Lecture :..... 15

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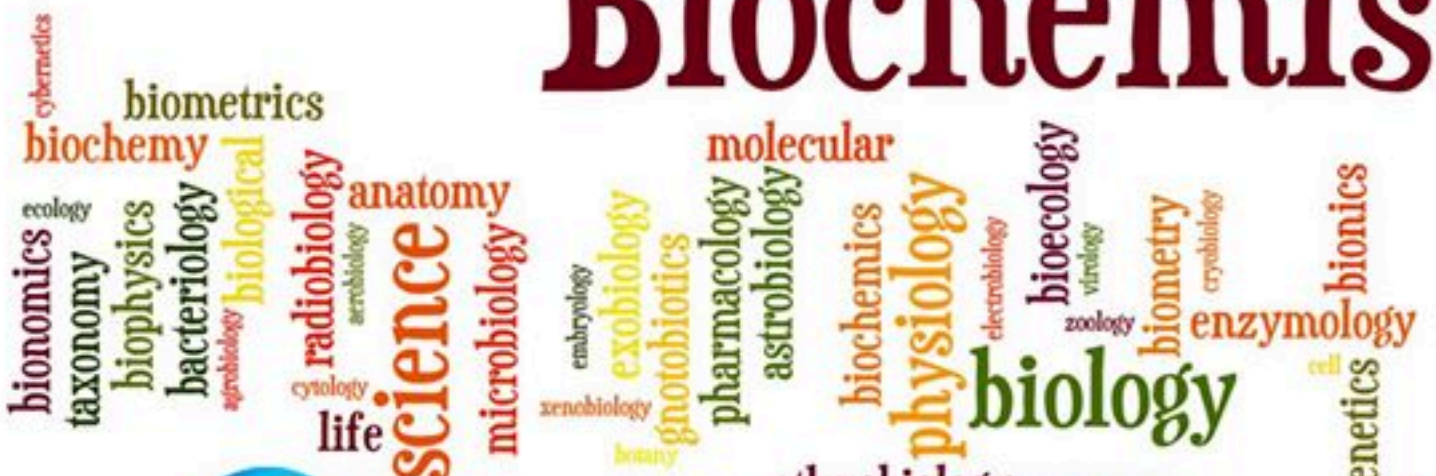
☐ Slide ■ Sheet

Medical Committee
The University of Jordan

Biochemistry



Mousa Suboh

Nucleic Acids

This lecture is meant to be a refreshment, it's a brief introduction to nucleic acids since we'll be given a full course about it next year in molecular biology.

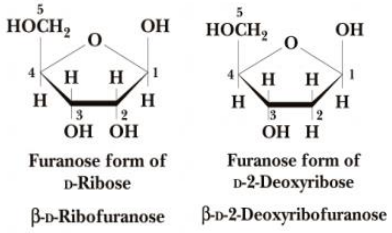
One last lecture and you'll be done with the midterm material inshaAllah :)

* What are the nucleic acids?

Molecules that store genetic information needed in cell growth and reproduction mechanisms.

- Nucleic acids contain 3 types of structures:

1) Nitrogenous base: purines and pyrimidines. Adenine, guanine, cytosine, uracil, and thymine.
2) Pentose (a monosaccharide); either ribose (in RNA) or deoxyribose (in DNA).
   On the anomeric carbon; H is directed downward and OH upward, on the 4th carbon atom the CH2O is directed upward, which means that the orientation of these two sugars is beta.



3) Phosphoric acid

RNA has lots of types and functions, that's why it can be found anywhere throughout the cell, however, DNA is found only in the nucleus and mitochondria.

*chemical composition:

The nitrogenous base is linked to the carbohydrate which is linked to the phosphoric acid.

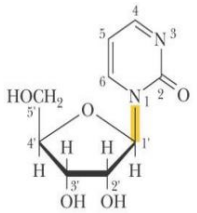
Carbon #1 (anomeric carbon) in the carbohydrate binds with the nitrogen of the nitrogenous base by a **glycosidic bond** (between the carbon and the nitrogen).

Carbon #5 of the sugar is linked to the phosphate group by a **phosphoester bond**.

Carbon #2 of the sugar has some modifications; in **RNA** it has a (*hydroxyl group OH*) while in **DNA** we remove the oxygen (*contains only H*) and that's why it's called **deoxy-ribonucleic acid**.

The total charge of nucleic acids is **negative** because of the presence of sugars with the the phosphate group attached to it; this always produces negative charges and. That's why the positively charged ions such as Mg^{2+}, Na^{+} and peptides can associate with nucleic acids. DNA (negatively charged) that is present in the nucleus is associated with positively charged proteins called Histones.

* Nitrogenous bases:



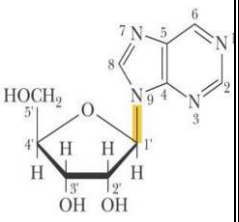
β-N_1-glycosidic bond in pyrimidine ribonucleosides

You have to be familiar with the structure of each base, and to be able to differentiate between them.

- **Purines** (shorter name→ two rings) the glycosidic bond is between the anomeric carbon and nitrogen #9,

- **Pyrimidines** (long name→ one ring) the glycosidic bond is between the anomiric carbon and nitrogen #1.

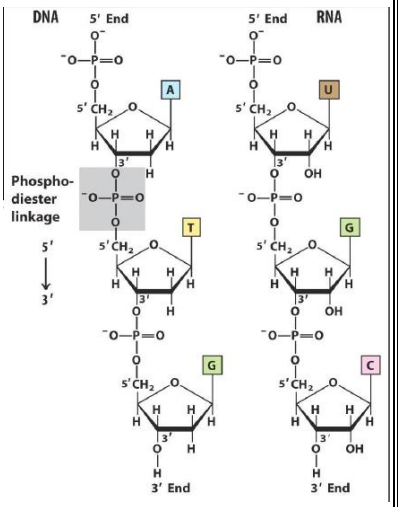
- So the anomrric carbons bind N1 of pyrimidines and N9 of purines.



β-N_9-glycosidic bond in purine ribonucleosides

* **Nucleoside** is composed of a nitrogenous base (either purine of pyrimidine) that is bound to a sugar (either ribose or deoxyribose). And we name them by putting the name of the nitrogenous base and adding the suffix *–osine* for **purines** and *–idine* for **pyrimidines**. For example, we have adenosine, guanosine, cytidine, and uridine.

* The **nucleotide** is a nucleoside that's bound to a phosphate group. And we have *monophosphate*, *diphosphate*, and *triphosphate*. We name them by adding the suffix *–ylate* to the name of the nitrogenous base. Ex: adenylate, guanylate, cytidylate and uridylate.



The chemical linkage between monomer units (nucleotides) in nucleic acid (the polymerization process) is done by bonds that are present on the phosphate group. The phosphate group of a single nucleotide is bound to carbon #5 by a phosphoester bond, and it binds also to carbon #3 of the next sugar forming a **phosphodiester bond**.

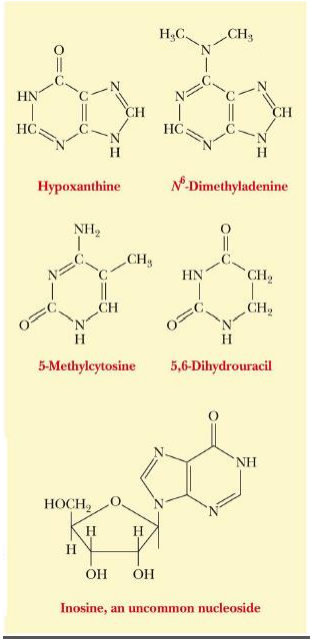
* make sure you go over the table in **slide #7**, just to be familiar with the naming and types of nucleosides and nucleotides.

TABLE 2-2 Terminology of Nucleosides and Nucleotides

		Bases			
		Purines		Pyrimidines	
		Adenine (A)	**Guanine (G)**	**Cytosine (C)**	**Uracil (U) Thymine [T]**
Nucleosides	in RNA	Adenosine	Guanosine	Cytidine	Uridine
	in DNA	Deoxyadenosine	Deoxyguanosine	Deoxycytidine	Deoxythymidine
Nucleotides	in RNA	Adenylate	Guanylate	Cytidylate	Uridylate
	in DNA	Deoxyadenylate	Deoxyguanylate	Deoxycytidylate	Deoxythymidylate
Nucleoside monophosphates		AMP	GMP	CMP	UMP
Nucleoside diphosphates		ADP	GDP	CDP	UDP
Nucleoside triphosphates		ATP	GTP	CTP	UTP
Deoxynucleoside mono-, di-, and triphosphates		dAMP, etc.			

* Nitrogenous bases have two types:

1) **Purines**: Adenine and guanine. (shorter name → bigger size)
2) **Pyrimidines**: cytosine, thymine and uracil. (longer name → smaller size)



We also have other nitrogenous bases which are much less common that the ones mentioned above. Those other bases are present in **RNA** not DNA, specifically in the tRNA. Some examples are hypoxanthine, dimethyl adenine, methyl cytosine, dihydrouracil and inosine. You should memorize the structures of the 5 basic bases (slide #8), since you'll be asked to label the modifications on the less common ones.

For example:

- The uracil has a double bond between two carbons in its chain (you have to memorize this), but in the dihydrouracil we've broken that double bond by adding two hydrogen atoms → dihydro.
- When we add a methyl group to the cytosine it's then converted into 5-methyl cytosine. So if you are familiar with the structure of cytosine, you'll easily recognise the addition of the methyl group.

*Other nucleotides:

- Xanthine, hypoxanthine and uric acid are important in purine metabolism. Uric acid causes a disease that is spreading wildly especially in the Jordanian society, which is gout داء النقرص . That happens when the uric acid forms crystals and accumulates in the joints causing severe pain.

- Methyl adenine, the addition of the methyl group is on nitrogen #6.

- 5-methyl-cytosine & N4-methyl cytosine

- pseudouracil: it differs from the uracil in the location of attachment to the ribose. The uracil since it's a pyrimidine should bind to the ribose on carbon #1, however, it binds on carbon #5, that's why it's called pseudouracil.

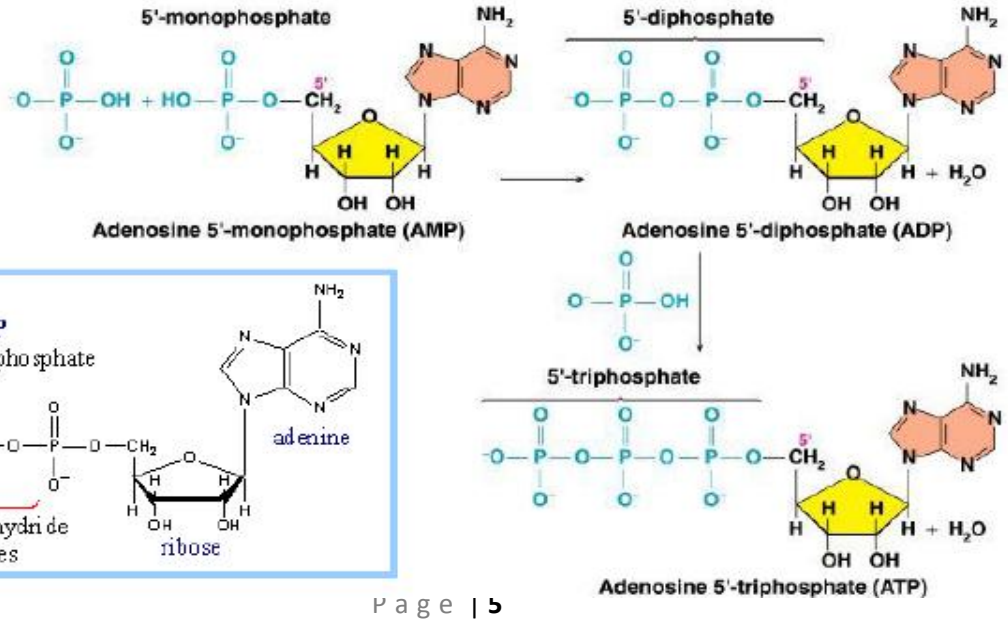
- 1,3,7-trimethylxanthine (caffeine) : it's a xanthine molecule that has 3 modifications (3 methyl groups) on 3 nitrogen atoms (#1,3,7).

*** How does caffeine awakens us?**

Adenosine (adenine +ribose "nucleoside; without phosphate") works as a neurotransmitter to promote sleepiness receptors, so the binding of adenosine to its receptor makes us feel sleepy. Caffeine blocks the interaction, by binding to the sleepiness receptor, which will prevent adenosine from binding to the receptor, as a result, you won't feel sleepy after drinking a cup of coffee.

* AMP, ADP and ATP:

The difference between them is in the number of phosphate groups. The phosphate groups are joined together via **phosphoanhydride bonds**. While the bond between the ribose and the base is called glycosidic bond.

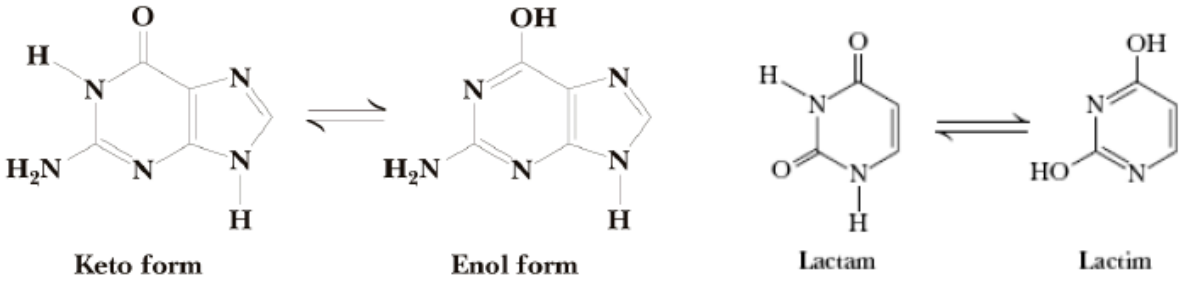


* Properties of pyrimidines and purines:

1. Keto-enol tautomerism:

- Tautomerisation is an isomerisation reaction, the bases regularly form double bonds with oxygen and next to it is a single bond with nitrogen {keto form; lactam}. But an isomer of this structure can have a single bond with the oxygen - by adding a hydrogen atom (forming a hydroxyl group instead of a carbonyl group)- and forming a double bond with the nitrogen {enol form; lactim}.

Because nitrogen #1&3 have pKa greater than 8, on neutral pH=7, we find most of the bases in the keto form. So the keto form is the more predominant form for the bases at neutral pH (physiological pH; close to 7).



- A mutation can be caused due to a tautomerisation reaction, so usually normal DNA is composed of nitrogenous bases in the keton form (physiological form). The consequence of the tautomerisation reaction affects the hydrogen bonding. In normal DNA, the adenine binds to the thymine (A=T), guanine with cytosine (C≡G), via hydrogen bonding between these bases while sugars form the backbone.

Nitrogenous bases have nitrogen and oxygen atoms, if the oxygen changed from being **an electron donor** (double bond; keto form) into **a proton donor** (OH; enol form), and the nitrogen changed from being a proton donor (N-H; keto form) into a single nitrogen (enol form) this will change the hydrogen bonding between the nitrogenous bases, so it might cause cytosine to bind with adenine instead of guanine, because it

becames a hydrogen bonding donor not a hydrogen bonding acceptor as it used to be at certain points. Same story can happen with guanine causing it to bind with thymine, and all of this will result in a huge problem in the base pairing (miss pairing; **tautomeric shift mutations**).

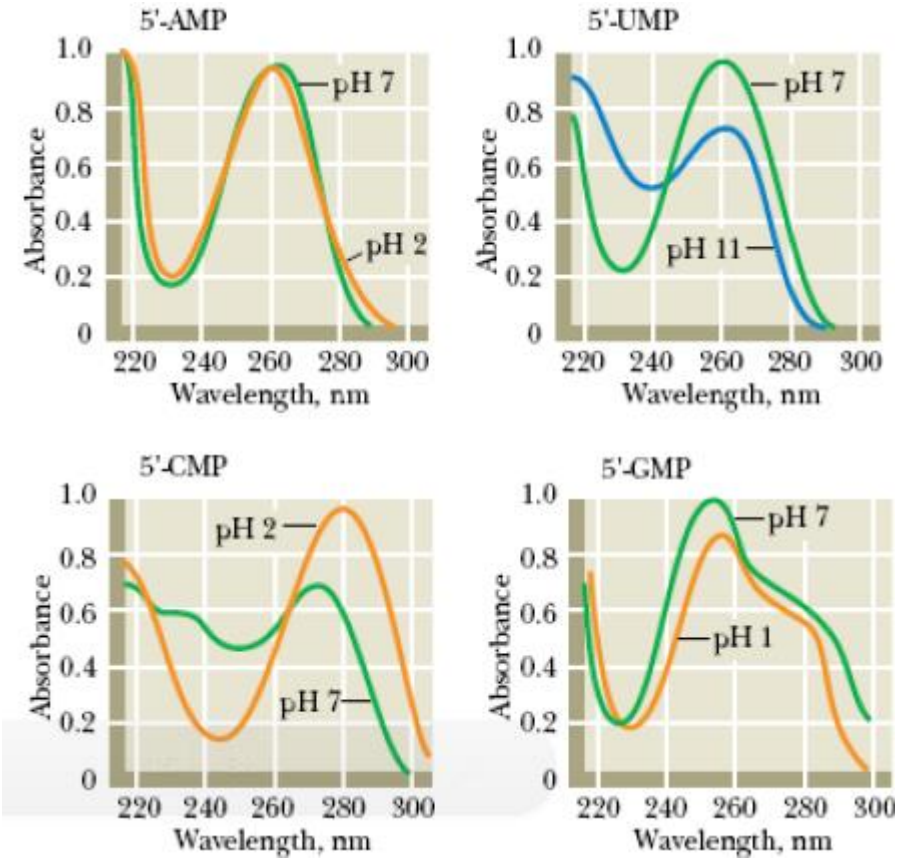
2. Acid/base dissociation:

The nitrogenous bases can be affected by the pH value, by affecting the protonation state of the nitrogen and oxygen atoms and will accordingly affect the hydrogen bonding between the bases. If the pH value increased higher than the pKa value of the amino group, it will lose its hydrogen, so it won't be able to form hydrogen bonds.

3. Strong absorbance of UV light:

If we expose the nitrogenous bases to UV light, we'll observe a peak in the absorbance degree. Any cyclic structure has strong absorbance for UV light around 280 nm. You put a sample of the structure you want to study and place it in a chamber then expose it to light (UV, infrared or regular light) you'll notice a reflection of that light in certain shape according to the structure placed in the chamber, we can consider this to be as an imprint of each substance.

- As you can notice in slide #15, 4 different substances with certain shape for each one, although they're somehow similar to each other, they differ at some points.



- The peak of absorbance for the structures (AMP, UMP, CMP, and GMP) is around 260 nm. The peak for most ring structures is around 280 nm, for example proteins with high amount of tyrosine and tryptophan (ring structures; aromatic). So the peak for most ring structures is around 280 nm, while in nucleosides and nucleotides it's around 260 nm.

- This property is used to make examination, experiments on DNA or RNA. When the amount of base increases the peak of absorbance increases, there's more quantity of this material (high concentration).

- When the absorbance reaches 1.0 on 260 nm from **double stranded DNA** means that you have 50 micro gram/mL, if the sample is **single stranded DNA** this means that you have 30ug/mL, and if it was **single stranded RNA** this means that you have 40 ug/mL. When you place any nucleic acid in a buffer solution (water) and expose it to light, it'll give you a specific shape, the value of the peak of absorbance (could be 1,2,3…) so if the peak was 1.0 it indicates the concentration of each one, when the absorbance reaches 1.0 and we were studying a DNA sample;

the concentration of that sample is 50 ug/ml. if we were studying single stranded DNA; having the absorbance of 1.0 means that the concentration is 30 ug/ml, and if we were studying single stranded RNA; it'll mean that the concentration is 40 ug/ml.

A_{260} of 1.0	dsDNA	ssDNA	ssRNA
	50 ug/ml	30 ug/ml	40 ug/ml

- Each nucleotide has its own peak, and we can distinguish them from one another.

- The difference is that the **double stranded DNA gives a lower absorbance than the single stranded DNA at the same concentration.** It's *because of the hydrogen bonds between the bases in the double stranded DNA*, so the bases cover each other and this won't give you the full reflection of the light. If you open the two strands from each other, this will give more reflection of the light resulting in higher value of absorption. If I had single stranded and double stranded DNA of the **same absorbance**, the amount of the double stranded DNA will be higher.

  - Same concentration → single stranded DNA will have higher absorbance
  - Same absorbance → double stranded DNA will have higher concentration

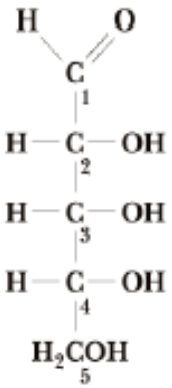
- Question:
  What is the concentration of a sample of double stranded DNA that was diluted 1:10, and the absorbance is 0.1?
  If the absorbance equaled 1.0 → you have 50 ug/ml
  If the absorbance is 0.1 → you have 5 ug/ml
  However the sample was diluted 1:10, so we must multiply the concentration by 10, and the answer is then 50 ug/ml.

* Pentoses of nucleotides:

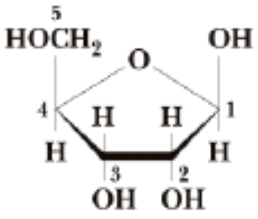
Two types: ribose, deoxyribose.

- When you are naming, you put a' (prime) which means that there's a sugar and the number indicates where that sugar is binding.

- The stereochemistry of pentoses is always beta (cis).

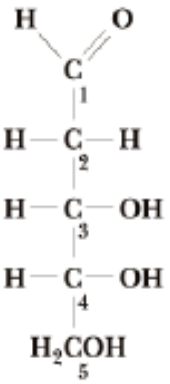


D-Ribose

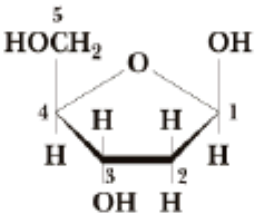


Furanose form of D-Ribose

β-D-Ribofuranose



D-2-Deoxyribose



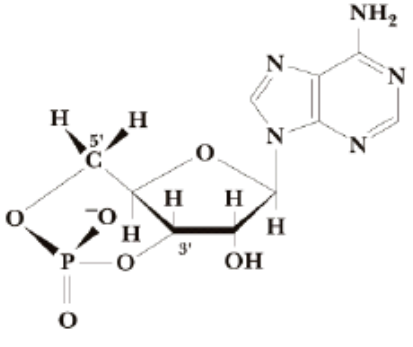
Furanose form of D-2-Deoxyribose

β-D-2-Deoxyribofuranose

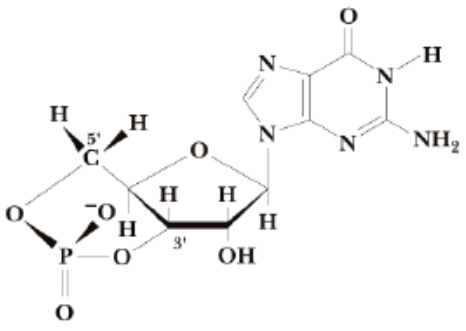
* Functions of nucleotides:

- ATP is an energy carrier, most of the mechanisms that happen in the body are accomplished by the use of ATP (central energy molecule).

- cyclic nucleotides; some nucleotides are cyclic, such as, AMP and GMP → cyclic AMP and cGMP, which work as messengers, signal molecules, they make regulation for cellular metabolism and reproduction. So they are regulators and messengers. Cyclic nucleotides are signaling molecules while non-cyclic nucleotides participate in energy donation.



3',5'-Cyclic AMP



3',5'-Cyclic GMP

- **ATP** is the **central energy molecule**.

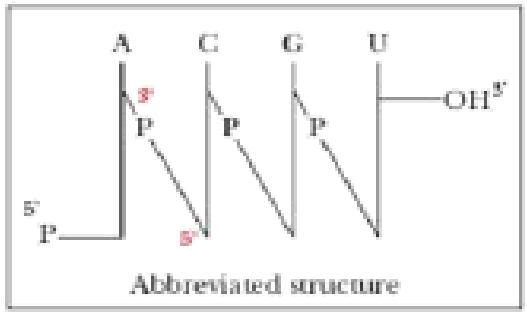
- **GTP** gives the same amount of energy provided by ATP, but it's specific for **protein synthesis** causing better localization and specification.

- **CTP** drives **lipid synthesis**.

- **UTP** drives **carbohydrate metabolism**.

* Polymerization:

We bind the **3'C** to the **5'C** by a **phosphodiester bond**, and the direction is **5' to 3'**. **How are they linked? 3'C from one ribose is separated from the 5'C of the next ribose by a phosphate group which forms the phosphodiester bond.



- Two types of enzymes are engaged in this process:

1) Polymerases
2) Ligases

- The **phosphate pKa** is around **zero**, which means that at any pH it'll lose its hydrogen (always deprotonated). So the phosphate group is always negatively charged, making the nucleic acids (DNA, RNA) always negatively charged. That's why one of the properties of DNA is that it's a negatively charged molecule.

* Classes of nucleic acids:

- DNA: only one type, one purpose; which is carrying genetic information.

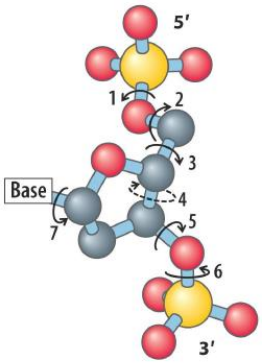
- RNA: 5 types with 5 functions; each RNA has a specific function.

- Ribosomal RNA: the structural one, gives ribosomes their shape and function.
- tRNA binds with the amino acids needed in the translation process.
- messenger RNA carries the message from DNA to the ribosomes.
- small nuclear RNA
- small non-coding RNAs

* DNA structure:

DNA can't fit in the cell unless it's very compact and folded around itself, this way it can fit inside the nucleus or the prokaryotic cells. DNA is an antiparallel double helix, the backbone bonds are phosphodiester bonds, while the side chain bonds are hydrogen bonds between the bases.

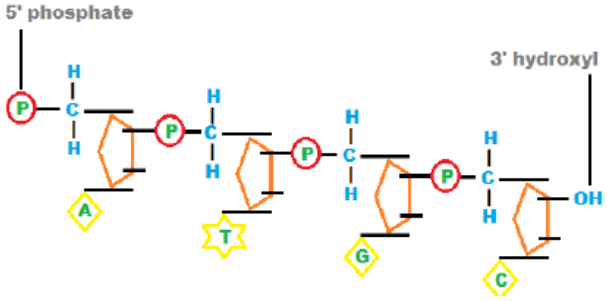
Specific base pairing; Chargaff's rule (adenine and thymine are linked by two hydrogen bonds, guanine and cytosine are linked by 3 hydrogen bonds)



Stability of the DNA is accomplished through the extensive number of hydrogen bonding that can occur. Flexibility of the DNA is achieved through the multiple bond rotations. (refer to **slide #23** to see the figure showing bonds numbered 1-7 that rotate).

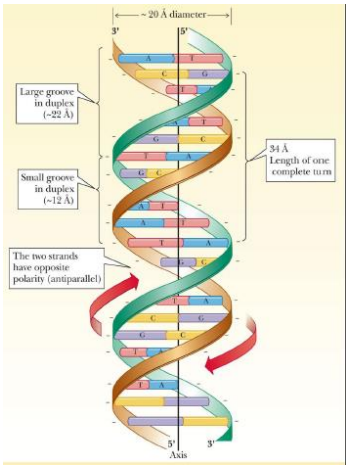
* DNA structural levels:

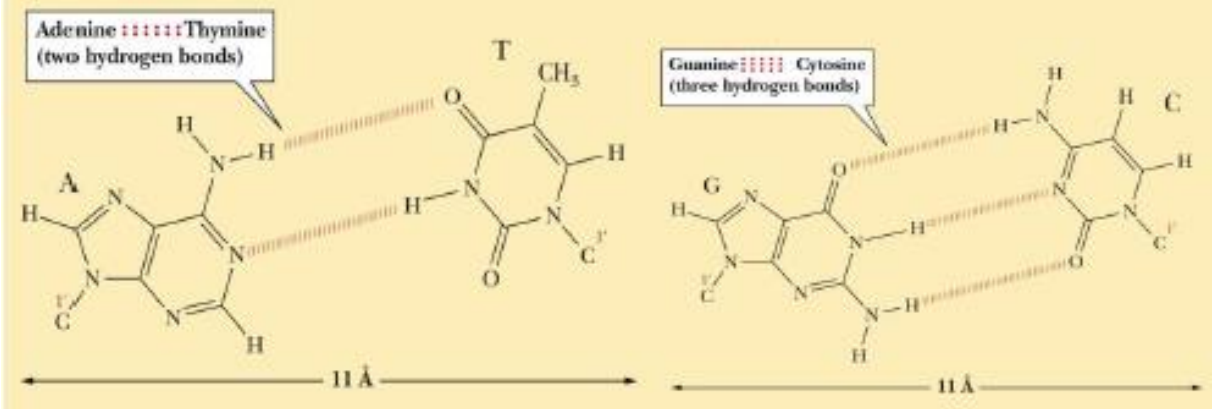
1) **primary structure**: it's the same as the primary structure of proteins, it is a sequence of nucleotides that are bound together by phosphodiester bonds. By convention, when you read the primary structure (the bases) you must start from left to right (5' to the 3').



System of notation single letter (A,T,C and G) for indicating the type of nitrogenous base, and if it was unprecedented with (d) this means that this is the deoxy form of the nucleotides.

2) **secondary structure**: when two strands rotate around each other it gives us the so-called double helix model that was suggested by Watson and Crick. It's composed of two antiparallel strands coiled in a right handed helix. The base pairing is (A=T, C≡G). The rotation of the two strands results in leaving spaces (grooves) between them, and we have small groove (minor) and large groove (major).





❖ *Forms of DNA:*

- B-DNA: most common one (physiological one), it's a right handed helix with a diameter of 11A, and 10 base pairs per turn.
- A-DNA: it's also right handed, but it differs in having 11 base pairs per turn resulting in a larger diameter (wider than B-DNA), and it hasn't been found in vivo (inside the human body), it can present outside.
- Z-DNA: it can be found in vivo, but it's left handed helix, plays a role in gene expression, and it's narrower than the B-DNA.

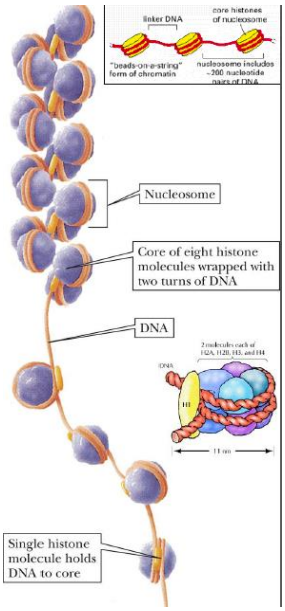
❖ *Features of DNA:*

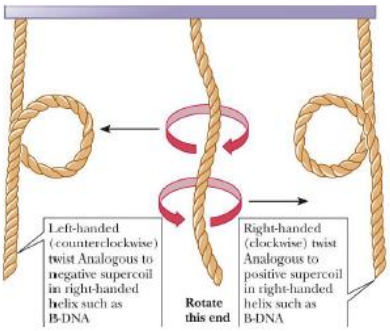
✓ Base stacking:

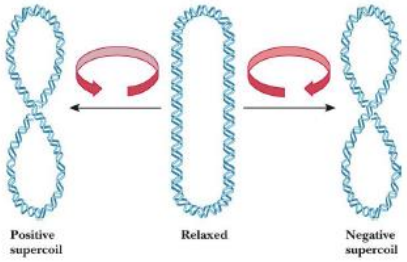
- Bases of the same chain are above one another and hydrogen bonding occurs horizontally between the two chains. The bases are hydrophobic, so when they are close to each other, they make hydrophobic interactions between each other(van der waals interactions) in order to become more stable which is better for the DNA. So now we have bonding that is horizontally (hydrogen bonds) and bonding that is vertically (hydrophobic interactions), what happens in the DNA is that the bases that are above each other rotate for about 32 degrees (**propeller twist**) in order to become closer to each other and maximize the hydrophobic interactions (van der waals forces). But this rotation affects the hydrogen bonds and makes them less optimal; having kinks instead of being straight.
- Base stacking means that the bases above each other make hydrophobic interactions between them and become closer to each other, forming twists around 32 degrees resulting in propeller-twist. Hydrogen bonding is then affected on the expense of hydrophobic interactions.

- The base stacking process has large energy, because the hydrophobic interactions are really strong, that's why if we had C≡G above C≡G that will have 61kJ/mol, and the amount of energy is always larger than the energy given by ATP (7.3kJ/mol) even the least amount of energy given by the hydrophobic interactions is double that of ATP.

3) **tertiary structure**: it's the supercoiling of the secondary structure double helix. DNA can coil in different ways than the double helix, it's either anti-clockwise (positive supercoil), or clockwise (negative supercoil) and it's driven by enzymes such as DNA gyrase in the bacteria and topoisomerases in eukaryotic cells







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Supercoiling in the nucleus:

DNA rotates around positively charged proteins called **Histones**. Histones are octoral in shape, 8 proteins (8 subunits) rotated around each other. These 8 subunits are: two H2A, two H2B, two H3, two H4. The two adjacent histones are linked together via *linker histone (H1).* Since histones are positively charged they help in stabilizing the DNA that is negatively charged. Each bead (DNA wrapped around one histone core) is called **Nucleosome**.

* Denaturation of DNA:

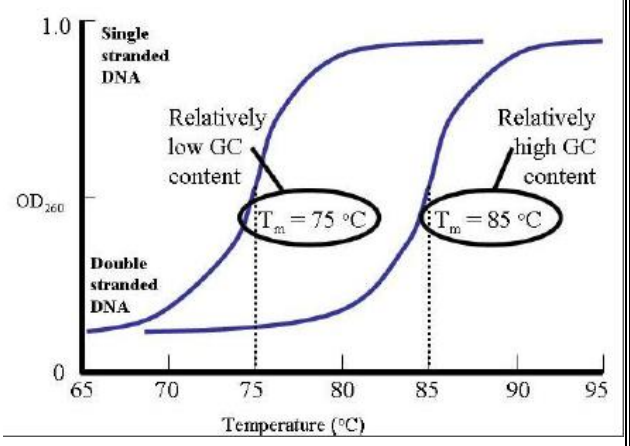
It's when the double stranded structure is broken down, producing 2 single stranded DNA molecules. Most denaturation processes in proteins are irreversible, while most of those in DNA are reversible. Denaturation of DNA occurs as a result of **heating** which causes the hydrogen bonds to become far away from each other and the final result will be 2 single stranded molecules. **They can be brought back together (renaturation) by slow cooling.**

As we discussed earlier, that single stranded DNA has higher absorbance than double stranded DNA, because the bases become fully exposed to the light (hyperchromicity; chrome means color, so the color is increased by increasing the absorbance due to the breaking down of the double helix).

- ✓ When does denaturation occur?
  When the heat is increased higher than the melting point (it's the point when achieved the strands will open from each other). The two strands begin to separate from one another when the temperature is around the melting point, further denaturation occurs as a result of increasing temperatures, at the end you'll end up with two single strands.

✓ What affects the denaturation process?

- ➢ The G≡C content, the higher the G≡C content, the more hydrogen bonds you need to break in order to separate the two strands (since they're connected via 3 hydrogen bonds) and the higher temperature you need to achieve the full breakdown.

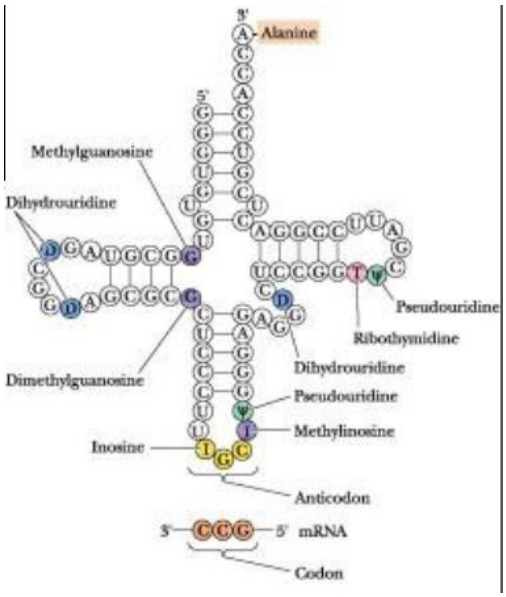


- pH
- salt and ion concentration
- destabilizing agents; alkalines, urea, formamides.

* RNA:

It contains the same nitrogenous bases in DNA except that thymine in DNA is replaced by uracil in RNA.

* Types of RNA:



- tRNA: The smallest of the 3,

~75 base molecule, it contains modified bases (found only in tRNA), it's single stranded. Its secondary structure may seem to be double stranded, but it's single stranded, contains only one 3'-end and one 5'-end. Hydrogen bonding forms a structure called **stem**, but the **loop** (circular structure) that is formed is not due to hydrogen bonding, that's why we have structures of the tRNA called stems and loops. The loops are the areas that contain modified bases (any base rather than the regular ones).

- **ribosomal RNA**: maintains ribosomes structure & provides sites for mRNA binding & protein synthesis.

- **messenger RNA**: it carries the message (coded genetic information) from the DNA in the nucleus to the ribosomes, relatively small amounts & very short-lived.

- **non-coding RNAs**: molecules that aren't related to proteins, such as, small nuclear RNAs which are found in association with the proteins, these complexes are called small nuclear ribonucleoproteins (snRNPs)

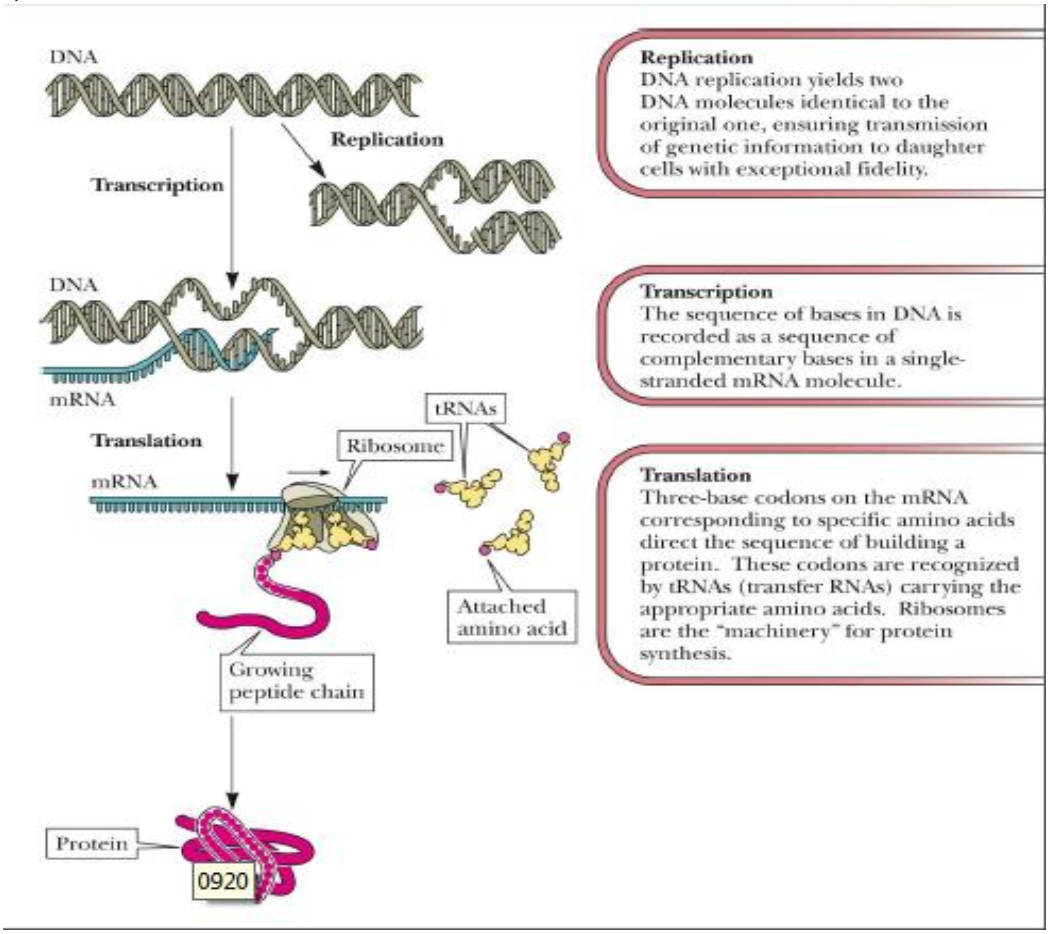
and they do certain processing on the mRNA after it leaves the nucleus to improve the gene transcription process.

- **microRNAs**: natural molecules, occur in the cell. They regulate the translation process, they can either make it faster or slower.

- **small interfering RNAs**: structures that can inhibit the mRNA. They can be manufactured in the body or taken from outside. They're widely used in research, to block certain genes by making a small interfering RNA that's complementary to the mRNA of the wanted gene, and once you place it in the cell it will inhibit that mRNA from gene translation. So it regulates the translation.

* Information transfer in the cell:

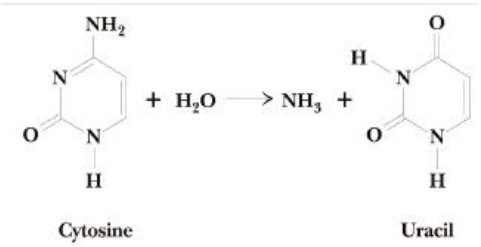
The DNA produces mRNA via gene transcription, and by gene translation we can synthesize proteins. This process in the cell is known as: **Central dogma of biology**.

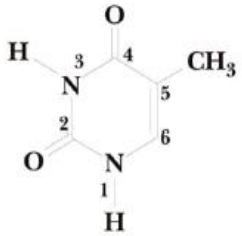


* DNA & RNA differences:

1. Thymine and uracil:

Certain mutation in the DNA converts the cytosine into uracil. So if the DNA contained uracil at normal conditions, you won't be able to know whether the uracil is original or it's caused by a mutation. So any uracil found in the DNA is considered a mutation and it's broken down and replaced by a new cytosine.

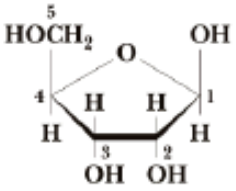




- ✓ The only difference between cytosine and uracil is in **the amine group**. That's why the mutation that converts cytosine into uracil is very common.

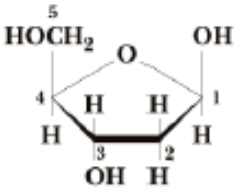
2. The 2'-deoxy sugar:

Deoxyribose is more stable, while ribose is less stable because it contains a **hydroxyl group** that can form bonds and react with enzymes so it's less stable. Therefore, removing the oxygen from the hydroxyl group makes it more stable. We want DNA to be more stable and the RNA less stable because RNA functions temporarily while DNA is permanent that's why it contains deoxyribose.



Furanose form of D-Ribose

β-D-Ribofuranose



Furanose form of D-2-Deoxyribose

β-D-2-Deoxyribofuranose

Eid Mubarak :)

Best of luck :)