CYTOLOGY

Premed 2018 - JU

◉Sheet ○Slides

Number

3 (Molecular bio.)

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RNA Molecule :

¶ It consists of long, unbranched chains of nucleotides joined by **phosphodiester bonds between the 3'OH of one pentose and the 5' PO_4^- of the next.**

¶ The pentose unit is beta- D -ribose (It is 2-deoxy-D-ribose in DNA).

¶ The pyrimidine bases are **uracil** and cytosine (thymine and cytosine in DNA).

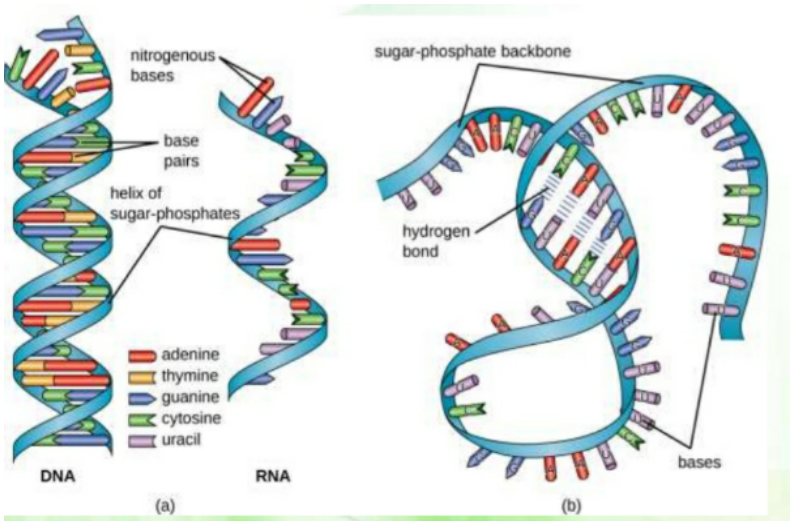
¶ in general, RNA is single stranded (DNA is double stranded) .

¶ **RNA does not have a precise structure, but it can fold on itself forming hydrogen bonds within the same Molecule.**

It can form a specific 3D structure by making a specific hydrogen bonds in each type of RNA Molecule.

-->> NOTE :

RNA MAINLY is single stranded in our cells, but we can find it as a double strand.



Types of RNA :

¶ Different types of RNA molecules **do not code for proteins but they have really important functions on cells.**

¶ we have a large RNA molecules like : **ribosomal RNA (rRNA), Transfer RNA (tRNA), small nuclear RNA (snRNA) and small nucleolar RNA (snoRNA).**

And we have small non_coding RNA (small ncRNA) like : **mRNA, siRNA, piRNA and long ncRNA.**

NOTE: the doctor said that we dont have to memorise all the schedule co'z later on we will take it in details.

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

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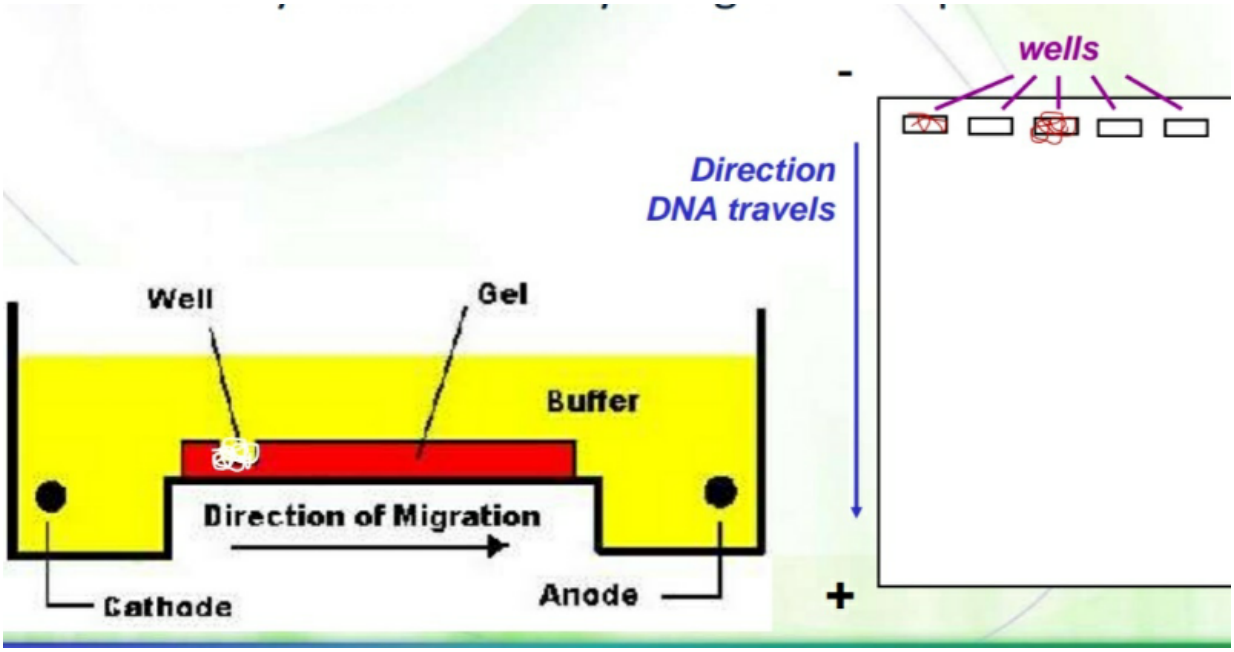
 # we have 2 techniques to study DNA :

Gel electrophoresis

(*electro* means by electrical current, *phoresis* means separation).

¶ The length(size) & purity of DNA molecules can be accurately determined by the gel electrophoresis.

We use this technique to seperate Different sizes of DNA fragments through a gel by an electrical field.



The idea is :

1_We put a Different samples of DNA in wells that we have made in a gel

(it means that in the first well we can add one sample of DNA and another sample in the next well)

Pay attention that one sample contains **thousands or millions of DNA fragments Not** one Molecule of DNA.

2_ then we expose the gel in an electrical field and the DNA fragments will move through the gel from cathode (- ve charge) to anode (+ve charge)

Why?? Co'z DNA fragments are negatively charged.

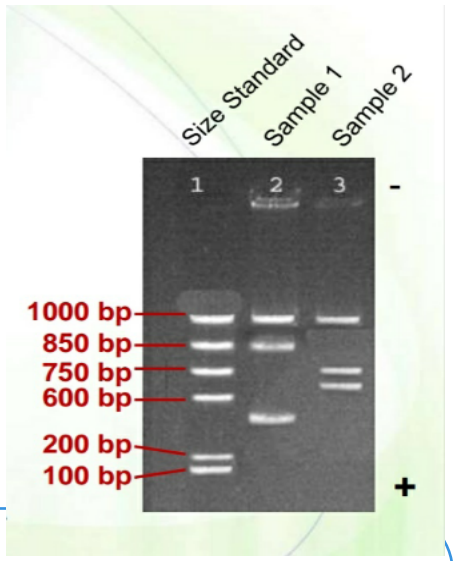
3_ the DNA fragments will move according to size

The small DNA fragments move faster and the large fragments move slower

In other words, the small fragments move long distance than the large ones.

(the gel contains a network inside it that keep the larger fragments in)

4_ we compare the DNA samples with **size standard** (which is a sample that contains DNA fragments of certain sizes and you know what the sizes are), and we use it to determine the length of the examined DNA Molecule .



bp : base pair

Important note :

The DNA runs as bands, each band does'nt contain one DNA Molecule, it contains millions of DNA fragments of the same size (they can be of same or Different type),,, they mix togather, run as same speed and stop at same point,, also, they get stained togather.

** from this technique, we get benefit of two things :

1- Does the sample contains DNA fragment?

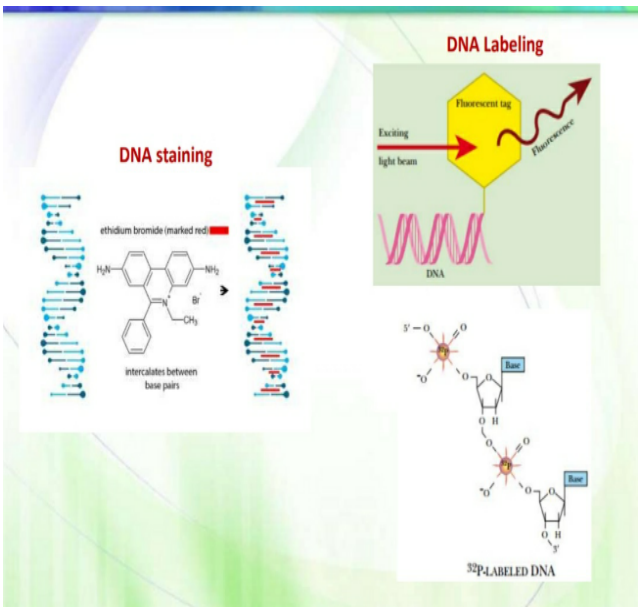
2- what are the sizes of DNA fragments?

But we can't know the types or sequences of DNA fragments.

Differences between staining and labeling

DNA staining :

Means colour it by adding chemicals that interact with DNA and this chemicals give a colour (painting), Like : ethidium bromide.



DNA labeling "for observing DNA" :

By adding fluorescent tag that seems like a lamp on DNA that flashes a light (DNA lights up) or using radioactive phosphorus (^{32}p) (DNA emits radioactivity).

**** Denaturation of DNA means that DNA loses the non covalant intermolecular forces (hydrogen bonds between the 2 strands) so it loses the structure.**

*** if we cool the sample of DNA single strands it will return to its original structure and that depends on base pairing and complementary to each other, we call it " Renaturation".**

Hybridization :(something that forms from 2 different things)

¶ DNA from different sources can form double helix as long as their sequences are compatible (hybrid DNA) " complementary base pairing".

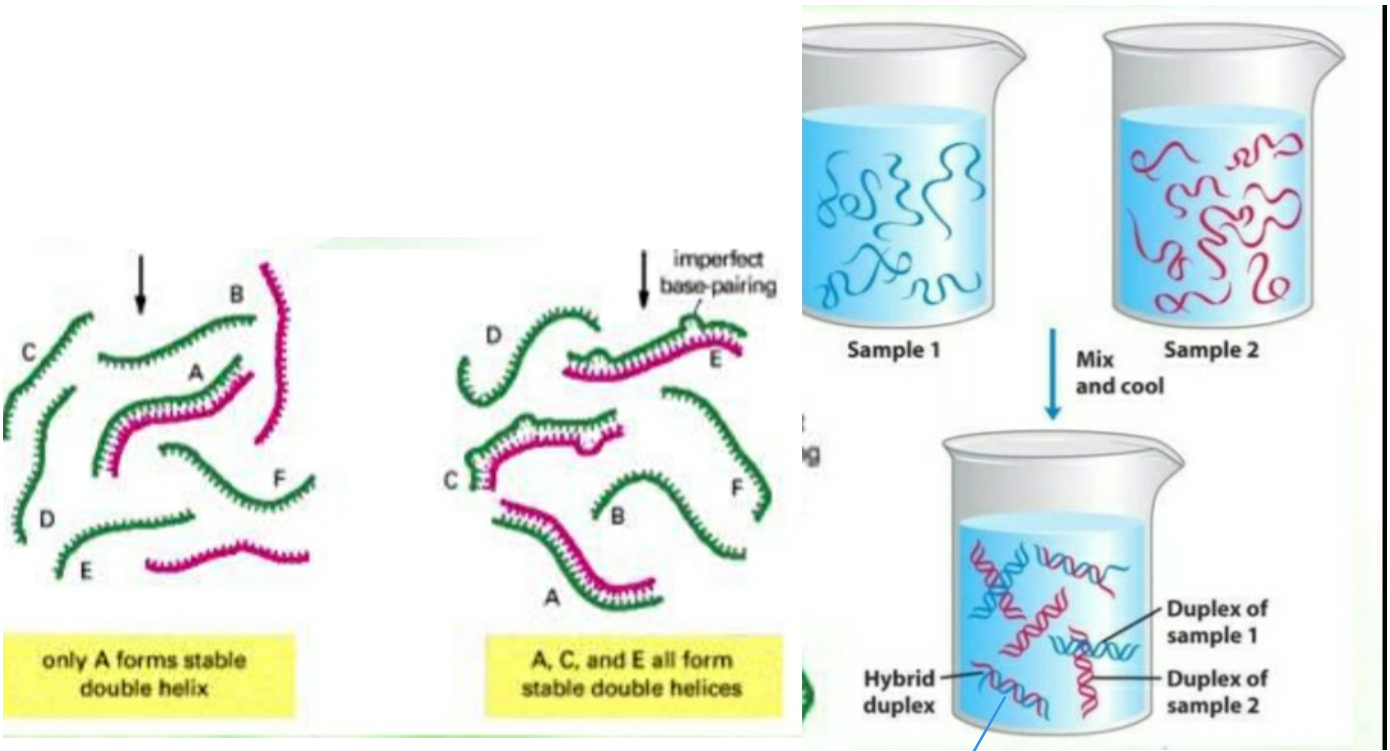
¶ Hybridization can be imperfect.

¶ Hybridization reactions can occur between any two single stranded nucleic acid chains provided that they have complementary nucleotide sequences.

¶ Hybridization reactions are used to detect and characterize specific nucleotide sequences.

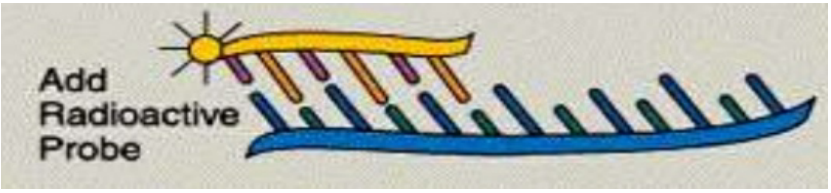
****(as long as they have enough hydrogen bonds we can conform the hybrid).**

**** we can control with specific conditions to allow the imperfect Hybridization to take place or to prevent it to take place by changing the temperature, ionic strength of solutions, GC content, etc.**



here, the presence of hybrid duplex co'z the diff. Strands are complementary

Probes



** a prob is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence. (20 nucleotides)*

** Hybridization reactions use labeled DNA probes to detect larger DNA fragments.*

** The idea is :

Firstly, we label the prob, we can add radioactive Molecule or fluorescent tag and wherever it goes we can see the flash light and knowing the exist of a prob.

We use millions of probes of the same sequences and we add them to DNA sample.

And because the hydrogen bonds are reversible (they're non covalant), they form, detach and reform, so it will be a competition between a prob and the two strands in forming hydrogen bonds between each other, because there are more prob Molecules, they will win in a competition, the region that have a sequence that is complementary with a prob would form double strand interaction with prob, but doesnt mean that the whole DNA is single strand, only small region in DNA strand will be hybridazed by a prob.

If there's a signal that exists, it means that there's a Hybridization between prob and DNA strand (they're complementary).

Good luck :)