Genetics & molecular biology

◉ Sheet ○ Slide

Number:	1
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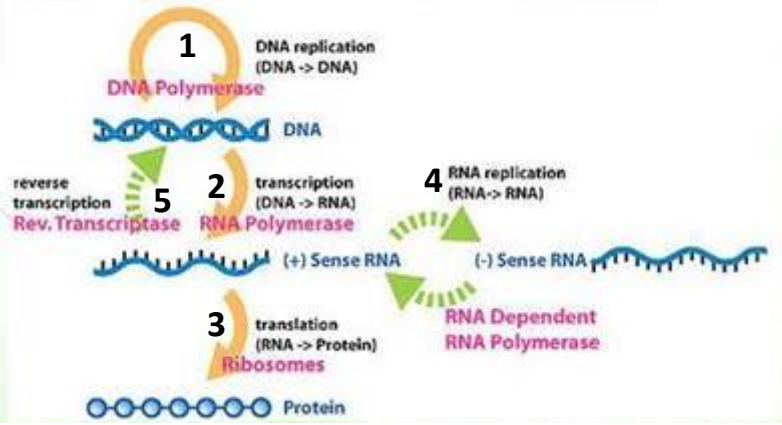
**Difference between Molecular Biology and Genetics:

Molecular Biology: is a fancy term of biochemistry. It is the science that deals with DNA, RNA and their composition of nucleotides.

Genetics: is the science that deals with genotypes (genetic components) and phenotypes (the appearance of an individual), it also deals with chromosomes.

**Central dogma of molecular biology:

Dogma means the main idea that a study is based on. The dogma of molecular biology is exemplified in the figure below:

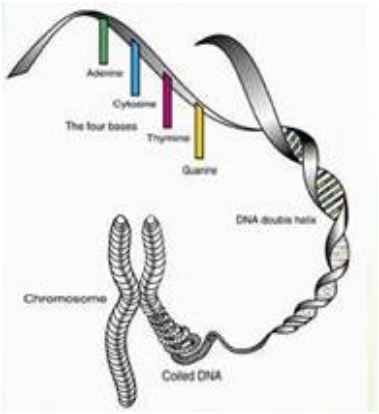


1)From DNA we can make another copy of DNA in a process called **DNA replication**, and the enzymes that are responsible for this process are called **DNA polymerases**.
2)DNA can be used as a template to generate RNA molecules in a process called **transcription**, and the enzymes that are responsible for the process are called **RNA polymerases.**
3)From RNA (especially mRNA) we can make proteins in a process known as **translation** with the need of **ribosome**.
4)RNA can be used as a template to produce other copies of RNA in a process called **RNA replication**, and the enzymes that are responsible for that process are called **RNA polymerases**.
5)Also, RNA can be used as a template to produce DNA molecules in a process known as **reverse transcription** with the need of enzymes known as **reverse transcriptases**.

We don't have reverse transcriptase in our bodies, so we aren't able to produce DNA from RNA molecules. This is found in viruses (ex. HIV viruses).

Nucleic Acids: Polymers made up of repeated monomers called nucleotides.

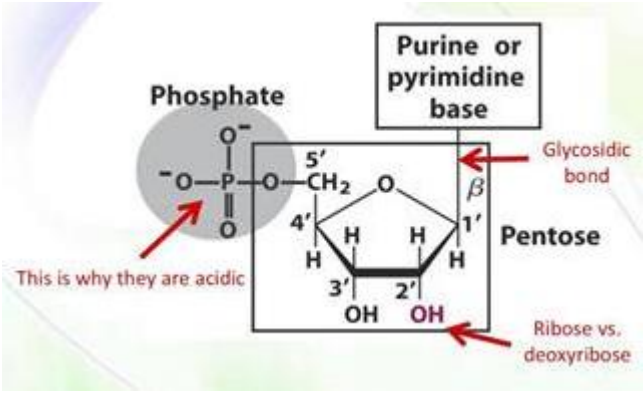
This figure shows a DNA molecules that is composed of a very long sequence of nucleotides. The DNA strand can be coiled to produce what is called “a chromosome” which makes up our genome. And a genome is the total component of DNA in a cell.



Nucleotides are made of 3 components:

pay attention to the numbering of carbons

The prime (‘) is used to differentiate between the carbons of the sugar molecule and the carbons of the nitrogenous base.



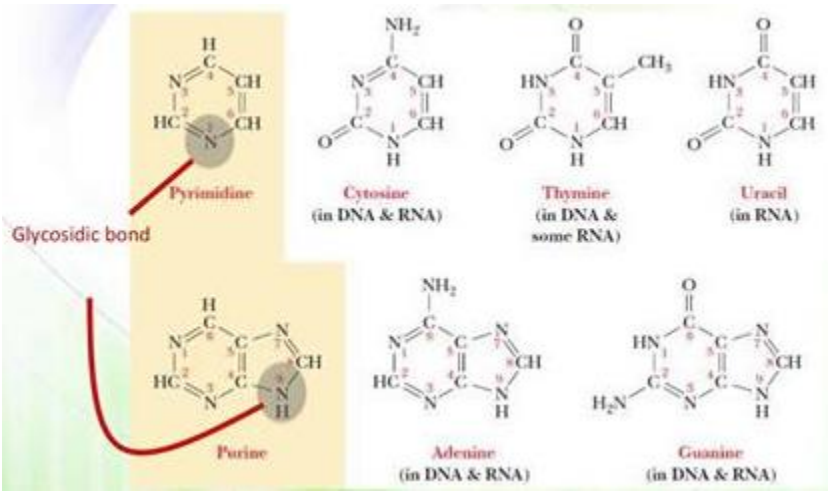
1) Sugar: it is a 5-carbon sugar (pentose)
It is either a ribose (in RNA)
(**C#2** is linked to an –OH group)
Or a deoxyribose (in DNA) (C#2 is not linked to an –OH group but is linked to -H).

2) Nitrogenous base: linked to the sugar molecule at **C#1** via a glycosidic bond (which is a bond between the anomeric carbon and another chemical compound) and since it is rotated upwards then we refer to it as β)

3) Phosphate group: linked to the sugar at **C#5**. This group is negatively charged.

Even though a DNA molecule contains billions of nucleotides that are negatively charged producing repulsions along the DNA strand, they are highly stable due to the association with positively charged ions (Na+, Mg++) or peptides with positively charged side chains or even Histones which are positive proteins; masking the negative charges.

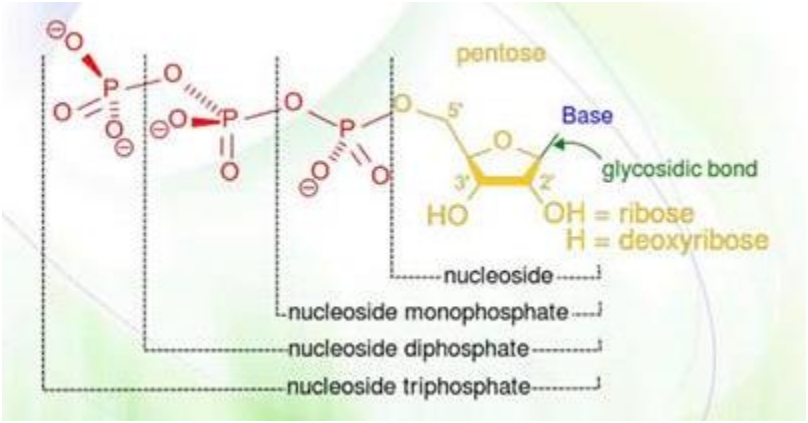
** Nitrogenous bases:



Structures are not for memorizing

We have 2 classes of these bases:

-Purines: double-ringed structures (Adenine, Guanine), they link via N#9.
-Pyrimidines: single-ringed structures. (Cytosine, Thymine, Uracil), they link via N#1.
-Thymine is only present in DNA and not RNA, while Uracil is found in RNA and DNA.



** So, as we said before, a nucleotide is composed of a sugar molecule bounded to a phosphate group at C#5 and a Base at C#1.

Nucleotide – Phosphate group = Nucleoside

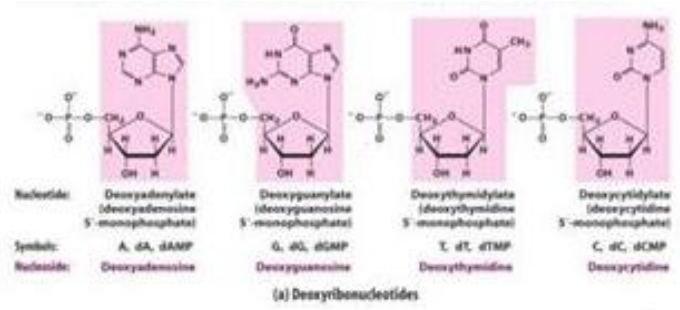
A nucleotide may either contain one phosphate group or two or three.

So, to indicate how many phosphate groups are there we use the word “nucleoside”

For example: a nucleotide with one phosphate group is called nucleoside monophosphate, a nucleotide with two phosphate groups is called nucleoside diphosphate and so on.

**Naming:

Naming of Deoxyribonucleosides/Deoxyribonucleotides:



Let's take the far-left example:

Adenine + ribose = Adenosine (Nucleoside)

Adenine + Deoxyribose = Deoxyadenosine (Nucleoside)

Adenine + ribose + phosphate = adenosine monophosphate (Nucleotide)

Adenine + Deoxyribose + Phosphate = Deoxyadenylate (Nucleotide)
(also can be named as deoxyadenosine 5'- monophosphate, in order to specify the number of phosphate groups and avoid misunderstanding as we mentioned before)

Adenine + Deoxyribose + 2 Phosphates = Deoxyadenosine 5'- diphosphate

Adenine + Deoxyribose + 3 Phosphates = Deoxyadenosine 5'- triphosphate

Naming of Ribonucleosides/Ribonucleotides:

Let's take the far-right example this time:

Cytosine + Ribose = Cytidine (Nucleoside)

Cytosine + Ribose + Phosphate = Cytidylate (Nucleotide)
(also can be named as Cytidine 5'-monophosphate, in order to specify the number of phosphate groups and avoid misunderstanding as we mentioned before)

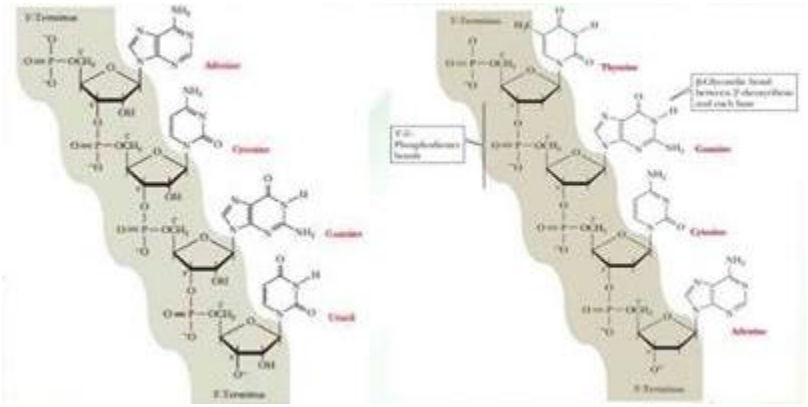
Cytosine + Ribose + 2 Phosphates = Cytidine 5'-diphosphate

Cytosine + Ribose + 3 Phosphates = Cytidine 5'-triphosphate

**Nucleic acid polymers:

The linkage between the nucleotides is **phospho-di-ester** bond. This link is between C#3 of the sugar with the phosphate group on C#5 of the below sugar. And this is how the elongation happens (elongation is always on the 3' end and we read the DNA sequence from 5' to 3')

In nucleic acid polymers we have **directionality** (same as the proteins) that is, we have 2 ends: the 5' end which is untouchable and the 3' end that we add a new nucleotide to it.



The molecule on the left: RNA (presence of Uracil and –OH group on C#2)

The molecule on the right: DNA (presence of Thymine and absence of –OH group on C#2)

Note: We can use the letter "d" to indicate a deoxyribonucleotide residue.

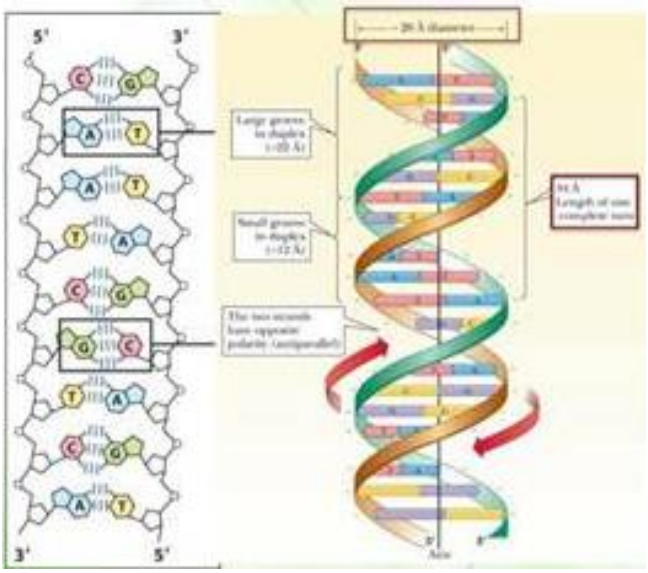
**DNA structure:

The structure was resolved in 1953 by Watson and Crick. It shows the following features:

1) Double stranded helical molecule.
2) The two strands are linked to each other by hydrogen bonds between the bases.

3) Pairing between the bases is specific A=T (**2** H-bonds), G≡C (**3** H-bonds)

*A strand with more (G≡C) pairing is more stable than a strand with (A=T).



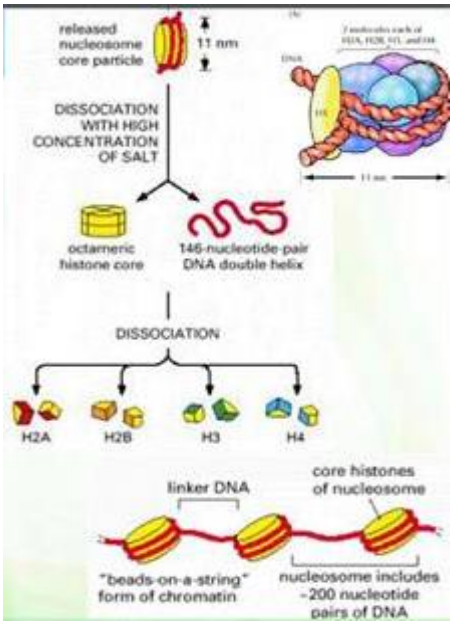
4) Backbone: sugar-phosphate
Sidechain: bases (oriented inwards unlike the sidechains\R-groups of amino acids in proteins).
5)**Antiparallel**: the two strands of the DNA molecule run in opposite directions complementary to each other, first strand 5' to 3' and the other one 3' to 5'.

*If it is not indicated in the exam question the starting and end points of the DNA molecule then eventually it is in the 5'→3' direction.

6)It is not a perfect helix, it contains major and minor **groovings**. The major grooves are the sites of interaction with the Histones.
7)The helical structure is highly **stable** and although it is stable it is also **flexible** (exactly like an electrical cable).

** DNA molecule must be packed tightly so that it fits in the nucleus. The packing is done with the help of proteins called Histones. And the resultant structure from DNA and protein is called a **Chromatin.**

Carefully study the figure shown to the right:



Histones (mainly composed of Lysine) are positively charged octamers, this means that a histone molecule is composed of 8 subunits, they are:

2 (H2A), 2 (H2B), 2 (H3), 2 (H4)

Nucleosome: DNA double helix wrapped twice around the histone molecule,

And in order to seal or fix the DNA that is wrapped **H1** is used.

This whole structure is called **Chromatosome.**

(Nucleosome + H1= Chromatosome)

The area between two histone molecules with DNA wrapped around it is called **linker DNA.**

** A feature of DNA is light absorbance. Even though it absorbs light, DNA is colourless.

The light that is absorbed is within the UV range and equals 260 nm.

We can take advantage of that feature by measuring the concentration of DNA or RNA in a given solution. (The more DNA found in a solution the more light that is absorbed). We use a certain device for that called photospectrometer.

Examples:

1)If we have a dsDNA solution fixed at 260nm and the resultant absorbance of light equals 1 then the concentration of the dsDNA molecules = 5o µg\ml.
2)If we have a ssDNA solution fixed at 260nm with a concentration of 30 µg\ml then the light absorbed= 1 unit.

dsDNA: A260 of 1.0 = 50 ug/ml

ssDNA: A260 of 1.0 = 30 ug/ml

ssRNA: A260 of 1.0 =40 ug/ml

What is the reason behind this difference? This is because the bases in the ssDNA are exposed and not stacked allowing the molecule to absorb more light, unlike the dsDNA with stacked bases oriented inwards, ***because the ring structure in the bases is responsible for light absorbance.***

Question: - what is the concentration of dsDNA sample diluted at 1:10 and the A260 is 0.1? and what is the con. Of the diluted sample?

Here, note that dilution took place so for sure there will be difference between the two values.

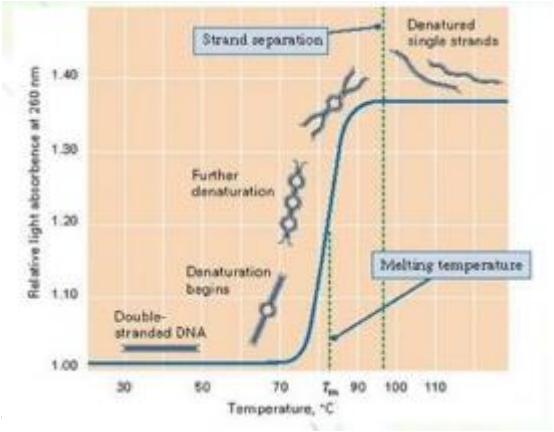
For the diluted sample: 0.1*50= 5 µg\ml.

For the DNA original concentration: 0.1*50*10= 50 µg\ml.

** Observation of denaturation:

We can break down the DNA molecule by separating the two strands from one another by a process called denaturation. It is done by increasing the temperature.

There is a term that is used here called "**melting temperature**" or simply (Tm): it refers to the temperature which 50% of DNA molecules are denatured and 50% are still double stranded.
It indicates stability.



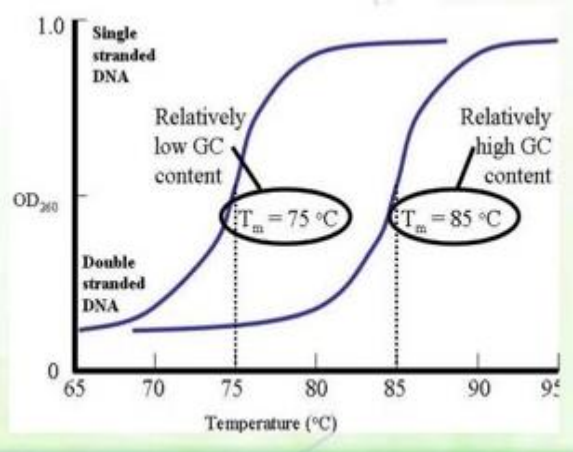
There are plenty of factors influencing Tm:

1)The length of the strand (directly proportional).
2)G-C content Hydrogen bonding and base stackin proportional)
3)pH: the higher the pH (alkaline environment) the less the Tm (inversely proportional).
4)Salts and ions: the ions mask the –ve charge increasing the Tm (directly proportional).

5)Destabilizing agents (inversely proportional).

The figure here shows a comparison between 2 strands of DNA having different composition of G≡C base pairing and its relation with Tm.

The line on the right shows the higher the G≡C in the molecule the higher the Tm. And the line on the left shows otherwise.



**RNA:

It is a long unbranched chain of nucleotides joined by phosphor-di-ester bonds between the 3'-OH of one pentose and the 5'-phosphate of the next.

In general, it is single-stranded with no precise structure, but sometimes it can fold on itself forming H-bonds within the same molecule.

As mentioned before, the pentose sugar is a ribose and the pyrimidine bases are uracil and cytosine.

This table shows different types of RNA molecules. ***not for memorizing***

Non-coding RNA		Length (nt)	Species	Function
Ribosomal RNA (rRNA)		120–4700	All	Translation
Transfer RNA (tRNA)		70–100	All	Translation
Small nuclear RNA (snRNA)		70–350	Eukaryote	Splicing, mRNA processing
Small nucleolar RNA (snoRNA)		70–300	Eukaryote, archaea	RNA modification, rRNA processing
miRNA	Small ncRNA	21–25	Eukaryote	Translational regulation
siRNA	Small ncRNA	21–25	Eukaryote	Protection against viral infection
piRNA	Small ncRNA	24–30	Eukaryote	Genome stabilization
Long ncRNA		several hundreds–several hundred thousands	Eukaryote	Transcription, splicing, transport regulation