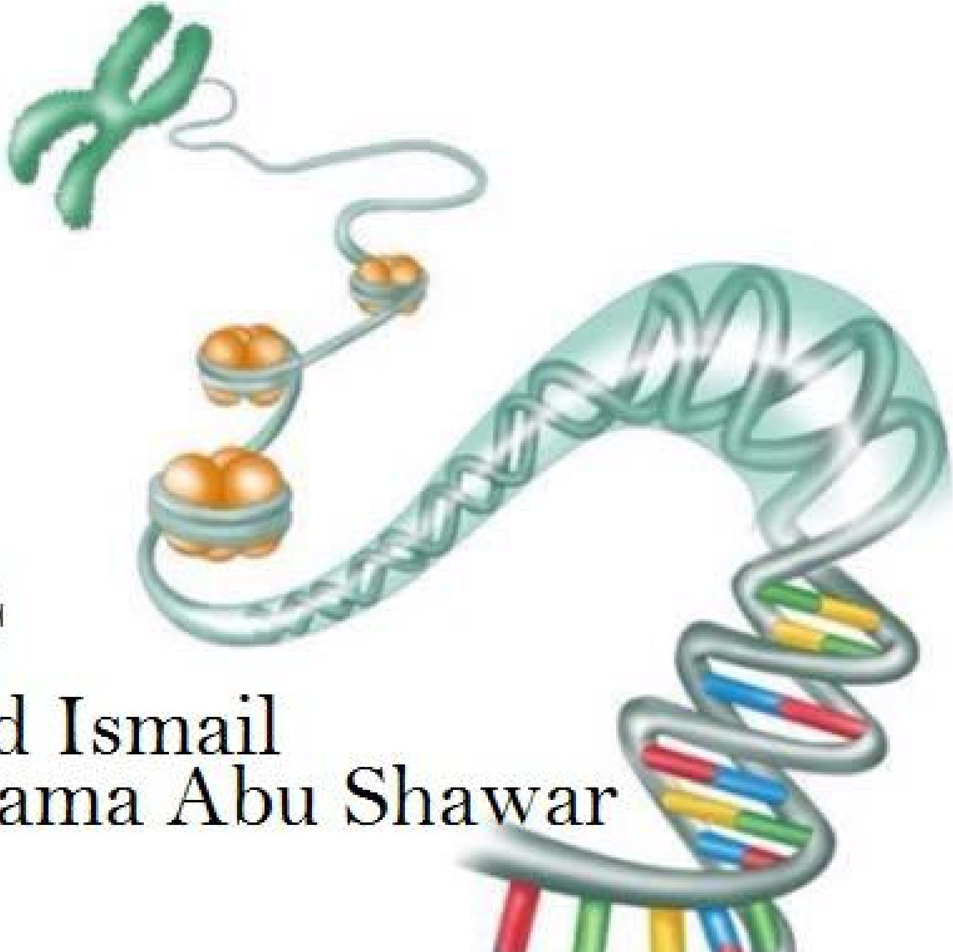
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other..................



Lecture # 14
Title:
Dr. Dr. Said Ismail
Done By: Osama Abu Shawar
Date:
Price:

Designed by Nadeen Al-Freihat

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Finally, we have started the most enjoyable and interesting medical science….. "Molecular Biology".

****

Introduction

This course consists of three related sciences:
1. Cell biology: the study of the whole cell containing the nucleus and DNA.
2. Molecular biology: the study of DNA structure, function, expression, mutation and genetic engineering.
3. Genetics: the study of the patterns of human inheritance and the association between the mutations and human diseases.

There are two fields of Molecular biology:
1. Basic Molecular biology: The basics of DNA molecule (structure, replication, transcription, translation……etc)
2. Applied Molecular biology: genetic engineering (recombinant DNA technology), gene therapy and cloning.

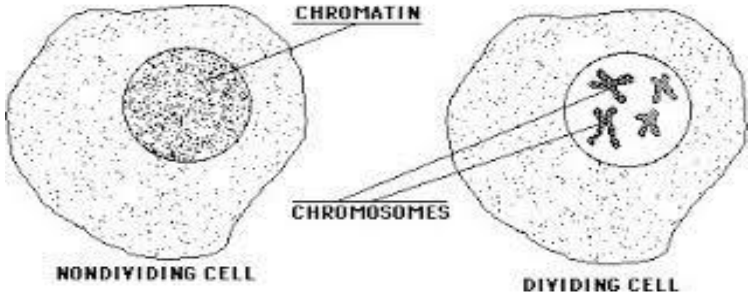
The doctor want to send some messages to us during the first lecture:
1. The behavior is separated from the genes, it associated with "nature versus nurture" debate.
If you wish:
Dr Walid fitaihi about "behavior vs genes":
https://www.youtube.com/watch?v=ws5IIpKZLEM
Dr Mohammed al-nabulsi about gays:
https://www.youtube.com/watch?v=_4yA6G0c1bc
2. All religions consider the cloning unethical issue. Simply, the cloning is possible but unacceptable.
3. Nobody around the world can tell you anything about the spirit because;
قال تعالى: (ويسألونك عن الروح قل الروح من أمر ربي وما أوتيتم من العلم إلا قليلا)

Nucleic acid structure:

In the past, there are lots of efforts and studies tried to discover the secret of the similarity between the offspring. In 1950, James Watson and Francis Crick discovered DNA, then the scientists can explain how these tiny molecule (DNA) is a responsible of the great complexity of the human when they discovered the codon.

Terminology:

Deoxyribonucleic acid (DNA): a carrier of genetic code (double stranded) made of phosphate , sugar and nitrogenous base
Ribonucleic acid (RNA): a mediator between DNA and protein (single stranded).
Chromatin: Unwound DNA condensed throughout interphase (period at cell cycle) to form chromosome.
Chromosome: A compact structure of nucleic acid (tightly packaged Parts of DNA).We have 46 chromosome each one of them contains a tightly packed part of the DNA .they are different in length for example chromosome Y is very small while chromosome 2 is huge .
Chromosome VS chromatin:



conclusion : if the DNA was condensed around histones and separated then it is a chromosome but If they were intermingled and mixed then it is chromatin .At most of the time the cell's DNA is in the form of chromatin and in a very short period of time during mitosis they become chromosomes

assume that the chromosomes are some balls of wool, and chromatin is the wool after unwinding and mixing these balls.
https://www.youtube.com/watch?v=4Jp9OxYxMVc

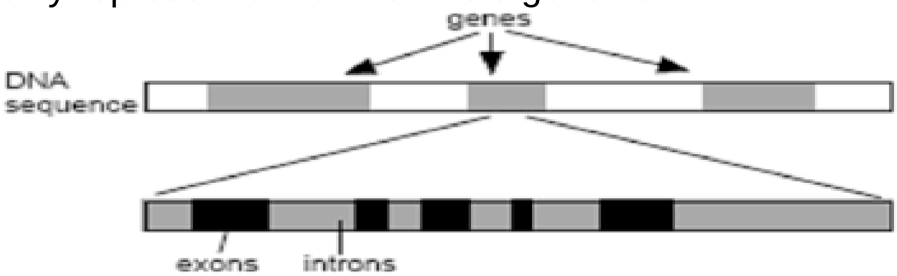
Gene: a sequence of DNA that encodes a specific protein.

genome: the whole genetic material of an organism (discussed later in this sheet).

A typical (diploid) human cell contains 46 chromosomes, whose total DNA is approximately 2 meter long! It is difficult to imagine how such a large amount of genetic material can be effectively packaged into a volume the size of a cell nucleus. To do so requires the interaction of DNA with a large number of proteins. So, DNA is associated with tightly bound proteins called histones.

Coding and non-coding region in DNA:

The coding regions in our DNA make 30%of the whole genome , the rest (70%) represent non-coding region. Surprisingly The non-coding regions can also exist within the coding regions (within the 30 %) and they are called introns, they make 25%, which are even higher in number than the coding ones (called exons) so the coding genes that are transcribed then translated only represent 5% of the whole genome !!



This junk DNA (non-coding region) function is unknown. Some say it's the result of accumulation of our evolution, but knowing how efficient our cells are in maintaining everything (the cell doesn't spend as much as 1 ATP without a proper reason) it's not likely that they'll keep as much as **95%** of DNA for no reason!!!.

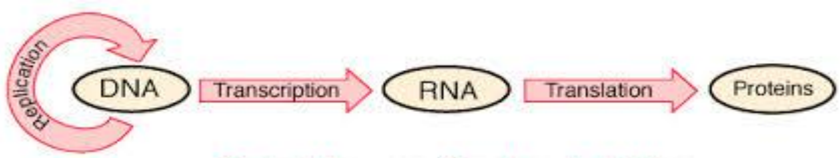
How did scientist explain the presence of the non-coding genes ? they explained it through the theory of evolution stating the human started from a bacteria then he became an algae so the bacterial genes were not necessary and became non-coding then we became fishes then reptiles then humans so we accumulated genes that are not necessary and became non-coding .This is absolutely non-sense because there is a type of worm it has junk DNA even more than humans so <u>it's not true</u> that as we go up in the ladder of evolution the junk DNA becomes more .

Most likely, these non-coding regions have another huge function that's unknown to us yet. As far as we know, DNA is a template that is translated to RNA to make protein, so another function can exist without our knowledge.

Our genome is still a mystery. Our genes (coding regions) make 5% of our DNA (30%, if you count the introns) and most of their functions are unknown.

If you still confused; assume that our DNA is a huge reference book contains 100 pages as meaningless introduction (junk pages=non-coding), then this book contains 10 pages about the basics of this science (coding), then contains other 100 pages about the failed experiments (junk pages=non-coding)…..etc

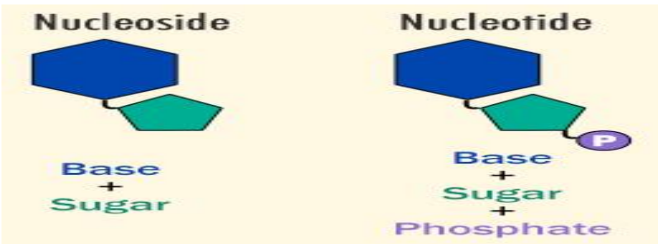
Central dogma of biology:



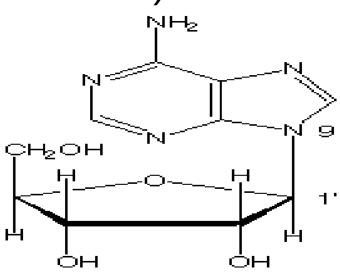
Central dogma of molecular biology

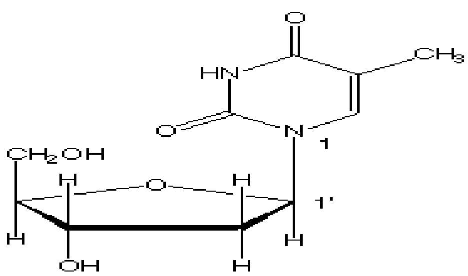
DNA is a valuable molecule, that can't exit from the nucleus so it is transcribed (copied) into RNA that is then translated in cytosol into functional proteins then RNA is degraded and that's a central dogma of biology.

Nucleotides:



Nucleoside consists of nitrogenous base and ribose "in RNA" or deoxyribose sugar "in DNA" linked together by glycosidic bond. The glycosidic bond formed between 1' carbon of the ribose sugar and N1 in pyrimidine or N9 in Purine (see below) .

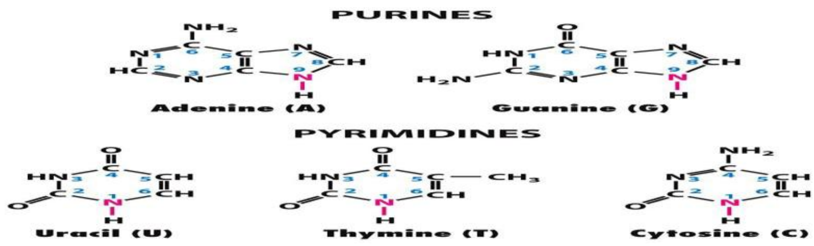

Adenosine


Deoxythymidine

Nucleotides are the monomeric (building) units of nucleic acids (DNA & RNA). Each nucleotide consists of nucleoside (base & sugar) and phosphate.

We can distinguish DNA from RNA by checking the 2' carbon in the ribose sugar. If it has (OH) then it's RNA. However, if it has (H) then it's DNA.

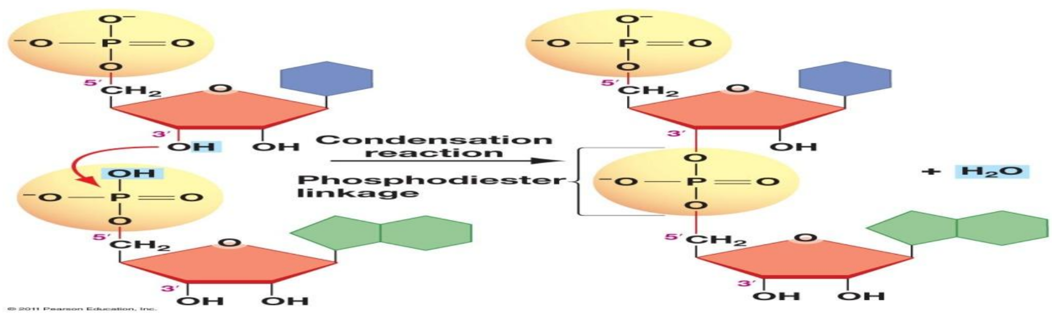
There are two types of nitrogenous bases:
Purine (double ring): adenine (A) and guanine (G).
pyrimidine (single ring): cytosine (C), thymine (T) and uracil (U).
DNA contains the Purine bases (A & G) and the pyrimidine bases (C & T) not uracil.
RNA contains the Purine bases (A & G) and the pyrimidine bases (C & U) not thymine.



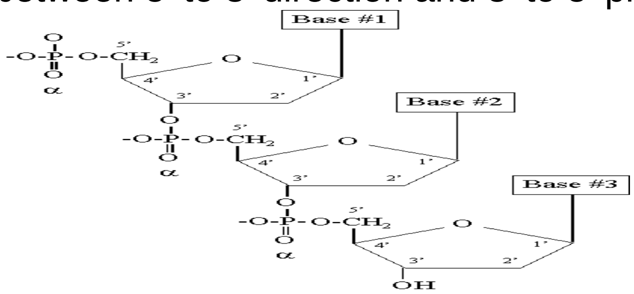
Phospho-di-ester bond:

DNA and RNA are linear sequences of nucleotides linked by 3' to 5' phosphodiester bonds. The phosphodiester bond is a strong covalent bond join 3-hydroxyl group of deoxyribose sugar of one nucleotide to the 5-hydroxyl group of deoxyribose of adjacent nucleotide through a phosphoryl group.

The nucleotides are joined together by a condensation reaction that releases pyrophosphate (two phosphate bound together), when pyrophosphate is cleaved, a big amount of free energy released and that's why this reaction start with tri-phosphate nucleoside but the mono-phosphate nucleoside is incorporated in the nucleic acid strand. The phosphate group has a negatively charged so it has a strong acidity and that's why it is called a nucleic acid.



What is the difference between 5' to 3' direction and 3' to 5' phospho-di-ester bond?



Now, assume that this (see above) small nucleic acid consist of just three nucleotides, look at its first nucleotide; you'll observe that its sugar bind on 3' carbon by ester bond with phosphate that also bind with 5' carbon on adjacent nucleotide by another ester bond; so it is called 3' to 5' phospho-di-ester bond.

Now, look again at first nucleotide you'll observe that it binds on its 5' carbon with free phosphate (unbound phosphate), now look at the last nucleotide, you'll observe that its 3' carbon bind with hydroxyl (OH) not phosphate; so any nucleic acid has the 5' to 3' direction. Also, the polymerization (building) process follows 5' to 3' direction.

Conclusion:……..when we said 3' to 5' phospho-di-ester bond, we mean that phosphate group bind with 3' carbon on the previous nucleotide and with 5' carbon on the following nucleotide, while when we said 5' to 3' direction we mean that (in any nucleic acid) first nucleotide has a free 5' phosphate and the last nucleotide has a free 3' (OH) group. Also, each strand has distinct charged 5' and 3' end, thus has polarity.

When you see TGAC, you should know that T is a 5' end while C is a 3' end (5' to 3' direction).

Nomenclature (VERY IMPORTANT):

Base	Nucleoside	Nucleotide
Adenine	Adenosine (Deoxyadenosine)	Adenosine mono\di\tri phosphate (dAMP\ dADP\ dATP)
Guanine	Guanosine (Deoxyguanosine)	Guanosine mono\di\tri phosphate (dGMP\ dGDP\ dGTP)
Cytosine	Cytidine (Deoxycytidine)	Cytidine mono\di\tri phosphate (dCMP\ dCDP\ dCTP)
Uracil	Uridine	Uridine mono\di\tri phosphate
Thymine	Deoxythymidine	Deoxythymidine mono\di\tri phosphate

DNA double helix:

DNA is a double-helix and has two strands running in opposite directions. Each strand is a polymer of subunits called nucleotides.

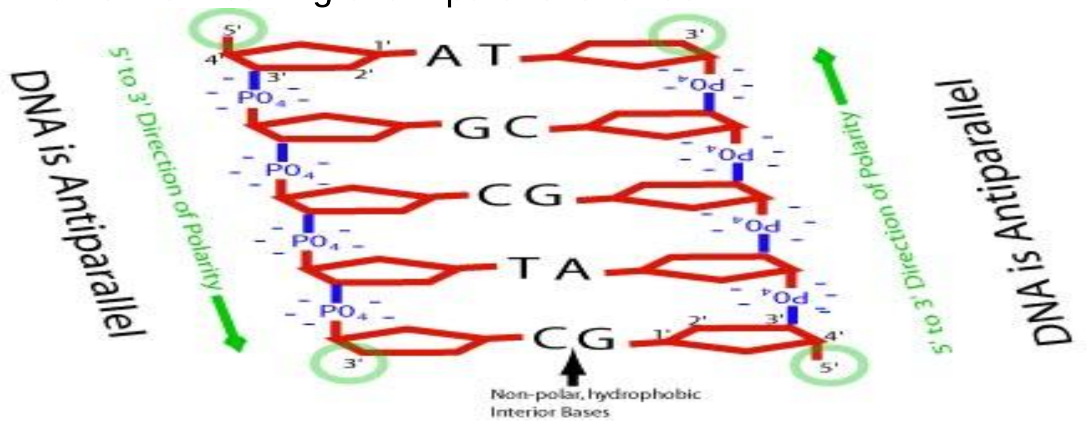
Each strand has a **backbone** made up of (deoxy-ribose) sugar molecules linked together by phosphate groups. So, each DNA contains the same backbone (deoxyribose + phosphate) but they differ from each other by the nitrogenous bases.

Base pairing (Chargaff's rules):

In the double-stranded DNA, the bases pair up; such that A always pairs with T and G always pairs with C. So, the two strands are complementary regarding the nitrogenous bases (A with T, C with G). So, in any double-stranded DNA; A=T and G=C (Chargaff's rule).

The A-T base-pair has 2 hydrogen bonds and the G-C base-pair has 3 hydrogen bonds. The G-C interaction is therefore stronger than A-T. So, the two strands of DNA bind to each other by hydrogen bonds (just).

What is the meaning of antiparallel strands?



The figure above showing the 2 anti-parallel strands. Being parallel means; they're side by side and having the same distance between them. **Anti** each other means run in opposite direction that the 5' end of one strand is paired with the 3' end of the other strand. Simply, look at the top of the figure, you'll observe that the left strand contains 5' end while the right strand contains 3' end, so they run in opposite direction.

DNA strands don't stay straight, the double stranded will take a helical shape (be twisted as زنبرك) they can take a right-handed (clockwise) or a left-handed (anti-clockwise) rotation. Most of our DNA, as humans, is **right-handed.**

There are three major structural forms of DNA; B form, A form and Z form. What is the difference between these structures?

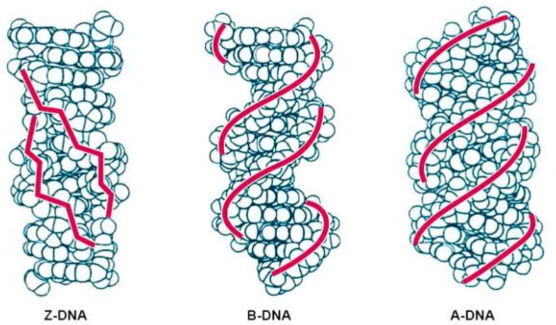


FIG. 12.9. Z, B, and A forms of DNA. The *solid red lines* connect one phosphate group to the next. (Modified from Saenger W. *Principles of Nucleic Acid Structure*. New York, NY: Springer-Verlag; 1984:257–286.)

DNA can be in the B, Z or A form depends on how compact the double helix is; that means DNA might be like an expanded spring "زنبرك مشدود"; its rings can be far from each other or like compressed spring "زنبرك مضغوط"; its rings compacted together.

Z is the most compact, then A, and B is a loose; this is determined by measuring the distance between one turn and the next. For example, in B form, the distance is 3.4 Angstrom between each turn and the next. All these forms are found in nature, but our DNA is in the B form.

DNA characteristics:

DNA Denaturation (splitting of two strands):

The two strands of DNA separate when hydrogen bonds between the paired bases are disrupted. Disruption can occur by heating or changing PH of DNA solution. DNA denaturation doesn't affect the tertiary structure at all, it only breaks the hydrogen bonds between the two strands which is very **reversible.** It differs from protein denaturation, which means the loss of tertiary structure "unfolding of the protein". Protein denaturation is irreversible so polypeptides will precipitate in the solution and **never** reanture.

Denaturation by heating:

It's done by heating the DNA to split the two strands (by breaking weak hydrogen bonds) and reversed easily by cooling them (renaturation). This can be done millions of times and will always rejoin.

Denaturation by changing PH:

Another way of denaturation is by putting DNA in an alkaline solution (raising the PH of the solution). When PH is raised, the phosphate group (the acidic group in DNA) will tend to lose its protons into the solution to act as a buffer. This will lead to increase of the acidity by increasing the negative charges of the phosphate group (PO4-2). When these negative charges appear strongly on the two strands, they will rebel "تنافر" each other.

Hybridization:

Reversing the denaturation is known as hybridization. It is the reassociation of the two complementary strands. Not only the two sister strands, but can be applied also to any two different DNA fragments that share a complementary to bind to each other, even a complementary RNA can bind to the DNA by hybridization, DNA-RNA hybrid is a very useful in genetic engineering but isn't found in the nature.

Melting temperature:

When DNA is heated, the temperature at which one half (50%) of the helical structure is separated is defined as the melting temperature (Tm).

Melting temperature depends on:

1. The type of bases that DNA has; the more G-C, the higher melting temperature is. Because there are three hydrogen bonds between G and C but only two between A and T, DNA that contains high concentrations of A-T denatures at a lower temperature than G-C rich DNA.
2. The length of DNA; the longer DNA, the higher its melting temperature. (More bonds need more temperature).

Genomes:

A genome means all DNA content in the cell.

E. Coli genome vs. human Genome:

Humans	E. Coli
46 chromosomes	Single chromosome
Linear (has a beginning and an end)	Circular genome
Associated with histons. It needs to be wrapped around them for packing (it can reach 2 m without them.)	Exists on its own. It doesn't have any associated proteins 'cause its DNA is small (2 mm).
25,000 genes 6 billions nucleotides	3,000 genes 3 millions nucleotides
2 copies for each gene (We have 23 pairs of chromosomes; diploid cells)	A single copy for each gene (A haploid cell).
Long distances between each coding gene and the next (have introns)	All its genome is coding (No introns)

our human genome shared by 93% with mice and 98% with monkeys but they have extra chromosome (probably by splitting our second chromosome). by the way, Darwin said that we and monkeys are just cousins!!, there are lots of evidences against and with Darwinism.

قال تعالى:

(شهد الله أنه لا إله إلا هو والملائكة وأولو العلم قائما بالقسط لا إله إلا هو العزيز الحكيم)

......نهاية العلم.......التوحيد......

Summary:

-Nucleic acids (DNA and RNA) are assembled from nucleotides, which consist of three components: a nitrogenous base, ribose sugar (pentose) and phosphate.

-There are two types of nitrogenous bases commonly found in nucleotides: purines A & G (double ring) and pyrimidines C & T & U (single ring).

-The nomenclature for the commonly found bases, nucleosides and nucleotides is most likely to be tested material.

-Nucleic acids are polymers of nucleotides joined by 3' to 5' phospho-di-ester bond.
-Some of the features of double stranded DNA include:
The two strands are antiparallel (opposite direction).
The two strands are complementary A with T (two H bonds) and G with C (three H bonds).
There are 3 major structures of DNA (B, A & Z) forms, but our DNA is B form (right-handed).

-DNA can be denatured by conditions that disrupt hydrogen bonding resulting in the melting of the double helix into two single strands that separate from each other.
Heat and alkaline PH are commonly used to denature DNA.

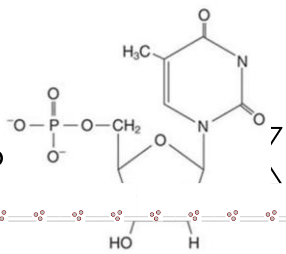
-Large DNA molecules must be packaged in such a way that they can fit inside the nucleus and still be functional, this occur by winding around histones.

<u>Quiz:</u>

Past paper Questions

- ✓ The glycosidic bond that exists in nucleosides is between:

A.3' carbon of sugar and N9 of adenine.
B. 1' carbon of sugar and N1 of guanine.
C. 5' carbon of sugar and N1 of cytosine.
D. 1' carbon of sugar and N9 of guanine.
E. 5' carbon of sugar and N1 of thymine.



✓ The following molecule resembles:
(A) Guanosine monophosphate
(B) Cytidine monophosphate
(C) Adenosine monophosphate
(D) Deoxythymidine monophosphate
(E) Deoxyadenosine monophosphate

Two questions from the recommended book (Mark's)

✓ The backbone of a DNA strand is composed of which of the following:
a. sugars and bases
b. sugars and phosphates
c. phosphates and bases
d. Nucleotides and sugars
e. nucleosides and phosphates

✓ In DNA, the bond between the deoxyribose sugar and the phosphate is which of the following:
a. A polar bond
b. An ionic bond
c. A hydrogen bond
d. A covalent bond
e. A van der waals bond

A question from Lippincott's review

✓ While studying the structure of a small gene that was sequenced during the human genome project. An investigator notices that one strand of the DNA molecule contains 20As, 25Gs, 30Cs and 22Ts. How many of each base is found in the complete double stranded molecule?
a. A=40, G=50, C=60, T=44.
b. A=44, G=60, C=50, T=40.
c. A=45, G=45, C=52, T=52.
d. A=50, G=47, C=50, T=47.
e. A=42, G=55, C=55, T=42.

A question need......."شوية حذاقه".......:P

✓ A medical student working in a great molecular biology laboratory at JU ☺ is asked by his mentor to determine the base composition of an unlabeled nucleic acid sample left behind by a former research technologist. The results of

his analysis show 10% adenine, 40% cytosine, 30% thymine and 20% guanine. What is the most likely source of the nucleic acid in this sample:
A. Bacterial chromosome
B. Bacterial plasmid
C. Mitochondrial chromosome
D. Nuclear chromosome
E. Viral genome

Explanations:

1 (D), **3** (B) & **4** (D): Easy, سهل (ester is a covalent bond)

2: you can answer the question easily after excluding three choices (A, C & E). Why? simply, because the figure showing single ring nitrogenous base (pyrimidine) while (A, C & E) suggest Purine base. Now, we have two choices (B & D), the figure showing deoxyribose sugar (not ribose). So, the answer is D.

5: the two DNA strands are complementary to each other, with A base-paired with T, and G base-paired with C. so, for example, the 20 As on the first strand would be paired with 20 Ts on the second strand, the 22 Ts on the first strand would be paired with 22 As on the second strand, and so forth. When these are all added together (20 As on the first strand + 22 As on the second strand= 42 As). Notice that, in correct answer, A=T and G=C. Answer is E.

6: A base compositional analysis oppose Chargaff's rule (%A=%T, %C=%G) so, it is indicative of single stranded (not double stranded) nucleic acid. All options listed except E are examples of double stranded DNA. Only a few viruses (Parvovirus; B19) have single stranded DNA. Answer is E.