GENOMICS AND KEMOINFORMATICS IN MEDICINAL CHEMISTRY

DNA ve yapısı

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What is genetics?

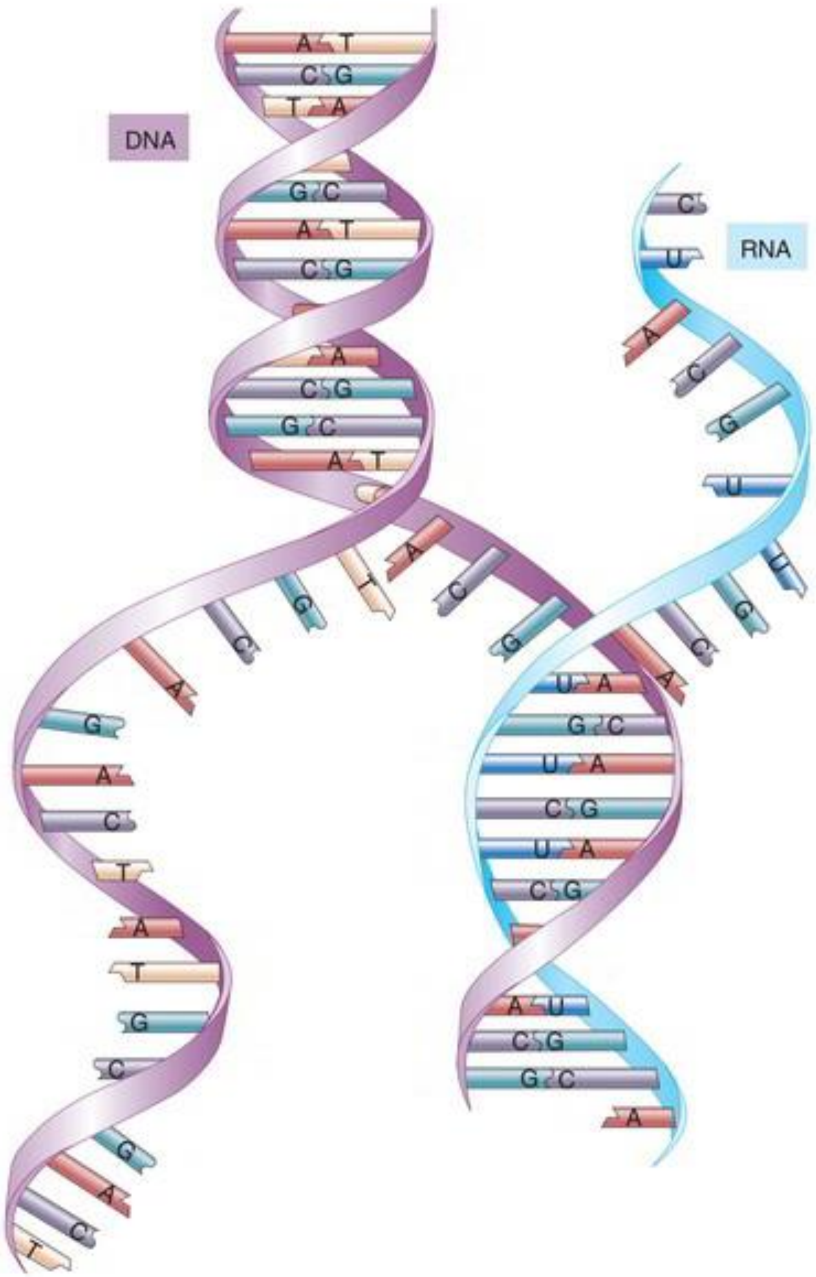
- Genetics is the scientific study of genes and heredity—of how certain qualities or traits are passed from parents to offspring as a result of changes in DNA sequence.

https://www.nigms.nih.gov/education/fact-sheets/Pages/genetics.aspx

Nucleic acid

- Nucleic acid is an important class of macromolecules found in all cells and viruses.
- The functions of nucleic acids have to do with the storage and expression of genetic information.
- Deoxyribonucleic acid (DNA) encodes the information the cell needs to make proteins.
- A related type of nucleic acid, called ribonucleic acid (RNA), comes in different molecular forms that participate in protein synthesis.

https://www.genome.gov/genetics-glossary/Nucleic-Acid

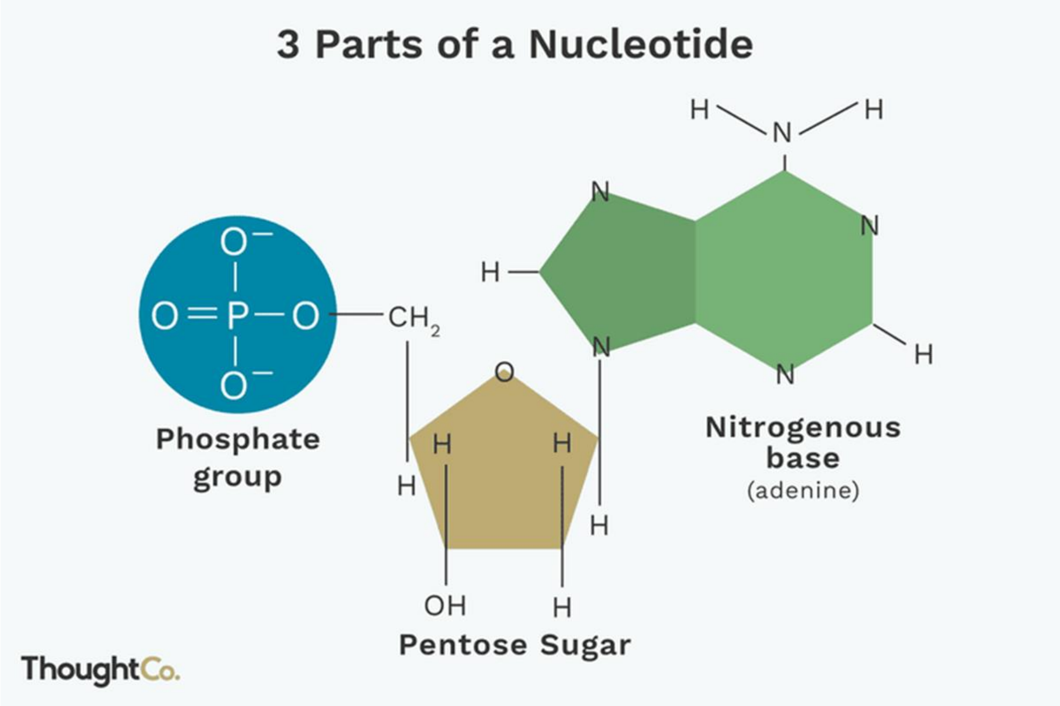


https://www.earthslab.com/physiology/rna-structure-function/

Nucleotide

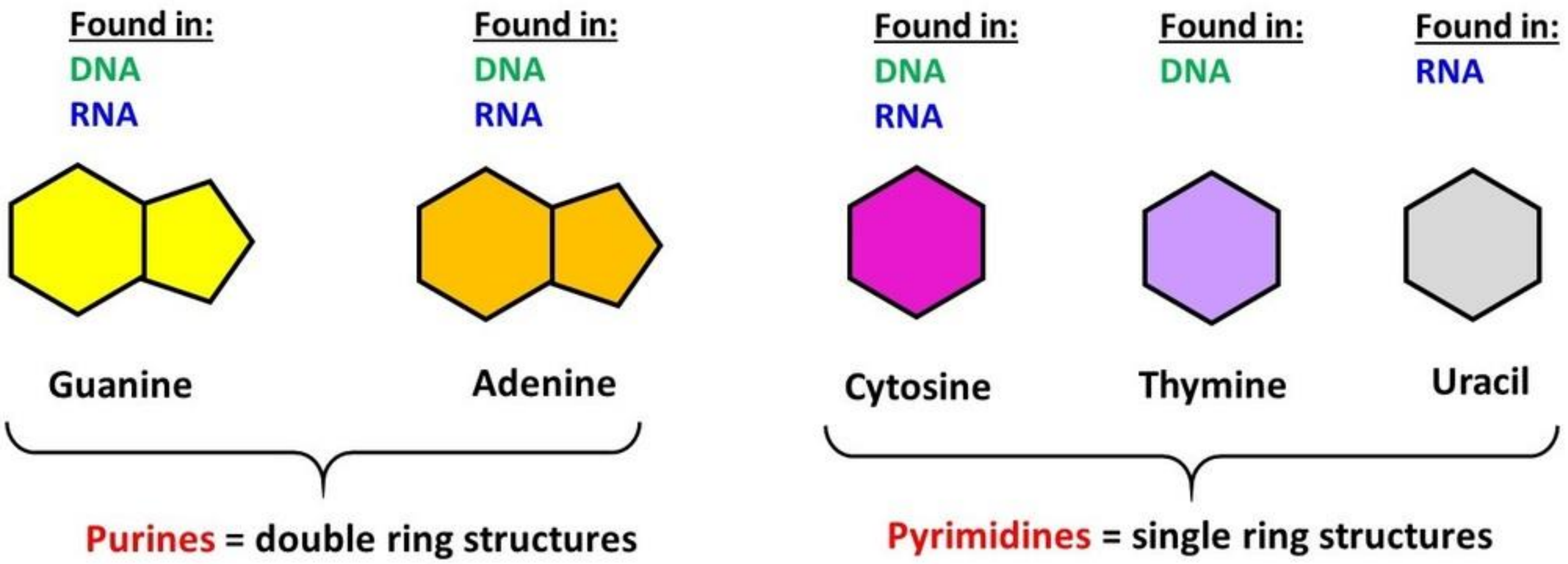
- A nucleotide is the basic building block of nucleic acids.
- RNA and DNA are polymers made of long chains of nucleotides.
- A nucleotide consists of:
    - a sugar molecule (either ribose in RNA or deoxyribose in DNA)
    - a nitrogen-containing base
    - a phosphate group

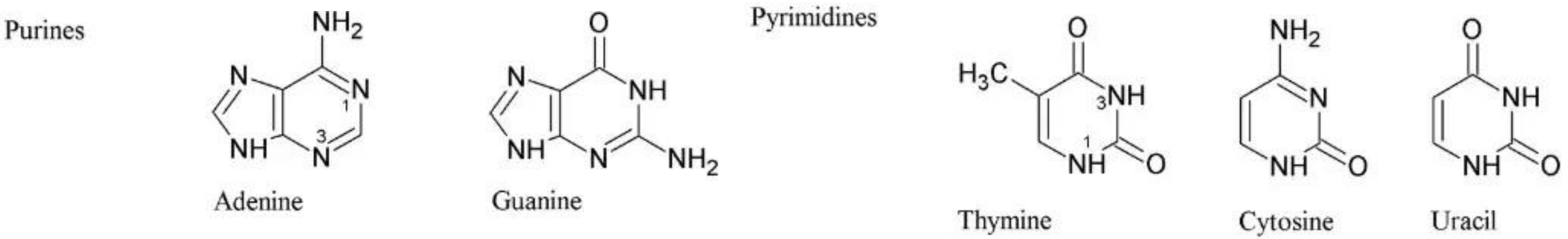
https://www.genome.gov/genetics-glossary/Nucleotide

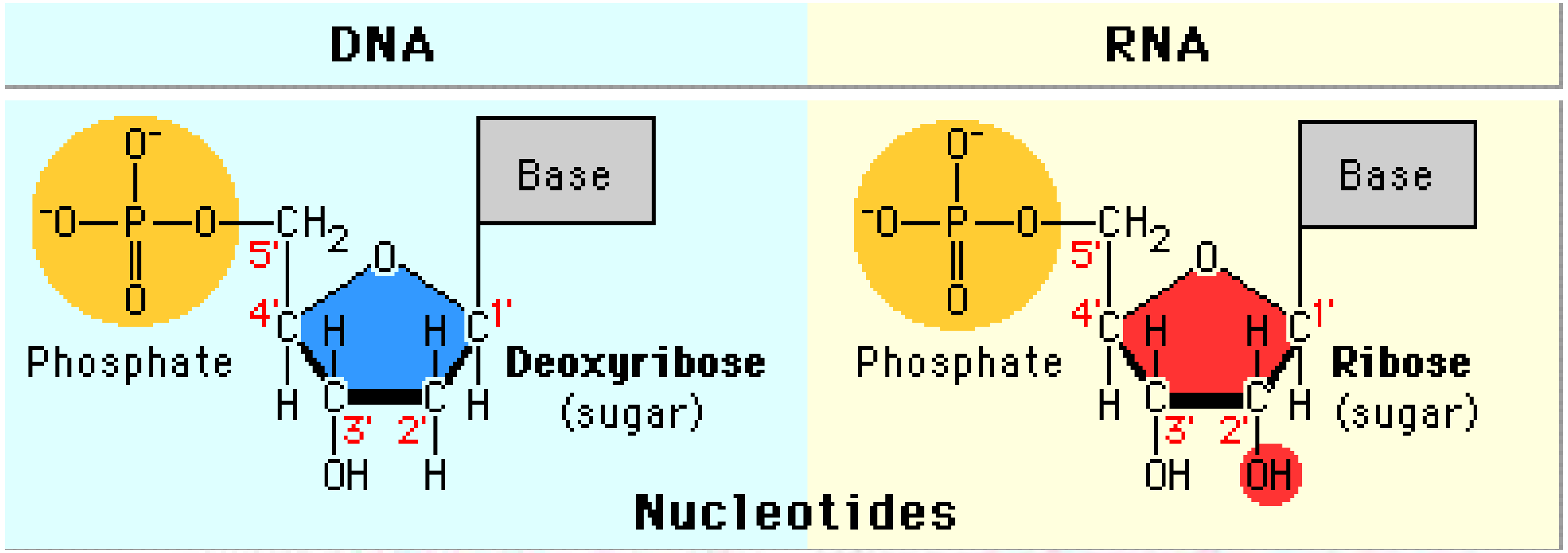


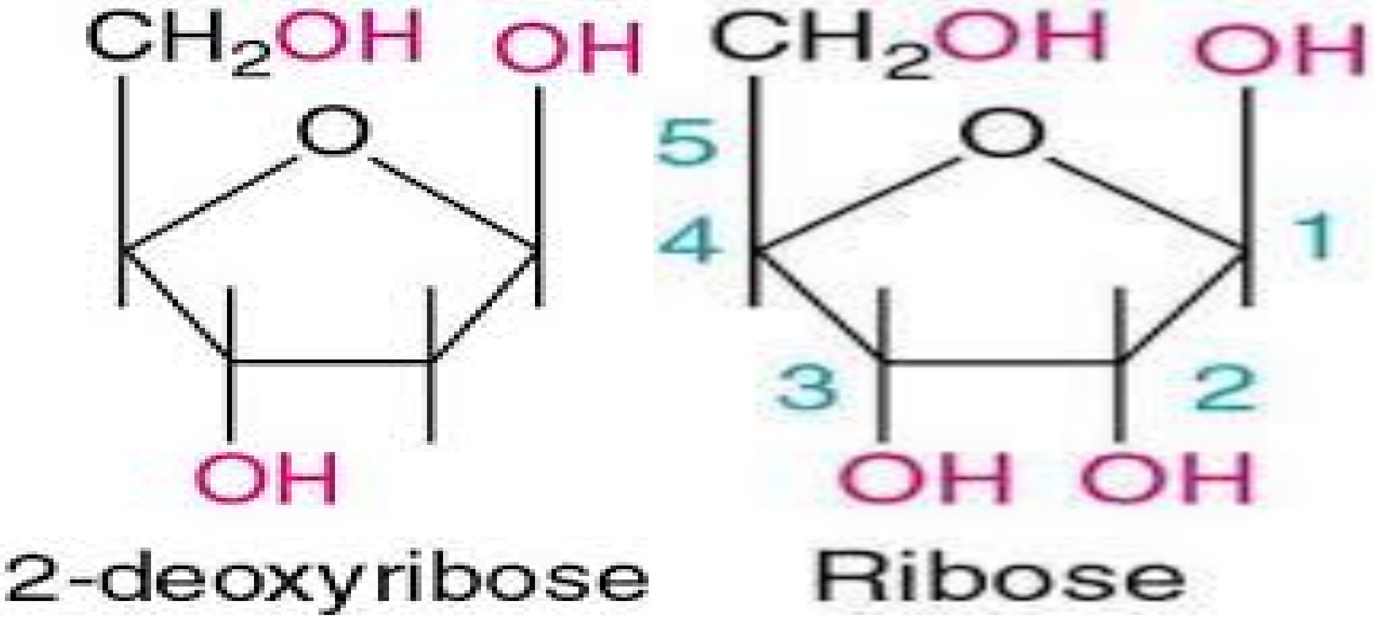
https://www.thoughtco.com/what-are-the-parts-of-nucleotide-606385

There are five nitrogenous bases in total:





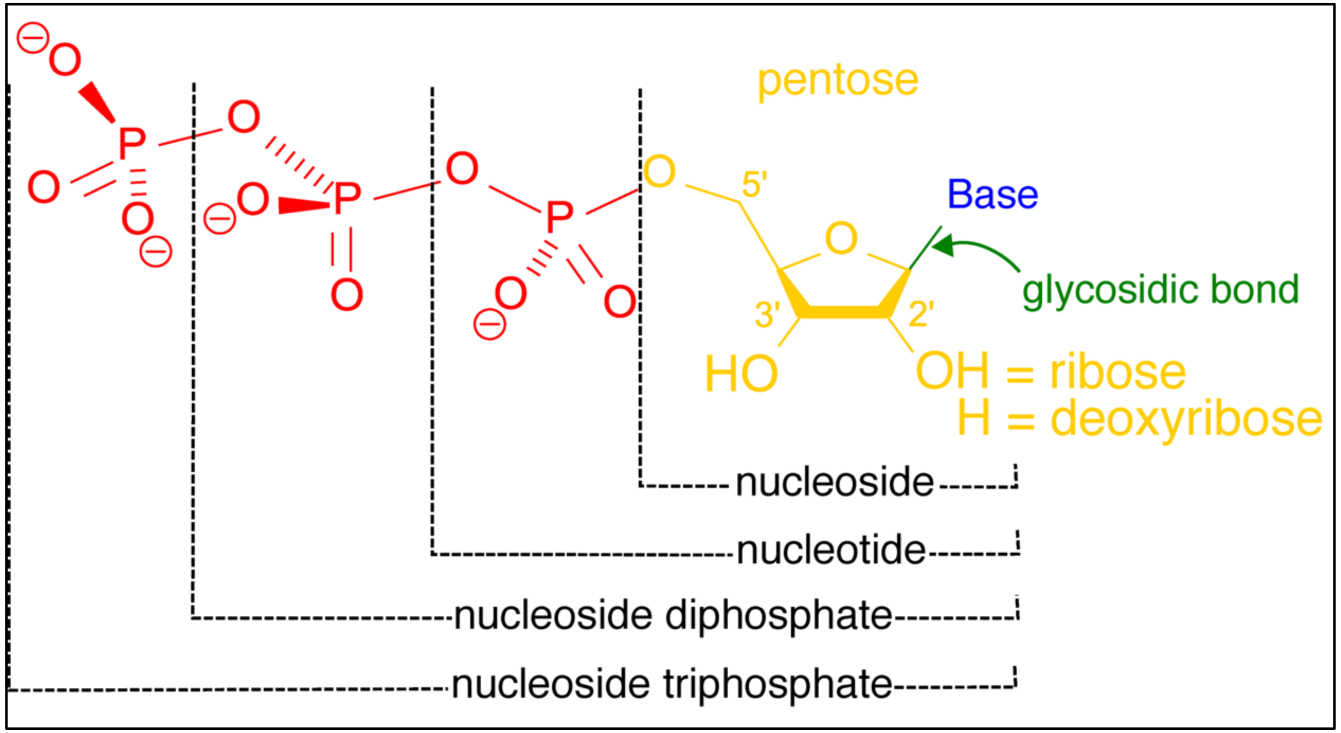


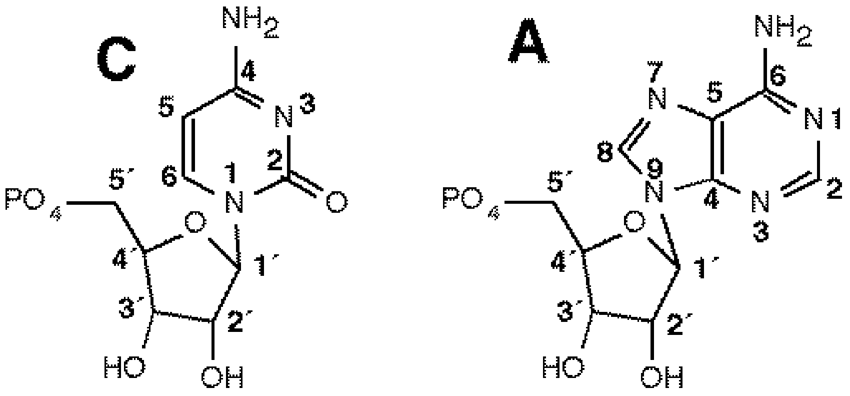


http://www.phschool.com/science/biology_place/biocoach/bioprop/ribose.html
http://pratclif.com/biologie-moleculaire/dna/Sugars.html

Nucleoside

- Nucleoside is a nucleobase attached to a sugar molecule usually a pentose sugar; ribose or deoxyribose.
- This linkage refers to as a beta-glycosidic bond. The significant feature of the nucleoside is that, if a nucleoside links with a phosphate group, eventually it becomes a nucleotide or a nucleoside monophosphate, which is the basic unit of nucleic acids.

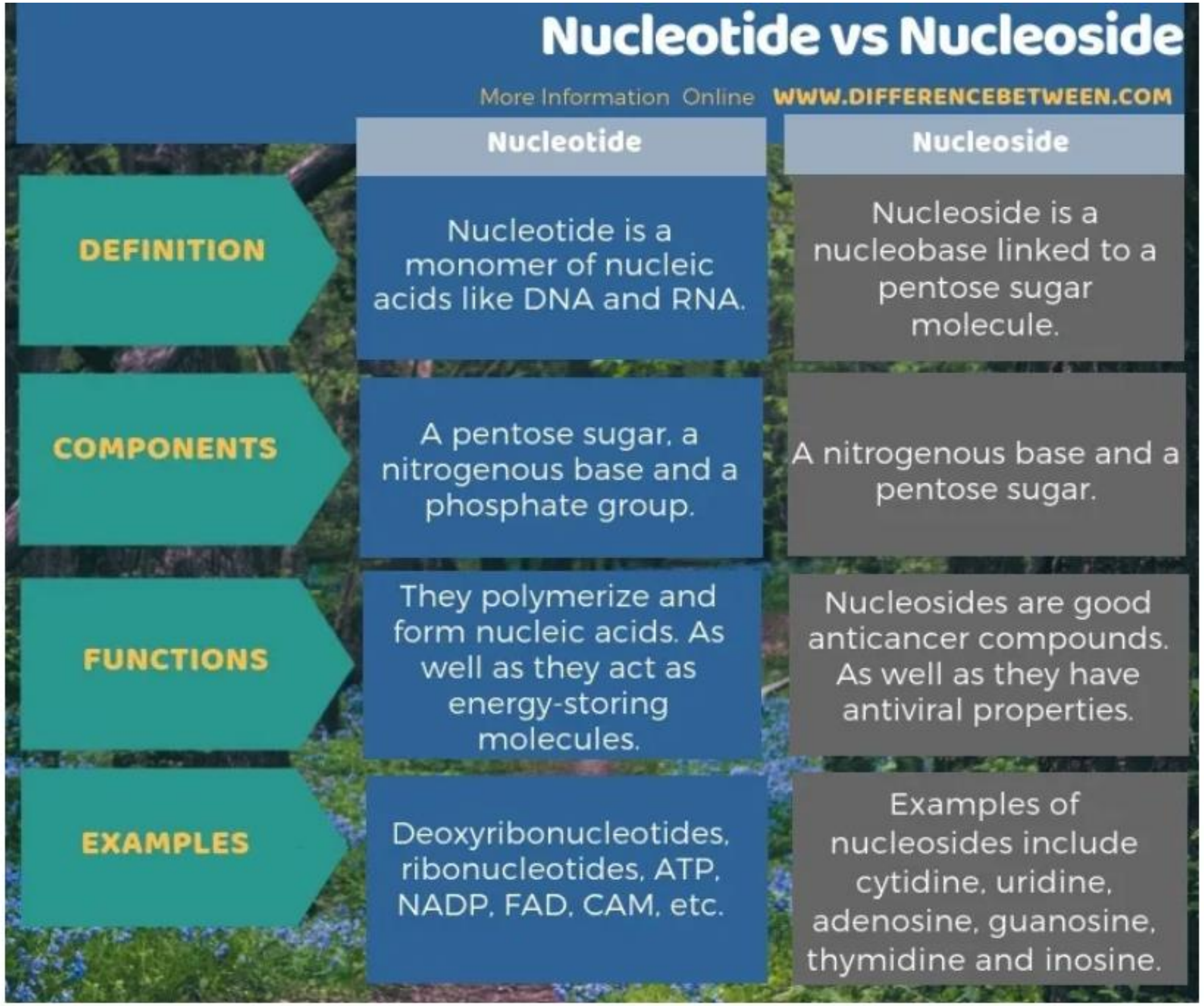




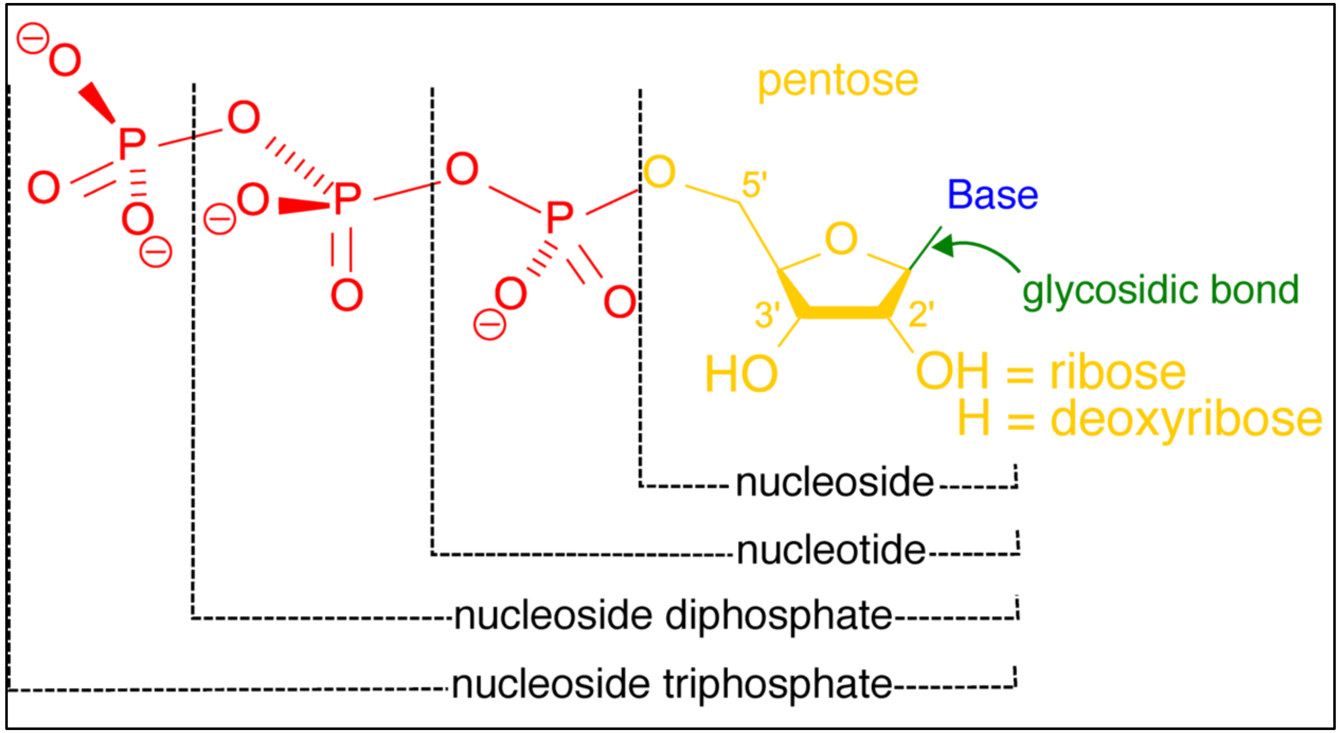
https://www.differencebetween.com/difference-between-nucleotide-and-vs-nucleoside/

Nucleotide and Nucleoside

- Nucleotide and nucleoside are important molecules. The key difference between nucleotide and nucleoside is the presence and absence of a phosphate group/s.
- Nucleotide has three components namely a pentose sugar, a nitrogenous base and a phosphate group while nucleoside has two components namely pentose sugar and a nitrogenous base.
- It lacks a phosphate group. Furthermore, nucleosides are good anticancer and antiviral substances while nucleotides are building blocks of DNA and RNA and some are energy molecules. However, malfunction nucleotides can cause fatal cancers too.



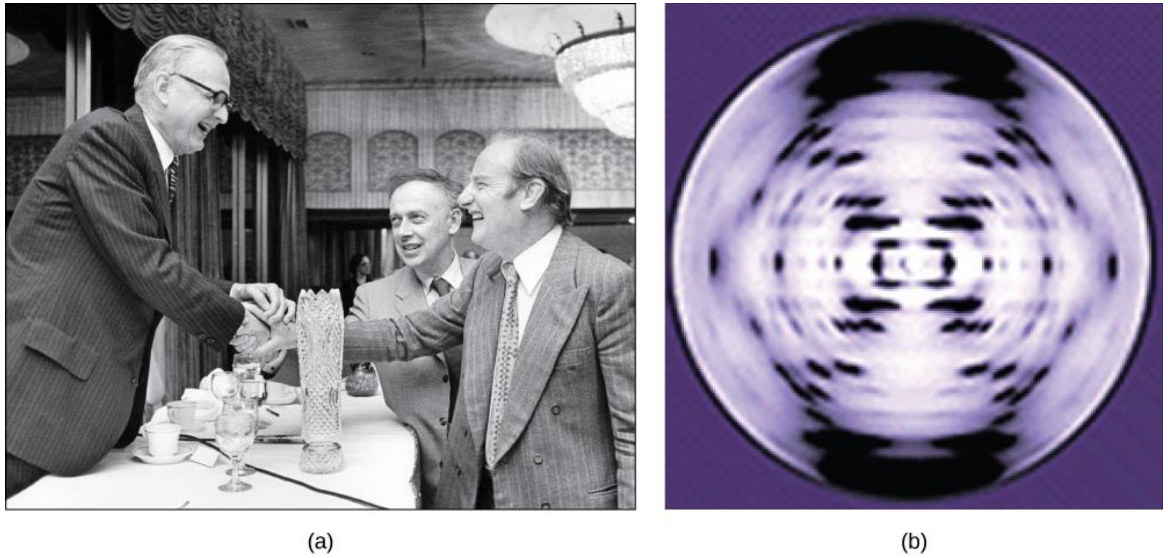
https://www.differencebetween.com/difference-between-nucleotide-and-vs-nucleoside/



- Nucleotides are mono, di or triphosphate esters of nucleosides.
- The phosphate group is attached to the 5'OH group of the pentose by an ester bond. This compound is called nucleoside-5'-phosphate or 5'-nucleotide.
- The type of pentose is indicated by a prefix such as 5'-ribonucleoside and 5'-deoxyribonucleoside.
- If a phosphate group is added to the 5'-carbon of the pentose, nucleoside monophosphate (AMP) is formed, if two phosphate groups are added, nucleoside diphosphate (ADP) and if three phosphate groups are added, nucleoside triphosphate (ATP) is formed.
- These phosphate groups provide the negative charges of nucleotides and nucleic acids.

DNA structure

- In the 1950s, to determine the structure of DNA:
- Francis Crick and James Watson (University of Cambridge, England)
- Linus Pauling and Maurice Wilkins, Rosalind Franklin
- Pauling had discovered the secondary structure of proteins using X-ray crystallography.
- Watson and Crick were able to piece together the puzzle of the DNA molecule using Franklin's data.

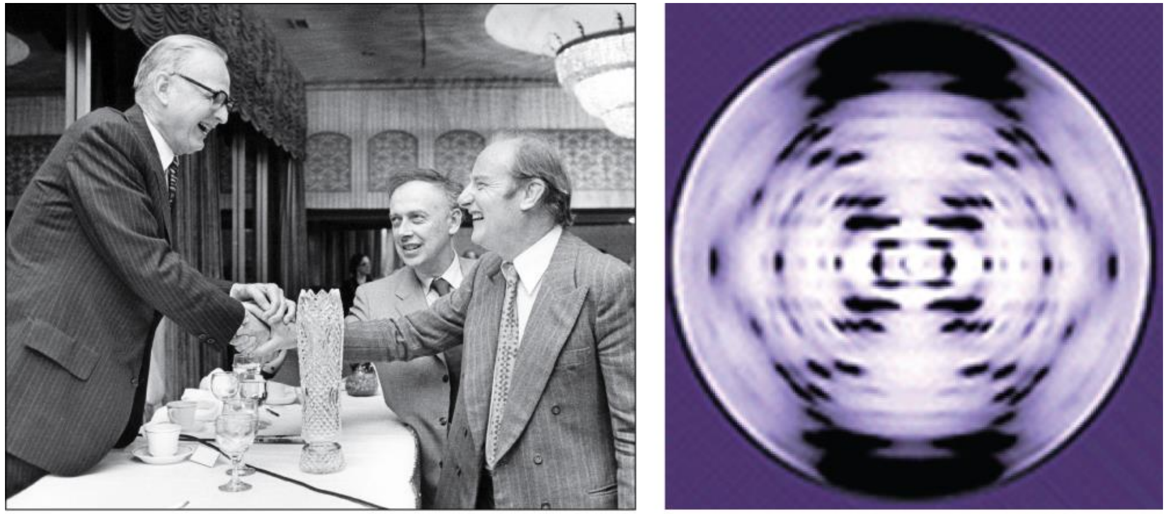


Pioneering scientists (a) James Watson and Francis Crick are pictured here with American geneticist Maclyn McCarty. Scientist Rosalind Franklin discovered (b) the X-ray diffraction pattern of DNA, which helped to elucidate its double helix structure. (credit a: modification of work by Marjorie McCarty; b: modification of work by NIH)

https://opentextbc.ca/biology/chapter/9-1-the-structure-of-dna/

DNA structure

- Watson and Crick also had key pieces of information available from other researchers such as Chargaff's rules.
- Chargaff had shown that of the four kinds of monomers (nucleotides) present in a DNA molecule, two types were always present in equal amounts and the remaining two types were also always present in equal amounts. This meant they were always paired in some way.
- In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Medicine for their work in determining the structure of DNA.



(a) (b)

https://opentextbc.ca/biology/chapter/9-1-the-structure-of-dna/

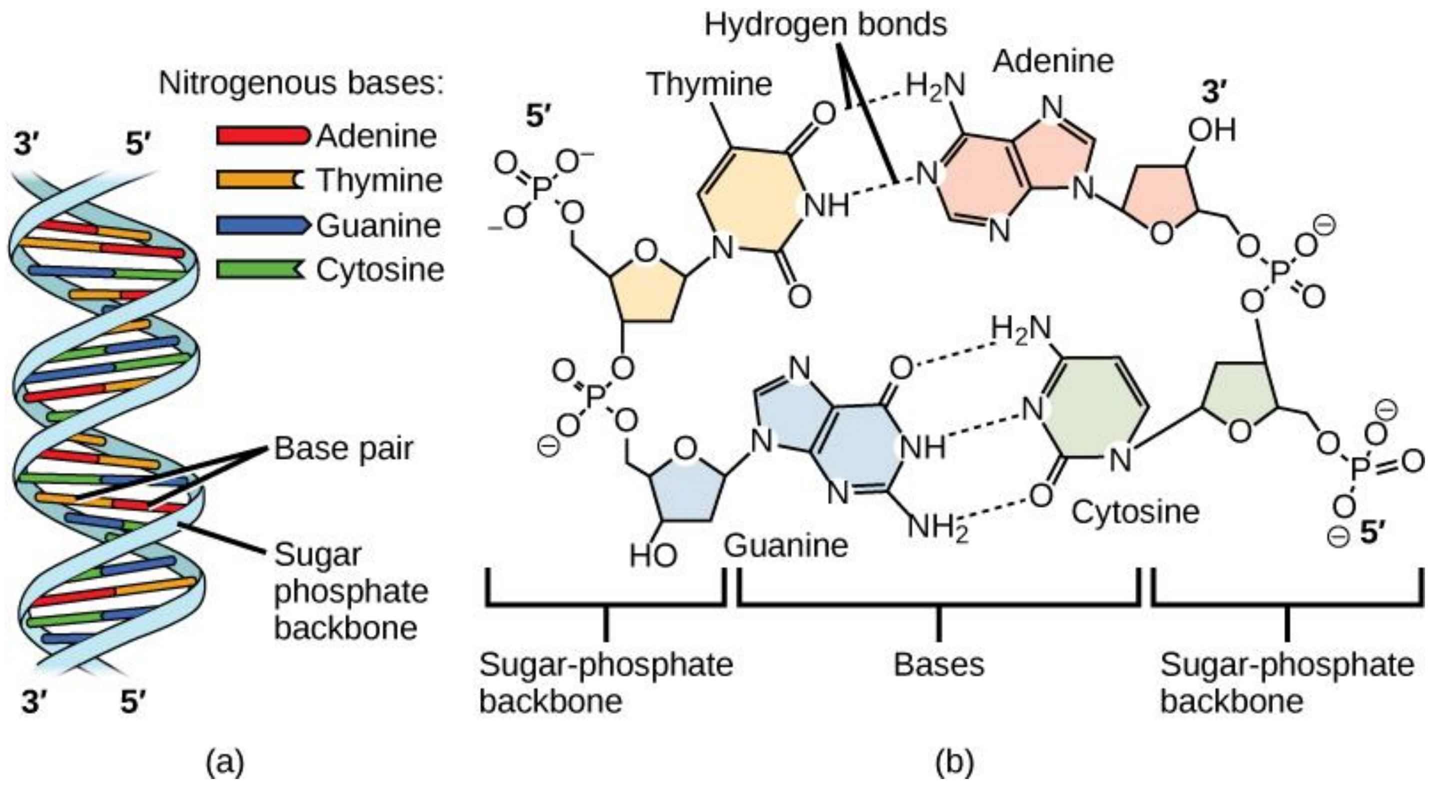
DNA

- *DNA=Deoxyribonükleic acid*
- DNA is a polydeoxyribonucleotide.
- DNA is found in the chromosomes of eukaryotic cell nuclei, mitochondria, and chloroplasts of plants.

DNA ;

- encodes the genes responsible for the structure and function of living organisms
- allows genetic information to be passed from generation to generation.

DNA structure



The carbon atoms of the five-carbon sugar are numbered clockwise from the oxygen as 1′, 2′, 3′, 4′, and 5′. The phosphate group is attached to the 5′ carbon of one nucleotide and the 3′ carbon of the next nucleotide.

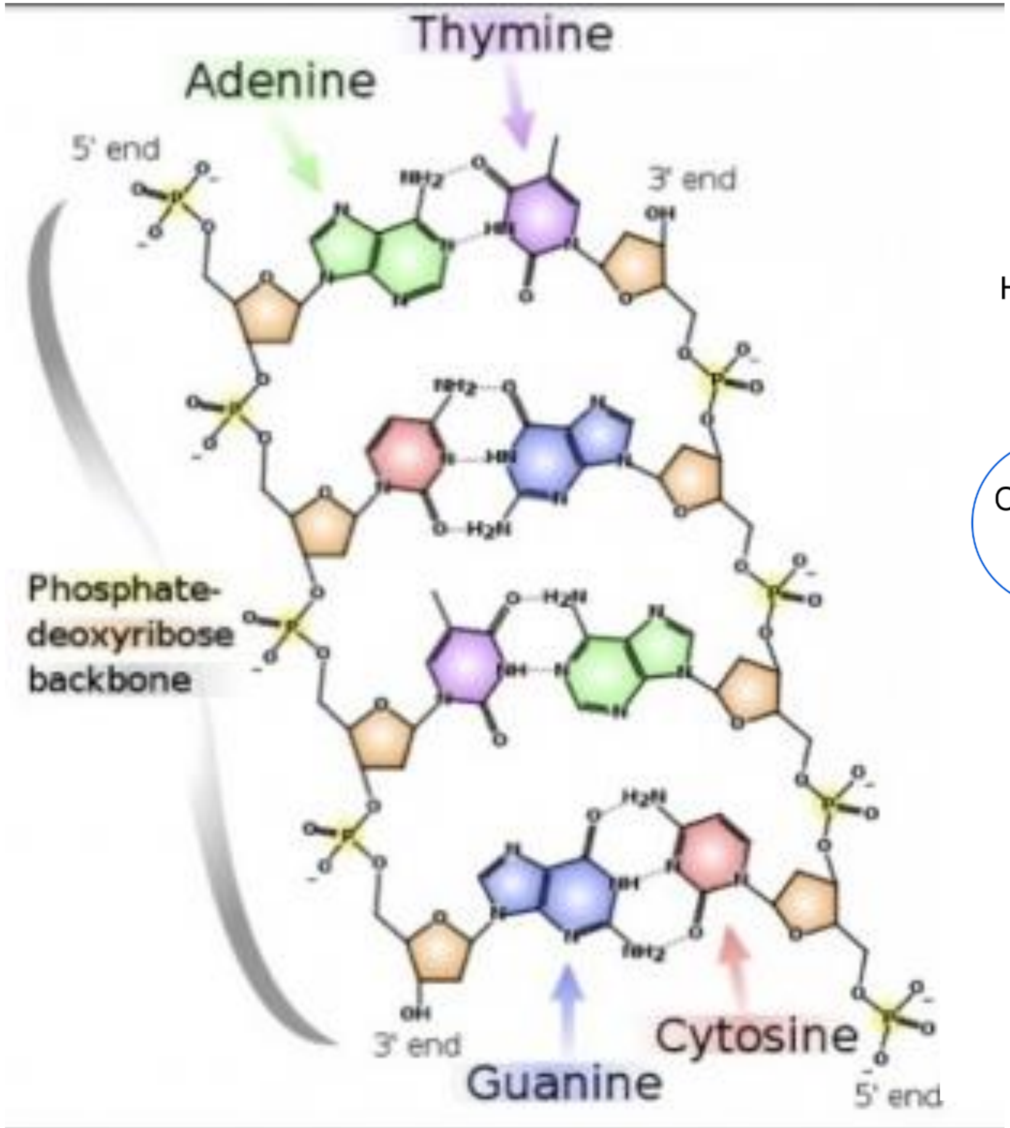
The phosphate group of one nucleotide bonds covalently with the sugar molecule of the next nucleotide, and so on, forming a long polymer of nucleotide monomers.

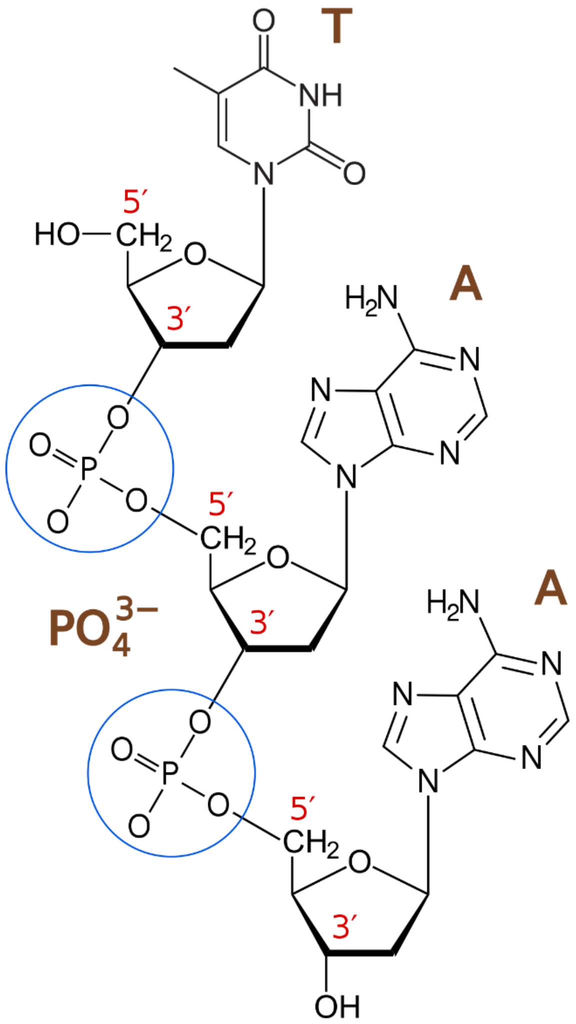
In its natural state, each DNA molecule is actually composed of two single strands held together along their length with hydrogen bonds between the bases.

https://opentextbc.ca/biology/chapter/9-1-the-structure-of-dna/

DNA structure

- DNA contains many monodeoxyribonucleotide which covalently bounded each other with a 3'-5' phosphodiester bond.
- DNA is made up of two strands that are twisted around each other to form a right-handed helix, called a double helix.
- There are 10 nucleotides in one complete cycle of DNA.
- The two strands are anti-parallel in nature; that is, one strand will have the 3′ carbon of the sugar in the "upward" position, whereas the other strand will have the 5′ carbon in the upward position.

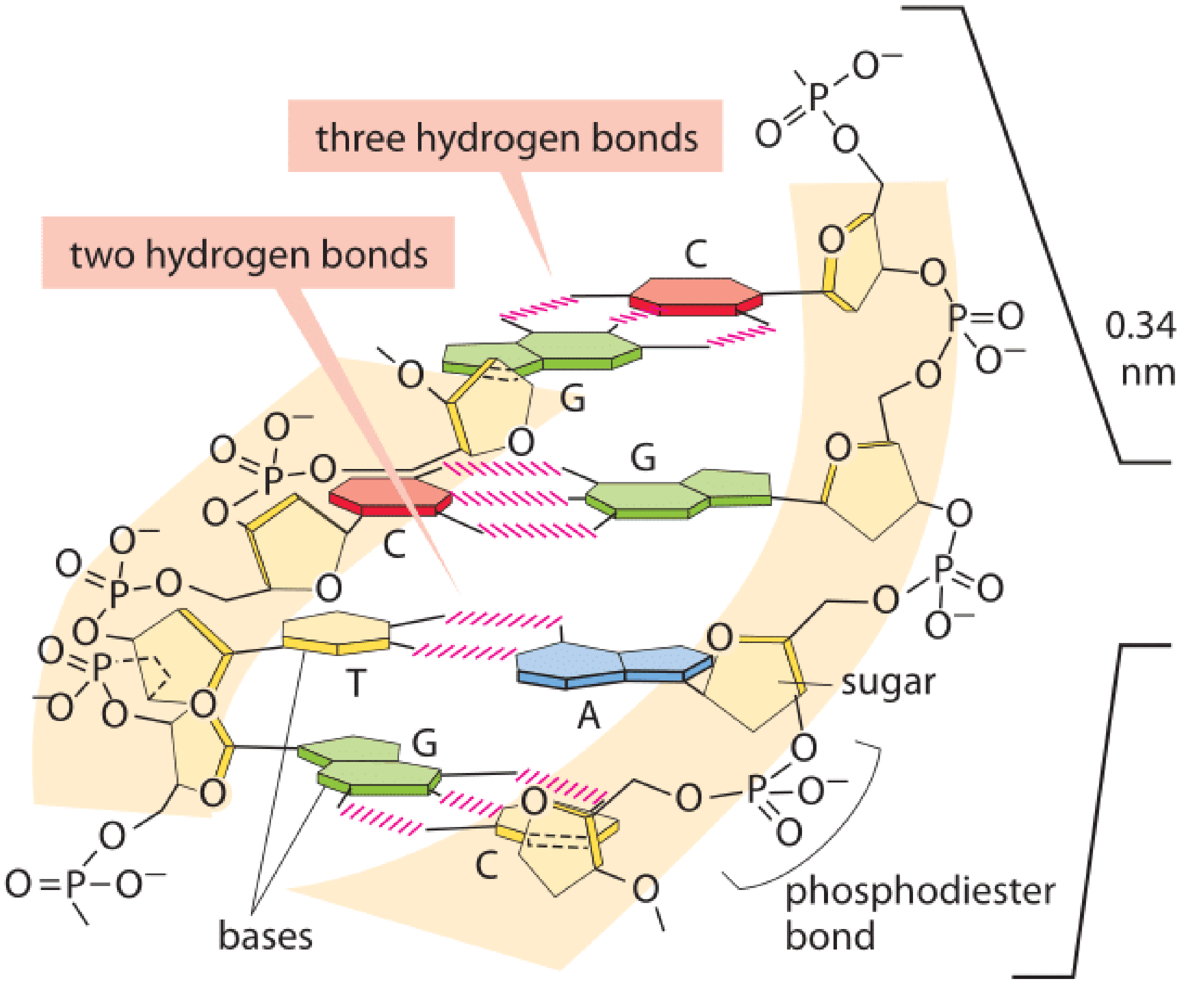


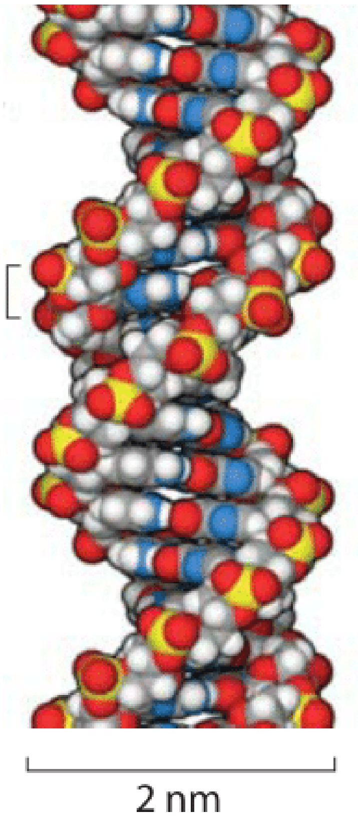


https://en.wikipedia.org/wiki/Phosphodiester_bond
https://opentextbc.ca/biology/chapter/9-1-the-structure-of-dna/

DNA structure

- Base-pairing takes place between a purine and pyrimidine: namely, A pairs with T, and G pairs with C.
- This is the basis for Chargaff's rule; because of their complementarity, there is as much adenine as thymine in a DNA molecule and as much guanine as cytosine.
- Adenine and thymine are connected by two hydrogen bonds, and cytosine and guanine are connected by three hydrogen bonds.
- The diameter of the DNA double helix is uniform throughout because a purine (two rings) always pairs with a pyrimidine (one ring) and their combined lengths are always equal.

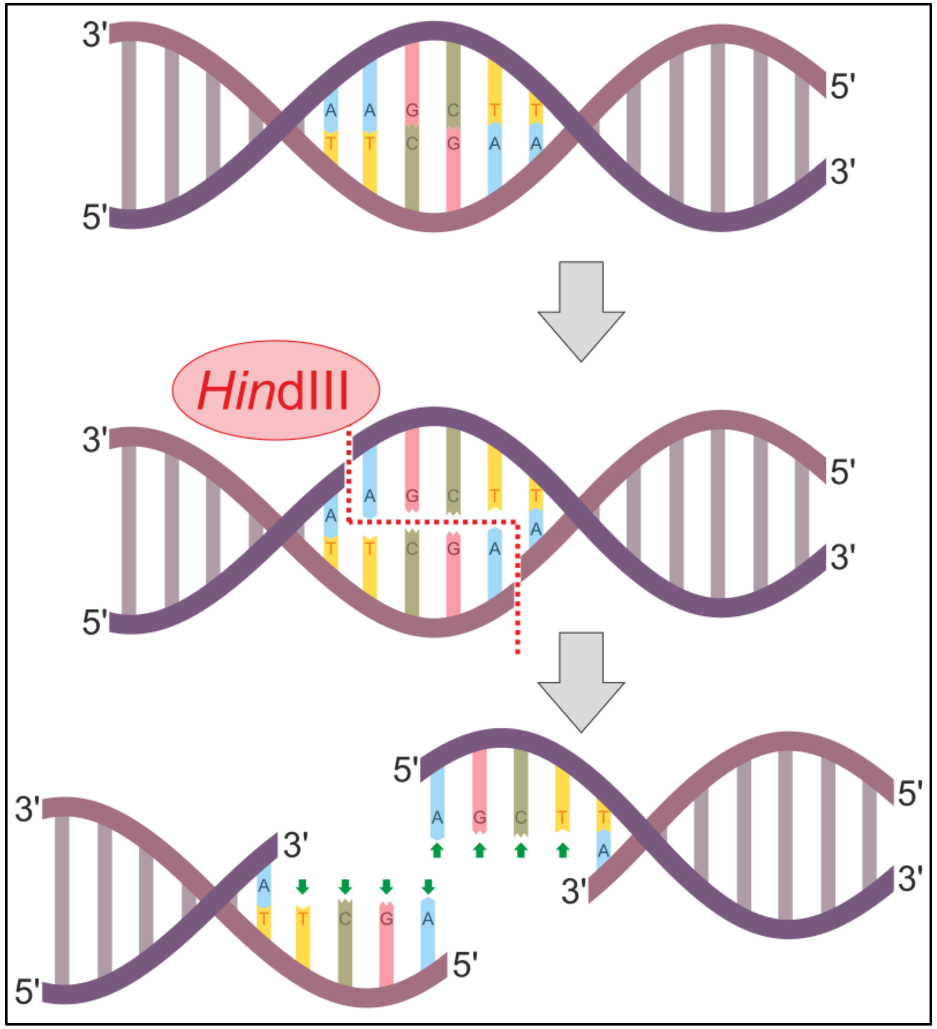




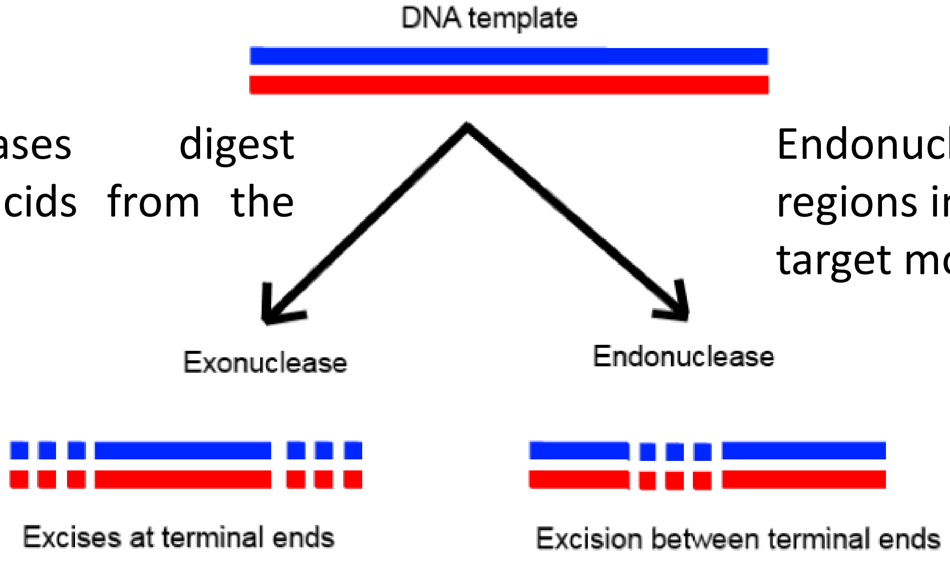
https://opentextbc.ca/biology/chapter/9-1-the-structure-of-dna/

http://book.bionumbers.org/what-is-the-energy-of-a-hydrogen-bond/

- Phosphodiester bonds between nucleotides in DNA and RNA can be broken either by chemical hydrolysis or by enzymatic hydrolysis.
- These bonds are resistant to pH and temperature changes and do not deteriorate.
- A nuclease is an enzyme capable of cleaving the phosphodiester bonds between nucleotides of nucleic acids.
- They are further subcategorized as deoxyribonucleases and ribonucleases. The former acts on DNA, the latter on RNA.



Exonucleases digest nucleic acids from the ends.



Endonucleases act on regions in the middle of target molecules.

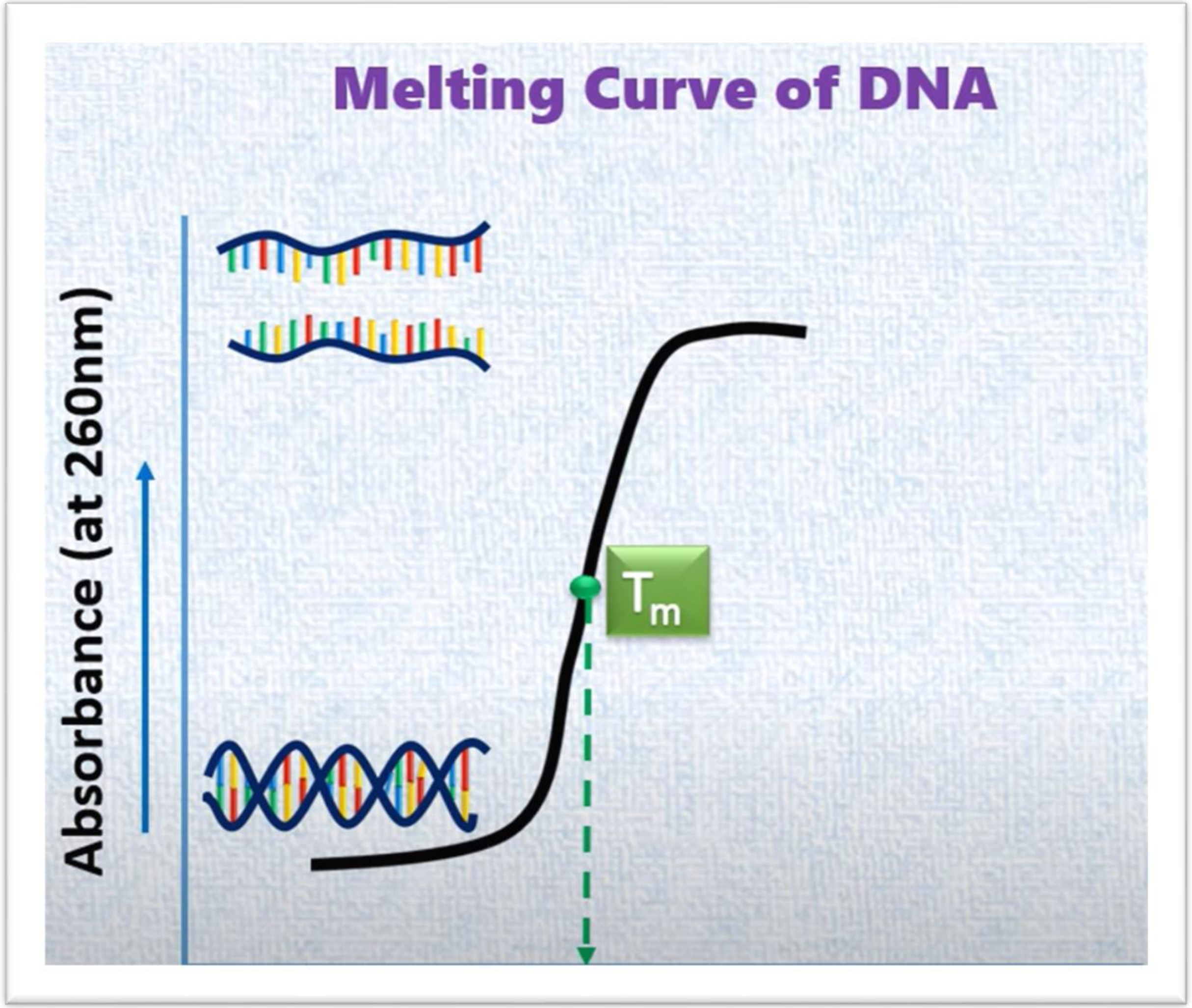
ttps://en.wikipedia.org/wiki/Nuclease#/media/File:HindIII_Restriction_site_and_sticky_ends_vector.svg
https://www.mbiology.com/2019/01/exonucleases.html

Melting temperature of DNA

- The temperature at which 50% of DNA is denatured is known as the melting temperature (T_m).

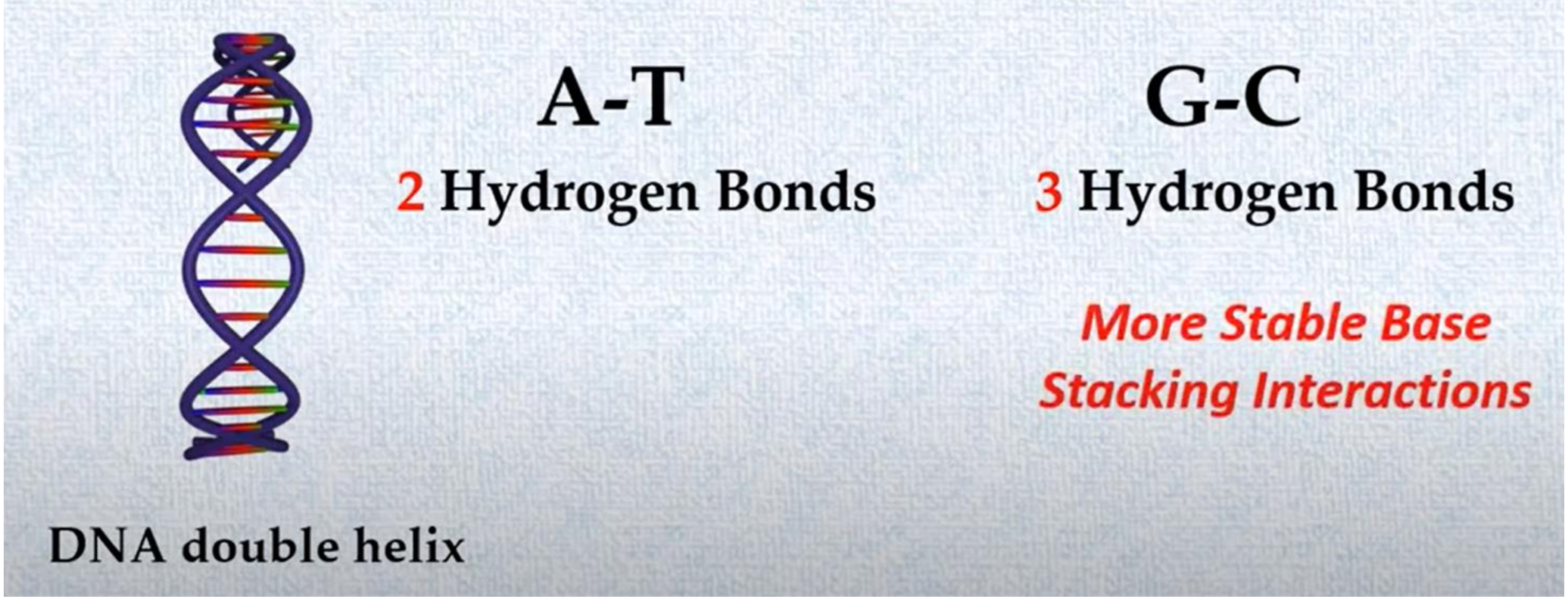
- Melting temperature of DNA is affected by 3 main factors:

    - Nucleotide content of DNA
    - Length of the DNA molecule
    - Ionic strength of the DNA solution



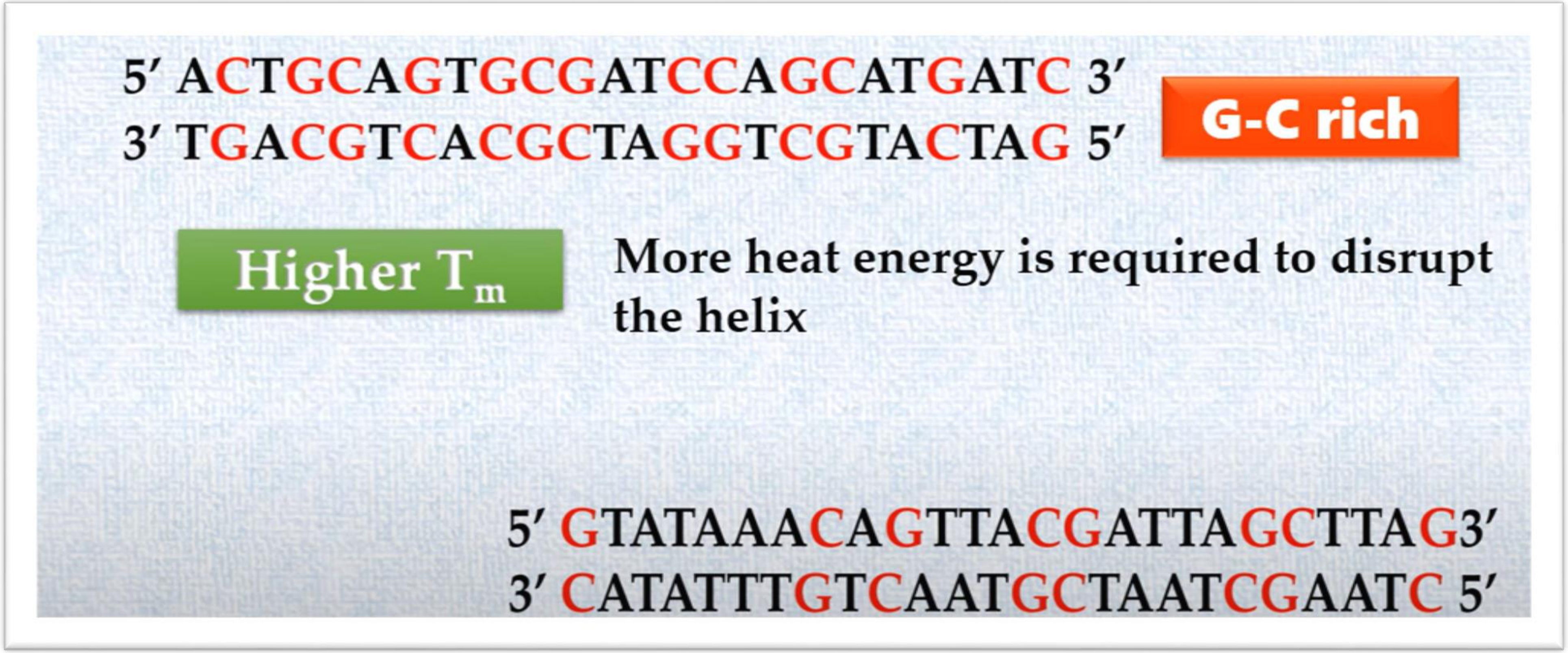
https://www.youtube.com/watch?v=NufigSvIfA4&app=desktop

1- Nucleotide content of DNA



1- Nucleotide content of DNA

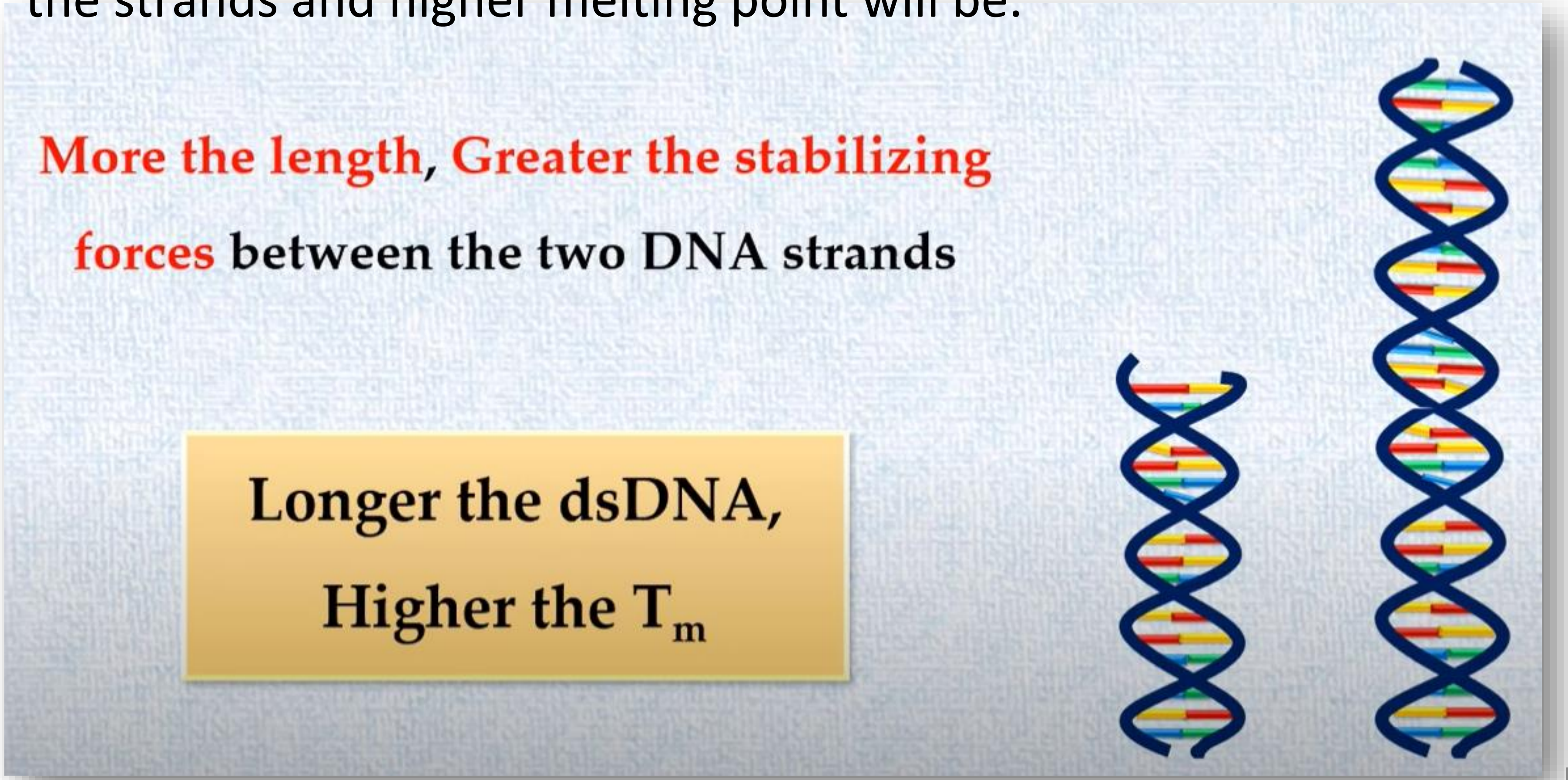
- DNA molecule which is G-C-rich will have higher melting point. Because, more heat energy is required to disrupt the stable base stacking interaction.



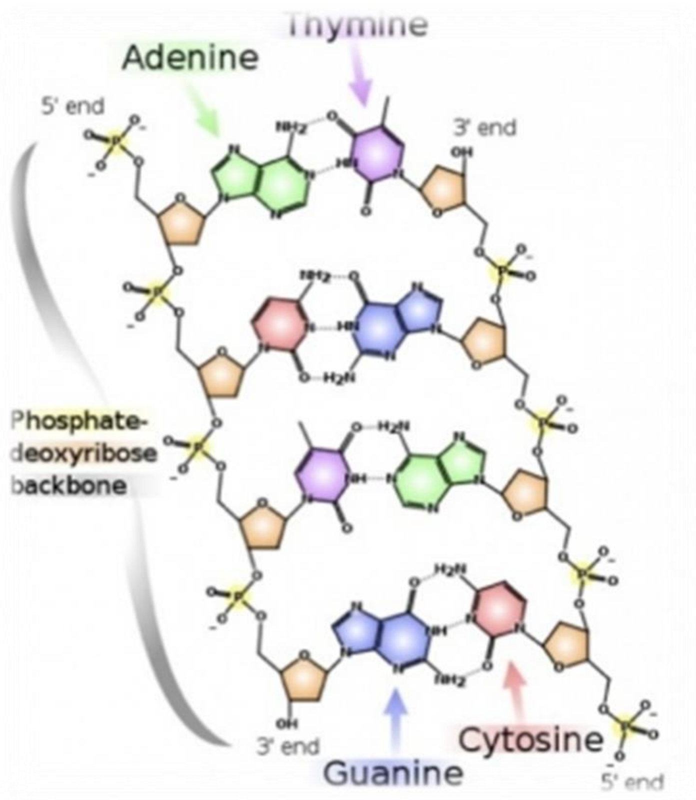
https://www.youtube.com/watch?v=NufigSvIfA4&app=desktop

➢Length of the DNA molecule;

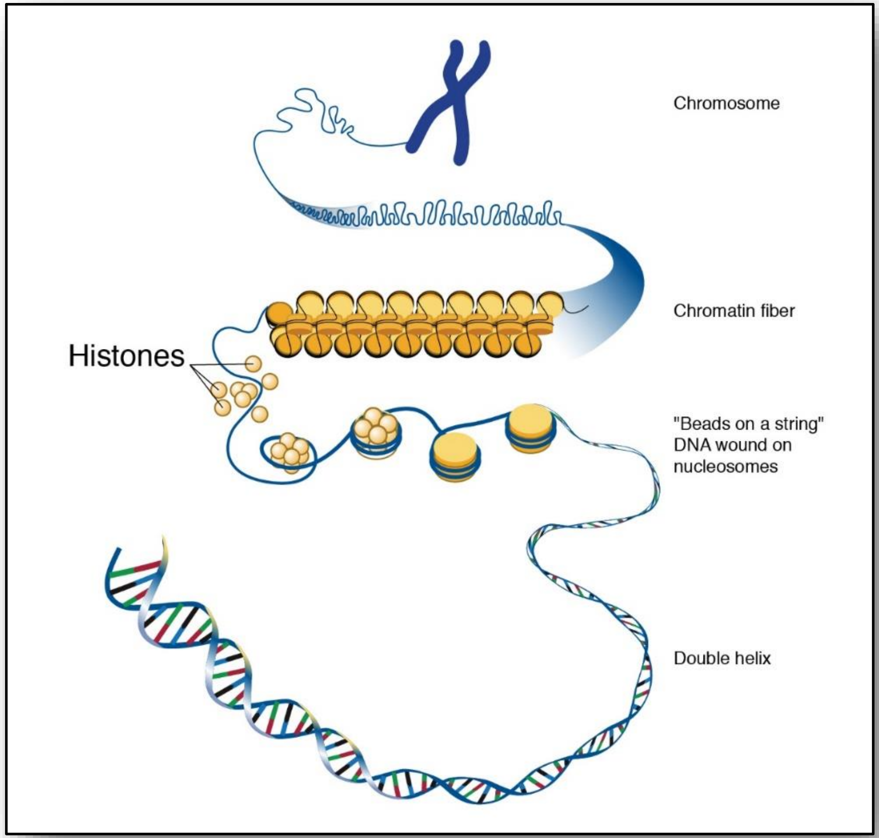
- A longer molecule of double-stranded DNA requires more energy to get disrupted as compared to a shorter molecule. Because, longer molecule has greater stabilizing forces between the two DNA strands. So, more heat energy is required to dissociate the strands and higher melting point will be.



3- Ionic strength of the DNA solution

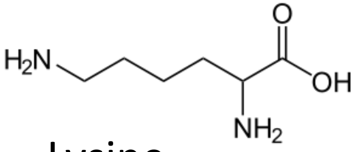


- Each phosphate group in DNA strand carries a negative charge.
- These negative charges on each strands repel each other.
- How do the two strands of DNA stay together?

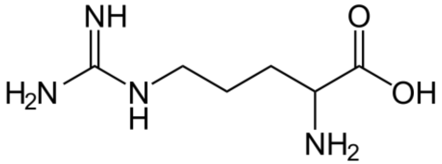


https://www.youtube.com/watch?v=NufigSvIfA4&app=desktop

Histones proteins play important role in compaction of DNA.
These proteins are rich of basic amino acids (arginin/lysin). Positive charge of these aa help in neutralizing the negative charges on DNA molecule.



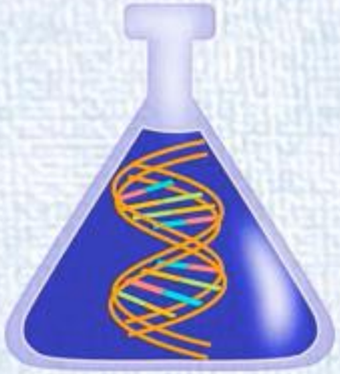
Lysine



Arginine

https://www.genome.gov/genetics-glossary/histone

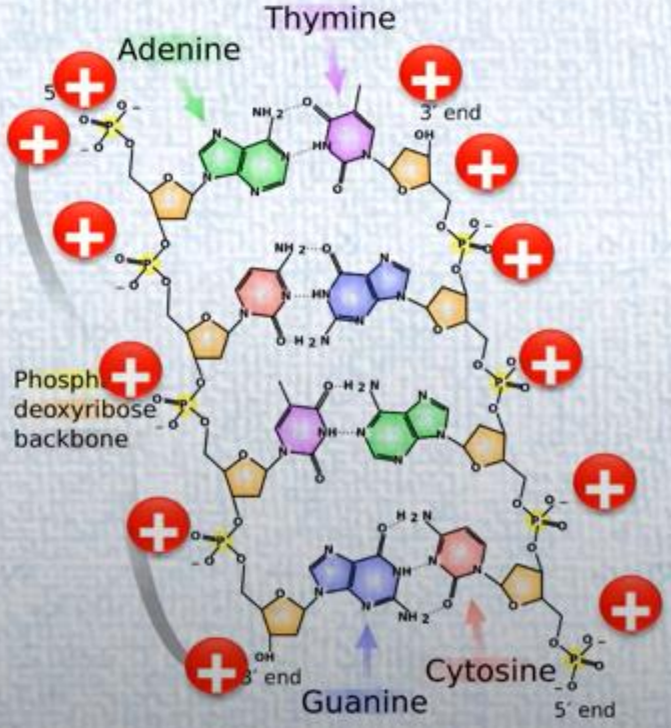
In the laboratory….



DNA solution

Positively charged ions e.g. Na^+ stabilize the DNA molecule in a solution.

Na^+ ions neutralizes the negative charge on phosphate groups.



DNA in solution becames stable

https://www.youtube.com/watch?v=NufigSvIfA4&app=desktop

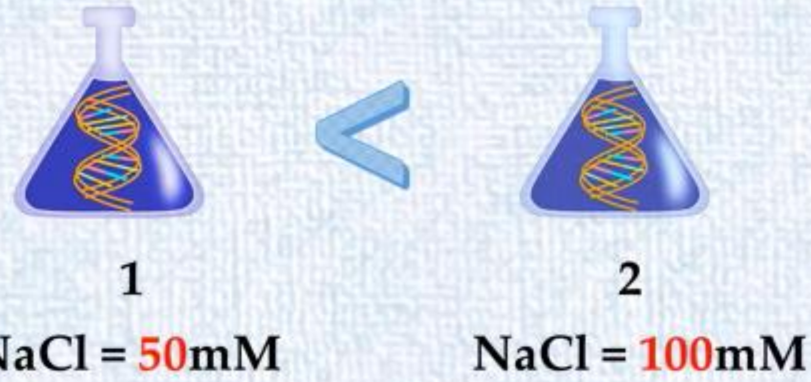
3. Ionic Strength of the DNA solution

Ionic strength refers to = Total ion concentration in the DNA solution.

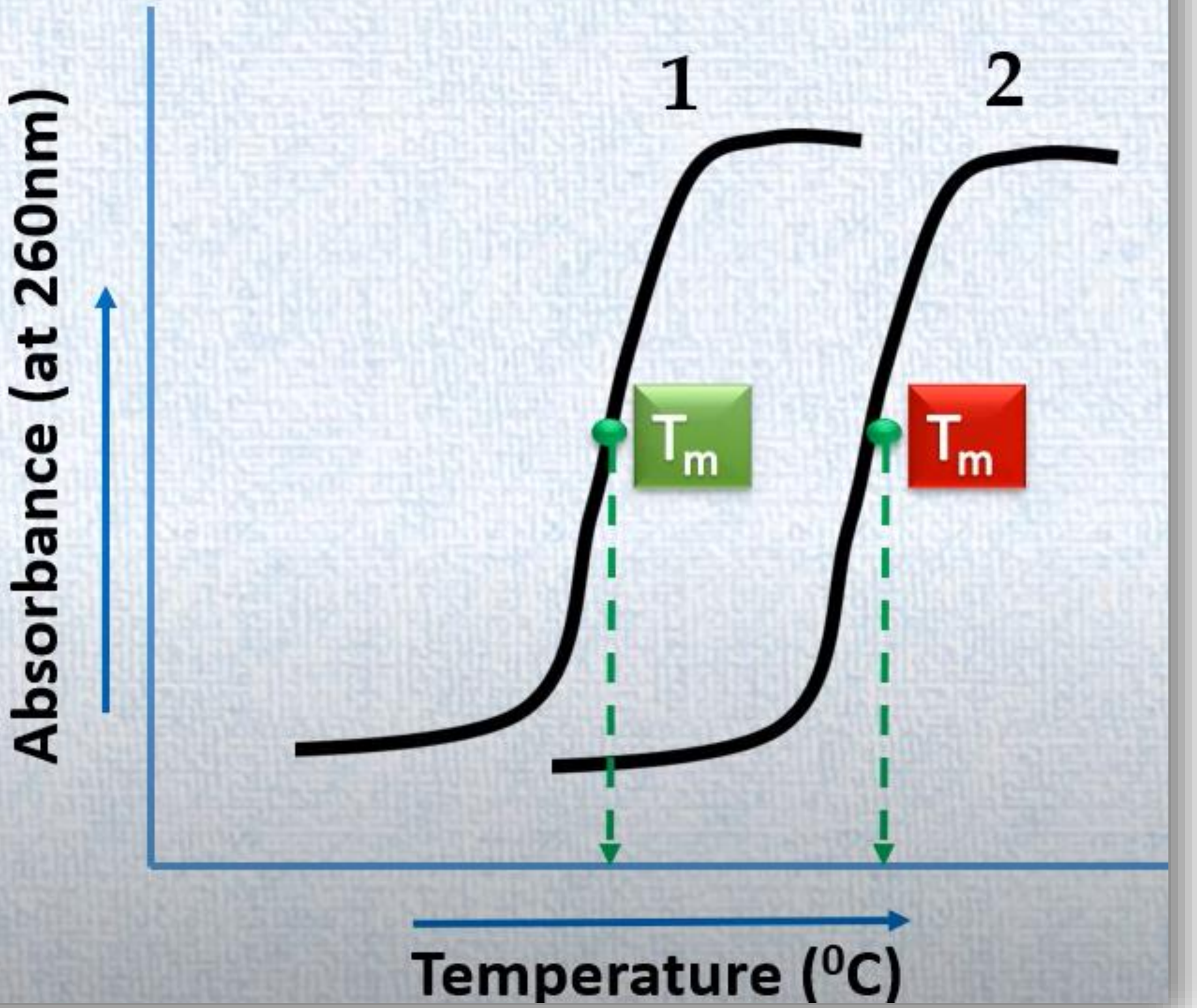
1	2
NaCl = 50mM	NaCl = 100mM
Less stable DNA molecules	More stable DNA molecules
Less heat energy required for denaturation	More heat energy required for denaturation

https://www.youtube.com/watch?v=NufigSvIfA4&app=desktop

3. Ionic Strength of the DNA solution



Higher the Ionic Strength, Higher the T_m

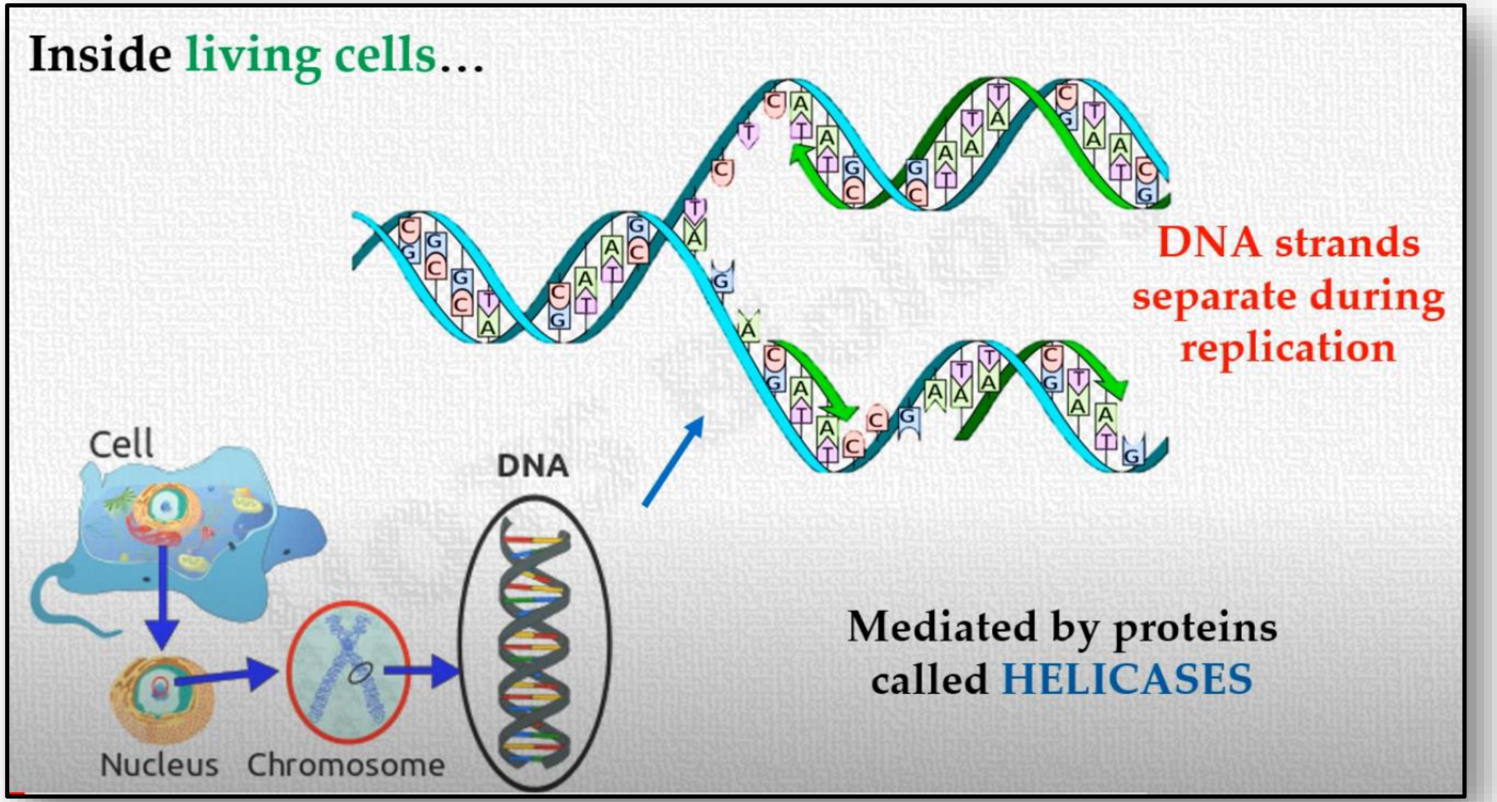


Melting temperature of DNA (T_m)

- Higher G-C content → Higher T_m
- Longer DNA → Higher T_m
- Higher ionic strength → Higher T_m

DNA denaturation

- Nucleic acid denaturation occurs when hydrogen bonding between nucleotides is disrupted, and results in the separation of previously annealed strands.
- This is done by proteins called as Helicases. Helicases are enzymes that bind and may even remodel nucleic acid or nucleic acid protein complexes. There are DNA and RNA helicases.



https://www.youtube.com/watch?v=XtXfHclIrxg
https://en.wikipedia.org/wiki/Denaturation_(biochemistry)

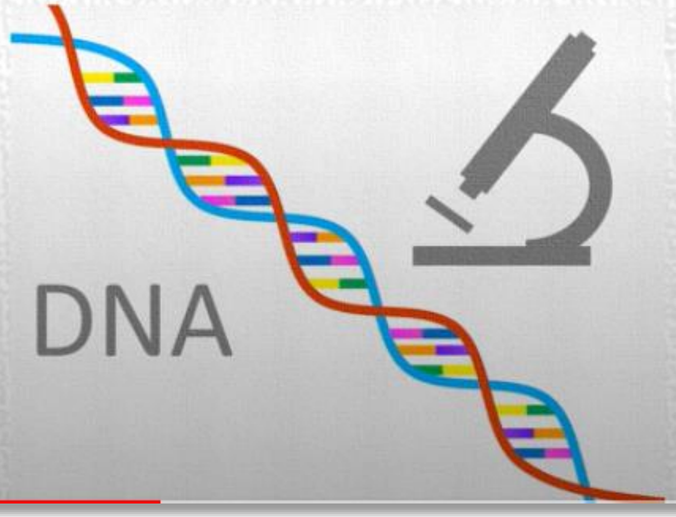
DNA denaturation

In the laboratory…

Separation of DNA strands can be done by…

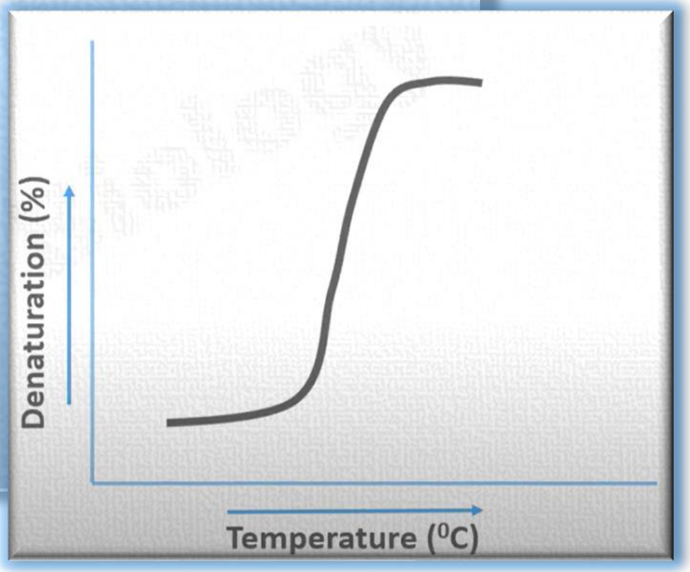
- When DNA solution is heated, hydrogen bond are disrupted. Double stranded Dna is seperated into single strands.

1. Changing pH



2. Heating

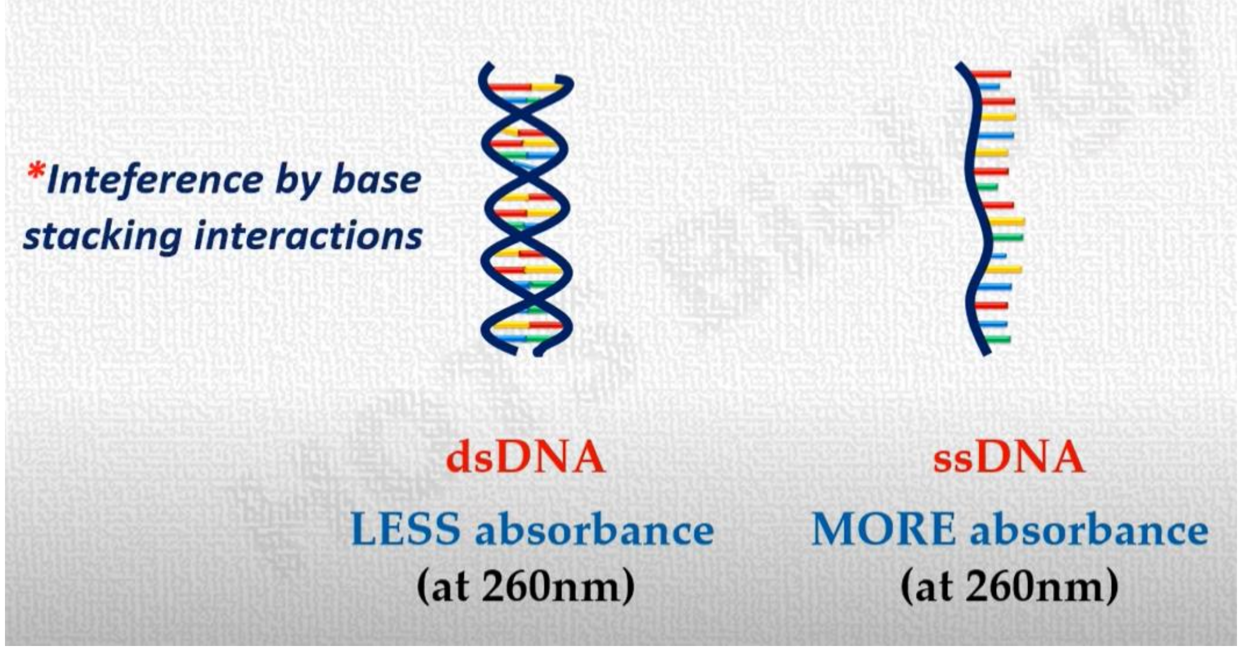
- As the temperature increases, the percentage of denaturation increases.

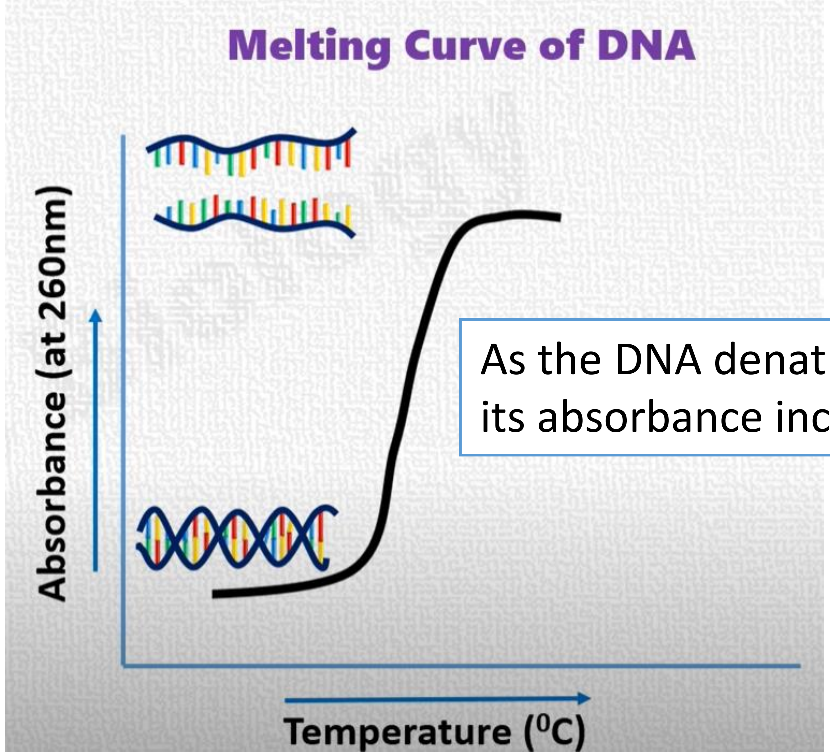


https://www.youtube.com/watch?v=XtXfHclIrxg

How to measure DNA denaturation?

- All nucleotide bases consist of aromatic rings. These aromatic rings absorb light in the ultraviolet (UV) range.
- All bases of DNA have strong absorbance at 260 nm.
- Double stranded DNA absorbs less light at 260 nm compared to single strand DNA. This is because base stacking interactions in DNA double helix interferes with the absorbance.





As the DNA denatures, its absorbance increases.

https://www.youtube.com/watch?v=XtXfHclIrxg

DNA Renaturation

- When the DNA solution temperature is lowered below its melting temperature, seperated DNA strands spontaneously reassociate to form double helix. This is known as DNA renaturation.
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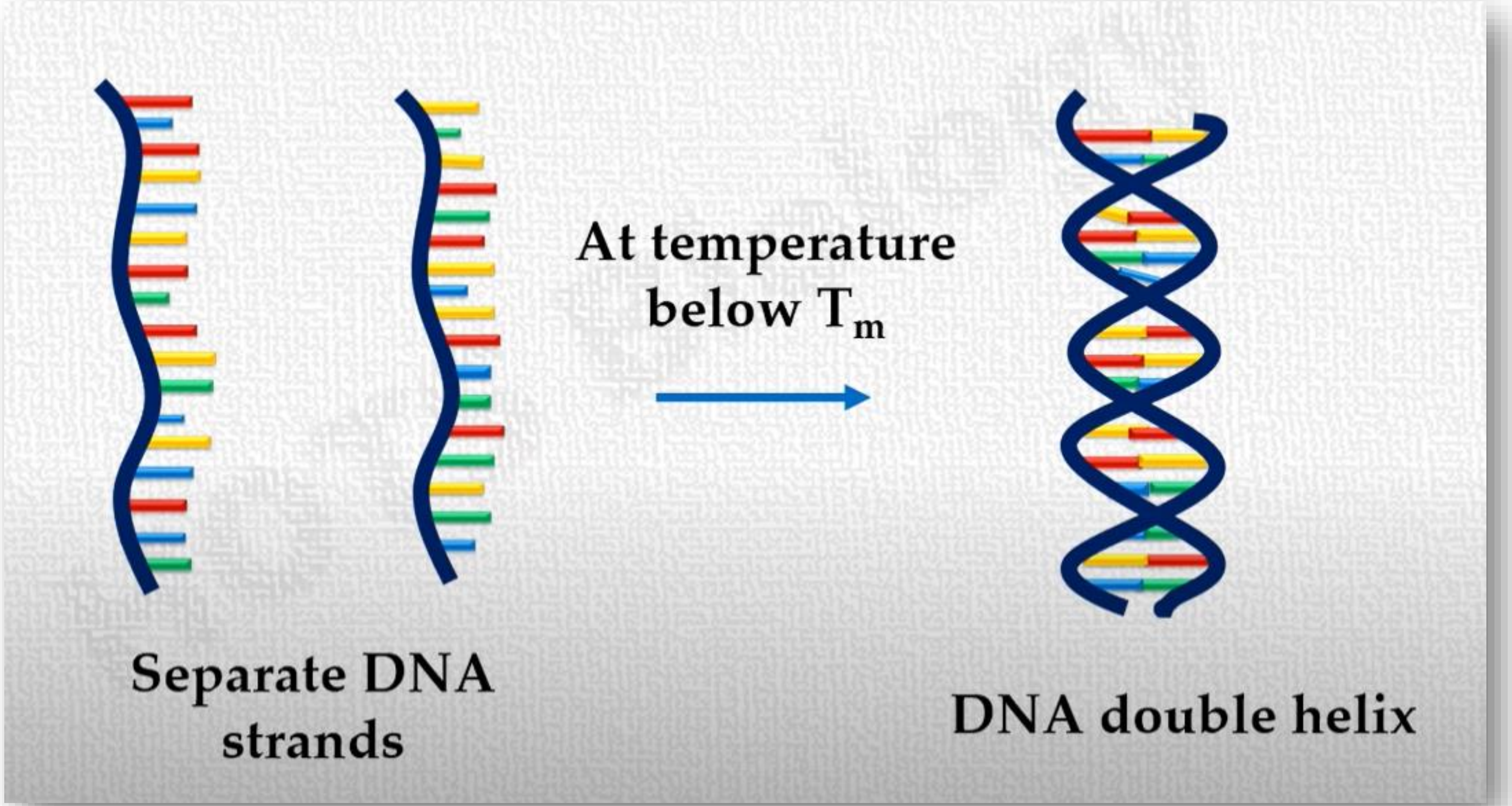

https://www.youtube.com/watch?v=XtXfHclIrxg