Lecture :..... 15

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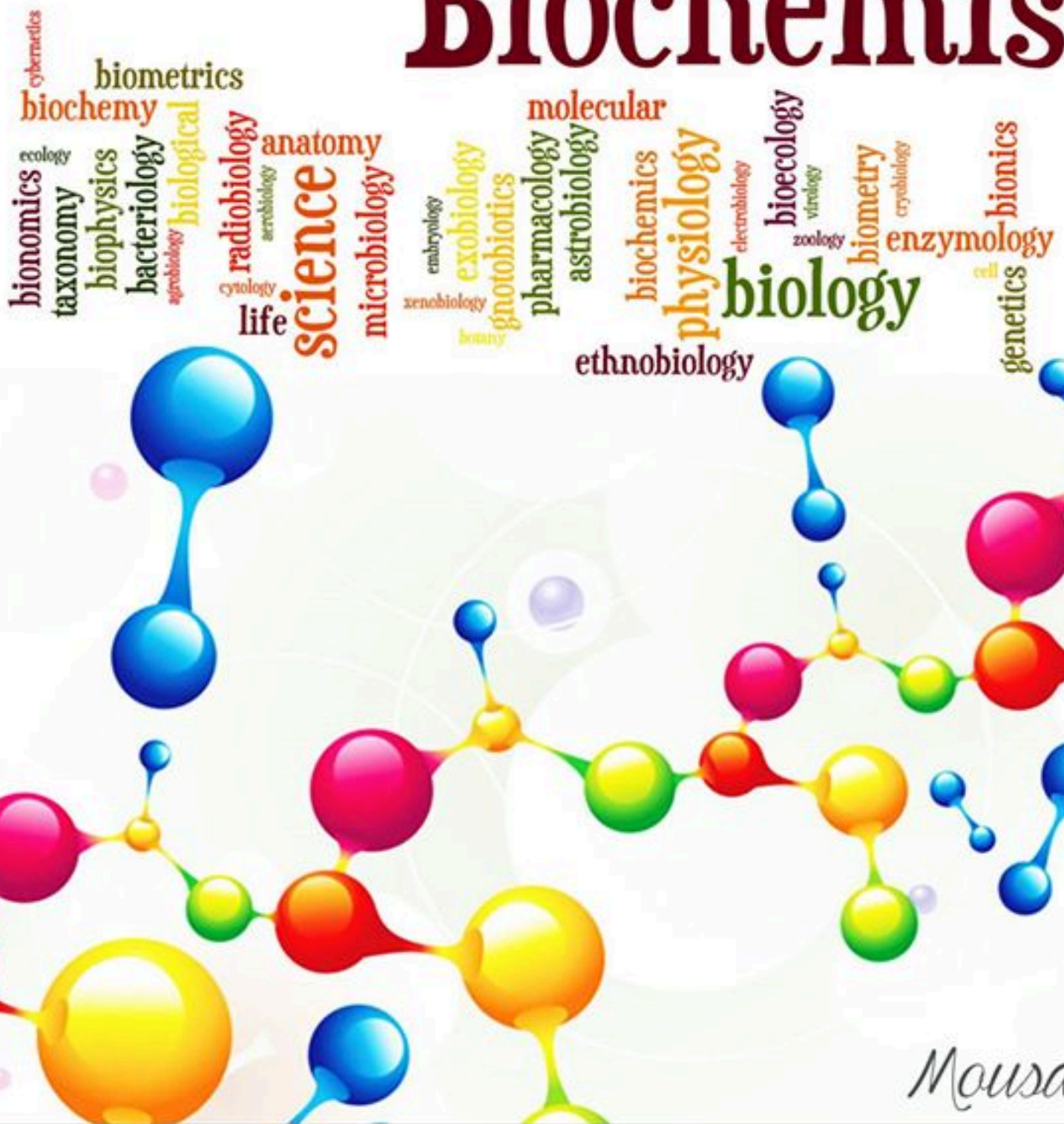
☐ Slide ■ Sheet

Medical Committee
The University of Jordan

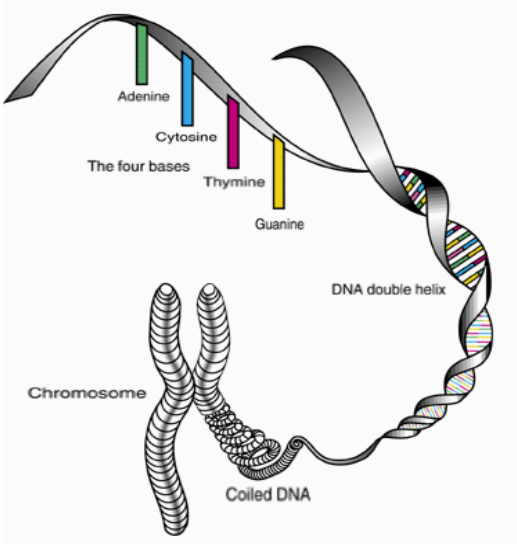
Biochemistry



Nucleotides and nucleic acid

This is the last lecture for this week ☺ I wish you a blessed Eid and remarkable marks in the mid exam ;) this lecture is talking about nucleic acids and nucleotides. Dr.Ma'mon said it's an easy lecture -Insha'allah- because we've taken all these things before... so here we go ☺

Nucleic acids have interesting structure; they have PRIMARY, SECONDARY and TERTIARY structures. Basically, the *primary structure* is the: order of nucleotides (just like amino acids and peptides), *the secondary structure* is: the 3D structure of the backbone, **which is the helical structure.** Then we have the tertiary structure which is: the supercoiling (in order to package DNA inside a nucleus it has to be coiled), THIS IS CALLED SUPERCOILING, which means that it has to wrap around itself and proteins in order to fit into the nucleotides!



Let's look at the nucleotides themselves

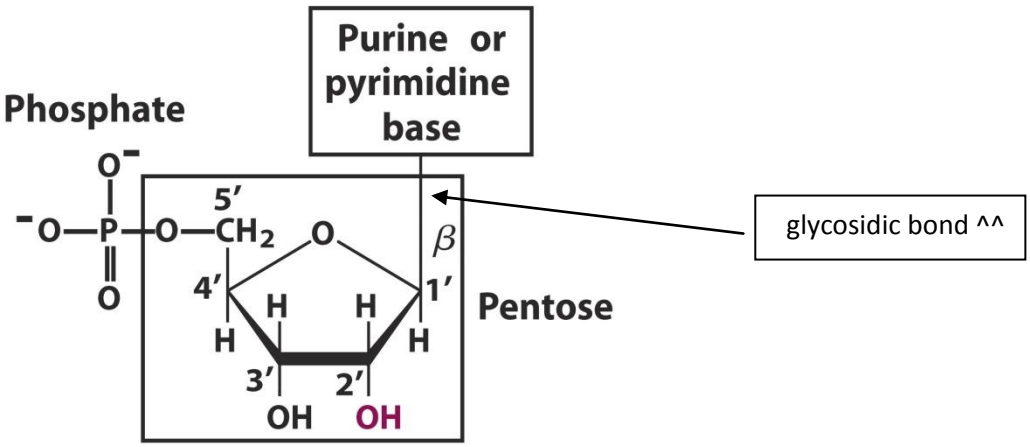
NUCLEOTIDES are made from three groups, and these groups are:

1-A ribose sugar: it can be either ribose or deoxyribose! because we have two types of nucleic acids: DNA and RNA (DNA has deoxyribos, RNA has ribose), (DNA the "D" for deoxy and RNA the "R" for ribose). The difference between ribose and deoxyribose is at carbon #2 where it's either a hydroxyl group (ribose) or just a hydrogen in case of a deoxyribose :D

2. The purines or pyrmadines bases are the second components of the nucleic acids, these are the nitrogenous bases. The link (connection) between the ribose and nitrogenous bases is a glycosidic bond :)

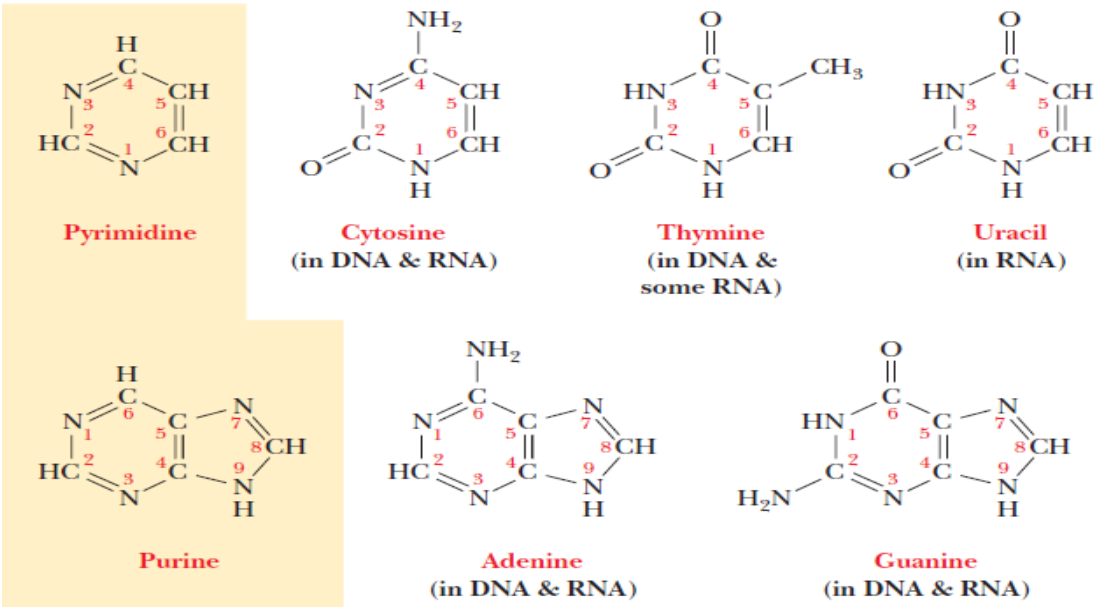
WHY WE CALL IT GLYCOSIDIC BOND? Because the link occurs between the anomeric carbon of the ribose and the base,:) more specifically it's not a n-glycosidic bond because the link occurs with the nitrogen of the base, so we have two types of bases: purines and pyrmadins.

3-A phosphate group: we can have 1, 2 or 3 phosphate groups! So we can have monophosphate, diphosphate or triphosphate. The presence of the phosphate group dictates that the overall molecule of DNA or RNA is acidic, it's negatively charged.



The presence of charged ions, like Mg or Na because they are positively charged they will interact with the phosphate group. In addition, you will have interactions between peptides (that have positive charges) with DNA or RNA. An example of this interaction between proteins and DNA is the interaction between DNA "nucleic acids" with histones in eukaryotic cells. Histones are positively charged and that's why it's a strong interaction.

These are the structure of bases, the glycosidic bonds take place between nitrogen #1 in pyrimidines and nitrogen #9 of purines, this is how you can differentiate between the purines and pyrimidines. Purine is a short word for big structure (a molecule with 2 rings) and pyramidine is a big word for a small structure (single ring molecule).



In terms on pyrimidines we have three different structures (or three different pyrimidins), we have Cytosine, Thymine and Uracil.

Cytosine is present in both DNA and RNA molecules, while thymine is present in only DNA and not in RNA normally. Uracil is present in RNA only.

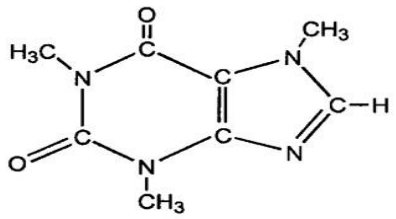
Purines (two ring molecule) have two types: adenine and guanine and both of them are present in DNA and RNA molecules (again: notice that the glycosidic bond takes place within the nitrogen number 9 of purines and nitrogen number one of pyrimidins).

Now, there are different types of nucleotides that exist in our system (just like amino acids, there are different amino acids that do not integrate in the structure of some proteins, same thing with nucleotides, some of them are not used to make nucleic acids). Examples include Xanthine and hypoxanthine which are components of

metabolic pathways of purines metabolism, and proteins for uric acid.

We have a modified form of adenine which is N6-methyl adenine and we have methylated cytosine. We also have pseudouracil, it has a ribose attached to carbon number 5 instead of the nitrogen number 1 of Uracil so this is known an psuedouracil (pseudo means fake).

And then we have Caffeine, it's a purine. Notice its structure below.



Caffeine

Note: 5-methyl-cytosine (epigenetic), what do we mean by epigenetic?

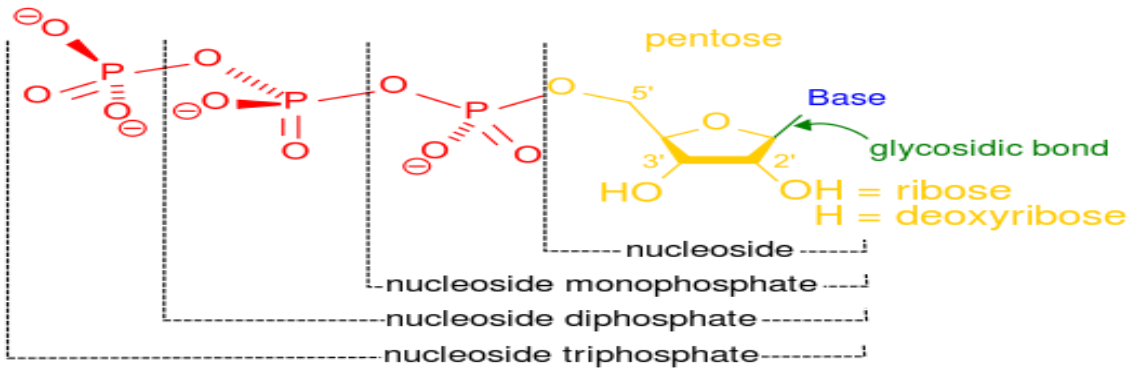
Sometimes we have differences among us..For example at certain positions in DNA I can have adenine but you can have Guanini, and this creates mutations and makes us individually different from each other, this is what we call طفرات جينية genetic differences or genetic mutations. So there is something called epigenetic (above genetics), what does epigenetic mean? That for example I may have a C and you have a C at the same position in DNA, the only difference is that my C is methylated and yours is not. If the DNA is replicated, it will be read as C in my DNA and

C in your DNA, but the difference is in the activity of the gene or the DNA itself, so my gene that contains the methylated C is not active, whereas your gene that contains C which is not methylated (regular cytosine) is active. That what makes us different from each other :) is that clear Guys? :P

And that's why we have identical twins. Identical twins have the same exact copy of DNA. The only difference between them depends on food or environment or activity, they can have epigenetic differences between each other.

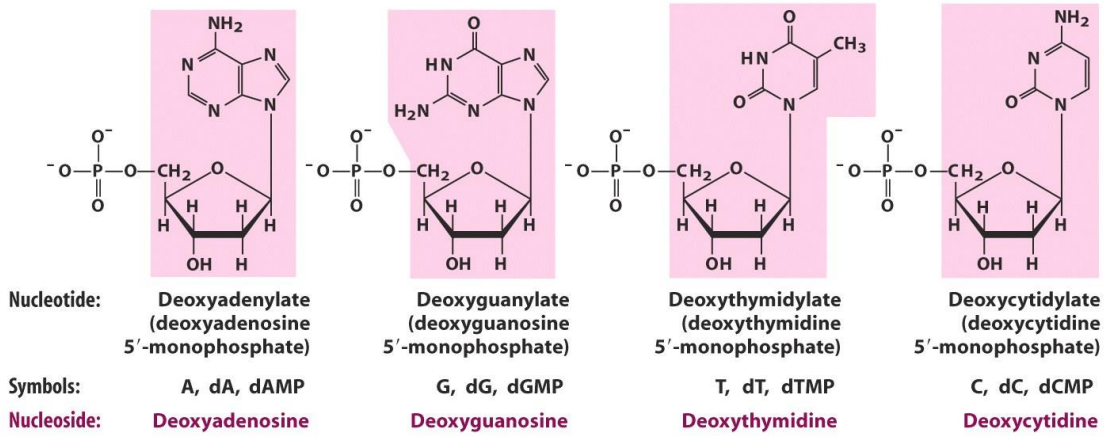
NUCLEOTIDES vs. NUCLEOSIDES

The difference between them is that a nucleoside is that molecule that contains the ribose sugar and nitrogenous base, but if we add a phosphate we'll convert the nucleoside to a nucleotide. Notice the figure in the next page.

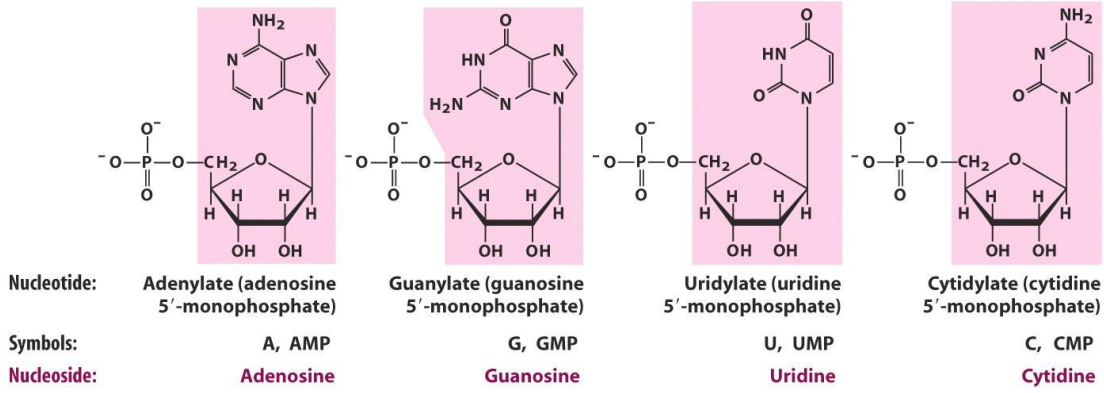


Notice the nucleoside, it contains a ribose and a nitrogenous base, it can be either ribose or deoxyribose, if we add a phosphate, it'll be called a nucleotide, and this nucleotide can have either 1, 2 or 3 phosphate groups, so

the nucleoside can be monophosphate, diphosphate or triphosphate nucleoside, so when you say nucleotide, you have to be specific, is it a mono, di, or tri phosphate? How many phosphate groups does it have? The picture above shows different types of nucleotides, you need to know the names of them. If you know what the base was then you can know the name of the nucleoside or nucleotide. Notice the figure below.



(a) Deoxyribonucleotides



(b) Ribonucleotides

In the figure above they are all monophosphate nucleosides, you need to know their names! By knowing the base name, for example: here is an adenine and its

connected to ribose and there is one phosphate (monophosphate).

Notice the figure above, the 1st nucleotide is deoxyadenylate, which means that it's a nucleotide, but if you say deoxyadenosine it means it's a nucleoside. If you say deoxyadenosine monophosphate it means that it's a nucleotide.

Deoxyguanylate: a nucleotide, deoxyguanosine: nucleoside, and deoxyguanosine monophosphate: nucleotide.

If you were asked to give the name for a given structure in the exam, you can follow these steps:

1. Look at the structure, if there was a ribose sugar, a base and a phosphate group, immediately you say this is a nucleotide. If there was no phosphate you say this is a nucleoside.

2. Look at the ribose sugar and determine is it a ribose or deoxyribose? What carbon we look at? Carbon number 2, if it has a hydroxyl group then the sugar is ribose, if it has a hydrogen then it's deoxyribose.

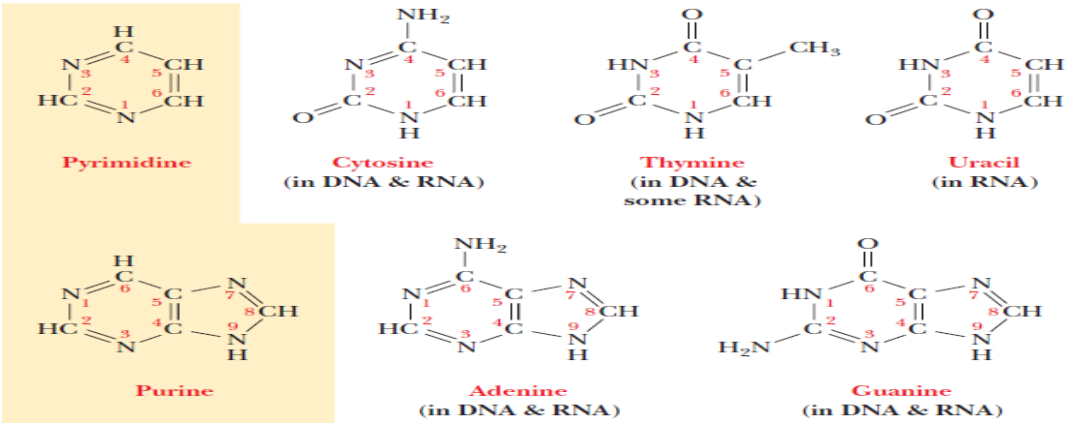
3. Look at the base itself, is it a purine or pyrimidine?

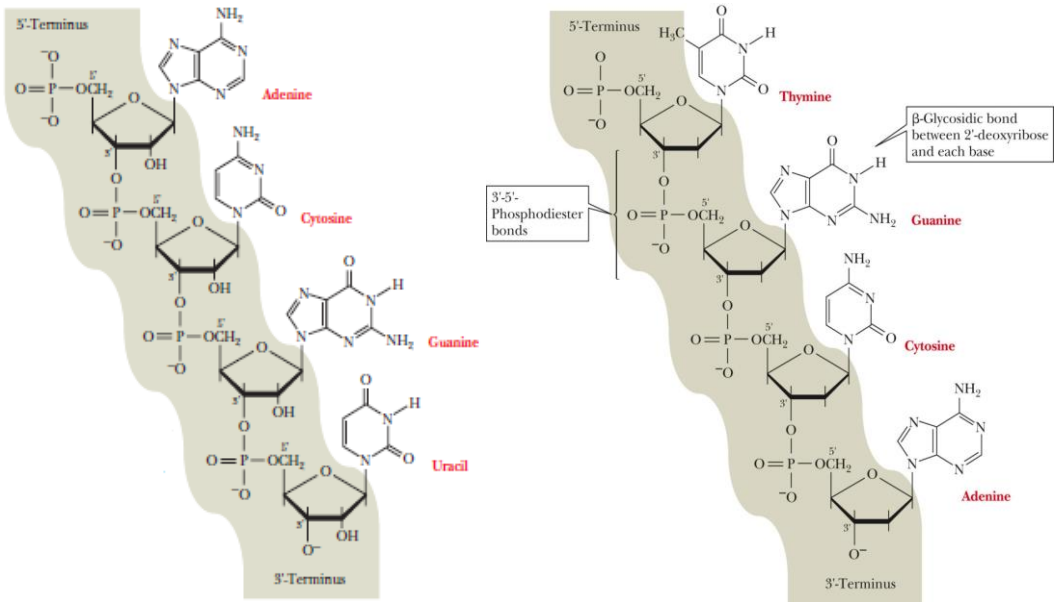
Basically, you solve the question by eliminating what is wrong.

At the end you decide if it's a purine or pyrimidine. If it's a purine you have to decide if it's a guanine or an adenine,

and if it's a pyrimidine you need to differentiate between ctyosine, thymine and Uracil (:

Example: the difference between thymine and cytosine is: Thymine has a ketone group (carbon #4) and the cytosine has an amine group (carbon #4) and so on.





The different nucleotides can be connected to form/polymerize DNA or RNA. that's why DNA and RNA are considered polymers, because they have these nucleotides connected to each other (monomers). The type

of bonds that connect two nucleotides together is known as **phosphodiester** bond.

So we have two different bonds: glycosidic bond and phosphodiester bond

There is also backbone for nucleic acids and the backbone basically is phosphate sugar, phosphate sugar, phosphate sugar and so on. The branches are the bases which extend sideways.

Notice something: there is directionality in synthesizing nucleic acids, just like proteins! Synthesis occurs in proteins at the C terminus, but in nucleic acid whenever we want to extend it we add nucleotides to one end of DNA, and this end is 3˚ end.

So there are two ends in DNA, the 3˚ end and the 5˚ end. The 5˚ end indicates that the carbon is carbon #5 of the ribose which is connected to the phosphate, and the 3˚ end is the one that has the hydroxyl group/Hydrogen, and whenever we want to extend the nucleic acid we add nucleotides to the carbon that is free (the 3˚ carbon), we do not extend it from the other side, so synthesis of DNA is always 5˚ to 3˚.

To differentiate between DNA and RNA -the two nucleic acids- we need to know if the ribose sugar is regular ribose or deoxyribose. So if you say dG, the small letter d represents the deoxy sugar (a hydrogen is there, not a hydroxyl group), so you say it's a deoxyguanosine which

means that this molecule is actually DNA, G indicates that the nucleotide has guanine.

We can also say d(AGCT) and also dAdGdCdT.

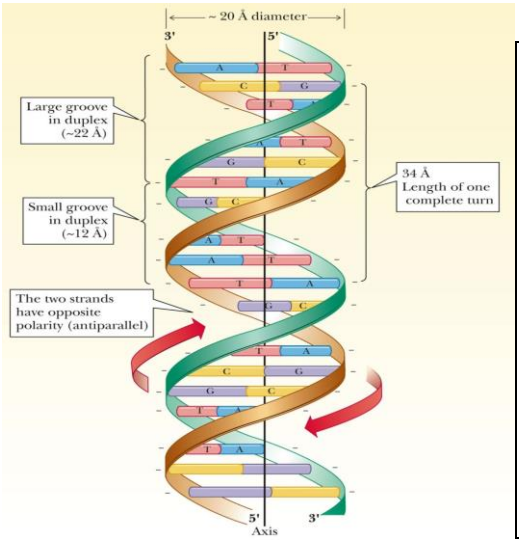
DNA structure

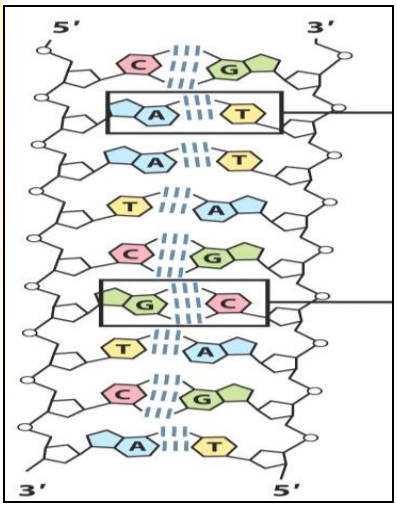
In 1953 a revolution happened in science, we have two scientists: Watson and Crick, they only knew that DNA was the genetic material, and it was the molecule responsible for heredity, and they knew that: 1) the number of A = the number of T and 2) the number of G = the number of C and 3) the number of purines = the number of pyrimidines, but it's not necessarily true that A=G or C=T.

They also determined that the structure of DNA is complementary and it's a double helix, which means that it's composed of two strands, intertwined around each other, but it's not perfect intertwining! And they noticed it's complementary (The two strands are complementary, which means that whenever you have A at one nucleotide, there is T on the other nucleotide. :') They also noticed that they are anti-parallel: one strand starts with the 5° end and ends with the 3° end and the other strand starts with 3° end and ends with 5° end.

Notice the backbone (phosphate, sugar, phosphate, sugar… and so on) and they are straight but extending

sideways you have the bases themselves





DNA STRUCTURE

DNA is a stable molecule, the two strands are strongly connected to each other, mainly because of the formation of hydrogen bonds between the two different bases -the complementary bases- so the number of hydrogen bonds between C and G = 3 and the number of hydrogen bonds between A and T = 2.

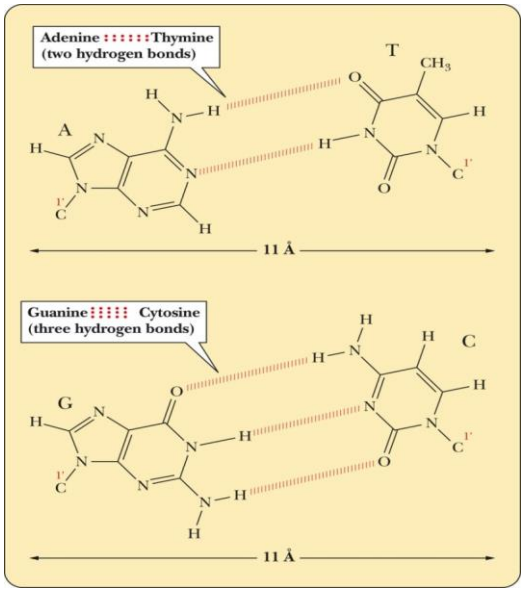
Even though it's stable, it's a flexible molecule which means that you can really bind it .it can be bent easily, like the electricity wire you can't break it or cut it, but you can bind it very easily. :)

Also, there are things known as grooves (grooving) and these grooves are like inserts into the DNA structure. You have major groove: it's relatively larger and the minor groove which is smaller (the minor and major groove are relative to each other). :)

These grooves are important when it comes to DNA interaction with proteins, so when you have a protein that wants to interact with the DNA it mainly interacts at the major groove not at the minor groove, why is that? Because the protein fits easily in the major groove.

In DNA there is a turn and the distance between two nucleotides in a full turn is 34 angstrom **Ă** or 3.4 nanometers, also the diameter between two nucleotides is about 20 angstrom **Ă**

Base pairing between the different nucleotides, for A and T is two because of the formation of the hydrogen bonding, and 3 between the C and G.



CHEMICAL FORCS IN NUCLEIC ACIDS.

Now we will talk about the chemical forces that stabilize or destabilize the DNA structure. First we will talk about hydrogen bonding -between the different bases (3 VS 2). But we also have the ionic interactions between the

phosphates, and this interaction is actually a repulsive one, this is one factor that destabilizes the DNA structure and that's why when it comes to ionic interactions with the phosphates like Mg or Na which are positively charged they interact with the phosphates, these ions can stabilize the DNA structure itself because they reduce the repulsive forces between the phosphates.

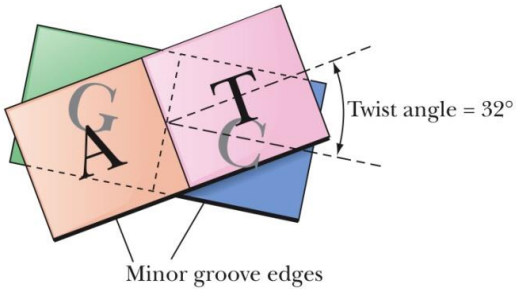
What are certainly important when it comes to stability of DNA is the Van Der Waals interactions and the hydrophobic interactions between the bases. So we have these bases which are quite hydrophobic because of the aromatic ring structure in them, the way they look and the way they are organized. And there is this twist in their structure -the bases - the base stacking is the hydrophobic interactions and the Van Der Waals interactions between the two bases, so if you have these two bases on the top of each other, there is a twist, so this rotation maximizes the hydrogen bonding between the two bases but the overlap - the hydrophobic interactions between the two- is not perfect because of the twist. :)

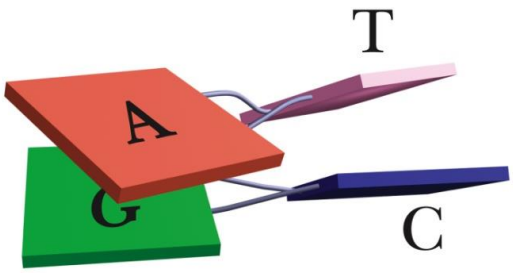
In addition, these bases are exposed to water, especially if they are exposed to the minor groove (:

Use your imagination to imagine the repulsive forces in base stacking; this is how the doctor explained it:

You have these bases, there is a little twist -horizontal twist- it's perfect base pairing between the complementary bases but because of the presence of water in the minor groove

that causes repulsion as well!:) As a result bases are not really perpendicular to each other but if you look at the slides you will notice that the bases are perpendicular to each other or to the backbone but this is not the case, because there is another twist known as propeller twist which means that you have this twist right here and then you have another tilt or twist, this tilt right here doesn't make the base pairing very optimal between this base and that base but it maximizes the -base stacking- hydrophobic interactions between the bases and it also removes water -repulsive forces of water- which makes the interaction stronger between the bases.





- Maximal base pairing, but
- Not optimal overlap of the bases.

The edges of the bases that are exposed to the minor groove unfavorably come in contact with water in this form

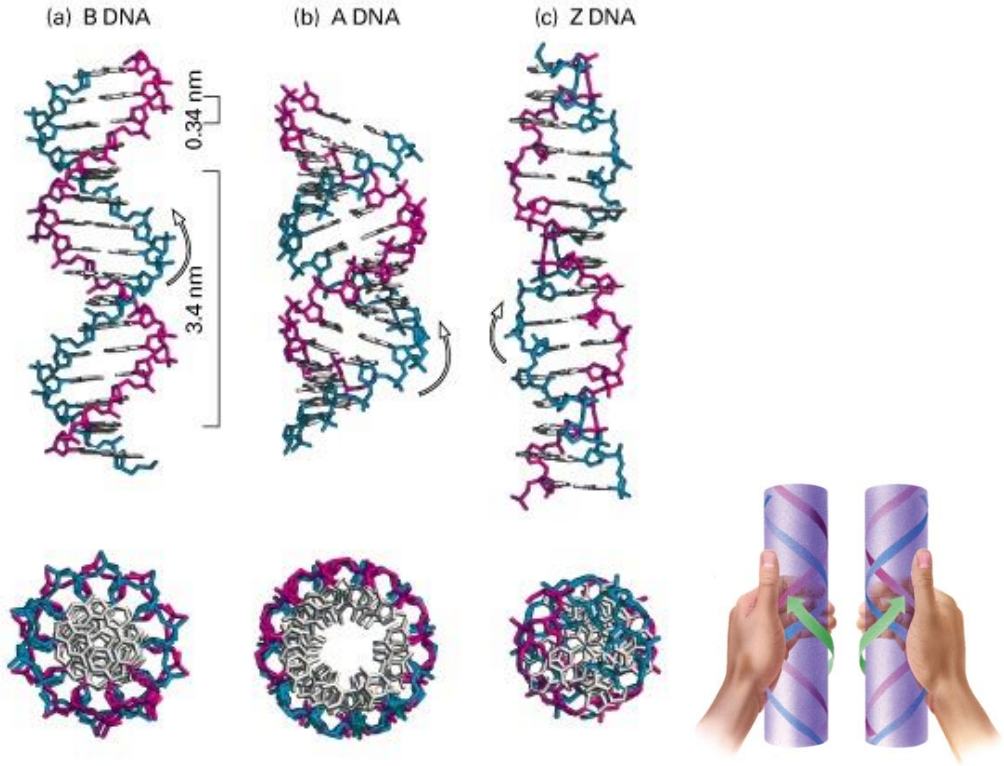
- The base-pairing distances are less optimal.
- Base stacking is more optimal.
- Water is eliminated from the minor-groove contacts with the bases.

We repeat again: the bases on top of each other, and hydrophobic interactions and van der Waals interactions between the two which are actually twisted...a part of the twist -not mentioned in the book- is the repulsive forces of phosphate ions because there is repulsion between the two. So there is a twist and it creates the helical structure of DNA. Again this twist maximizes the base pairing between the complementary bases! But it minimizes the hydrophobic interactions between the two bases that are stacked on top of each other ..that's why when water comes in, it makes repulsive forces with the bases and that's why there is another tilt knows an propeller tilt/twist, and it maximizes the base stacking also eliminates water from the DNA structure.

Looking at the DNA structure we have three forms of DNA structure, the natural form that you study in books is known as the B FORM.

The B form is right handed which means that the direction of the strands goes upward and to the right and it rotates and the bases are perpendicular to the backbone. Another form is called the A-DNA: it's wider in diameter and it has 11 bases per full turn vs. 10 bases per turn in the B form, which means that the A DNA is compressed and it's wider, and in the A-DNA the bases lie at an angle so they are not perpendicular and it's right handed as well. But we have an interesting form of DNA, it's called the Z –DNA, it differs a

lot from the two other forms, it's left handed and occurs when there is alternating purine-pyrmindine sequence (purine-pyrimidine-purine-pyrimidine- purine-pyrimidine…) it takes this Z-DNA structure. As well as sequence of methylated DNA. It's narrower/slimmer compared with B and A DNA.



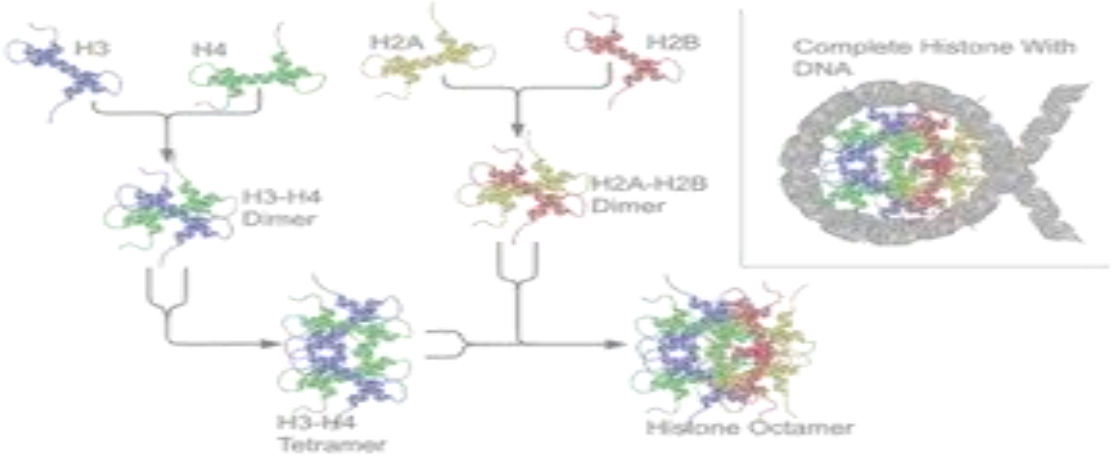
DNA coiling

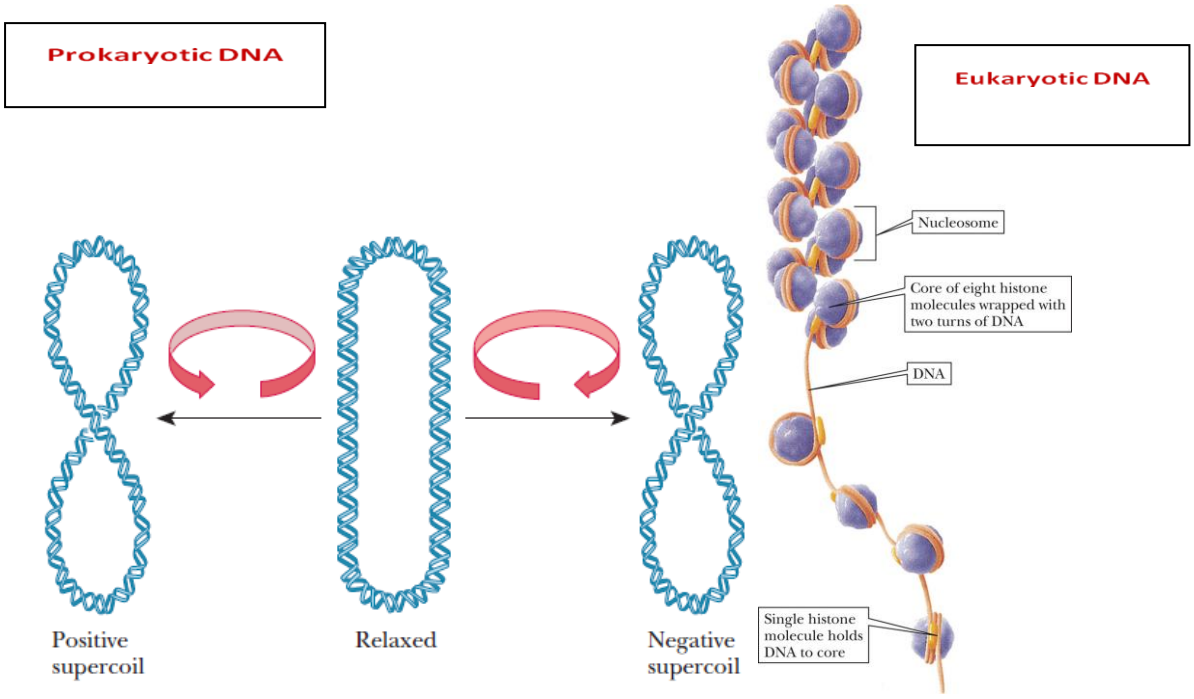
Basically, that the DNA must be wrapped, condensed, coiled. Coiling in Eukaryotic DNA takes place around histones (proteins that are positively charged) you find the DNA wrapped around these proteins.

Now if you take one of these histones, a structure called nucleosome. This nucleosome contains DNA and an octamer which means that you have 8 different proteins of

two types, the proteins are H2A, H2B, H4, H3, and you have two of each, a total of four different proteins! Forming with the DNA what is known as the Nucleosome and what helps this interactions between the histones and the DNA is that DNA is negatively charged and Histones are positively charged.

This figure is from the Internet

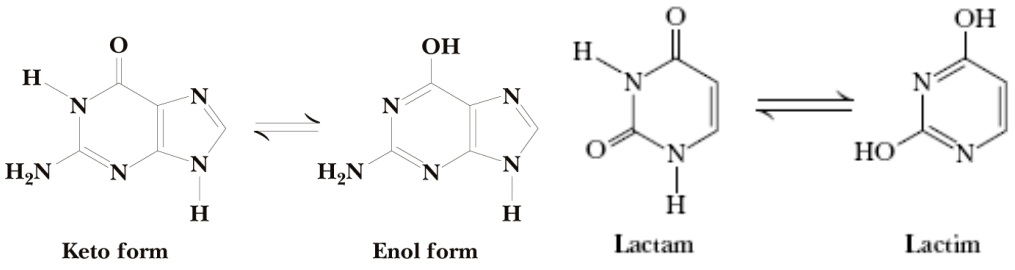




Now, between any two histones there is another histone: which is loosely bound DNA and it's called histone 1. :)

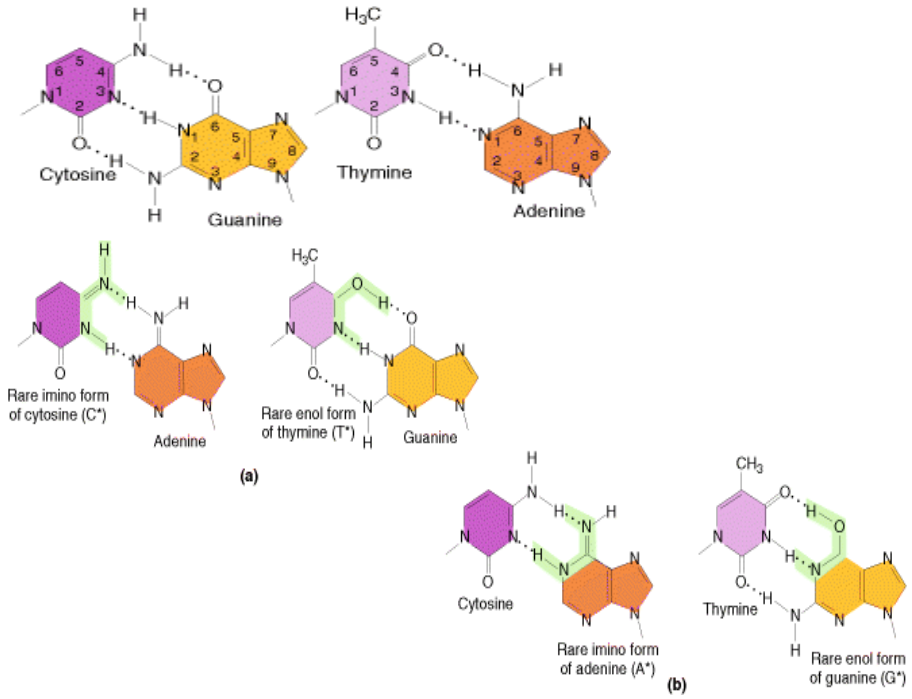
Keto-enol tautomerism

There are important chemical formations of purines and pyrimidines, one of them is known as **Keto-enol tautomerism, tautomerism** means that there are two constitutional isomers and they are interconverted, for example: you can convert glucose to fructose, these are constitutional isomers, one of them is an aldose and the other one is a ketose. So we have similar formations with nucleotides. We can have a keto-form of a nucleotide, and whenever you have this structure right here like an amide bond (in the figure below) you can have the double bond switching from carbon and oxygen to carbon and nitrogen, and this switching changes a lot of things, because it changes the hydrogen bonding characteristic of nucleotides. This switching from one form to another changes the hydrogen bonding between the bases.



There are different forms of these structures like Lactam and Lactim.

This causes mispairing between the bases. If you have for example an enol form of Guanine in this case you will have the Guanine pairing with Thymine instead of Cytosine and that can cause mutations later on when DNA is copied. Similarly you have Adenine and Thymine, say you have an Enol form of Thymine, that results in interaction or hydrogen bonding between Thymine and Guanine instead o f thymine and Adenine.



So tautomerism can result in mispairing as a result of Hydrogen bonding.

Acid-Base dissociation: It means that at different pH there will be different protonations between the different groups, that includes the amino groups, the nitrogens and

oxygens in the rings and that can result in changing the pairing between the bases.

Light absorbance of nucleic acids

What's important about this is that DNA can absorb light as a result of the aromatic ring of the purines and pyrimidines, and the peak absorbance is at about 260 nm that is at (UV light) .That's why DNA doesn't have any color. But did you even notice that double stranded DNA can absorb light at different quantities VS single stranded DNA or even single stranded RNA? They all absorb light at 260 nm but single stranded DNA in loop absorbs more light that double stranded DNA.

EXAMPLE: What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1unit?

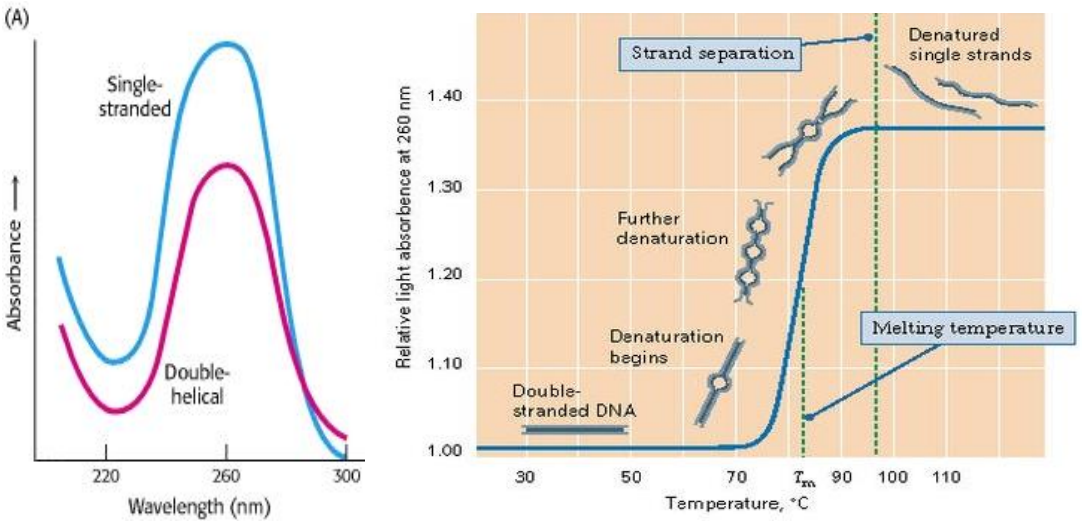
DNA concentration = 0.1 x 10 x 50 µg/ml = 50 µg /ml

Doctor's explanation for this example: if i have 50 microgram/ml DNA it means that it absorbs one unit of light and at 0.1 * 50 microgram/ml BUT because I'm diluting my sample at 1:10 I multiply it by the dilution factor! So as a result, you say it's 0.1 times the dilution factor times 50 µg /ml equal the concentration of DNA. The reason why we have this difference in absorption is because of base stacking. In the double stranded DNA we have more base stacking than in the single DNA.

An important property of DNA is denaturation which means that the two strands that are connected to each

other via hydrogen bonds can be separated from each other by heating. So if we heat DNA, the two strands will tend to separate. We can observe this DNA denaturation by taking different observations at different temperatures. So the same amount of DNA when its single stranded it absorbs more light than the double stranded DNA. So the same amount of DNA for example at temperature 40,50 or 60 still double stranded but if I increase the temperature the same amount of DNA absorbs more light →denaturation!

-The melting temperature is the temperature when 50% of the DNA is denaturated, just like the TM or P50 of hemoglobin, it's the point when hemoglobin is 50% saturated with oxygen.



And there are many factors that influence DNA denaturation. One of them is G-C pairing; so if you have more G's and C's in a DNA it will denature at higher temperature but why? Because we have more hydrogen bonds between G and C so it will take more energy to

separate the two strands from each other. Also because there is more or stronger base stacking in G-C pairing than A-T. In addition we have factors like pH, salt ion concentration as well as the presence of the stabilizing agent.

Now let's say I have two DNA fragments and these two fragments have the same length and they are in the same conditions except that one of them is and has higher G-C pairing than the other one. The one with more G-C contents will be denatured at 85 degrees whereas the other one at 75.

Central dogma of biology

There is something known as the central dogma of biology which says that DNA is copied to make another copy of DNA or it can be used to make RNA and RNA is used to make protein. This is the central dogma of biology; it's more like a philosophy of molecular biology. It's really important for the DNA to be copied otherwise life will stop! Making a copy of DNA is called DNA replication, making RNA out of DNA is known as transcription, and making a protein out of RNA is called translation. The difference between transferring information in eukaryotes vs. prokaryotes is that: in prokaryotes we have no nucleus, so we can perform transcription and translation at the same time and place! So we are making the RNA and the protein all at the same time. But this can't take place in eukaryotes because first of all there is a nucleus in

eukaryotes where transcription takes place, but the translation takes place in the cytoplasm, so they occur in two different places. The 2second reason is that once we make RNA it must be processed. For example some pieces are removed and they are called the introns -introns are bad- and the exons which are used to make the protein are connected to each other, and this must be done before the protein is made.

Types of RNA

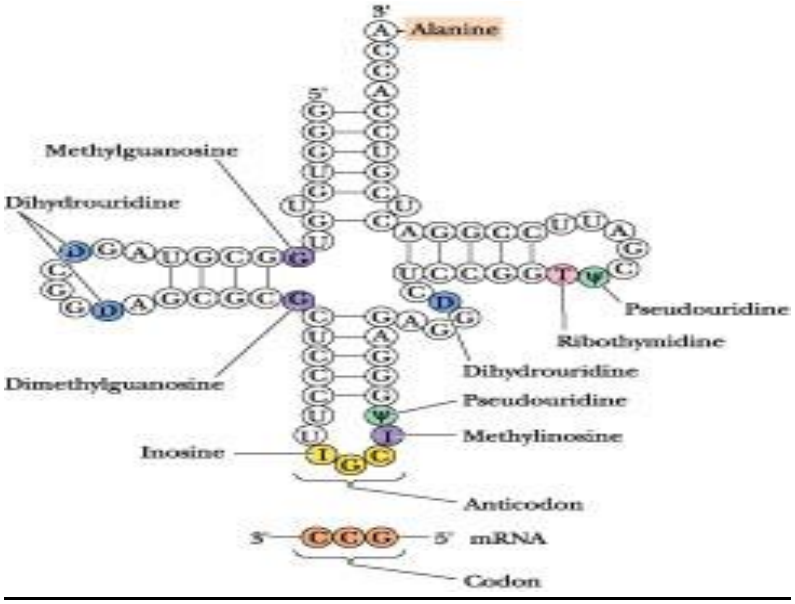
The Roles of Different Kinds of RNA

RNA Type	Size	Function
Transfer RNA	Small	Transports amino acids to site of protein synthesis
Ribosomal RNA	Several kinds—variable in size	Combines with proteins to form ribosomes, the site of protein synthesis
Messenger RNA	Variable	Directs amino acid sequence of proteins
Small nuclear RNA	Small	Processes initial mRNA to its mature form in eukaryotes
Small interfering RNA	Small	Affects gene expression; used by scientists to knock out a gene being studied
Micro RNA	Small	Affects gene expression; important in growth and development

The ribosomal RNA known as rRNA: Makes up huge mass of ribosomes which are the factories that make the proteins and they also perform the enzymatic catalysis (the synthesis of protein is not driven by proteins but driven by RNA molecules).

Transfer RNA: small molecule made up of 75 bases,there are different tRNA molecules and they differ by a certain sequence known as anticodan and the type of amino acids that is connected to tRNA. It's made of structures known

as stems and loops. Stems have hydrogen bonding between the bases in the single strand whereas loops don't have any hydrogen bonding between the bases.

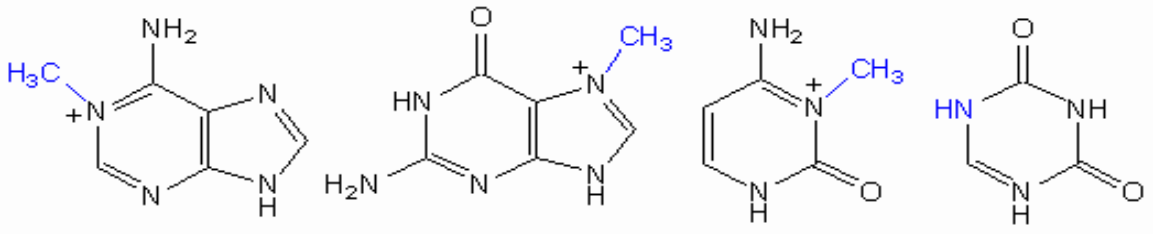


Modified nucleotides in tRNA

What's interesting about tRNA that it contains modified bases not the ones that are normally found in RNA and DNA.

So these are examples of modified bases (the figure below).

Examples of modified bases found in tRNA:



1-methyladenine (m^1A) 7-methylguanine (m^7G) 3-methylcytosine (m^3C) pseudouracil (Ψ)

Other RNA molecules

We have other types of RNA molecules and they include:

1. Small nuclear RNA (snRNA): which are involved in RNA processing in Eukaryotes. They occur in eukaryotes but they also have complexes with proteins making structures known as ribonucleoprotein particles.

2. Micro RNA molecules (miRNA): which are small RNA molecules that control and regulate the translation and the synthesis of proteins.

3. Small interfering RNA (siRNA): which are man-made(synthetic) and they also controls protein synthesis inside cells.

GOOD LUCK ☺