University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Introduction to

BIOCHEMISTRY

Lecture #: (.....9.....)

■ Sheet ☐ Slides ☐ Other

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Lecture Outline:

1-Qucik Revision concerning the previous lecture of primary and secondary structure of DNA

2-The tertiary structure Of DNA .

3-DNA denaturation and renaturation

4-RNA and its types

5 – AAs classification

Qucik Revision

*Primary structure Of the Nucleic acid: It is the linear arrangement of the nucleotides in any DNA molecules according to number, sequence and type in the form of one strand

*Secondary structure: More than one strand how do they relate to each other.

** 3ry Structure :

3-D arrangement of all the atoms in any nucleic acid molecule ; commonly referred to as super-coiling in space (in prokaryotes). Super-coiling decreases the space they take in space. If this coiled DNA linked

to any other structure which is not nucleic acid like Carbohydrates and proteins then it will be the quaternary structure of DNA . this type is present in eukaryotes.

* Supercoiling: further coiling and twisting of DNA helix.

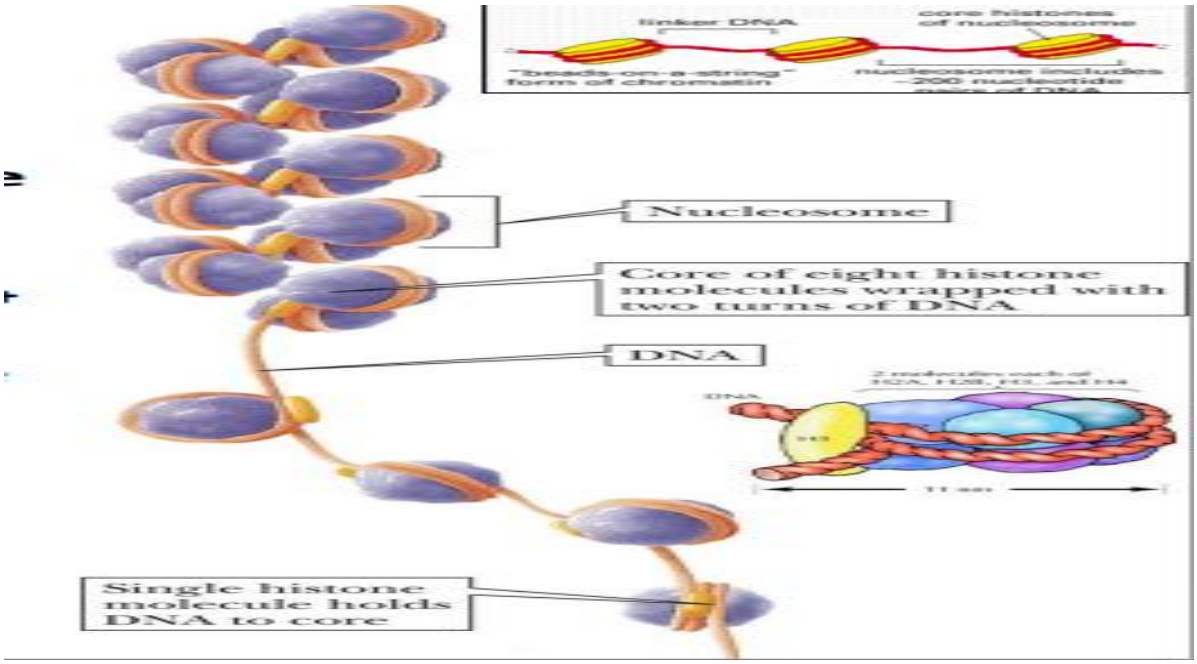
* Circular DNA: a type of double-stranded DNA in which the 5’ and

3’ ends of each stand are joined by phosphodiester bonds. Prokaryotes

**In Bacterial cells {Prokaryutic} is differnt than {eucaryutic} cells .the tertiary structure is achieved by the process of super coiling to fit the area ..Eg. E-Choli as a reference for Bacterial cell it is 2000 nm In length while the circular DNA molecule is about 1.6 Million nm in Length ➔ It must be supercoiled to fit the space. The process of supercoiling is done through several enzymes such as topoisomerases and gyrases.

- IN Eucaryutic cell . DNA is link to protein Called Histones ... Which are proteins heavily loaded with +vely charged (A.A) Amino Acids Mainly Arginin . So why we need this heavily loaded (+vely charged linking protein ? Hence DNA posses a (-ve) charge come from the phosphate which is Always (-evely charged ..Remeber Pka of phosphate ≈ 0 so always dissociated since the ph is nearly 7.4 Ph> Pka ➔ it must be dissociated . Histones plays a rule in making an interaction between the (-ve) and (+ve) . in addition to make a neutralization of the charge.

- There are five main different types of histone proteins : they are; H1, H2A, H2B, H3, H4.
- H1 is unique with a different story as doctor said … every histon other than H1 make 2 carbons ➔ 2 of H2A , 2of H2B ,2H3 and 2 of H4 .The total is 8 . 8 Proteins wrap with each other in specific way making an opomer. a segment of DNA is coiling around these 8 molecules of histons then A nucleusome is formed .it is like a bead (العقدة) and by this way the DNA is formed in the Eukaryotic cells The DNA Linking between each bead and other called linker DNA . H1 Is the linker histone (A protein associating with the linker DNA . here is the illustration.(refer to slides for more clear picture …



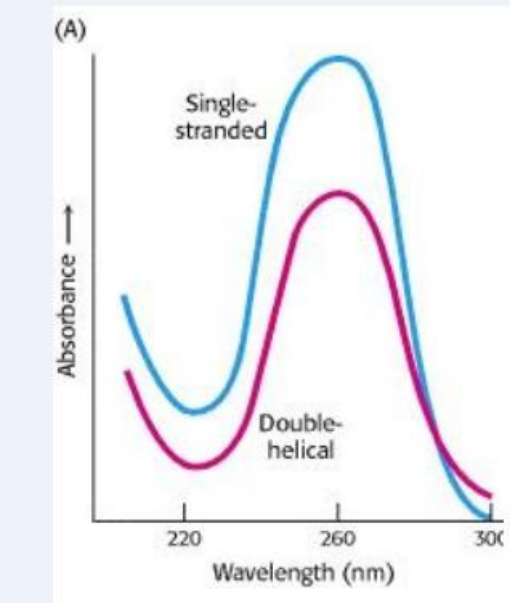
DNA denaturation

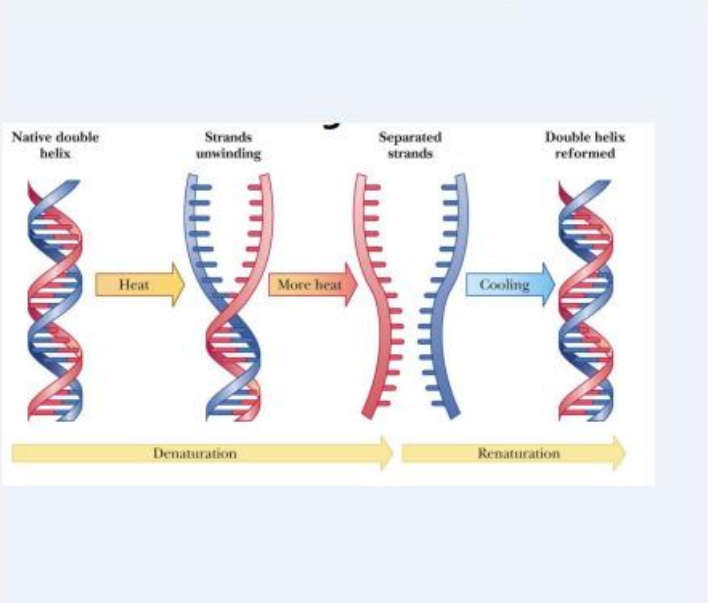
- It is a process in which the DNA or a protein is losing the 3-D shape of the structure represented by unwinding of DNA strands leaving the primary structure of molecule you have, either a nucleic acid or a protein . e.g When DNA double helix is heated ➔ it will split resulting in 2 separated strands . How do they split? with heating the hydrogen bonds between the nitrogenous bases in DNA strands will be broken this will result in restoring the 1ry structure .(Remember the hydrophobic interactions between the nitrogenous bases was responsible for forming the spiral double helix structure) .

  ** Renaturation (Annealing): This is done by until restoring the two complementary strands to re-associate into a perfect double helix. (this is because denaturation's cause was heating.
- How can we decide weather this DNA is separated or not. Flashback to UV light absorbance of DNA. DNA has a certain absorbance of UV light > caused by the Aromatic molecules of Nitrogenous bases and it is around. Denaturation of DNA can be observed by measuring absorbance at 260nm.in the normal Non denatured DNA The 2 strands are connected to each other in a spiral way .sometime the UV lights can't hit the bases . some of them won't face the light but once we denature them into 2 free strands ➔ All the nucleotides will face the light ➔ the absorbance increase and this property called the hyperchromicity

(Chroma) means color .so increase in absorbance .

- The Mid Point of denaturation process called the melting point. The melting point of any DNA molecule is affected by length of DNA, how many bases it has and what the content of these bases.
- How do the different types of DNA differ from each other in melting temp throughout their contents? *simply, Because between C≡G there are 3 hydrogen bonds while between A=T they are 2 ➔ DNA which contains high C≡G content , will get higher melting point. Consequently, the more Bonds in between the more heat you need to separate them because you need more energy to the higher amount of bonds.





RNA

What is the difference between RNA and DNA?

1-The pentose unit is β-D-ribose (it is 2-deoxy-D-ribose in DNA)

2- The pyrimidine bases are uracil and cytosine instead of the corresponding Thyamine and Cytosine in DNA

3-Mainly, RNA is single stranded but it could form a double stranded molecule and that's occured rarely as in viruses .(N.B.DNA is double stranded All the time) .

*Consist of long, unbranched chains of nucleotides joined by

phosphodiester bonds between the 3'-OH of one pentose and

the 5'-OH of the next.

*Classification according to the function and structure :

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

- T-RNA is the smallest in compare with R-RNA and m-RNA. t-RNA carries Amino Acids at its 3' end in the process of translation. when m-RNA go to the ribosome then the t-RNA put the amino acid resulting in protein synthesis. t-RNA is T shape molecule .it is Single-stranded.
- R-RNA :it is found on the ribosome Found in ribosomes (protein synthesis) they are result proteins. it constitutes almost 60-65 % of content of ribosome (mass)
- M-RNA ; it carries the message from the DNA toward the ribosomes where the translation is occurred Carries coded genetic information .it is Relatively small amounts & very short-lived
- Sn-RNA : found in the nucleus of the eukaryote they are small molecules 10-200 bases in length . it forms complex with protein called - small nuclear ribonucleoprotein particles (snRNPs) and they are important in regulation of transcription . *Micro RNAs : they are of 2 classes . natural produced by the cell . they aid in regulation of translation they are very helpful

especially in researches about cancer .

**Small interfering RNA :(siRNA)Synthetic, not natural and Translation regulation.

How to determine the function: isolate it then its effect will be determined .how that is occurred? excision of DNA from the genetic material which produce specific protein. then you will discover that the cell has no ability at all to express the protein of interest and you will determine the function of protein at the end and this process called knock out ,they are costly and takes a long time. In small interfering RNA, you can see the mRNA from which the DNA is produced and in the gene itself instead of isolating it we can see the sequence of bases included in it what the M-RNA produced that will formed to synthesize protein, you design a small piece of this RNA to be complementary to the mRNA so after injection in the cell it will bind to the mRNA. if it is bond the M-RNA then it will not be able to go to ribosome and get expressed ==> so no protein formed ➔ u can study effect while no protein is there this procedure is called knock down .. so any mRNA produced we bind it to other complementary RNA and it make for them degraded without completing the process of protein synthesis .

Light absorbance of nucleic acids

The peak absorbance is at 260 nm wavelength & it is constant

dsDNA: A 260 of 1.0 = 50 ug/ml

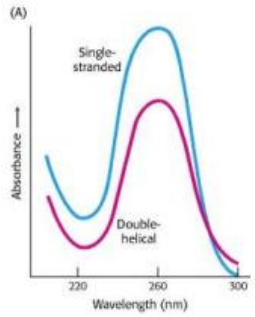
 ssDNA: A 260 of 1.0 = 30 ug/ml

 ssRNA: A 260 of 1.0 =40 ug/ml

So that means 1 Absorbance unit of a double stranded DNA = 50 ug/ml

While 1 Absorbance unit of Single stranded DNA = 30 ug/ml

Finally 1 absorbance unit of single stranded RNA =40 ug/ml



*+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

Analysis of numbers . (.1) is the is the Absorbance reading from the spectrophotometer . (10) is the dilution factor . since it is Double stranded in the question so 1 unit absorbance = (50) ug in ➔ Concentration = Dilution factor *Absorbance reading * 1 abs unit or (standard conc) of wanted nucleic acid from the sample .

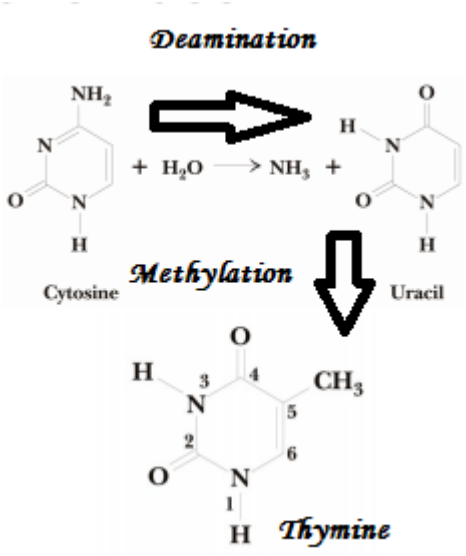
DNA and RNA Differences

1-Thymin in DNA vs Uracil in RNA

Why is it Uracil in RNA and Thymine in DNA?

* Cytosine can be converted to uracil through deamination reaction (Removing NH3 group) ,deamination of cytosine will form Uracil. That is considered to be a kind of mutation in DNA. now, suppose there is uracil in DNA just like RNA , repair enzyme come and remove Uracil and put cytosine instead. If DNA contains Uracil in origin the Repair enzyme will not differentiate between the Normal Uracil or the Uracil come by mutation and it will keep on replacing it with thymine.

*thymine is produced by methylation of uracil .so thymine is modified Uracil . Repair enzymes just look for unmethylated Uracil



2- Sugar in DNA is Deoxy while in RNA is Ribose .

What the difference btw them: DNA sugar is not oxygenated while the

ribose in RNA is oxygenated

How do deoxyribose in DNA correlate to it's function , the ribose of RNA ,either?

-OH groups (2' and 3') in RNA make it more susceptible to hydrolysis reaction more than DNA which lacks 2'-OH that serves (stability) for it because it make the genetic material and we don't need it to be degraded and going into reactions as what's happened with RNA . always More functional group mean more undergoing reaction .

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Protein structure and fuction:

* 50 % of body's dry weight is protein indicates their importance.

* Wide range of different functions in relation to type. (refer to slides to get more example for each type of protein and it s function.)

* Proteins are polymers made of monomers called amino acids

* Amino acids consist of backbone and side chain. In nucleic acids the backbone is Phosphate with the pentose sugar while the side chain is the N- Base. In Amino Acid the backbone consist of α carbon connected to carboxylic group, amine group, and to hydrogen. The side chain is the R group which makes the distinct amino acids. The amino acids obtained by hydrolysis of proteins differ in respect to R (the side chain)

• The properties of the amino acid vary as the structure of R varies

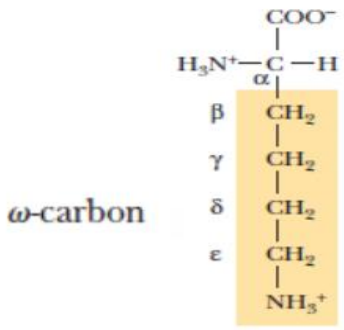
*Thee are lot of amino acids but we got just 20 naturally occurring amino acids in humans can be found in human proteins .

* AAs are 3-D molecule we do represent them in 2-D by α carbon with 4 different groups (chiral Carbon). it can make stereoisomers. -L and -D isomers because since we got chiral carbon . All Naturally occurring AAs in Human are of the L type. Remember: the Carbohydrates naturally occurring are of the D-type.

* D-L configuration make s a differencnce since stereochemistry add complication to function generally. e.g Carvon

* if it in L-type it is found in spearmint it gives the smell and taste of it .But in carawy it is D and it do the same with it .

*we start naming the carbon in R group of AA from the α carbon (α>β>γ>δ>ε) but the last carbon always named Omega .



**Names and codes of AAs:

- You have to identify the structure of all 20 AAs .
- Common question: structure contains more than amino acids connected to each other by peptide bond . you have to identify each amino acid in the oligopeptide or the poly peptide .
- Refer to slide for the 3 letter coding of amino acids

*How Amino acids are classified?

We classify AAs according to R groups in addition we can classify them according to the polarity through the R groups.
* can we differentiate between polar and non polar molecules? Polar Should have Strong electronegative atom in its structure including O,N, And S . They give strong electronegativity which can bind hydrogen of other molecules .the Non polar they are mostly composed of hydrocarbon content or ring structure (hydrophobic) any ring structure is hydrophobic in its nature unless it is attached with hydrophilic molecules. so there is a hydrophilic part of it and the other is hydrophobic.

- The R Groups are divided into Polar or non polar , the polar also divided into charged and uncharged . In addition the Charged subdivided into (+vely charged (basic) and (-vely chaged AA (Acidic)
- Negatively charged AAs are 2 :
- 1-Aspartic aicd(Asp) (2c) and Glutamic acid (3C)
- 2-=eve;y charged (Basic) : arg(3 Ns inside chain) ,His(5 membered ring 5 with +ve charge so easy to be spotted) ,Lys(1 N in R) ((refer to slides for the structure

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