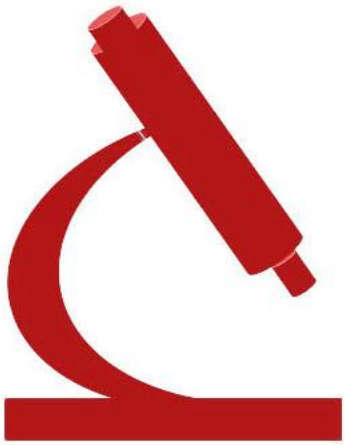

CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

2 (Molecular Bio)

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Last thing we talked about was **Nucleic Acid Polymers**

- When **elongating** the DNA strand, we add free nucleotides to **C number 3** of the **pentose sugar** of the **last nucleotide** at the 3' end of the DNA strand.
- There is polarity due to presence of two ends, 5' and 3'.

- **Ribo-** in the term "Ribonucleic acid-RNA" indicates that it consists of Ribose sugar.

- **Deoxyribo-** in the term "Deoxyribonucleic acid-DNA" indicates that it consists of deoxyribose sugar.

- **Oligo-** in the term "Ribooligonucleotide" means **Short.**

  - **Oligonucleotide** is a short polymer that consists of 3-10 nucleotides (monomers).
  - **Oligopeptide** is a short polypeptide that consists of 3-10 amino acids (monomers).
  - **Oligosaccharide** is a short polysaccharide that consists of 3-10 monosaccharides (monomers).

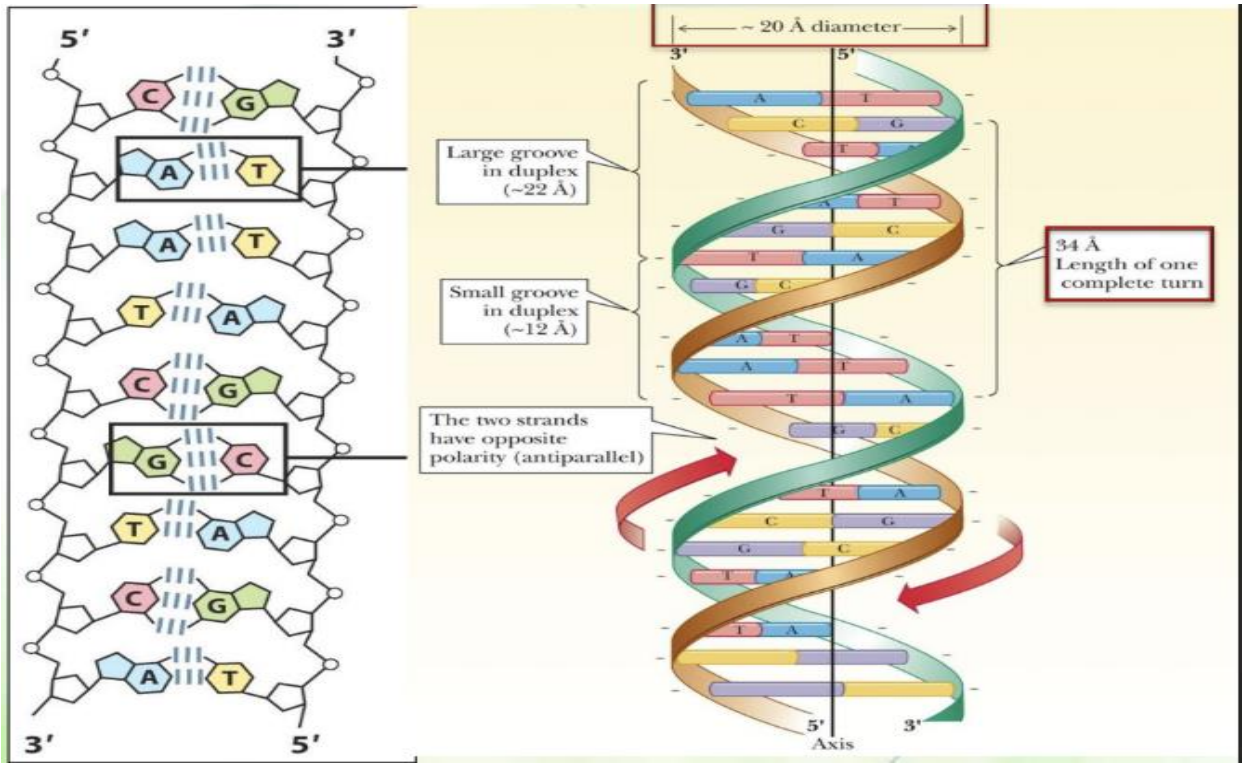
- A letter **d** can be added to indicate a **deoxyribonucleotide residue.**
  - for example, dG is substituted for G.
  - The deoxy analogue of one example of a ribooligonucleotide would be d(GACAT).

- **DNA Structure:** first discovered by scientists Watson and Crick

Characteristics of DNA:

- Antiparallel
- Stable
- Flexible
- Grooving
- Stability vs. flexibility
- Specific base-pairing
  A = T; G = C; Pur = pyr
- Complementary
- A double helix
- Backbone vs. side chains

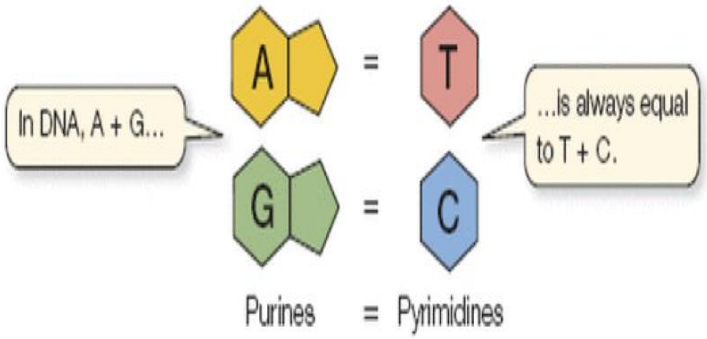
Watson and Crick drew two structures in their scientific journal, one of them was drawn by hand. This structure is the structure of DNA that we know **(figure shown below)**



Chargaff, the scientist who analyzed the DNA molecule and broke it down.

He thought of the following rules that are called (**Chargaff Rules**) which state:

- Number of A = Number of T
- Number of G = Number of C
- Number of Purines = Number of Pyrimidines doesn't necessarily mean A+T=G+C.
  It means A+G=T+C

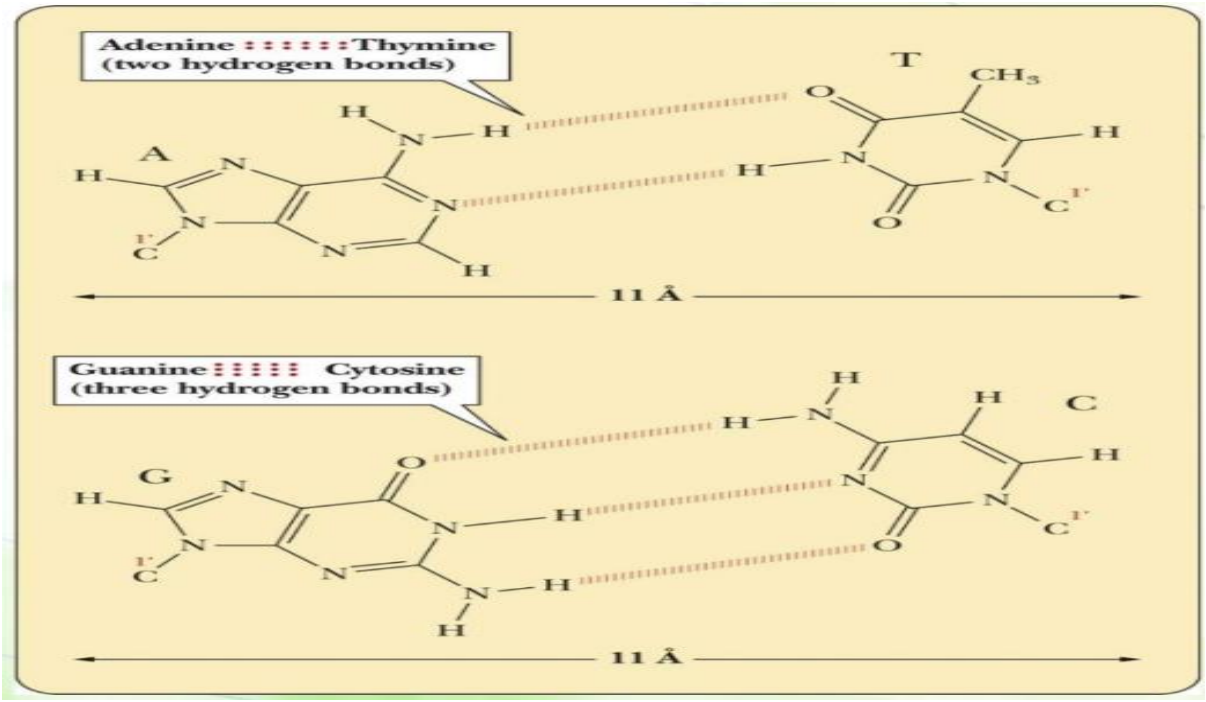


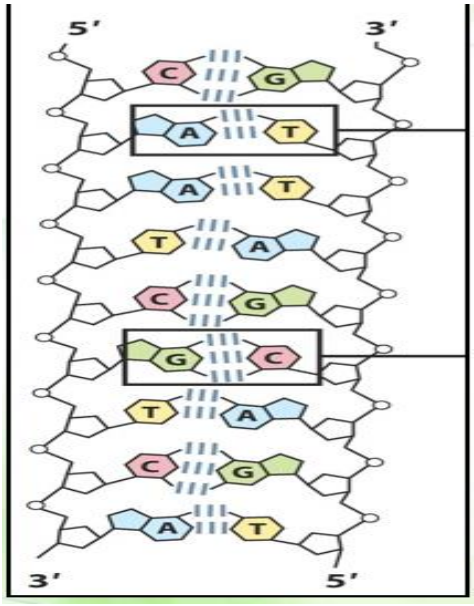
➢ **DNA is a double helix:**

Made up of two strands that appear to be twisted in a helical patter, forming a "duplex"

➢ **Base pairing:**

- A always pairs with T:
  forming **2 hydrogen bonds**
- C always pairs with G:
  forming **3 hydrogen bonds**





How is base pairing possible?

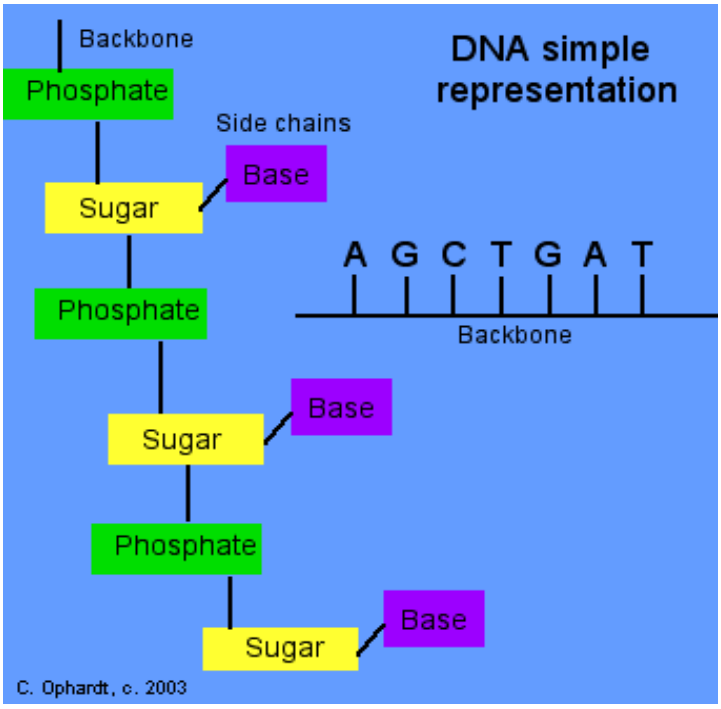
One strand of DNA must be **Complementary to the other,** which means base pairing is Complementary.

(A is complementary to T, G is complementary to C)

➢ **Backbone vs. side chains:**

DNA has a backbone and side chains (Like a tree and its branches)

- **The backbone:** Consists of linked phosphates and sugars.
- **Side chains**: Are the nitrogenous bases, and are ALMOST perpendicular to the backbone.



NOTE: bases are oriented/ pointing **inwards**.

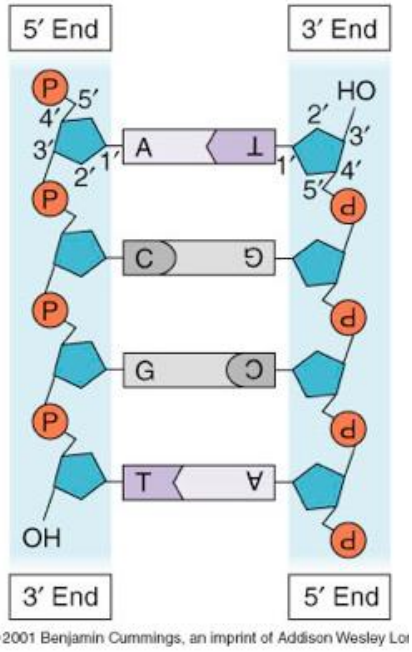
(Unlike the alpha helix structure in a protein, where amino acid side chains point OUTWARD)

➢ **Strands of DNA are *anti-parallel*:**

- One strand has the 5' end on top and the 3' end at the bottom
- The second strand has the 3' end on top, and 5' end at the bottom

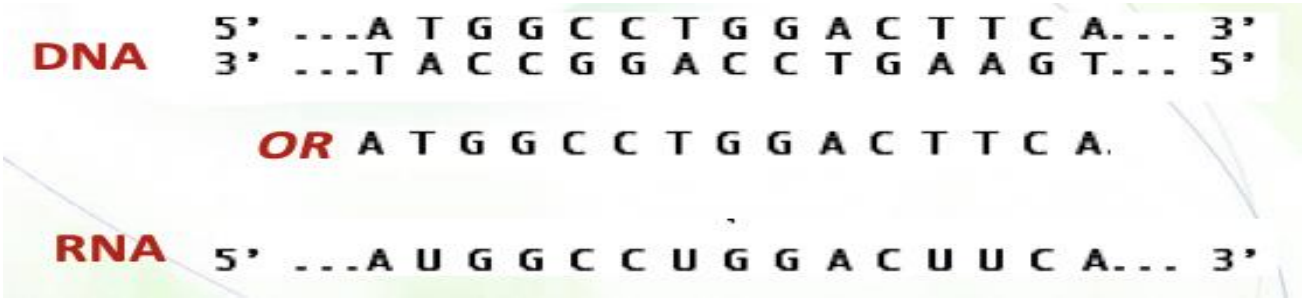
WHY is it antiparallel?

This is the most stable structure of DNA. It is the structure that requires the least amount of energy to keep it stable.



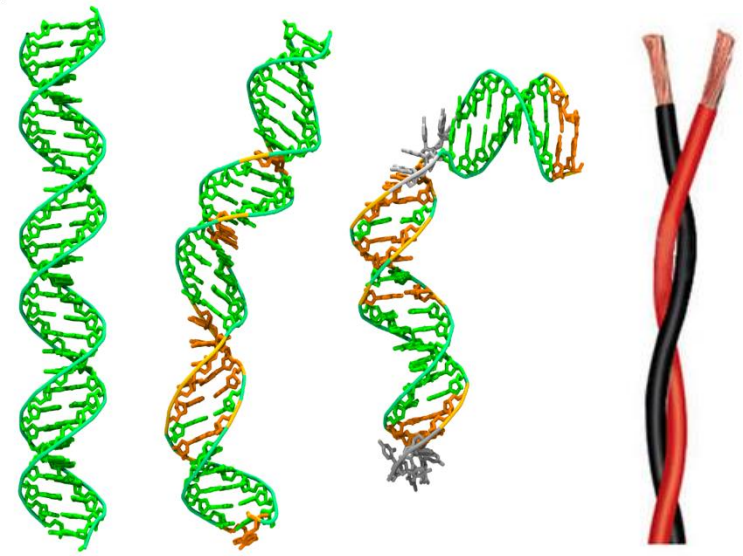
NOTE: We always read starting from the **5' end,** unless it's indicated that you must read it from 3', or are asked to read it starting from **3'.**

Figure above: both strands are read as **ACGT,** since they are read starting from 5'.



NOTE: The professor says he won't necessarily give you the sequence of both strands. He will only give you one and ask for the other. You must keep in mind that knowing the sequence of one strand, you can figure out the sequence of the second **COMPLEMENTARY** strand.

- **DNA is flexible, yet stable.**



DNA is stable although hydrogen bonds formed between bases are weak.

How are they stable?

The collection of noncovalent bonds (Hydrogen bonds, electrostatic interactions, hydrophobic interactions) all together makes DNA very strong.

Although they are strong, they are still flexible (Similar to electrical wires) **-figure above-** and are bendable (bended easily).

- **DNA isnt a perfect helix** due to the presense of bases sticking out.
  Due to that imperfection, DNA has two distinct structures:
  Major and Minor **grooves** .

  When DNA twists or rotates, the grooves rotate along with it.

Function of Major and minor grooves:

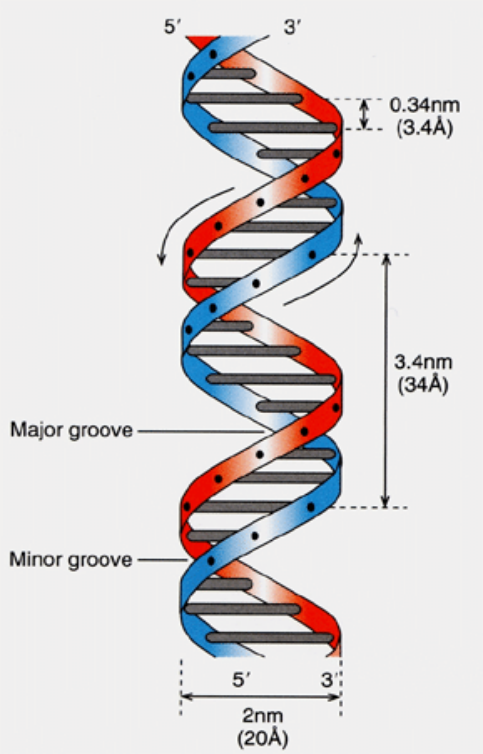
Allow interactions between DNA and proteins.

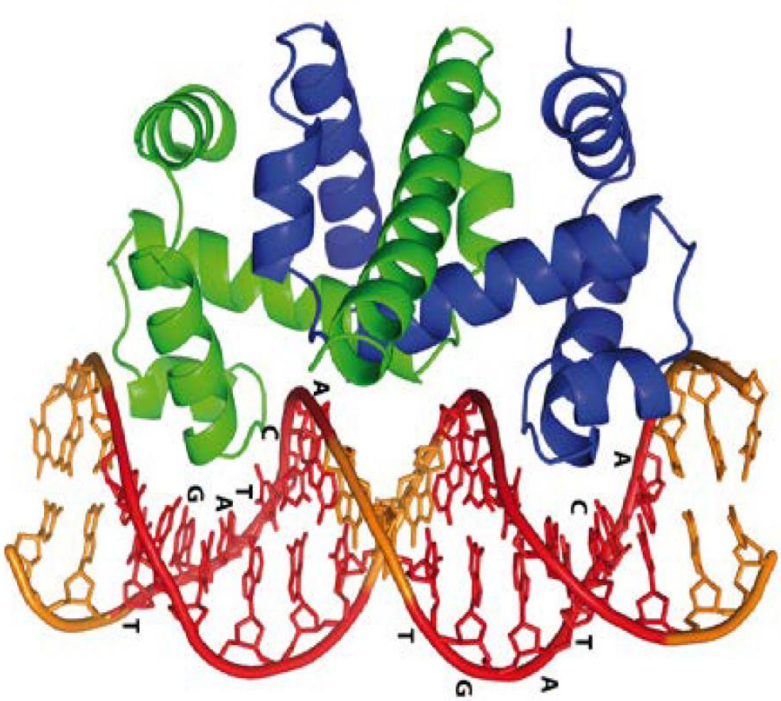
Major groove: Larger than the minor groove with bigger spaces

Minor groove: Smaller than the major groove with smaller spaces

- **How does the protein interact with DNA through grooves?**

Proteins have 2 "arms" to interact with DNA. One "arm" goes into a major groove, while the second arm goes into another major groove.





- **How do these interactions take place?**

Specific non-covalent interactions between protein's amino acids and bases of the DNA.

Specific: because the protein attaches to specific parts of the DNA and amino acids of this protein will only interact with a specific sequence of DNA.

- **Proteins prefer interacting with the major groove.** Why?
  1. Major groove is larger than the minor groove
  2. Major groove has more spaces
  3. Bases are more exposed in the major groove than in the minor

NOTE: No protein acts on its own. It must interact with another molecule (like DNA) in order to perform its specific function

- **DNA structure is Dynamic and changeable.**
  (there are different structures of DNA that have been discovered other than the structure we know)

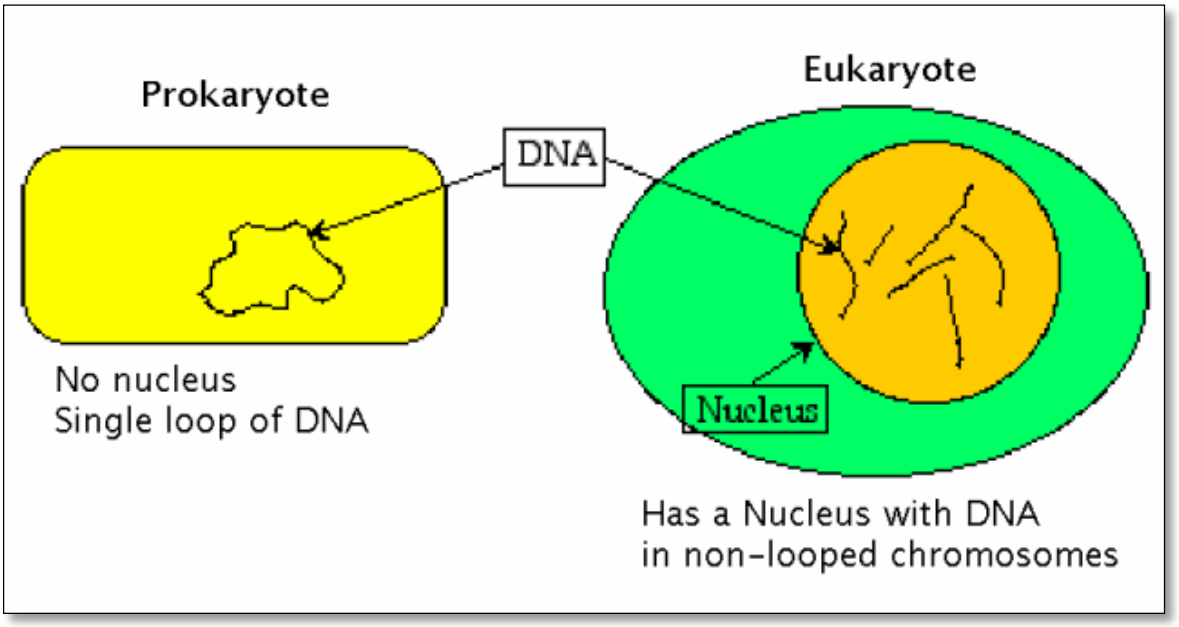
- **Human genome**

DNA is organized into linear Chromosomes, which are 46 In number (23 pairs of chromosomes)

  - Females: Have 22 pairs of chromosomes and 2 X chromosomes
  - Males: Have 22 pairs of chromosomes with 1 X and 1 Y chromosome

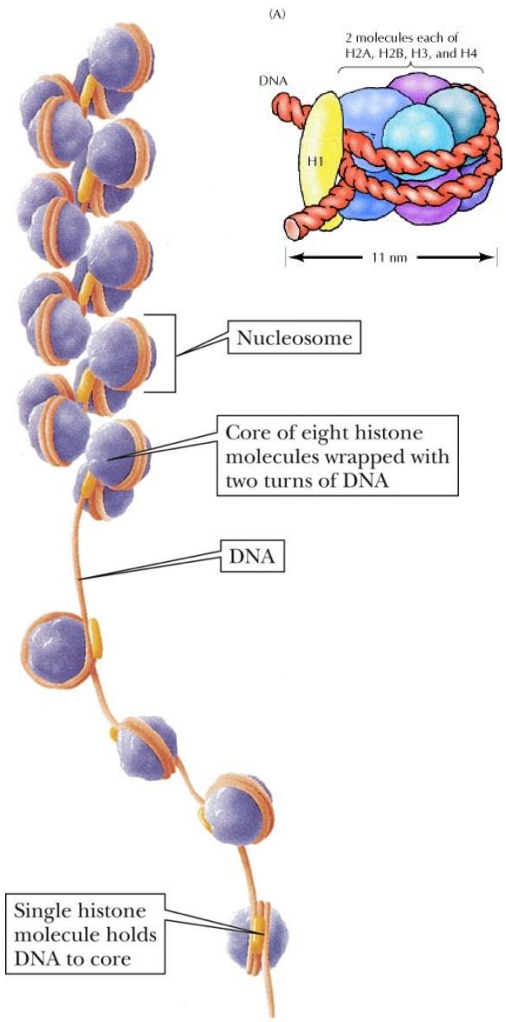
- **In prokaryotes:**

They only have a single loop of **circular DNA** in the cytosol (our DNA is located in the nucleus).



➢ **In eukaryotes:**

DNA is coiled to become more compact and is coiled or twisted around protein structures called histones, which package DNA.



➢ **Terms to keep in mind:**

Chromosome: Single unit of genetic material (highly condensed)

Chromatin: DNA + Histones

DNA: Double helix (double stranded).

Nucleosome: DNA coiled around histones twice + Histone + Linker DNA (free DNA that is not linked to histones)

Chromatosome: Histone + DNA coiled around it + H1 (will be explained below)

Octamer: has 8 subunits (ex: histone)

Types of protein duplets (2 units each) that make histone octamer:

1. H-2A
2. H-2B
3. H-3
4. H-4

NOTE: Histone binding to DNA isn't specific

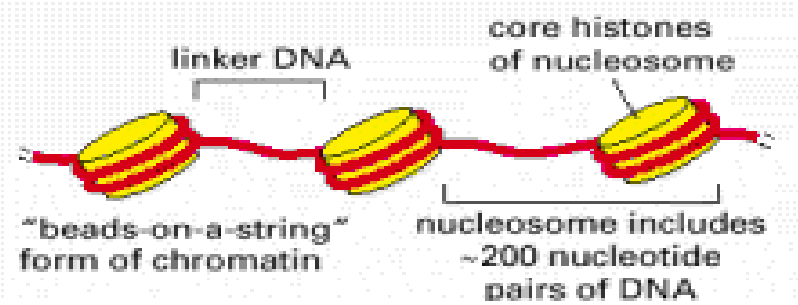
It only relies on the presence of negative phosphate groups, to neutralize the histone's positive charge.

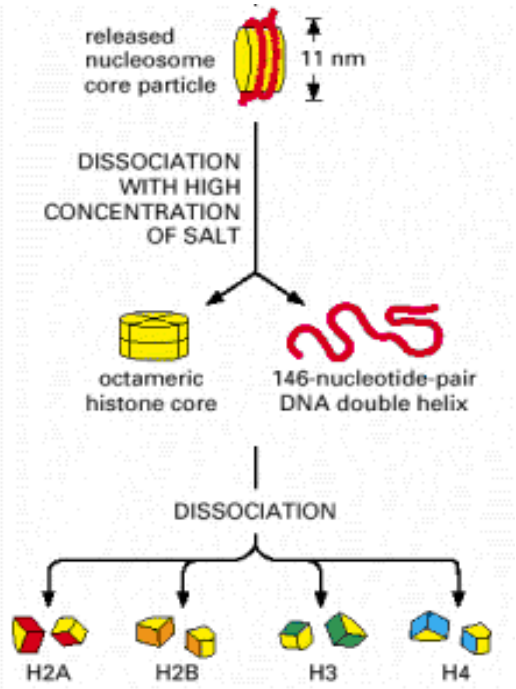
NOTE: Chromatin does not exist in prokaryotic cells as they do not have histones and DNA is not packaged there.

➢ **Why does it rely on phosphate groups?**

Histones have positively charged amino acids (Lysine and Arginine) and need the negatively charged phosphate to bind to.

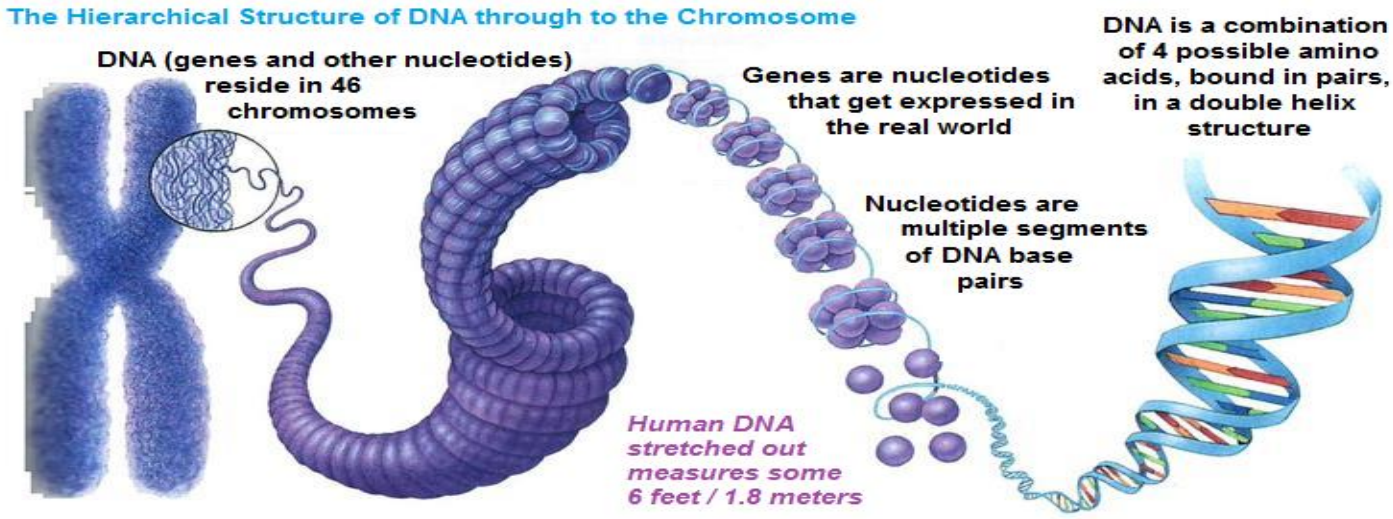
- A linker DNA/spacer region connects the octamer DNA complexes.





H1: Bound to the octamer and wrapped DNA,
forming the **chromatosome.**

- H1 is used as a lock to prevent flexibility of the octamer-DNA complex, and keeps it intact (متماسك)
- H1 maintains the DNA and makes it smaller or more compact to fit better in the nucleus (يضبطه)
- H1 locks the coiled DNA on the histone to maintain the structure

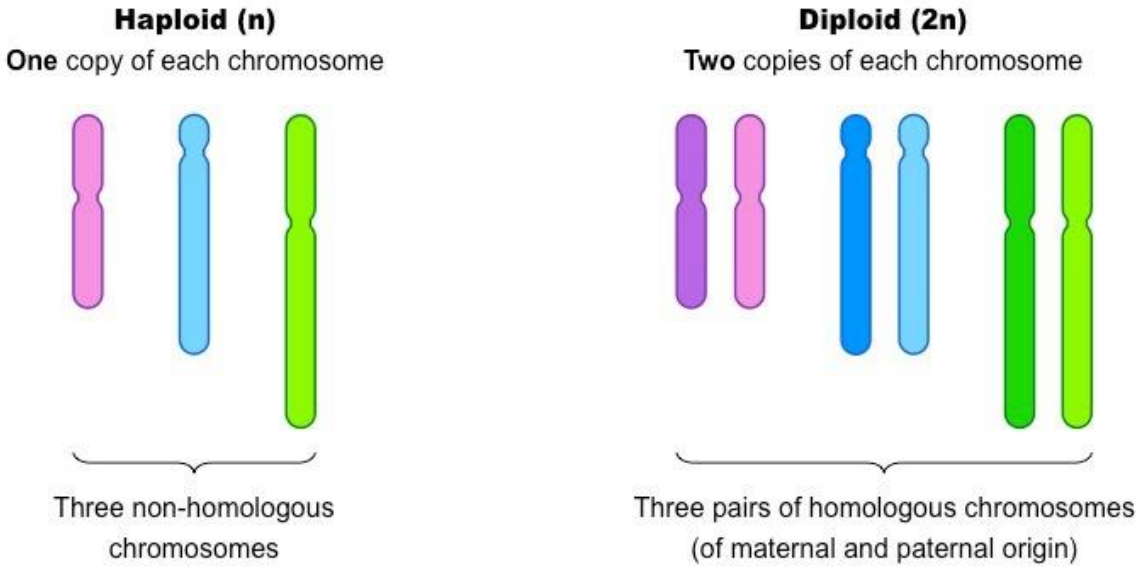


****In the figure above 'amino acids' should be changed to 'nucleotides'.**

➢ **REMEMBER**

- We are **diploid**.
- Some of our cells are haploid (Gametes which include sperm and egg)
- Our haploid cells become diploid once fertilization occurs/ takes place.

Homologous chromosomes: Two copies of the same chromosomes. (Homo: Same)



➢ **One property of DNA is that it can absorb light.**

How is it possible that DNA is colorless although it absorbs light?

- DNA absorbs color in the UV range, and we are unable to see UV light.

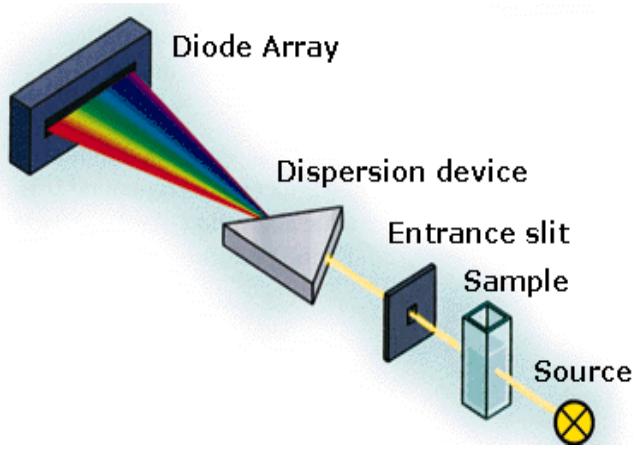
Why does DNA appear as white strands in solution?

- Positive ions interact with DNA to form the white color that we see.

NOTE: When we say "DNA solution"/ "DNA" in general, we don't mean ONLY one molecule of DNA, but millions of these molecules in one solution

➢ **How much DNA is there in solutions?**

1. Take a 5ml sample of blood to extract DNA from the sample
2. Using a **Spectrophotometer:**
    1. UV light hits the DNA molecule
    2. DNA absorbs the light that hits it
    3. A detector is used to measure how much light is absorbed.



3. We use the value obtained from the detector to determine the concentration of DNA in the sample solution.

How?

- There is a **Constant** that states: If 1 unit of **light** is absorbed, 50 µg/ml of **DNA** is found in solution. The absorbance of nucleic acids at 260 nm wavelength (A260) is constant (A260 corresponds to the light absorbance)
- A260 of 1.0 = 50 µg/ml

For example:

1. If 0.5 unit of light is absorbed, what is the concentration of DNA in this solution?

   using **(1 unit= 50 µg/ml),** 0.5*50= 25 µg/ml DNA

2. If 0.1 unit of light is absorbed, what is the concentration of DNA in this solution?
   0.1*50 µg/ml= 5 µg/ml DNA

NOTE: As DNA concentration increases, the amount of light absorbed increases

➢ **Measuring the concentration of DNA in a diluted solution**

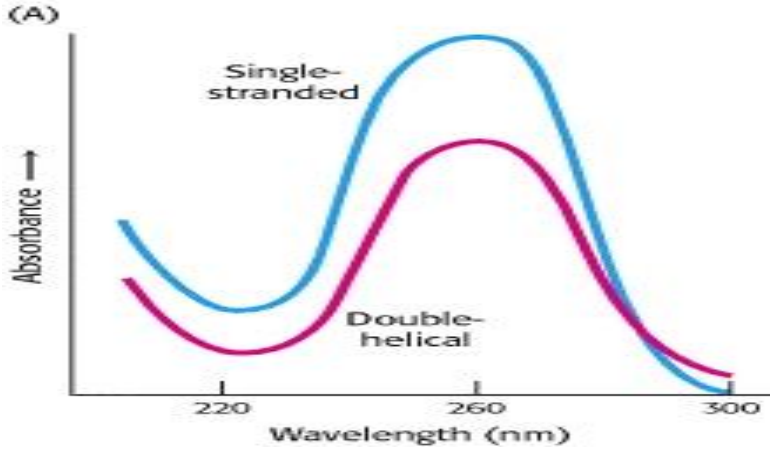
Sometimes DNA is very concentrated in a solution, but the device has a limit to how much DNA is can detect, so we dilute the sample with Water.

- If the diluted sample absorbs 1 unit of light, then the concentration of DNA in the Diluted sample is 50 µg/ml

- If you dilute 1 ml of the solution with 9 ml of water, it means the solution is 1:9. With a dilution factor of (1+9=10). If the concentration of DNA in this diluted solution is 50 µg/ml, then what is the concentration of DNA in the original concentrated solution?

  Concentration of DNA in the original solution (concentrated) =
  10 (dilution factor) x 50 (conc. of DNA in the diluted sample) = 500 µg/ml DNA

NOTE: Single stranded DNA absorbs more light than DNA that's double stranded

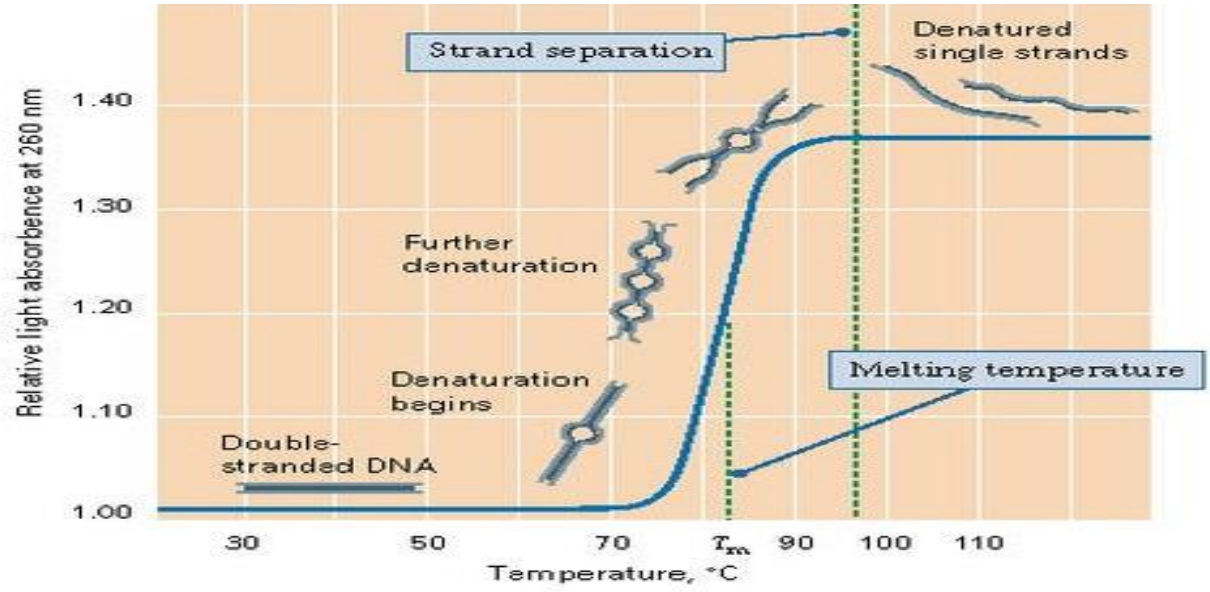


(details not required).

- **Denaturation:** Loss of hydrogen bonds (when molecules lose noncovalent interactions). In other words, when double stranded DNA becomes single stranded. (By heat, for example)

- Scientists love the number **50**, they always use it as a mark or indication.

In this case, look at the figure, and notice that the 50% mark is the melting point, and the point at which 50% of the DNA is single stranded, while the other 50% of the DNA is double stranded.



- Figure above shows the transition temperature, or melting temperature (Tm), and the light absorbance as temperature increases.
- Tm approximately = 82

- **Factors that affect Tm (melting temperature/ 50% mark):**

1. **Length of DNA:**

- Longer DNA means there are more hydrogen bonds
- The presence of more hydrogen bonds requires More energy needed for denaturation (Double strand → Single strand)
- Therefore, Longer DNA →Higher melting point/ temperature (Tm)

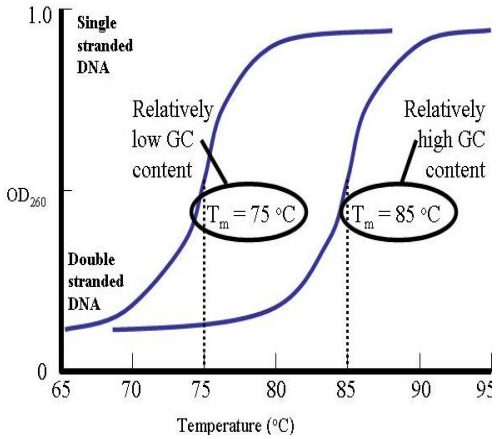
2. **G & C content**:

- Guanine and Cytosine pairs form a triple hydrogen bond between them (G≡C)
- The (G≡C) hydrogen bond requires more energy to break

NOTE: regarding **"base stacking"** mentioned in the slides, the professor said that details aren't required whatsoever, and that base stacking **means:**

→ the state of bases being on top of each other and interacting with one another to form hydrophobic interactions that affect DNA stability.

- Therefore, an increase in G and C content → Higher melting point (Tm)



The figure on the left shows the relationship between GC content and Tm (melting temperature)

3. **pH:**

- Extreme pH affects ionization state
- Effect in ionization state affects stability of DNA molecules
- Extreme pH (whether extremely high OR low), leads to lower DNA stability
- Less stable DNA requires less energy to denature
- pH (extreme)→ Lower melting point (Tm)

4. **Positive Ion concentration (ex: Na+, K+, etc...)**

- An increase in the concentration of positive ions masks the -ve charge of the DNA (masks the negative charge of Phosphate groups)
- A decrease in negative charges means there is less/ no repulsion
- Less repulsion leads to an increase in DNA stability
- An increase in DNA stability means more energy is required for DNA denaturation
- Therefore, an increase in concentration of +ve ions→ Higher melting temperature (Tm)

5. **Destabilizing agents (alkaline solutions, formamide, urea)**

- These agents lower DNA stability by disrupting hydrogen bonds
- As DNA stability decreases, less energy is required for denaturation
- Therefore, destabilizing agents→ Lower Melting temperature (Tm)

☺ GOOD LUCK