She carries out this work in the company of many co-protein factors that recruit each other and form a complex with it initiation factors. These factors are denoted TFIIA, TFIIB, etc... for Transcription Factor for RNA polymerase II. They correspond to general transcription factors because they assemble on all promoters used by RNApol II.

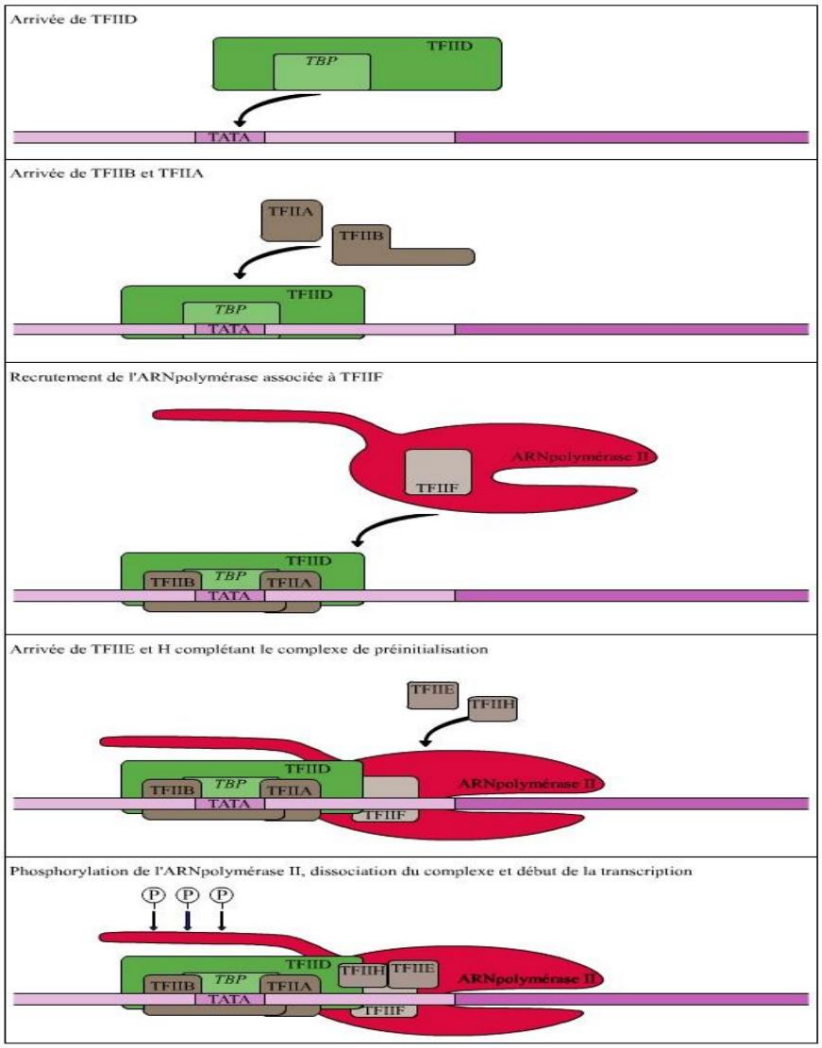


Figure 40: Setting up the initiation complex.

TBP (TBP = Tata box-binding protein) is the
first protein that recognizes a specific sequence of the initiating DNA of the transcription (the TATA box).

The TFIIB factor (TFII = Transcription Factor for RNA polymerase II (General Factors of transcription for RNA polymerase II); appears to be involved in precise site selection initiation (nucleotide from which transcription takes place).

The TFIIH factor has several enzymatic activities, including helicase activity.
allowing the opening of the DNA double helix at the promoter, and an activity
kinase responsible for the phosphorylation of the C-terminal tail of RNApolymerase II.

This phosphorylation results in a modification of the three-dimensional structure of RNA polymerase leading to the dissociation of the initiation complex and the start of the transcription.

2.1.2. Elongation

As RNA polymerase II moves along the template strand of DNA, Complementary RNA nucleotides are paired with DNA nucleotides. RNA polymerase II joins RNA nucleotides in the 5' to 3' direction.

The RNA/DNA association is not as stable as the DNA helix. Therefore, only the new part of the mRNA molecule is associated with the DNA; the rest is hanging on its side.

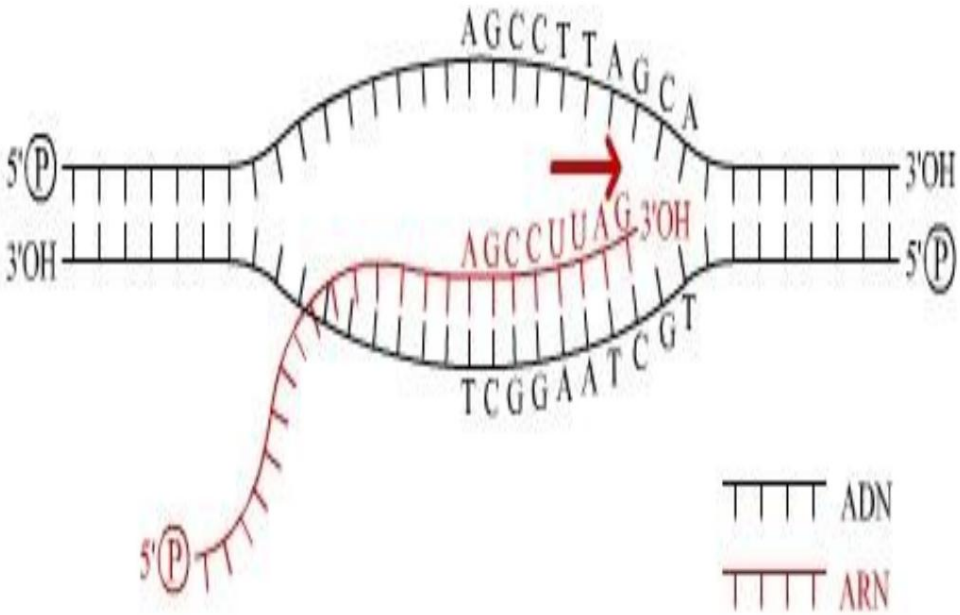


Figure 41: The elongation phase of transcription.

RNA polymerase reads the template strand (or antisense strand) which is complementary to the coding strand. (or sense strand) from its 3' end to its 5' end. The newly synthesized RNA strand is therefore identical to the coding strand of DNA, except for uracils and riboses.

2.1.3. The termination

RNA pol II is also equipped with termination protein factors, and thus recognizes one or more termination signals carried by the progressively traversed strand and which announce the end of transcription on the template DNA strand (TTATTT for example, sometimes

(also further downstream ATACAAC...). She soon stops her transcription work and releases the pmRNA that she has just assembled.

2.1.4. Maturation of pre-messenger RNA (Formation of messenger DNA)

The primary transcript is not used as is for protein synthesis (translation). It must undergo modifications that meet several requirements (increased half-life, (sequence modification). All these modifications are made as the progression of pre-mRNA synthesis in the nucleoplasm.

There are three main types of modifications, each catalyzed by enzymes of protein or ribonucleic nature.

Post-transcription: RNA modification

Enzymes inside the nucleus modify pre-mRNA in various ways specific before genetic messages can be sent to the cytoplasm.

During mRNA processing, the two ends of the primary transcript (RNA not having (still modified) are usually altered.

Furthermore, in most cases, certain internal sections of the molecule are cut, and the remaining parts attach together.

These modifications help to form the mRNA, which is ready to be translated.

There are three modifications:

2.1.4.1. The addition of a 5' cap

1. The 5' end of the pre-mRNA is closed with a modified form of a nucleotide of guanine, thus forming a *5' cap.*

It takes place at the very beginning of the transcription, before the chain has more than 30 nucleotides. It consists of adding a guanine nucleotide to the 5' end of the RNA followed of its methylation on nitrogen 7 of the base, as well as of the methylation at 2' of the ribose of one or the first two nucleotides of the primary transcript. The distinctive feature of this addition lies in the type of binding involved. As a result, the 5' end of the mRNA does not carry the three usual free phosphoric acids, but from a GMP, which limits the reactivity of this end and its recognition by exonucleases (protection against degradation).

This structure acts as a protective cap for the 5' end of the mRNA. It is also necessary for the export of mRNA to the cytoplasm and for the binding of the latter to the small subunit of the ribosome during the initiation stage of translation.

2.1.4.2. Polyadenylation (tail addition):

At the 3' end, an enzyme adds 50 to 250 adenines, thus forming a *poly-A tail.*

ÿ The 5' cap and the 3' poly-A tail share several functions important:

- They facilitate the export of mature mRNA molecules from the nucleus.
- They help protect mRNA from degradation by enzymes hydrolytics.

Once the mRNA reaches the cytoplasm, the two structures help the ribosomes to attach to the 5' end of the mRNA.

2.1.4.3. Excision and splicing:

Large portions of the pre-mRNA molecule are removed in a cutting and to stick, called **RNA splicing.**

Most genes and RNA transcripts have long nucleotide spans non-coding regions, that is, regions that are not translated. Most of these Non-coding sequences are between coding segments of the pre-mRNA.

Non-coding segments are known as *introns,* while segments

The coding elements are the *exons.*

ÿ The excision of introns is carried out by means of a formation known as "lasso".

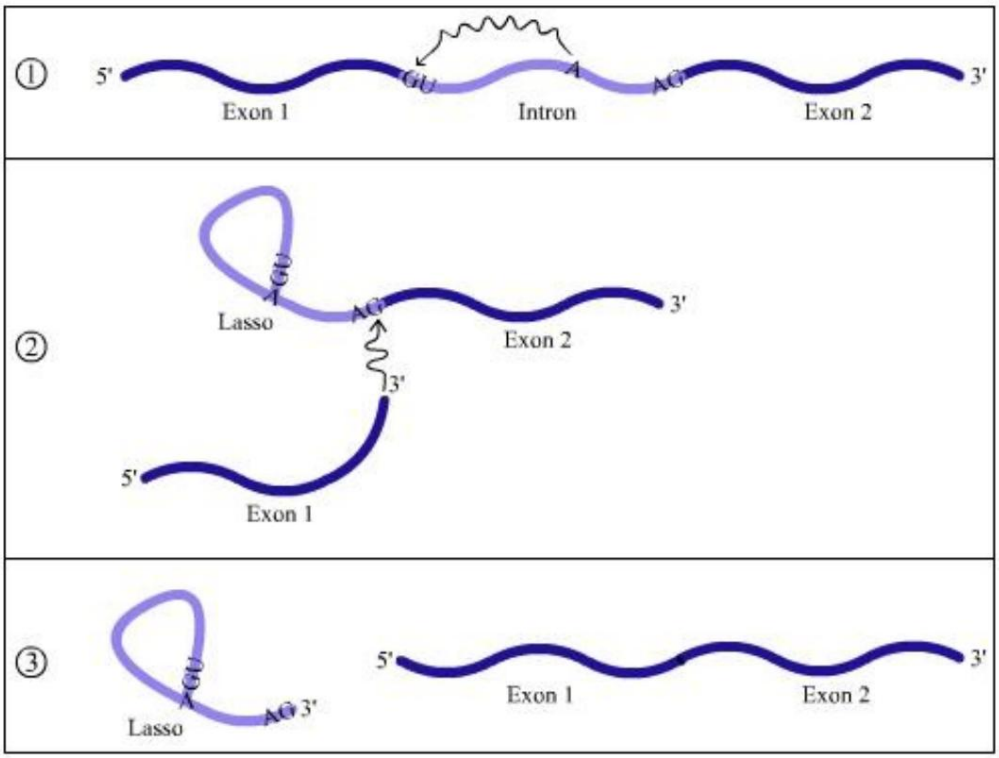


Figure 42: Intron excision by lasso formation: overview of the mechanism.

Excision-splicing is performed by the reaction of a nucleotide to adenine (A) located in the intron with a guanine nucleotide located at the 5' end of the intron. This results in the separation of the intron with exon 1 (located upstream) and the formation of a lasso-like structure internal to the intron. Next, the 3' end of exon 1 reacts with the 5' end of exon 2, allowing splicing. of the two exons and the release of the lasso which will be degraded by ribonucleases.

3. The Translation

Translation is the decoding of the mRNA message into a chain of polypeptides. Translation takes place on ribosomes that are free in the cytoplasm, or attached to the endoplasmic reticulum. endoplasmic.

3.1. The genetic code

The genetic code is a code that allows the conversion of a sequence of nucleotides (DNA). then RNA) in a sequence of amino acids (proteins). The code involves the bases A, C, T, and G as well as the 20 amino acids.

A group of **3 bases or triplet of bases in mRNA codes for 1 amino acid.**

These 3 mRNA bases are called ***codons.***

The genetic code has different characteristics:

- **The genetic code is universal.** Indeed, each amino acid has one or several codons, and this at the level of a multitude of prokaryotic living organisms and eukaryote.

  This characteristic allows the transfer of genes from one species to another = genius genetics: The introduction of genes into a bacterium or the introduction of genes into a multicellular organism

- **The genetic code is redundant (or degenerate).** Several codons code for one same amino acid: there are 64 codons and 20 amino acids. Often these are the the first two nucleotides of the codon that define the amino acid, the redundancy is therefore due to the third nucleotide of the codon.

The genetic code is non **-overlapping.** The nucleotides of a codon do not participate than the code of a single amino acid, so the next amino acid will be coded by the The next codon present on the mRNA. This is called the **reading *frame .***
frame).

The code **has a punctuation system.** The initiation codon is the AUG codon. (GUG for my mitochondria) and the termination codons are the UAA codons (ochre), UAG (amber) and UGA (opal). The UGA (opal) codon is not present in mitochondrial level.

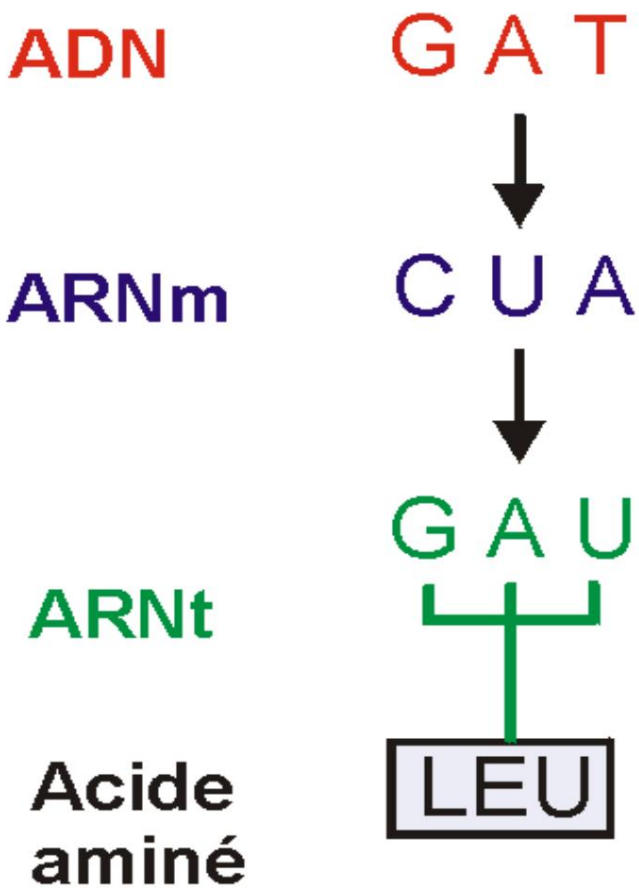


Figure 43: The genetic code

3.2. Variation in the genetic code

Mitochondria use a genetic code different from the universal genetic code, used by the vast majority of living organisms. In fact, there are 17 genetic codes. different, including 11 for the mitochondria of different organisms. It should be noted that the Associations between species and mitochondrial genetic code are constantly evolving according to sequencing of new mitochondrial genomes. For example, code no . 5, called "code mitochondrial disease in invertebrates is confirmed for species such as Caenorhabditis elegans or the fruit fly (with the exception of the absence of the AGG codon), but for example, it is not the case for sea urchins which have their own code (no 9).

3.3. The " *wobble* " effect: lopsided matching

Wobble ***pairing,*** literally "unbalanced pairing", is a method of non-pairing canonical between nucleic bases, which is observed primarily in RNA. We find in

in particular the base pairs G–U, I–U, I–A and I–C, which can play an important role in the secondary structure of RNAs. They differ from Watson-Crick pairs by the nature of the bases and hydrogen bonds involved.

Wobble matching plays a very important role in code translation genetics. They do, in fact, partially compensate for the disparity in the number of codons. (61) and the number of amino acids (20), using lopsided, or ***wobble (game)*** pairings ***(in English),*** at the first position of the transfer RNA anticodon, which allows a even tRNA to recognize several synonymous codons.

This hypothesis was first formulated by Francis Crick in 1966.

Inosine (symbol I), a modified base frequently found in first
The position of the tRNA anticodon is particularly important, as it allows for...
pairings with uracil (U), adenine (A), and cytosine (C). The G–U pair is also __
very common in many RNA structures.

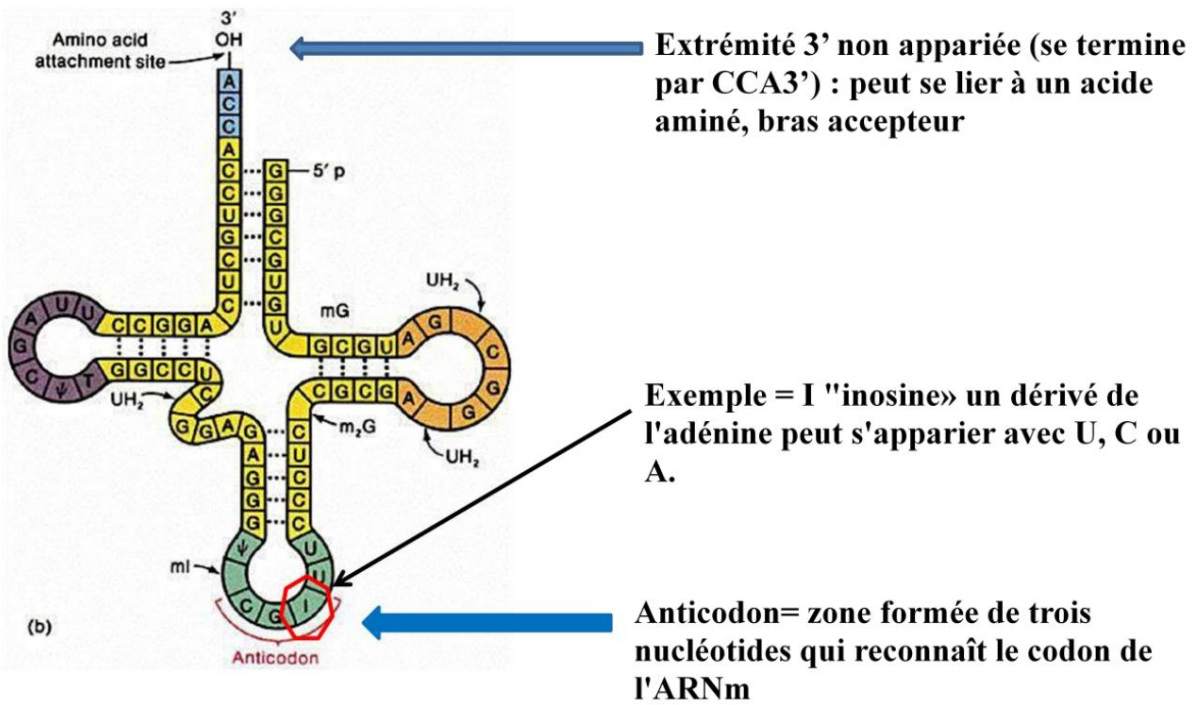


Figure 44: tRNA active sites

3.4. Translation of genetic information

Translation is the decoding of the mRNA message into a chain of polypeptides. Translation takes place on ribosomes that are free in the cytoplasm, or attached to the endoplasmic reticulum. endoplasmic.

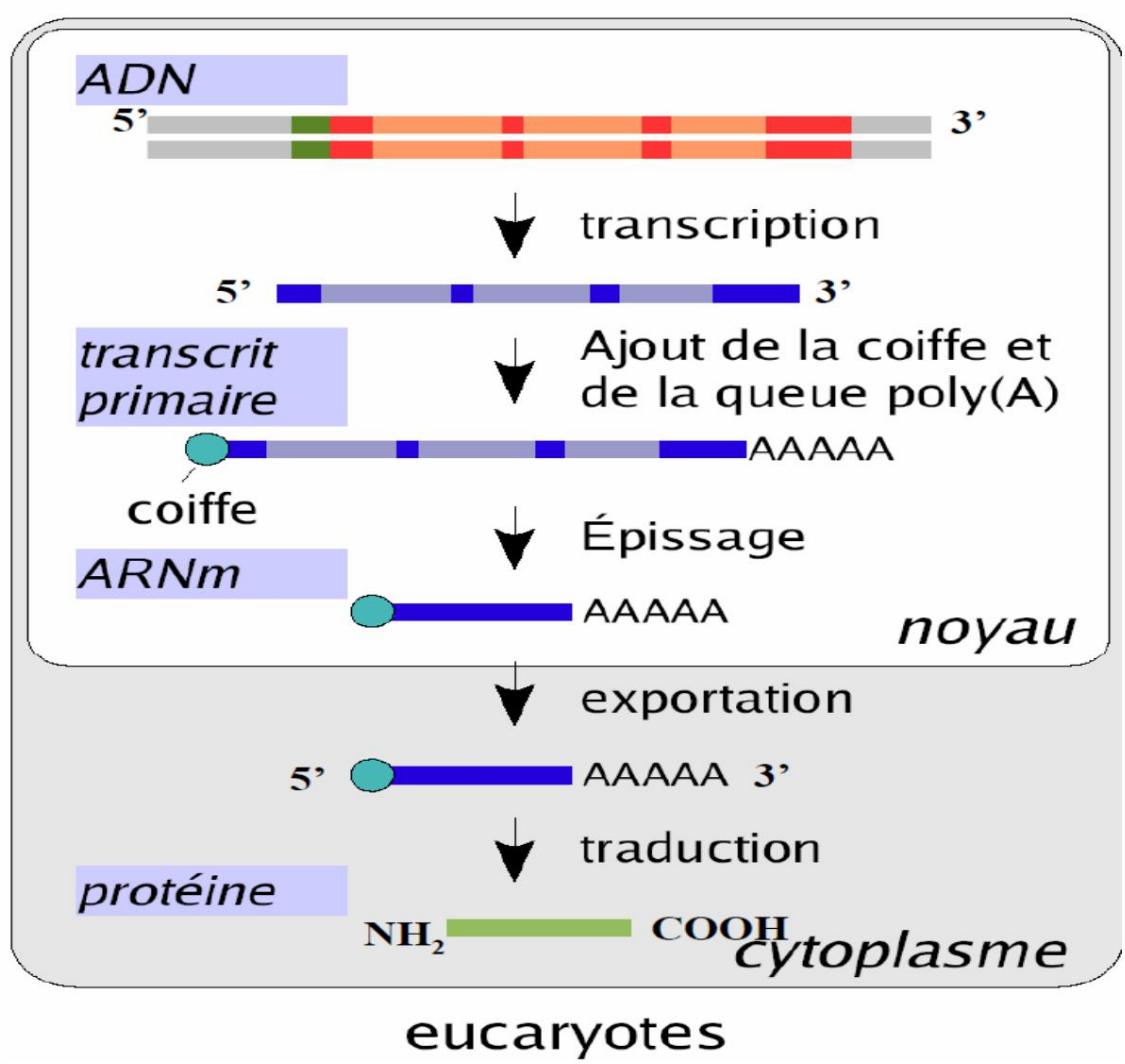


Figure 45: From transcription to translation of genetic information

3.4.1. The actors in translation:

- mRNA: codons
- Aminoacyl-tRNAs with anticodons
- The different enzymes

• Ribosomes

• The auxiliary factors that assist the ribosome at each stage

ÿ GTP: its hydrolysis provides energy

3.4.2. Ribosomes

These are ribonucleoprotein particles composed of:

–Proteins

–RNAr

Most of these proteins and rRNAs have a strictly structural role and are not involved in protein synthesis

The complete eukaryotic ribosome (60% RNA): 80S density is composed of:

Small subunit of 40 S

–1 18S rRNA

–33 proteins

Large 60S subunit

–1 28S rRNA

–A little 5S RNA

–A small RNA of 5.8 S

–49 proteins

Ribosomes behave like a mobile factor that moves along a matrix.
It provides the environment that allows for the control of the mRNA/Aminoacyl-tRNA interaction ·

The ribosome is composed of different active sites:

–P site or donor site (polypeptide site) spanning both subunits

–Site A: acceptor site (of aminoacyl-tRNA) spanning both subunits

–Site E: site through which the tRNA, having been stripped of its amino acid, leaves the ribosome

– Peptidyl transferase site

–A peptide exit domain

–mRNA binding site

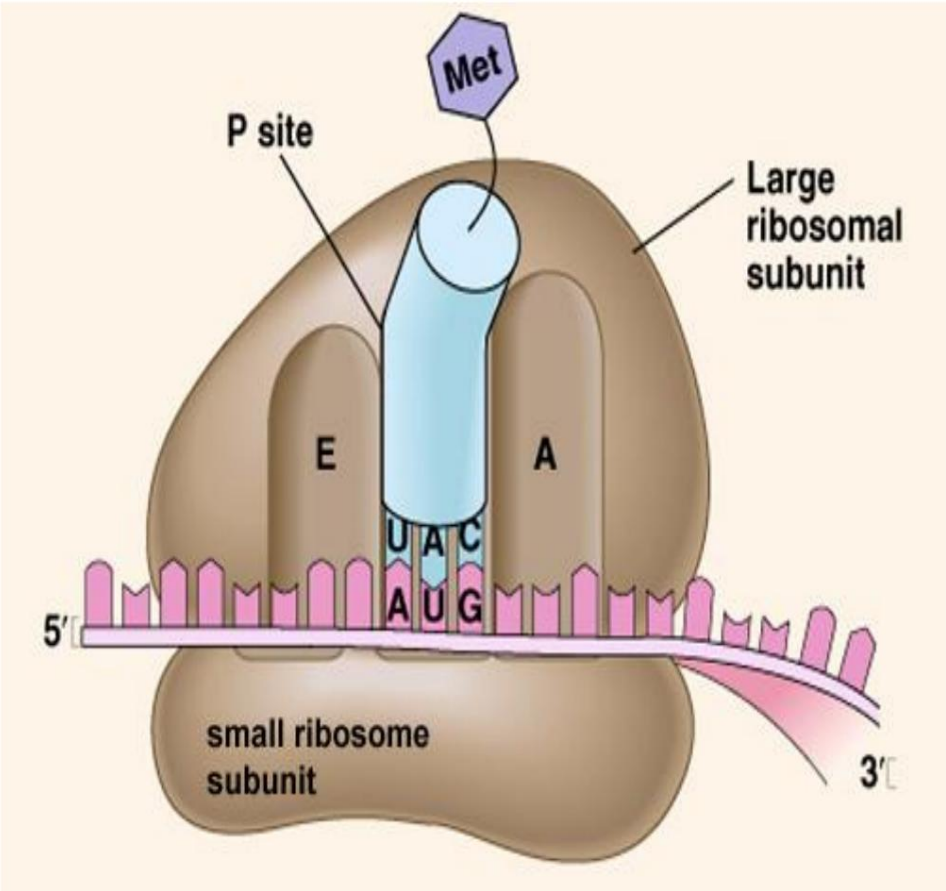


Figure 46: Ribosome active sites

3.4.3. tRNAs

Each tRNA is characterized by its anticodon. A tRNA does not carry just any amino acid: it depends on the anticodon, Ex.

AAA tRNA always carries the amino acid PHE

GAU tRNA always carries the amino acid LEU

- The amino acid is attached to the correct tRNA by the enzyme *aminoacyl-tRNA synthetase*

- The tRNA loaded with its amino acid is called **aminoacyl-tRNA.**

Each tRNA is synthesized like mRNA from special genes in DNA (still some genes that do not code for proteins)

3.4.3.1. Aminoacyl-tRNAs with anticodons:

There are several types *of aminoacyl-tRNA synthetase.* Each can attach one amino acid. specific to a particular tRNA.

The enzyme's active site recognizes: a particular amino acid AND a particular anticodon.

The enzyme links the amino acid to the tRNA

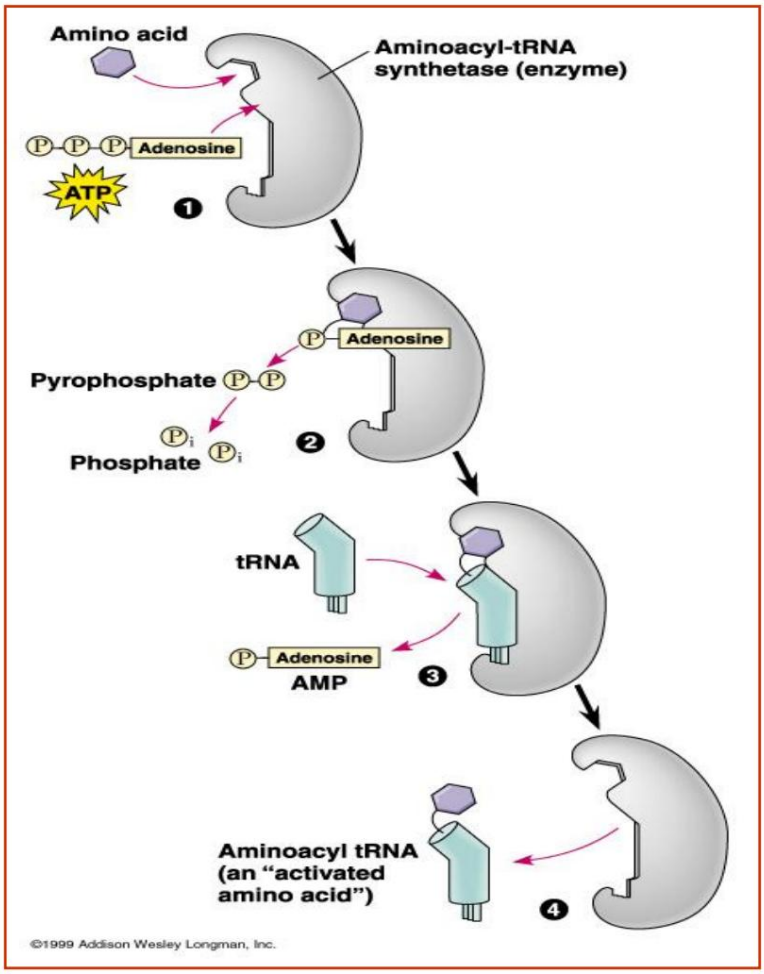


Figure 47: Aminoacyl tRNA

3.5. Translation Mechanism

3.5.1. Initiation

In eukaryotes, the small subunit recognizes an mRNA thanks to its 5' cap, migrates until the AUG where it will be joined by the large subunit.

When the large subunit joins the complex, the initiating aminoacyl-tRNA is found in site P. Site A is available for the entry of the complementary aminoacyl-tRNA of second codon of the gene.

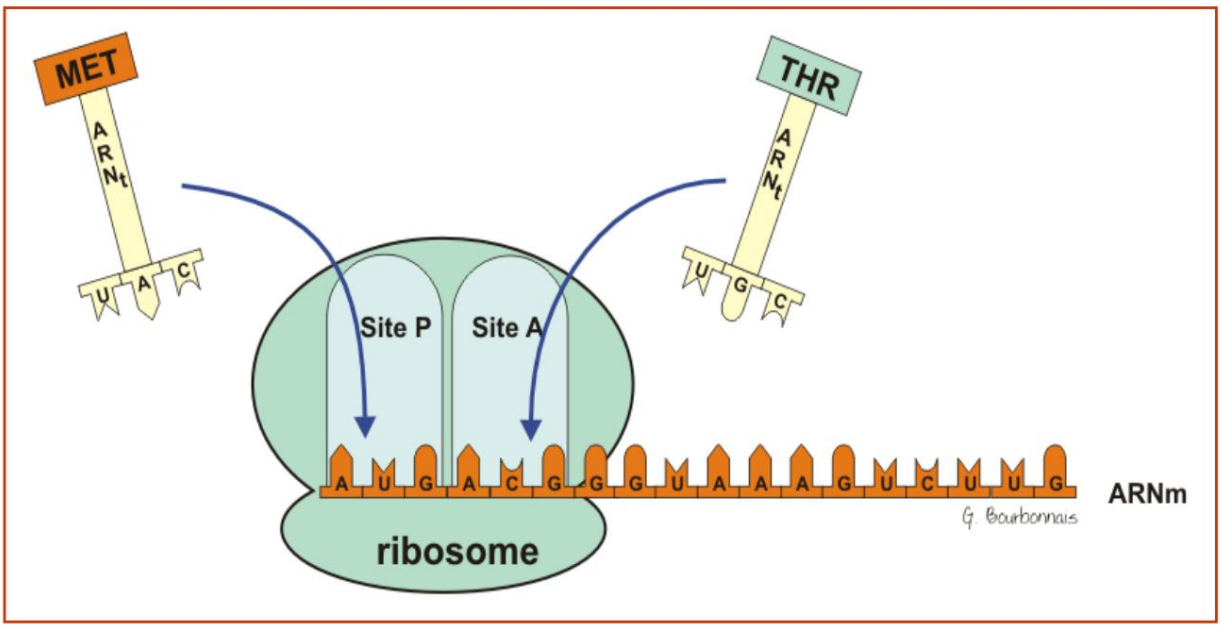


Figure 48: Initiation of translation

3.5.2. Elongation:

-Includes all reactions between the synthesis of the first peptide bond and the addition of the last amino acid

-Amino acids are added one by one to the polypeptide chain. The addition of an amino acid is a very quick step.

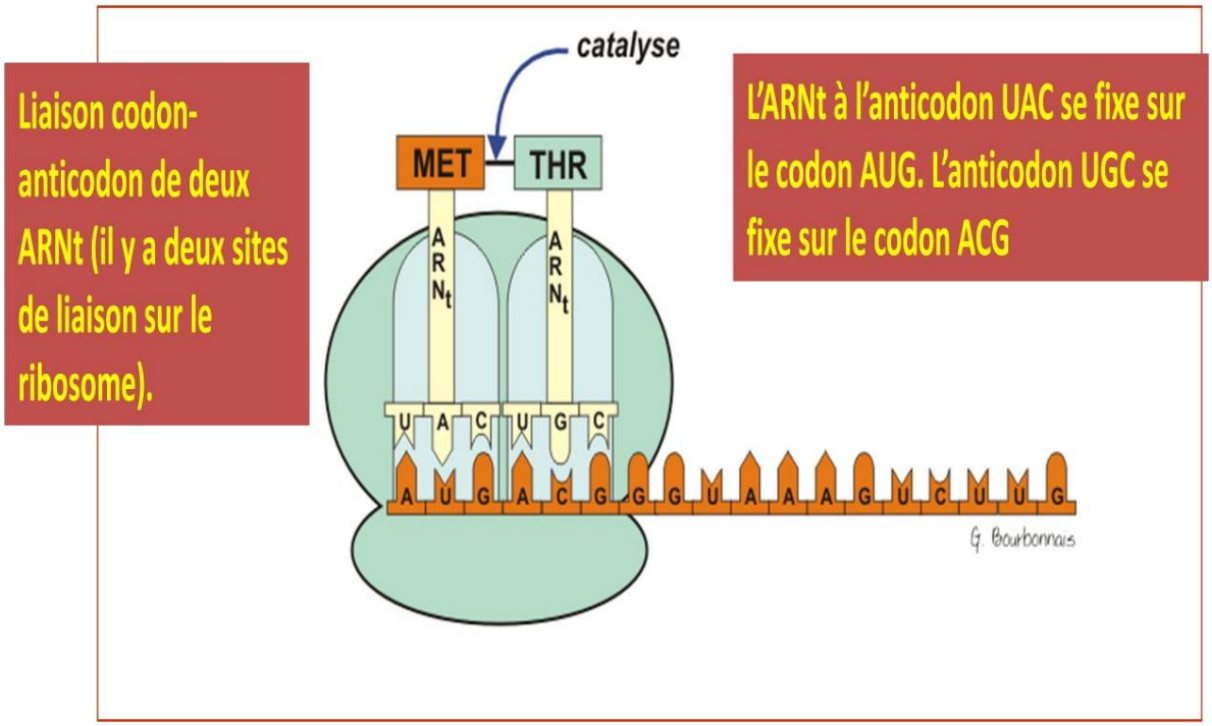


Figure 49 : Translation elongation

The ribosome is complete at the initiation site

- Starting a cycle:

–1 aminoacyl-tRNA enters the A site via an elongation factor (EF-Tu in the bacteria, eEF-1a in eukaryotes) in the presence of GTP

–GTP enables connection to site A

–Any aminoacyl-tRNA can enter the A site

–Site P is occupied either

–Either by the initiating aminoacyl-tRNA

–Either by a peptidyl-tRNA

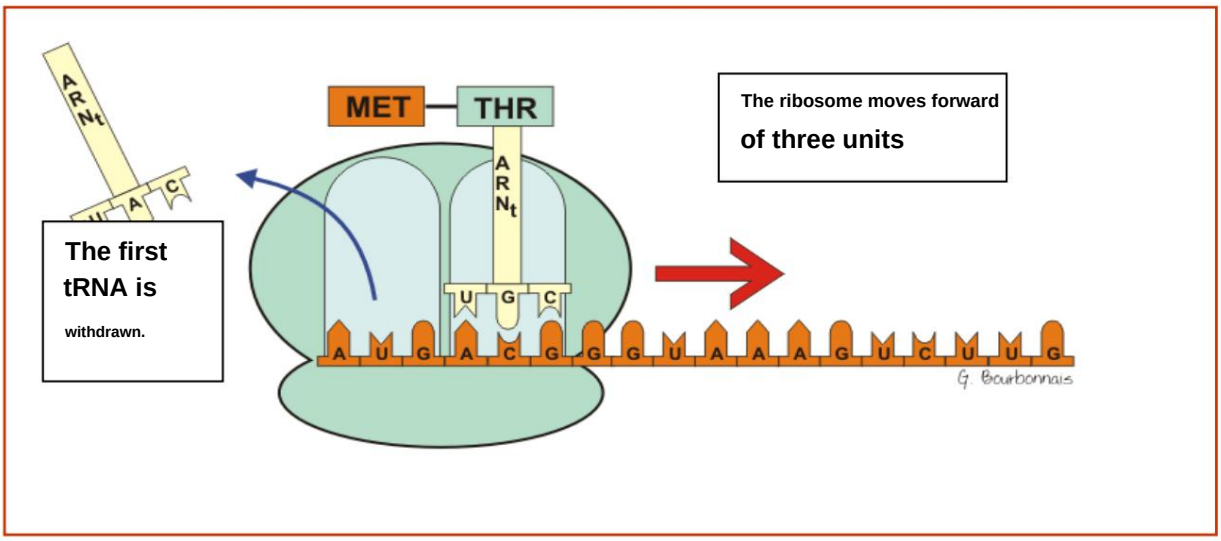
ÿ **Transfer of the polypeptide or of the formylated methionine attached to the tRNA of the site P on the aminoacyl-tRNA present in site A**

ÿ **Synthesis of the peptide bond by the activity**

peptidyl transferase of the large ribosomal subunit.

- Then comes the translocation of the ribosome, which moves three nucleotides on mRNA expulsion of the unloaded tRNA P site: entry of a new one peptidyl-tRNA, the A site is emptied , monitoring of aminoacyl-tRNA positioning corresponding to the following codon.

- Need for GTP, Elongation Factor eEF-2 in eukaryotes



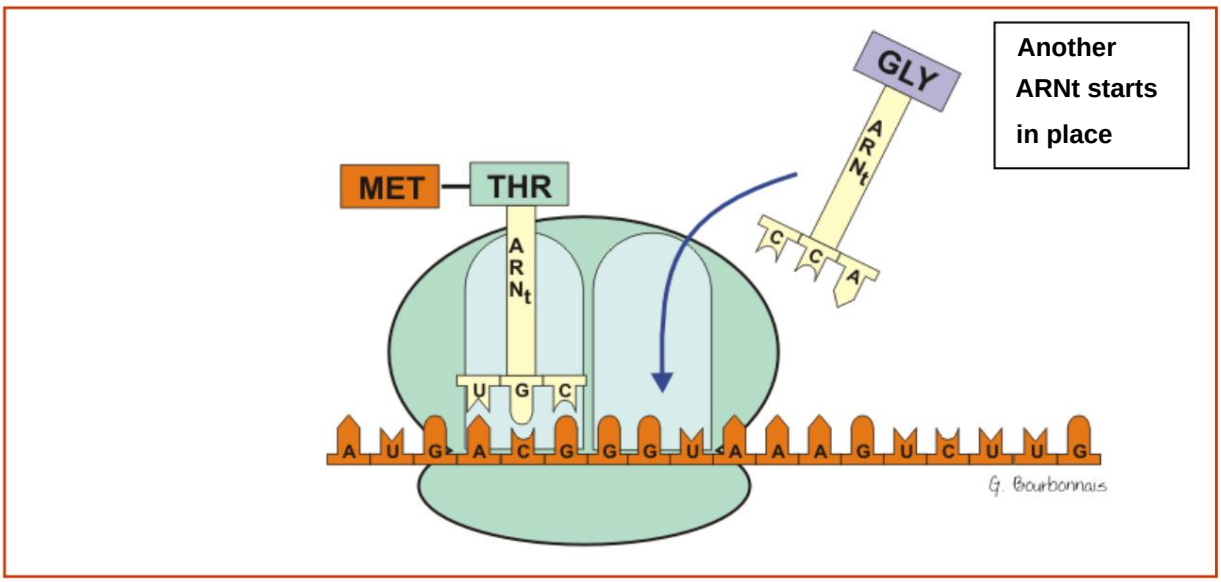


Figure 50: Progression of the elongation

3.5.3. The termination

Encompasses the reactions necessary to release the complete polypeptide chain; In At the same time, the ribosome dissociates from the mRNA.

All mRNAs end with the codon (triplet of bases) UAA, UAG or UGA = STOP codons (No corresponding tRNA).

When the ribosome reaches a STOP codon, an enzyme *(termination factor)* is released there. attaches and detaches the mRNA from the ribosome.

The synthesized protein enters the endoplasmic reticulum where it will take its final form.

Figure 51: Translation completion

1. Review of the structural organization of chromatin

Around 1865, Gregor Mendel, an Austrian monk and botanist, conducted experiments on the peas, which will form the basis of formal genetics, allowing us to identify laws of transmission hereditary traits. Chromosomes were then discovered in 1879 by the cytologist German WaltherFleming, who described them during cell division thanks to improvement cell dyes, without explaining their role (Figure 52).

The genetic material contained in eukaryotic cells is compacted into a nucleoprotein structure called chromatin, from the Greek "khroma" and which means "Colored." Chromatin was highlighted towards the end of the 19th century for its affinity to certain dyes. It carries genetic information and consists of a nucleoprotein complex comprising DNA, histones and non-histone proteins.

The fundamental unit of chromatin is the nucleosome, which consists of an octamer. histone containing two copies of the four histones H2A, H2B, H3 and H4 around which wraps around 146 base pairs (bp) of DNA; the whole forming a cylinder 11 nm in diameter and 5.5 nm in height. The nucleosome is the first level of DNA compaction in the nucleus. It regulates DNA's access to fundamental cellular processes such as replication, recombination, transcription and DNA repair.

The structure of the nucleosome was solved by X-ray crystallography at a resolution of 7 Å in 1985 and at 2.8 Å about ten years later. The nucleosome forms a cylinder around which 1.65 turns of DNA are wound in a left-handed superhelix. At the level Atomic, the interactions between DNA and histones are of the hydrogen type involving mainly positively charged lysines and arginines and the phosphate skeleton of negatively charged DNA. The nucleosome is one of the most stable complexes under certain conditions physiological, but this does not make it a static structure. On the contrary, it has been demonstrated lately, this structure is subject to variations in its composition and its conformation, sometimes with altered histone composition and stoichiometry that may ranging from an octasome, hexadome, tetramers and even a hematoma.

Such variations will have a tremendous impact on various cellular processes.

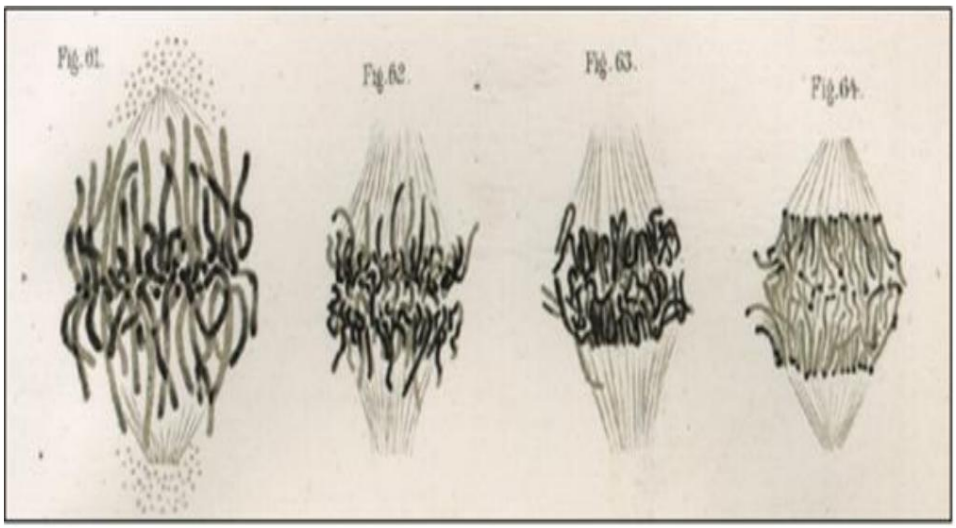


Figure 52: Schematic representation of mitosis by W. Flemming in 1882

1.1. Histones or canonical histones

Histones are crucial proteins in the organization of chromatin. Histones are basic proteins associated with DNA. They play a major role in the compaction of DNA and its organization into the chromatin structure. Histones consist of five main subunits: H1, H2A, H2B, H3 and H4.

a) Histone H1: This histone is associated with the part of the DNA located at the exit of the nucleosome, thus contributing to the stability of the chromatin fiber.

Histone H1, or linker histone, differs from the other histones H2A, H2B, H3, and H4. possesses a globular domain of approximately 90 amino acids and two unstructured extensions in N- and C-terminal. There is no structure of a nucleosome containing histone H1, but it appears to be located where DNA enters and exits the nucleosome. Its inactivation, unlike The reaction to other histones is not lethal; however, it results in a global alteration of the structure. of chromatin, suggesting a role in the formation of higher-order structures of the chromatin.

b) Histones H2A and H2B (or **canonical histones):** They form a dimer and participate actively involved in the formation of the nucleosome, the basic unit of chromatin.

c) Histones H3 and H4 (or **canonical histones) :** These histones are also involved in nucleosome formation and play an essential role in chromatin organization.

1.1.1. Structure of canonical histones

The canonical histones H2A, H2B, H3 and H4 are small proteins whose weight molecular weight varies between 11 and 20 kDa and its primary structure is very rich in amino acids basic. They have two structurally and functionally distinct areas: the N-terminal tails and the histone fold (HFD).

Histones contain a highly conserved globular domain (HFD) comprising 3 helices. ÿ (ÿ1-3) linked together by two small loops (L1 and L2) (Figure). The histones H2A-H2B and H3-H4 dimerize via their HFD to form a "handshake" structure (figure). Under physiological conditions of salts and in the absence of DNA, two H3-H4 dimers associate to form a tetramer (H3-H4)2 to which the two dimers (H2A-H2B) will bind to form an octamer of histones ((H3-H4) + (H2A-H2B))2.

Each histone has unstructured N-terminal regions, called N-tails terminals, which do not appear on published crystallographic structures. H2A and H2B These regions, on the other hand, have a C-terminal tail. These regions consist of approximately 20 to 35 residues. represent 20% of the total histone residues. They have the particularity of being oriented towards the outside of the nucleosome and do not participate in its structure.

In contrast, the N-terminal tails of H4 and H2B are important for formation of higher-order chromatin structure. These regions are highly accessible and the residues The molecules that compose them will be chemically modified by specific enzymes. modifications will impact various biological processes by serving as a pattern of recognition by different factors or by modifying the structure of chromatin.

Noticed :

The genes encoding canonical histones are expressed only in S phase of the cell cycle and in a replication-dependent manner. They are encoded by several genes and expressed massively during the S phase in order to meet the high demand for histone

resulting from DNA replication. Messenger RNA (mRNA) of histone genes canonicals contains, in 5' and 3', very short untranslated sequences and a 5' heading like all eukaryotic mRNAs transcribed by RNA polymerase II. However, these are the The only mRNAs that do not contain a polyadenylated tail at their 3' end, they instead possess a secondary structure in characteristic and highly conserved loop stem which allows their regulation.

The SLBP (Stem-loop binding protein) targets this stem loop and regulates translation and the degradation of histone mRNAs.

2. Histone variants

The incredible diversity of chromatin is also illustrated by the incorporation of histone variants in chromatin. Indeed, although histones represent proteins Among the most conserved throughout evolution, there are non-allelic histone variants. distinguishes between "canonical" variants and so-called "replacement" variants.

The study of chromatin as a carrier of epigenetic information has been particularly focused on DNA methylation and histone modifications. A level of complexity has been added in recent years with the discovery of versions alternatives to canonical histones. These paralogs, which are called histone variants, share broadly the same structure as canonical histones; however their primary sequences are relatively different (Figure 53).

Histone variants are found in all eukaryotes and appear to have specific roles at the level of specific regions of the genome.

Unlike canonical histones, which are deposited behind the fork of replication, histone variants are involved in a replacement process in very specific conditions. Some variants are incorporated into specific sites for replace canonical histones. Their depositions are carried out in a way independent of replication, outside of the S phase of the cell cycle.

Substitution of the canonical histone with a histone variant creates a nucleosome variant possessing a different structure and physico-chemical properties.

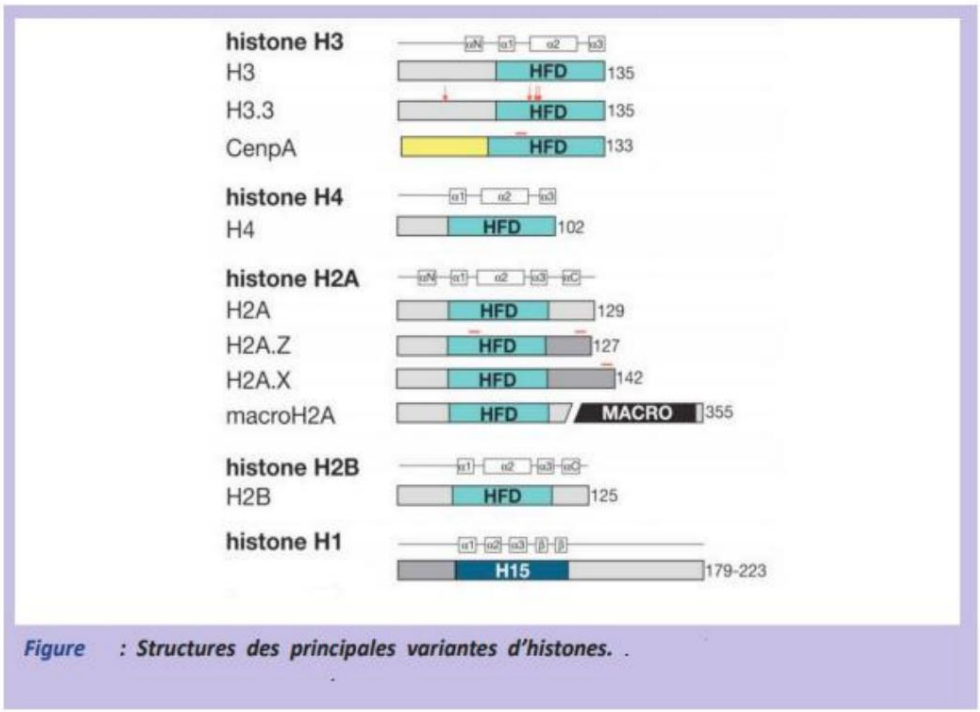


Figure 53: Structures of canonical histone variants

2.1. Variants of histone H3

The number of histone H3 variants differs depending on the species. distinguishes five variants in mammals: H3.1 and H3.2 are the replicative forms of H3, H3.3 is a replacement form that is expressed outside the S phase of the cell cycle, CENP-A is a paralog of H3 found in the regions centromeric (Palmer et al., 1991) and H3t, which is a testicular-specific variant and is not found in somatic cells (Govin et al., 2005).

Human H3.1 and H3.2 are 99% identical, while H3.3 is 96% identical. at H3.1, diverging by only five amino acids. Human CENP-A is very divergent and shares only 46% identity with H3.1 (Hamiche and Shuaib, 2012).

2.1.a. CENP-A or CenH3.

During mitosis, chromosome segregation requires alignment of all chromosomes are aligned at the cell's equator. This alignment is due to the attachment microtubules from the mitotic spindle to the centromeres, and this process is controlled by the kinetochores.

No consensus DNA sequence could be identified in the regions centromeric. However, variants of histone H3 from the CenH3 family, including CENP-In humans, Cid in Drosophila and Cse4p in yeast have been found in all centromeres in eukaryotes. Furthermore, the inactivation of CENP-A leads to poor kinetochore formation and segregation problems chromosomes in yeast, fruit fly and mammals (Sullivan, 2002). CENP-A inactivation in mice causes problems in mitosis and a embryonic lethality, which argues in favor of an important role for this variant in mitosis (Howman et al., 2000).

All members of the CenH3 family have a highly conserved C-terminal region and a variable N-terminal portion. The C-terminal portion appears to be the source of the targeting. from CENP-A to centromeric chromatin (Sullivan et al., 1994; Henikoff et al., 2000) while the N-terminal part is involved in the interaction with the kinetochores (Chen et al., 2000). CENP-A is deposited independently of replication, This results in a random distribution of CENP-A on the daughter chromosomes. It is not known I don't know how CENP-A is distributed on the daughter centromere.

However, there are several factors that will target CENP-A on the centromere. The CATD is a domain in the HFD region of CENP-A that directs it towards the centromere (Black et al., 2004; 2007) and the CRC RSF is necessary for the localization of CENP-A on centromeres (Perpelescu et al., 2009). Table 1 summarizes the distribution variants depending on chaperone proteins and remodeling complex with their implications in cancers and other genetic diseases.

TABLE 1: Histone variants, genomic distributions, functions and proteins Chaperones or remodeling complexes: Implications in cancers and other genetic diseases

Histone core	Distribution génomique	Fonction générale	Chaperons et remodeleurs	Fonctions des chaperons et des remodeleurs	Associée aux cancers		Associée aux maladies génétiques humaines
					Dérégulation dans les cancers	Rôle dans les cancers	
Famille H3							
CENP-A	Globale (centromère)	Identité du centromère, stabilité du génome	HJURP (chaperon)	Déposition au niveau des centromères	Amplification ou surexpression	Mauvaise ségrégation chromosomique, instabilité chromosomique	ND

2.1.b. H3.3.

The histone variant H3.3 is very close to the canonical histone H3; there are only... four
different amino acids in Drosophila and respectively five and four amino acids Different amino acids with H3.2 and H3.1 in mammals. H3.3 is deposited throughout the cell cycle while H3 is deposited during replication, which suggests a different function (Ahmad and Henikof, 2002).

- **H3.3 accumulates at active transcription sites.**

H3.3 is found at transcriptionally active loci in Drosophila, which
This suggests that H3.3 is associated with transcriptionally active chromatin. H3 and H3.3 share the same post-translation modification sites, but they are not not associated with the same type of modification; H3.3 contains modifications typical of active chromatin such as K4 and K79 methylation and acetylation of K9 and K14 (McKitrick et al., 2004). Indeed, post-transcriptional modifications of Chromatin has an impact on gene regulation. Post-modifications
The translational properties of histones are covalent. They alter electrostatic charges. histones and they change their structural properties and/or modify the interactions of their extremities. There are several types of modifications: acetylation, the methylation, phosphorylation, sumoylation, ADP-ribosylation, ubiquitination, the biotinylation (figure 54)

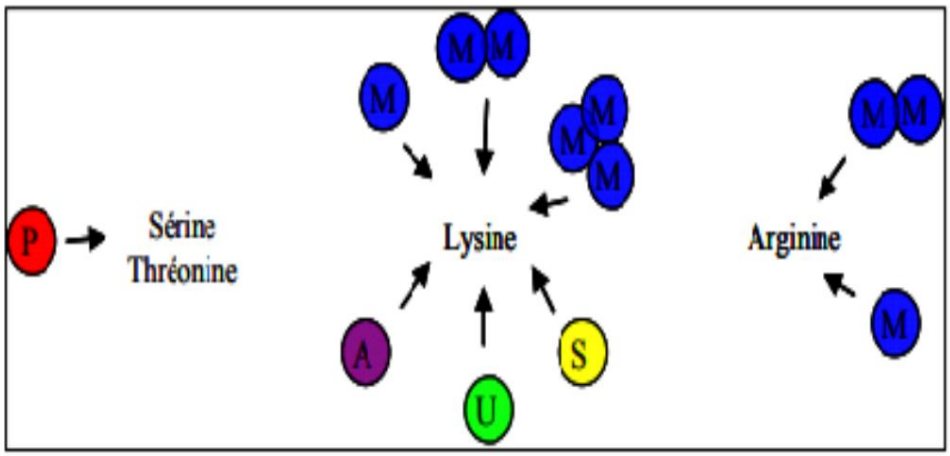


Figure 54: Different types of post-transcriptional histone modifications

The accumulation of this data reinforces the idea that H3.3 is important in transcription activation, however H3.3 is also the H3 variant predominant in differentiated cells that do not divide in vertebrates

The deposition of H3.3 in germ cells occurs independently of replication. H3.3 is deposited during decondensation of the nucleus of Sperm in Drosophila and mice. Knowledge of the mechanisms of Deposition at these specific H3.3 sites will allow the precise functions of H3.3 to these sites.

- **H3.3 Deposition**

HIRA was the first described chaperone responsible for the deposition of H3.3. Purification of proteins associated with H3.1 and H3.3 in cells of mammals reveal the presence of two different chaperones: CAF-1 for the assembly of H3.1 during replication and HIRA for H3.3. The pre-complex H3.3 deposition contains, in addition to HIRA, Cabin1 and Ubn1/2, Asf1 is a protein shared between H3.1 and H3.3 and which delivers the histones to the respective chaperones. HIRA is involved in the deposition of H3.3 during nuclear decondensation sperm in Drosophila, but is not required for H3.3 deposition in embryos or adult cells (Figure 55).

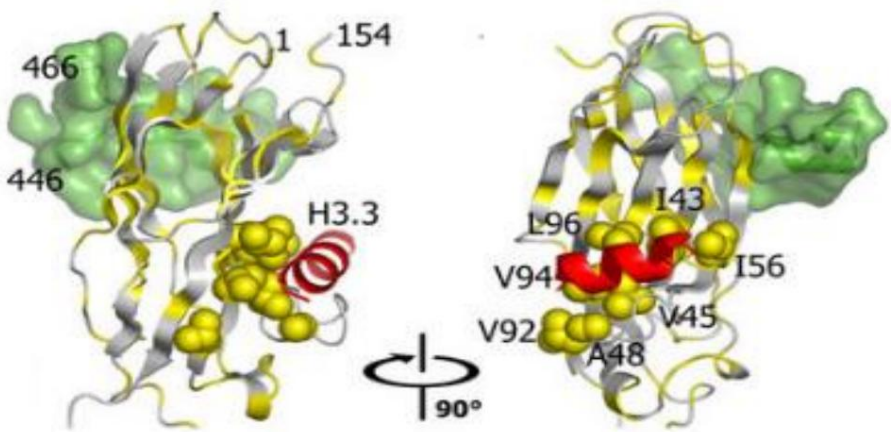


Figure 55: 3D linkage of HIRA regions and the C-terminal helix of histone H3.3 at the HIRA complex

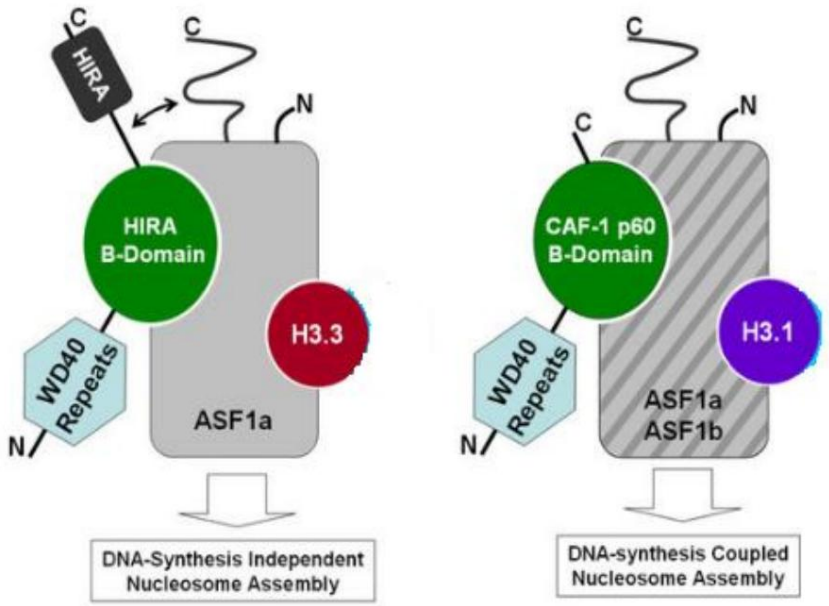


Figure 56: Model of the HIRA region and the C-terminal helix of histone H3.3 at the HIRA-B-domain/ASF1a complex

DAXX has been identified as another chaperone of H3.3 and this deposition is facilitated by ATRX. The DAXX-ATRX complex deposits H3.3 at the telomeres and of pericentric heterochromin as opposed to regions targeted by HIRA. DAXX-ATRX appears to regulate transcription at these sites, but the mechanism is not still known (Figure 56).

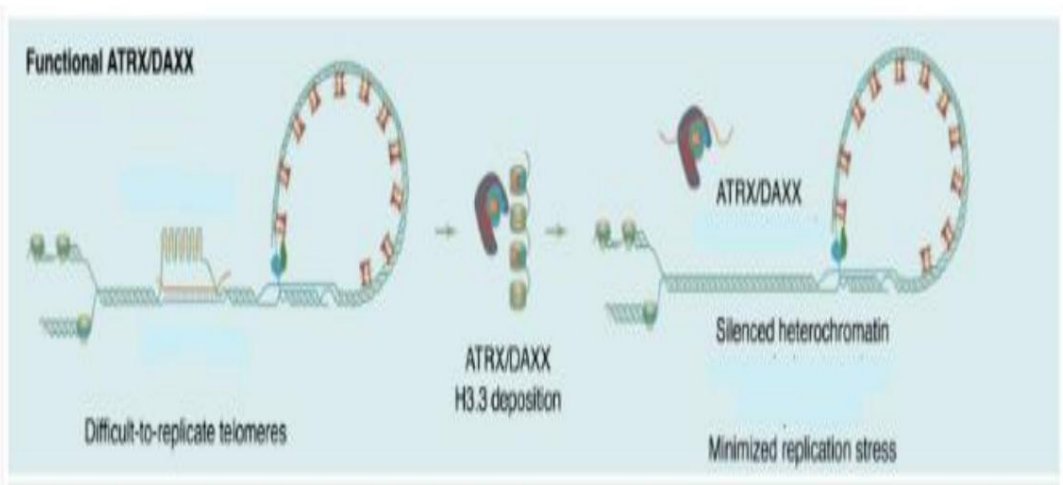


Figure 57: Function of ATRX/DAXX

The ATRX/DAXX complex suppresses ALT while maintaining the chromatin state and by reducing replication stress at the telomere level. In the absence of ATRX/DAXX, this results in replication stress (Figure 57). Table 2 summarizes the distribution of variants according to chaperone proteins and complex of remodeling and its implications in cancers and other genetic diseases.

TABLE 2: Histone variants, genomic distributions, functions and proteins Chaperones or remodeling complexes: Implications in cancers and other genetic diseases

Histone core	Distribution génomique	Fonction générale	Chaperons et remodeleurs	Fonctions des chaperons et des remodeleurs	Associée aux cancers		Associée aux maladies génétiques humaines
					Dérégulation dans les cancers	Rôle dans les cancers	
Famille H3							
H3.3	Globale (éléments régulateurs de gènes actifs, séquences répétées et hétéro-chromatiques	Activation de la transcription, dynamique de la chromatine	HIRA-UBN-CABIN1		Amplification et mutations faux sens (K27M, G34R/V/L et K36M) entraînant des substitutions d'acides aminés	Réponse transcriptionnelle à la signalisation pro-oncogène, dérégulation des états de la chromatine	ND
		Formation de l'hétérochro-matine, stabilisation des télomères	ATRX-DAXX (remodeleur et chaperon respective-ment)	Déposition sur l'hétérochro-matine	Mutation faux sens (avec potentiellemen t une perte de fonction) à la fois dans ATRX et DAXX	Suppresseur de tumeurs par le maintien de la structure des télomères	α -thalassé-mie liée à l'X avec retard mental (mutation ATRX)

2.2. Histone H2A variants.

This family of variants contains at least four subfamilies that differ in sequence and length terms. In mammals, the first subfamily encompasses H2A.1 and H2A.2 which are synthesized during the S phase of the cell cycle in parallel to replication.

The other two subfamilies, H2AX and H2AZ, are synthesized throughout the cell cycle, but at a very low level. The last subfamily comprising macroH2A.1 and macroH2A.2, which are approximately three times the size of H2A. Unlike the genes encoding the canonical histones, H2AX, H2AZ, and macroH2A are encoded by unique genes containing introns. The transcription of these genes

produces polyadenylated mRNAs with longer untranslated regions than canonical histones (figure 58).

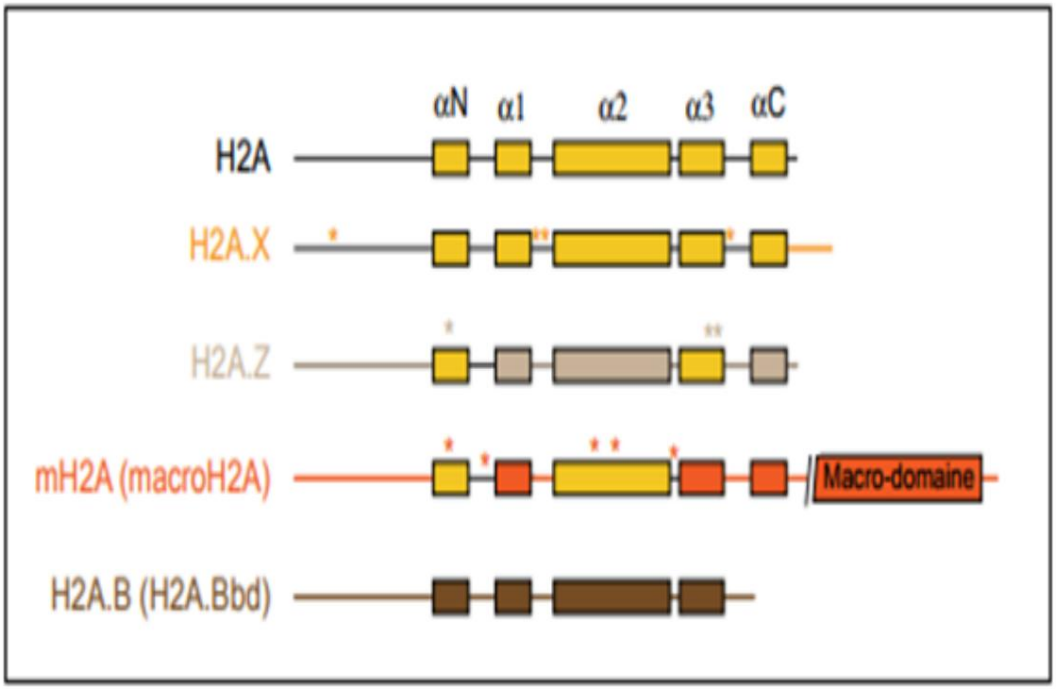


Figure 58: Variants of histone H2A in humans

2.2.a. H2AX.

H2AX is a variant of H2A that is present in a wide variety organisms such as mammals, plants, fungi and protozoa;
It has a very important function. This variant of H2A is characterized by the presence of a motif conserved SQ(E,D)(I,L,F,Y) in its C-terminal part.

- **H2AX in DNA repair.**

H2AX is the target of several covalent modifications such as acetylation, ADP Ribosylation and phosphorylation. The phosphorylation of serine 139 is important. for repair by NHEJ. The creation of double-strand breaks on DNA by ionizing radiation leads to rapid phosphorylation of H2AX (ÿ-H2AX) at at the level of the lesion sites. This results in a specific recruitment of proteins involved in DNA repair such as the MRN complex (Mrell/rad50/Nbs1), BRCA1 and p53BP1 (Schultz et al., 2000).

The use of a kinase inhibitor prevents the recruitment of these proteins, this which suggests that ÿ-H2AX is necessary for this process (Paull et al., 2000). Kinetics The phosphorylation of H2AX is similar to that of protein recruitment of the repair.

Phosphorylation of H2AX is carried out by kinases of the PIKK family: ATM and DNA-PK, which are used during breaks by ionizing radiation (Sif and al., 2004) and ATR in response to UV radiation (Ward and Chen, 2001).

Phosphorylation of H2AX does not affect nucleosome stability, but at a role in locating the factors involved in repair. Recently, it has been observed that another site (Y182) is phosphorylated on H2AX by WSRF (a member of the WICH complex). It is very important for adjusting chromatin structure and maintain the phosphorylation of S139 (Xiao et al., 2009).

Phosphorylation of H2AX induces recruitment of MDC1 which allows the arrival of a UBC13/RNF8 ubiquitin ligase complex at the break. The ubiquinol of H2AX and H2A is initiated by this complex and amplified by RNF168 (Doil et al., 2009; Stewart et al., 2009). But it is the ubiquitination of H2A that is crucial for the DNA repair.

- **H2AX in DNA recombination.**

Leaving aside DNA damage from external agents, H2AX is also required during recombination. Foci of ÿ-H2AX have been observed on Double-strand breaks occur during meiosis in germ cells. Similarly, The way ÿ-H2AX accumulates in the nuclei of B and T cells during development of the immune system. It targets the ÿ receptor locus, which is subject to recombination. V(D)J to form the variable regions of antibodies.

Deletion in mice of the gene encoding H2AX leads to an alteration of These recombinations suggest that H2AX is important during these processes (Petersen et al., 2001).

Finally, the accumulation of H2AX was also observed in the early stages. DNA fragmentation during apoptosis.

In addition to the independent replication processes described above, H2AX could play a role in controlling DNA replication. When the latter is inhibited by hydroxyurea, H2AX is phosphorylated by ATR and accumulates at the level stopped replication forks (Ward and Chen, 2001) (table3).

TABLE 3: Histone variants, genomic distributions, functions and proteins Chaperones or remodeling complexes: Implications in cancers and other genetic diseases

Histone core	Distribution génomique	Fonction générale	Chaperons et remodeleurs	Fonctions des chaperons et des remodeleurs	Associée aux cancers		Associée aux maladies génétiques humaines
					Dérégulation dans les cancers	Rôle dans les cancers	
Famille de H2A							
H2A.X	Globale (ensemble du génome)	Réponse aux dommages de l'ADN, remodelage de la chromatine	FACT (chaperon)	Déposition et échange d'histones	Mutation ou délétion	Suppresseur de tumeurs, par la prévention de l'instabilité du génome et des translocations d'oncogènes	Syndrome des cassures de Nijmegen de rupture de Nimègue, ataxie télangiec-tasie

2.2.b. MacroH2A.

The macroH2A variant differs from other H2A variants by its size Unusually, 372 amino acids, which is about three times larger than histone H2A canonical. The main characteristic of this histone variant is the domain globular macro in C-terminus. MacroH2A is the founding member of a large MacroH2A is a family of proteins containing macrodomains. It can be divided into two parts; the first third of the protein contains HFD and shares 64% homology with the H2A sequence.

The remaining two-thirds of the protein are characterized by the absence of HFD. Two subtypes of macroH2A have been identified to date; macroH2A1 and macroH2A2, each coded by a single gene containing eight exons. The gene coding for macroH2A1 produces two isoforms, named macroH2A1.1 and macroH2A1.2, resulting from a alternative splicing. MacroH2A1 and macroH2A2 share 84% homology in the histone domain, and 68% in the non-histone domain.

The macroH2A1.1 and 1.2 sequences only show about twenty acids Different amino acids in the non-histone domain. The non-histone region of the variants macroH2A is characterized by the presence of a leucine slide motif (leucine zipper), which is also found on transcription factors, suggesting a role

potential of these variants in the control of transcription (Pehrson et al., 1997). MacroH2A1.1 is found in quiescent cells, while macroH2A1.2 is generally expressed in proliferating cells, which implies a function different for these subtypes (Pehrson et al., 1997). Furthermore, macroH2A, which is present in both male and female cell nuclei, appears to be more concentrated in the region of the inactive X chromosome in female cells.

The association of macroH2A with the inactive X chromosome appears to occur following the extinction mechanism. A loss of macroH2A at the X chromosome inactive has been reported in several tumor cell lines, indicating a role potential in maintaining the inactive state of the X chromosome.

Recent studies have shown the involvement of macroH2A in the repression of transcription. The macro part of macroH2A represses transcription when it is at proximity to an active promoter (Ouararhni et al., 2006; Buschbeck et al., 2009). MacroH2A prevents the recruitment of transcription factors and remodeling by SWI/SNF when incorporated into nucleosomes (Angelov et al., 2003) (Table 4).

TABLE 4: Histone variants, genomic distributions, functions and proteins Chaperones or remodeling complexes: Implications in cancers and other genetic diseases

Histone core	Distribution génomique	Fonction générale	Chaperons et remodeleurs	Fonctions des chaperons et des remodeleurs	Associée aux cancers		Associée aux maladies génétiques humaines
					Dérégulation dans les cancers	Rôle dans les cancers	
Famille de H2A							
macroH2A1 (se présentant sous la forme 2 isoformes d'épissage : macroH2A1.1 et macroH2A1.2) et macroH2A2	Globale (hétérochro-matine facultative et constitutive)	Extinction de gènes, compaction de la chromatine d'ordre supérieur	FACT (chaperon) ATRX (remodeleur)	Éviction de macroH2A1 Régulation négative de la déposition de macroH2A1	Répression transcription-nelle, défauts d'épissage, amplification	Suppresseur de tumeurs par le maintien de l'hétérochromatin e et de l'identité cellulaire	ND

2.2.c. H2A.Bbd (or H2A.B)

Unlike macroH2A, which has a large C-terminal domain, H2A.Bbd (Barr body-deficient) contains a very short C-terminal domain and a domain truncated anchoring. It is 48% identical to H2A and lacks certain residues. conserved by other H2A variants. H2A.Bbd is absent from the inactive X chromosome fibroblasts and localizes in areas enriched in H4 acetylated on lysine 12, this which suggests a role in activating transcription.

In A431 cells, H2A.Bbd is exchanged faster than H2A (Gauier) et al., 2004), while in vitro NAP1 ensures the exchange of H2A.Bbd for H2A on Reconstituted nucleosomes (Okuwaki et al., 2005). Nucleosomes containing H2A.Bbd are relatively more relaxed and are not sensitive to remodeling by the complex SWI/SNF certainly due to the specific anchoring domain of H2A.Bbd (González-Romero et al., 2008).

H2A.Bbd has a smaller acidic region than H2A, which will inhibit the formation of the 30 nm fiber and reduces its ability to inhibit transcription (Zhou et al., 2007). It has It has been demonstrated very recently that H2A.Bbd is associated with active genes (Tolstorukov et al., 2012).

This new data reinforces the concept that H2A.Bbd is associated with a chromatin permissive for transcription.

Despite the accumulation of data demonstrating that H2A.Bbd forms a Chromatin promotes transcription, the exact function of H2A.Bbd is not known. Phylogenetic analyses of canonical histones and variants revealed that H2A.Bbd is a histone that evolved very rapidly in mammals, which This is not the case for other histones (Malik and Henikof, 2003).

The incorporation of H2A.Bbd into the nucleosome leads to a more unstable in the same way as a hyperacetylated nucleosome. H2A.Bbd is not affected by hyperacetylation of other nucleosome histones. It would appear that H2A.Bbd constitutes another mechanism for destabilizing the nucleosome that does not depend on acetylation (Eirín-López et al., 2008) (table 5).

TABLE 5: H2AB (or H2A.Bbd) histone variants, genomic distributions, functions and chaperone proteins or remodeling complexes: Implications in cancers and other genetic diseases

Histone core	Distribution génomique	Fonction générale	Chaperons et remodeleurs	Fonctions des chaperons et des remodeleurs	Associée aux cancers		Associée aux maladies génétiques humaines
					Dérégulation dans les cancers	Rôle dans les cancers	
Famille de H2A							
H2A.B	Testicule, cerveau (euchromatine)	Déstabilisation des nucléosomes, transcription et épissage d'ARNm	NAP1 (chaperon)	Assemblage et dissociation de H2A.B	ND	ND	ND

2.2.d. H2AZ.

The histone variant H2AZ has been found in organisms ranging from protists like Tetrahymena to higher eukaryotes, with approximately 90% homology to through these organisms. However, despite a similar structure, the sequence The H2AZ peptide exhibits only 60% homology with histone H2A (Figure 59). The H2AZ variant is called HV1 in Tetrahymena, HTZ1 or HTA3 in the yeast, H2AvD in Drosophila, H2AZ/F in humans.

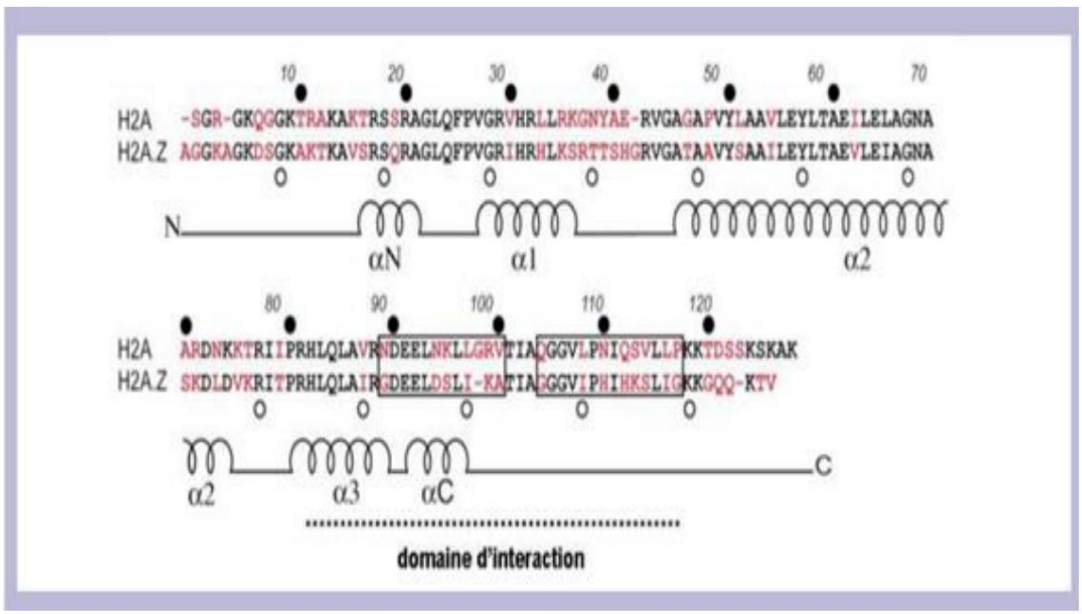


Figure 59: Alignment of the amino acid sequences of H2A and H2AZ. The secondary structure is shown below the sequences

- **Structural characteristics of H2AZ.**

The crystallographic structure of a nucleosome containing H2AZ has been solved and shows similarities to that of a nucleosome containing H2A, although the two proteins possess many differences in their peptide sequences (Suto and al., 2000). There are small differences between these structures, particularly at the level of the interaction domain at the H3-H4 tetramer.

In nucleosomes containing H2AZ, the overall shape of the domain The interaction of the tetramer is conserved, as are most of the hydrogen bonds. intermolecular, but the substitution of glutamine 104 by glycine causes the loss of three hydrogen bonds, destabilizing the interaction between H2AZ and H3. At the Unlike H2A, H2AZ has two histidine residues, capable of binding to an ion metallic in the tetramer interaction domain located on the surface of the nucleosome. The presence of this ion is likely to create an interaction with partners specific to H2AZ.

Other differences exist in the acidic zone formed by the acids amines of the H2A/H2AZ-H2B dimer binding domain located on the surface of This acidic region is probably involved in protein- proteins and with the basic tail of the neighboring histone H4 (Luger and Richmond, 1998). In the case of H2AZ, this area appears more extensive, resulting in a larger acidic surface area. important in octamers containing H2AZ (Figure 16) (Suto et al., 2000) (figure 60).

H2AZ appears to play a vital role in all organisms. The inactivation of The gene encoding H2AZ leads to a decreased growth rate in yeast and to the death in Tetrahymena, Drosophila, mouse and Xenopus (Liu et al., 1996; Clarkson and Saint, 1999; Jackson and Gorovsky, 2000; Faast et al., 2001; Ridgway et al., 2004).

The replacement of the tetramer anchoring domain of H2AZ by that of H2A This hinders the development of the fruit fly, highlighting the structural differences. major differences between H2AZ and H2A.

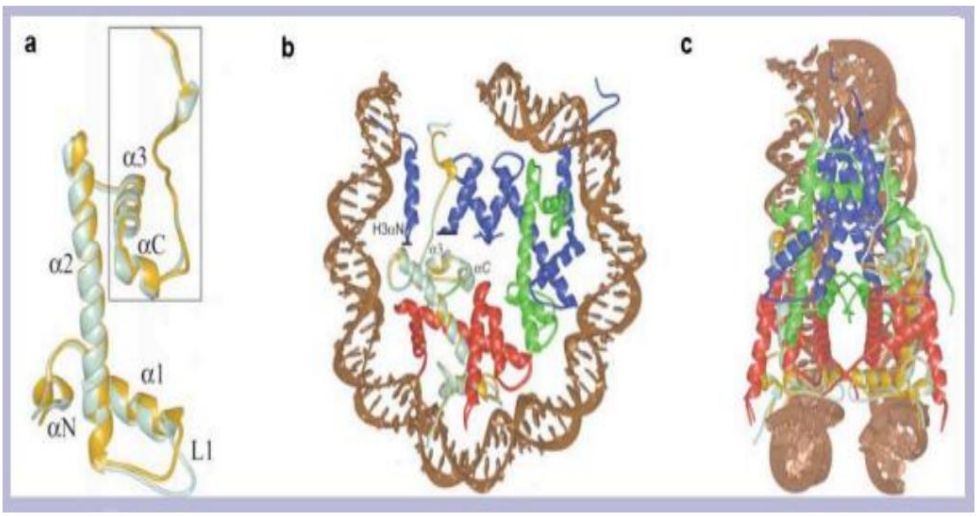


Figure 60: Comparison of nucleosomes containing H2A or H2AZ.

Drosophila embryos lacking H2AZ can be saved by expressing chimeric constructs, including the tetramer anchoring domain of H2AZ (Clarkson et al., 1999). All these data demonstrate that this domain is crucial in development. Studies in Tetrahymena have shown the importance of the acetylation of the N-terminal tail of H2AZ, which includes six lysines instead of three normally present on H2A.

Replacing lysine residues with arginine, which prevents Acetylation, or by glutamines, mimicking acetylated lysines, is lethal or leads to a rate reduced growth, indicating the need for H2AZ acetylation to ensure its cellular functions (Ren and Gorovsky, 2001; 2003).

Due to the proximity of the lysines to the N-terminal tail of H2AZ, it has been hypothesized that this domain might behave like a loaded patch. Reduction of the positive charge of the histone tail by acetylation could be translates to a reduced association of the H2AZ tail with DNA, which makes it more accessible for DNA-binding proteins.

Analysis by sedimentation and ultracentrifugation on chromatin Reconstituted H2AZ suggests that it has a destabilizing effect on histone- interactions intranucleosomal histone (Abbot, 2001).

The L1 loop domain, which modulates interactions with H2A in the The nucleosome itself is modified in the case of H2AZ in such a way that it can promote interaction with another H2AZ molecule. The pairing of an H2A- dimer H2B and an H2AZ-H2B dimer in the same nucleosome could lead to steric problems, and no stabilization between the two molecules can be formed (Suto et al., 2000).

It has recently been shown that nucleosomes containing H2AZ are exclusively homotypic (Luk et al., 2010; Weber et al., 2010), which demonstrates Once again, a nucleosome containing H2A and H2AZ is very unstable.

- **H2AZ and the activation of transcription.**

Initial observations in Tetrahymena showed that H2AZ was present in the transcriptionally active macronuclei and absent from the micronuclei. At At the start of conjugation, H2AZ is detected in the micronuclei just before activation. transcriptional, which links H2AZ to active chromatin (Stargell et al., 1993).

Immunofluorescence experiments on polytene chromosomes and Immunoprecipitation of chromatin in Drosophila has shown that H2AZ is distributed along the genome, but not randomly. H2AZ is localized on euchromatin and heterochromatin at the chromocenters of chromosomes polytenes. ChIP analysis shows that H2AZ is associated with transcribed genes and non-transcribed non-coding euchromatin and heterochromatin (Leach, 2000).

A similar study in yeast demonstrated that H2AZ is localized preferentially on the promoters of PHO5 and GAL1 (Sanisteban et al., 2000b). H2AZ was found specifically at these sites under repressive conditions, but significantly decreases or disappears after transcription activation, suggesting a role of H2AZ in establishing transcription. Furthermore, the deletion of the gene encoding for H2AZ compromises the activation of transcription.

Other experiments, in strains lacking the H2AZ gene, have shown a defective recruitment of RNA polymerase II and the TATA-binding protein box at the level of the promoter GAL1, advocating for a role for H2AZ in the recruitment of the

basic transcription machinery and its implementation. (Adam et al., 2001).

According to these observations, H2AZ could be involved, with ATP-dependent complexes chromatin remodeling and histone modification complexes, in the establishment of structures favorable to transcriptional activation. H2AZ could also serve to recruit the basal transcription complex.

- **H2AZ and transcription repression.**

Surprisingly, H2AZ was also involved in transcriptional repression (Svotelis et al., 2009). Overexpression of H2AZ is capable of restoring "silencing". in cells where Sir1 is reduced. ChIP studies have shown the presence of H2AZ on the HMR locus and telomeres; deletion of the gene encoding H2AZ results in a loss of repression of these loci (Dhillon and Kamakaka, 2000).

These results are consistent with the presence of H2AZ in non-regions transcribed and in heterochromatic non-coding regions in Drosophila (Leach, 2000) and in pericentric heterochromatin during the onset of development in mammals (Rangasamy et al., 2003).

It is therefore likely that H2AZ is involved in the activation and repression gene. Nucleosomes containing H2AZ are thought to be more stable than nucleosomes containing only canonical histones, which could mitigate the processes of transcription by preventing the movement of a histone dimer (Park et al., 2004). However, the exact function of H2AZ in activation and repression transcriptional remains to be determined (table6).

TABLE 6: Histone variants, genomic distributions, functions and proteins Chaperones or remodeling complexes: Implications in cancers and other genetic diseases

Histone core	Distribution génomique	Fonction générale	Chaperons et remodeleurs	Fonctions des chaperons et des remodeleurs	Associée aux cancers		Associée aux maladies génétiques humaines
					Dérégulation dans les cancers	Rôle dans les cancers	
Famille de H2A							
H2A.Z.1	Globale (régions régulatrices, hétérochro-matine)	Liaison aux complexes régulateurs, dynamique de la chromatine	SRCAP (remodeleur), YL1 (chaperon)	Déposition de H2A.Z	Amplifications et mutations faux sens de fonctions inconnues	Expression d'oncogènes, croissance cellulaire, transition épithélio-mésenchyma-teuse (TEM)	Syndrome de Floating-Harbour (FHS) /mutation de SRCAP
			INO80 (remodeleur), ANP32E (chaperon)	Éviction de H2A.Z, qui promeut les recombinaisons homologues			
			ANP32E (chaperon)	Éviction de H2A.Z, au niveau des lésions de l'ADN			
H2A.Z.2 (se présentant sous la forme de 2 isoformes d'épissage : H2A.Z.2.1 et H2A.Z.2.2)	Globale	Liaison aux complexes régulateurs, dynamique de la chromatine	YL1 (chaperon)	Déposition de H2A.Z	Amplifications et mutations faux sens de fonctions inconnues	Transcription de pro-oncogènes, prolifération cellulaire	Syndrome de Floating-Harbour (FHS) /mutation de SRCAP
			ANP32E (chaperon)	Eviction de H2A.Z			

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