University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: 1

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Subject: Introduction

Doctor: Mamoun Ahram

Price:

Date:

Before getting started with our material the doctor mentioned some information we should to know about:

1. The main reference for Doctor Mamoun's lectures is what he says in the lecture itself. He's going to mainly depend on Campbell and Farrell's Biochemistry for his material, specifying us the pages we need to know. The Doctor might also mention things that are not in Campbell, but he'll give us their references.

2. This course is divided into three parts given by three doctors:
   A. Molecular Biology by Dr.Mamoun
   B. Cell Biology by Dr.Diala
   C. Genetics b Dr. Mohammad Al-Khateeb

❖ What's the difference between molecular biology and genetics?

- Molecular Biology is biochemistry, but it deals with nucleic acids (DNA & RNA), and small mutations (a base is missing or added, and abnormal nucleic acid sequence).
- Genetics deals with transferring of phenotypes (appearance) among individuals, and mutation in chromosomes (extra chromosome so we are talking about a big chunk).

❖ **What are Nucleic Acids?**

Nucleic acids are polymers, and you know that a polymer is a molecule that is made of a repeated monomers. And in case of nucleic acids their monomers are called "nucleotides".

❖ These nucleic acids have 3 structures:

- **Primary structure:**
  Is basically the sequence of the nucleotides (nucleotide#1 followed by nucleotide#2... etc.)

- **Secondary structure:**
  Is the three-dimensional conformation of the backbone (as we say that the DNA is a double stranded helical structure).

- **Tertiary structure**:
  It deals with how the DNA, is coiled. For example it's a linear DNA, but it's coiled. So tertiary structure is about packaging.

❖ So as we said, the nucleotides are the monomers, the building units, of the nucleic acids.
And the nucleotides are composed of 3 molecular components (as seen in the figure below):

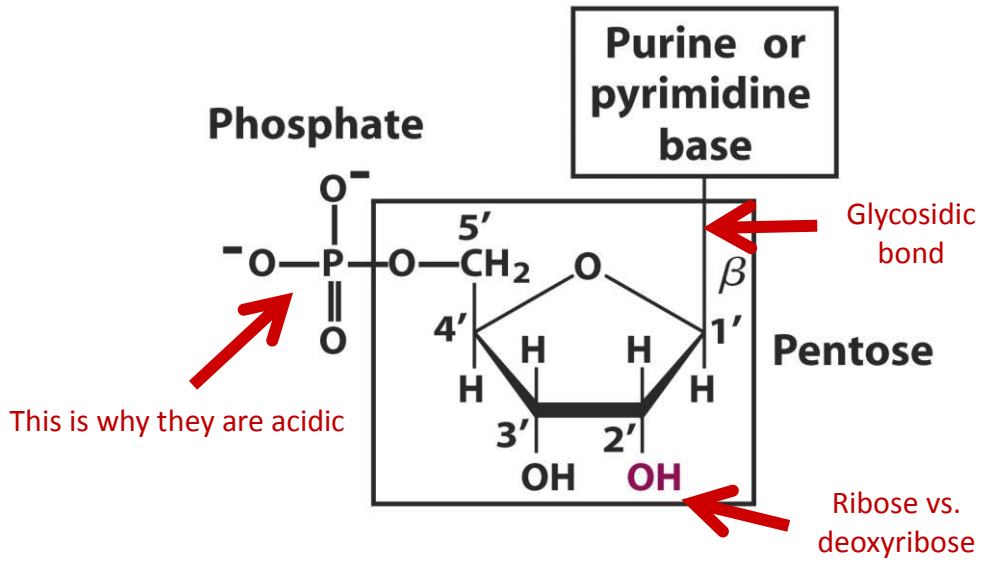
1. **Sugar:** a pentose (5 carbon sugar), and it could be:
   a) Ribose in RNA
   b) Deoxyribose in DNA

   And to distinguish between Ribose and Deoxyribose we should look at carbon #2 in the pentose sugar; if there is a hydroxyl group then it's a Ribose, and if there's only a hydrogen then it's a deoxyribose.

2. **Nitrogenous Base:** it's linked to carbon #1, in the pentose sugar, and forms a bond called a *glycosidic bond.* And we have two types of bases that we'll talk about in a bit.
   Remember: a glycosidic bond is the bond between some group and the anomeric carbon of a ring sugar.

The anomeric carbon in a ring sugar: is the group that was originally the functional group (the aldehyde or the ketone). And it is the carbon where the bond can be above the ring or it can rotate and become below the ring.

3. **Phosphate**: It can be 1, 2, or 3 phosphate groups, linked to the pentose sugar.
   Since this phosphate is negatively charged, so the nucleotide is negatively charged, and that means the whole nucleic acid is negatively charged as well. And it can form electrostatic interactions with positive charged ions, like Magnesium and Sodium ions.



❖ **So let's talk about the nitrogenous bases:**

There are two classes of bases that make up the nucleic acids, we call them:

a) Purines {Adenine & Guanine} → 2 rings
b) Pyrimidines {Cytosine & Thymine & Uracil} → 1 ring

Note: the Doctor will not ask us to distinguish between the different bases in the same class.

❖ **Nucleotides vs. Nucleosides:**

- The difference between them is that the *nucleoside* has a sugar and nitrogenous base *only*.
  I. Pentose sugar + Base= nucleoside
  II. Pentose sugar + Base + Phosphate = nucleotide

- And this difference has an advantage in term of naming, for example; Deoxyadenylate: from its name we can tell that it's a ***nucleotide*** which contains Adenine as a base, a sugar (Deoxyribose), and a phosphate, but we can't know the number of the phosphate groups!

- So we use another naming system, for example; Deoxyadenosine monophosphate: the word "Deoxyadenosine" refers to a ***nucleoside*** that has an Adenine base, and the deoxyribose sugar. And the "monophosphate" word means that this nucleoside has *one phosphate,* making it a ***nucleotide.*** So by this naming system we can know the exact number of the phosphate groups.

- Note: the covalent bond between two subsequent nucleotides, in the nucleic acid, is a **phosphodiester bond** whether it's a DNA or RNA.

❖ The nucleic acid structure is composed of:

a) **Backbone**:
   1. Sugar 2. Phosphate
b) **Branches:**
   The nitrogenous bases that are perpendicular on the backbone.

❖ **Nucleic acid polymer:**

A property of nucleic acids is that they have 2 ends which we can distinguish between them.

- These 2 ends are:
  1. 5 prime end (5') has a phosphate that is linked to carbon #5 in the sugar.
  2. 3 prime end (3') it's the free carbon #3 in sugar.

- Whenever we want to lengthen the nucleic acid then all we have to do is to form a phosphodiester bond between an incoming (another) nucleotide, with the 3’ end.

- So we can extend and elongate the nucleic acid from the 3' end only, and we don’t touch the 5’ end.

- If we say that the sequence of this nucleic acid is: 5'(AGTC)3' then here we’re indicating the 5’end and the 3’ end.

- And if we say: (AGTC) without indicating anything, then it should immediately come to your mind that “A” is the nucleotide at the 5’ end, and “C” is at the 3’ end of the polymer. So saying (AGTC) without indicating is just like saying 5’(AGTC)3’.

- We can also say: 3’(AGTC)5’. Where “A” is at the 3’ end, and “C” is at the 5’end.

- A letter “d” can be added to indicate a deoxyribonucleotide residue. For example, “dG” is substituted for “G”.

- The deoxy analogue of a ribooligonucleotide would be **d(GACAT).** Where “G” is nucleotide #1 in 5' end, and “T” in 3' end.

❖ **DNA structure:**

- Long time ago, Watson and Crick got the Noble prize for clarifying and declaring what the DNA structure is. And they came up with a model called “Watson and Crick Model”.

- This model tells us that the DNA structure is:

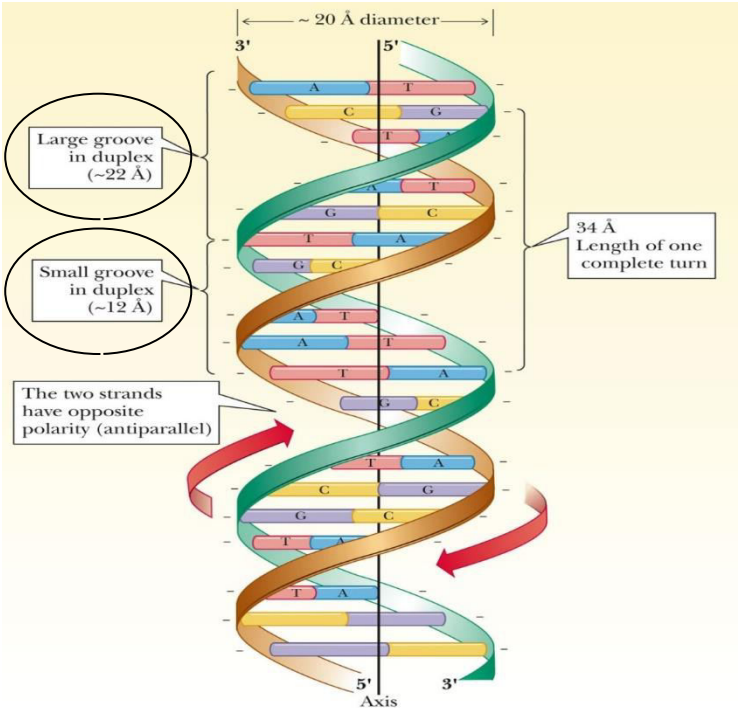
1. **A Double Helix & Complementary:**
   - “Double” stands for the 2 strands that are linked to each other by hydrogen bonds between nitrogenous bases (A-T) (G-C), so here comes the specific base pairing and complementary property.
   - G-C base pairing (3H bonds) is stronger than A-T (2H bonds) due to the additional hydrogen bond.
   - “Helix” stands for the helical structure, but it's not a perfect helix.

2. **Backbone:** (Sugar and Phosphate), **Side Chains**: Branches (Nitrogenous bases).

3. **Antiparallel** which means that the first strand is opposite to other stand (if one 5' – 3' the other will be 3' – 5')

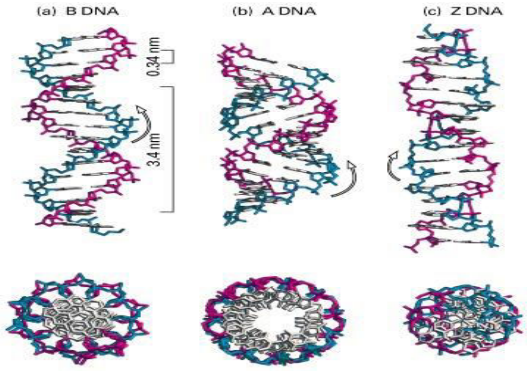
4. **Stable** but at the same time **flexible**, like an electrical wire. You can't break it, but you can bend it.

5. **Groovings:** remember we said the DNA is not a perfect helix, so looking at the pic to the right, you can see we have a 'major' groove and 'minor' groove. Proteins love 'major' grooves because it has enough space to interact with the DNA.
Note: In the same side of the DNA we have major then minor, major then minor etc. and it's opposite to the other side.



❖ **DNA forms:**
Later on, scientists have found that DNA have structures other than the Watson and Crick's Model, so their model got named as the "B-DNA form". And the other structures were named as: the "A-DNA form" and the "Z-DNA form".

*<u>NOTE</u>
Right and Left handed is a way for scientists looking at a molecule if the strands is going to the right or left.



- **B-DNA form:**
  a) The principal form of DNA that exists in our cells.
  b) Right-Handed.
  c) Base pairs are perpendicular.

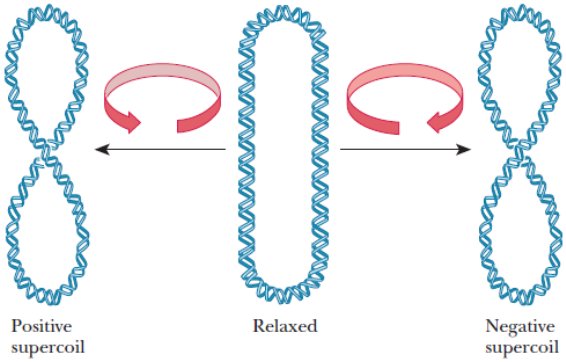
- **A-DNA form:**
  a) Has 11 base pairs per full turn.
  b) The A-DNA is compressed, so it's more base pairs, per turn, than the B-DNA.
  c) Wider than B-DNA (Looking from the top).
  d) Base pairs lie at an angle.
  e) Right-handed.

  * Doesn't exist in our cells.

- **Z-DNA form:**
  a) It occurs <u>naturally</u> in our cells when there are alternating purines and pyrimidines in the same DNA strand.
  b) Left-handed, so it goes upward toward the left.
  c) Longer, it has less bases pair per full turn than B-DNA.
  d) Narrower and slimmer than the B-DNA (looking from the top).

❖ **DNA coiling:** remember we said that the tertiary structure of nucleic acids is how the DNA is coiled.

- The bacterial DNA is circular so it looks like a rubber band in the relaxed form.
- And it can coil on itself, and this coiled DNA can be relaxed again with the action of enzymes; there are 2 enzymes that exist in our cells and even in bacterial cells.
- These two enzymes are called class I and class II <u>topoisomerases</u>. And topoisomerases are isomerase enzymes which change the structure of the molecule without changing the molecular component.



- The main function of topoisomerase is relaxing the DNA by making cuts.

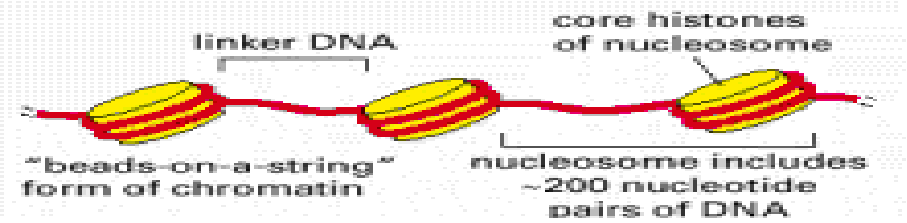
The 2 types of topoisomerase:
A] **Class I** topoisomerases cut the phosphodiester backbone of one strand of DNA.
B] **Class II** topoisomerases cut both strands of DNA.

- When we have our DNA tangled then the easier way to separate them is by cutting them by topoisomerase and reconnecting.
- Bacterial DNA doesn't have proteins associated with it, but our DNA (Eukaryotic DNA) have proteins.
- Eukaryotic DNA must be packaged.
  In one single cell the length of DNA is about 5 meters long, and this 5m must be packaged into the nucleus. Cells can do that by associating and binding the DNA with proteins known as **histones**, and <u>we call this combination of **histones** and DNA as Chromatin</u>.
- Let's talk about histones; scientists have found out that there are 5 types of histones. Let's discuss the first 4.

- They are known as: H2A, H2B, H3, and H4. And we have two of each, so we have a total of 8. They form a complex, we call it an octamer.

- This octamer forms something like a disc, and you have the DNA wrapped around it.

- Then you have what is known as a histone-free DNA that we call a linker DNA, because it links one octamer to the next octamer of histones and DNA wrapped around it. Just as seen in the pic below.

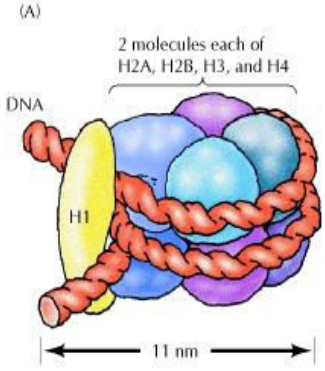


- We call the: Octamer + the DNA wrapped around it + the linker DNA = Nucleosome

- But how this DNA that is wrapped around the octamer is stabilized? It's stabilized by the 5th histone, known as H1.

- H1 locks the DNA to the histone octamer.

- Now we call the: histone octamer + the DNA wrapped around it + the H1 = Chromatosome. (So without the linker DNA).



❖ **Light absorbance of nucleic acids**

- Nucleic acids can absorbed light at a wavelength of 260 nm, which is in the UV range (we can't see it).

- But because of the presence of the ring structures of the bases in the DNA they can absorb light at 260 nm.

- As a result, we can measure the concentration of the DNA by knowing how much light it can absorb.

- For example:
    a) **dsDNA**: If I have a concentration of 50 ug/ml then it can absorb 1 unit of light. So I have a DNA sample, I hit it with light, and I measure how much light it absorbs, then if I find out that it absorbs 1 unit of light, I can say that the concentration of this DNA sample is 50 ug/ml.

    b) **ssDNA:** its concentration 30 ug/ml if it absorbs 1 unit of light.
    Note: ssDNA absorbs more light than dsDNA due to the free bases exposed.

    c) **ssRNA:** a 40 ug/ml of RNA can absorb 1 unit of light, so it's intermediate between dsDNA and ssDNA.

Q: What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1?
DNA concentration = 0.1 x 10 x 50 µg/ml = 50 µg /ml
(if you think the answer should be 5, notice that we have diluted the sample at 1:10, meaning that original sample is 10 times more concentrated that this new one).

*SPECIAL THANKS TO:
- محمد رسول القُضاه -