MOLECULAR AND CELL BIOLOGY

BIOC 131

PRESENATION BY:

1. Robert Endriaku
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QUESTION

DISCUSS THE MOLECULAR ASPECTS OF THE NUCLEUS.

1. DNA
2. RNA

OBJECTIVES

1. UNDERSTAND BRIEFLY ABOUT THE SUB-STRUCTURES OF THE NULCEUS.
2. DISCUSS NUCLEIC ACIDS – DNA and RNA.
3. UNDERSTAND THE DIFFERENCES BETWEEN DNA AND RNA.

THE NUCLEUS

BY: ROBERT ENDRIAKU

INTRODUCTION

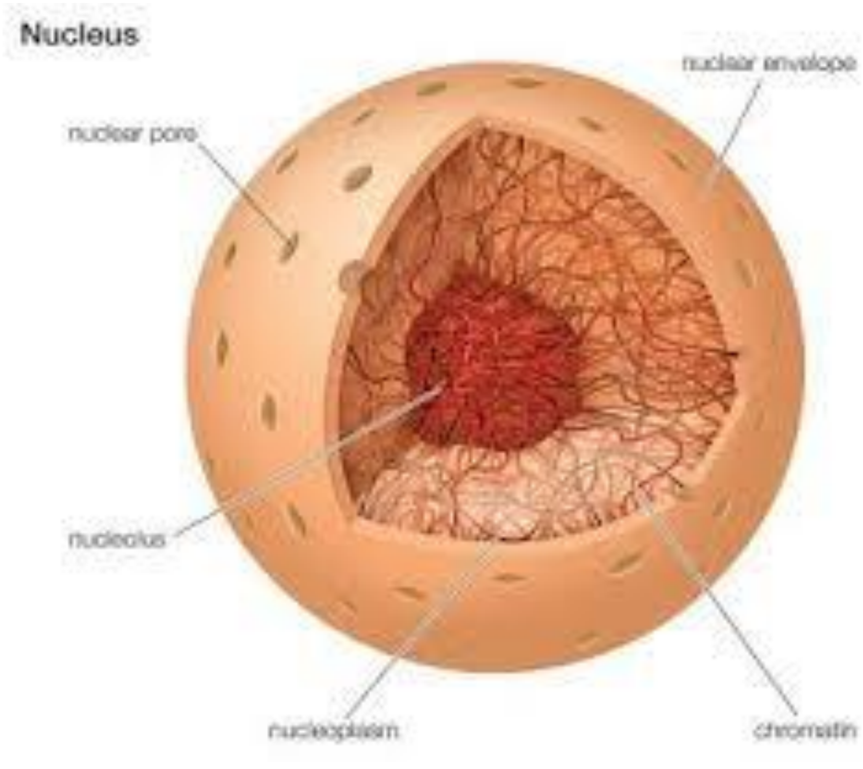
THE NUCLEUS

The nucleus was the first organelle to be discovered. The probably oldest preserved drawing dates back to the early microscopist Antonie van Leeuwenhoek (1632 – 1723).

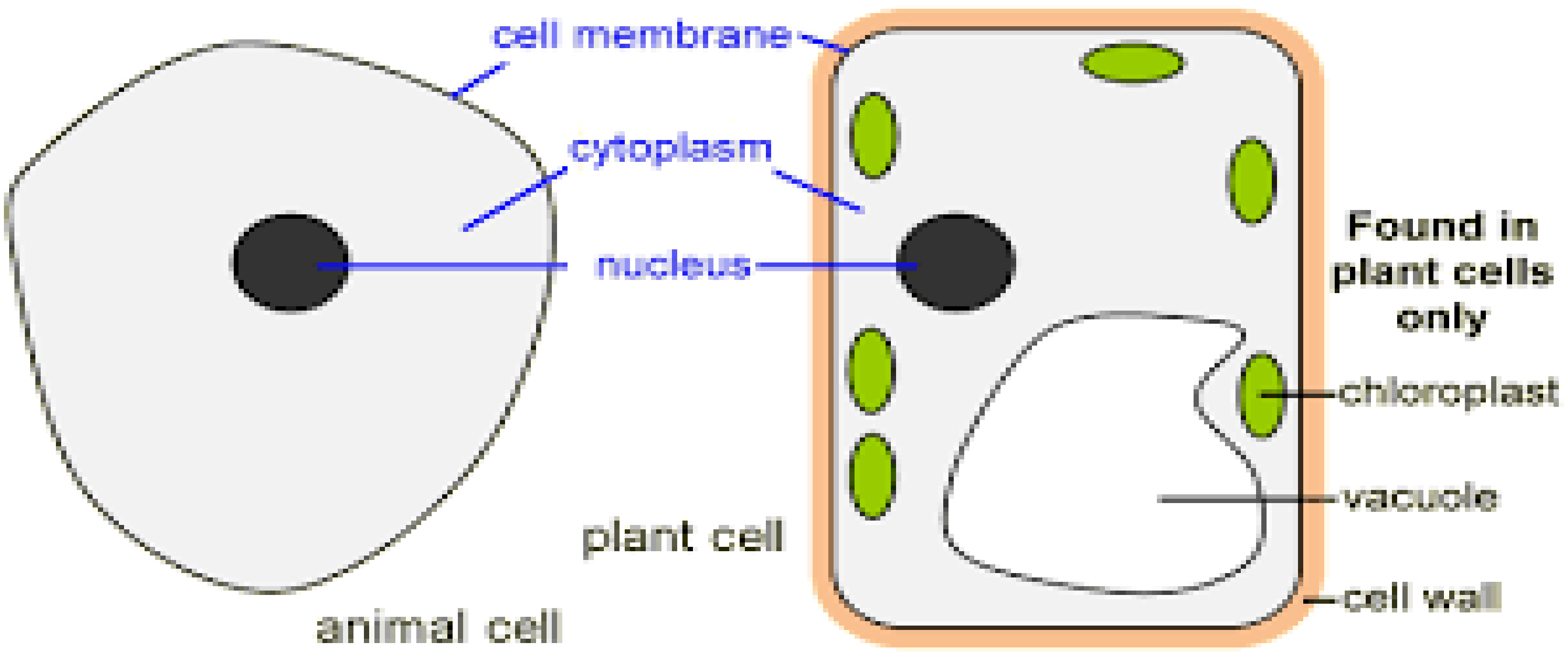
WHAT IS THE NUCLUES?

- The nucleus is a membrane-enclosed cell organelle containing the genetic material and various proteins.

In a nut-shell the Nucleus can be called the ‘Engine’ of the Cell.



THE NUMBER OF NUCLEI IN CELLS OF DIFFERENT ORGANISMS



Continuation…

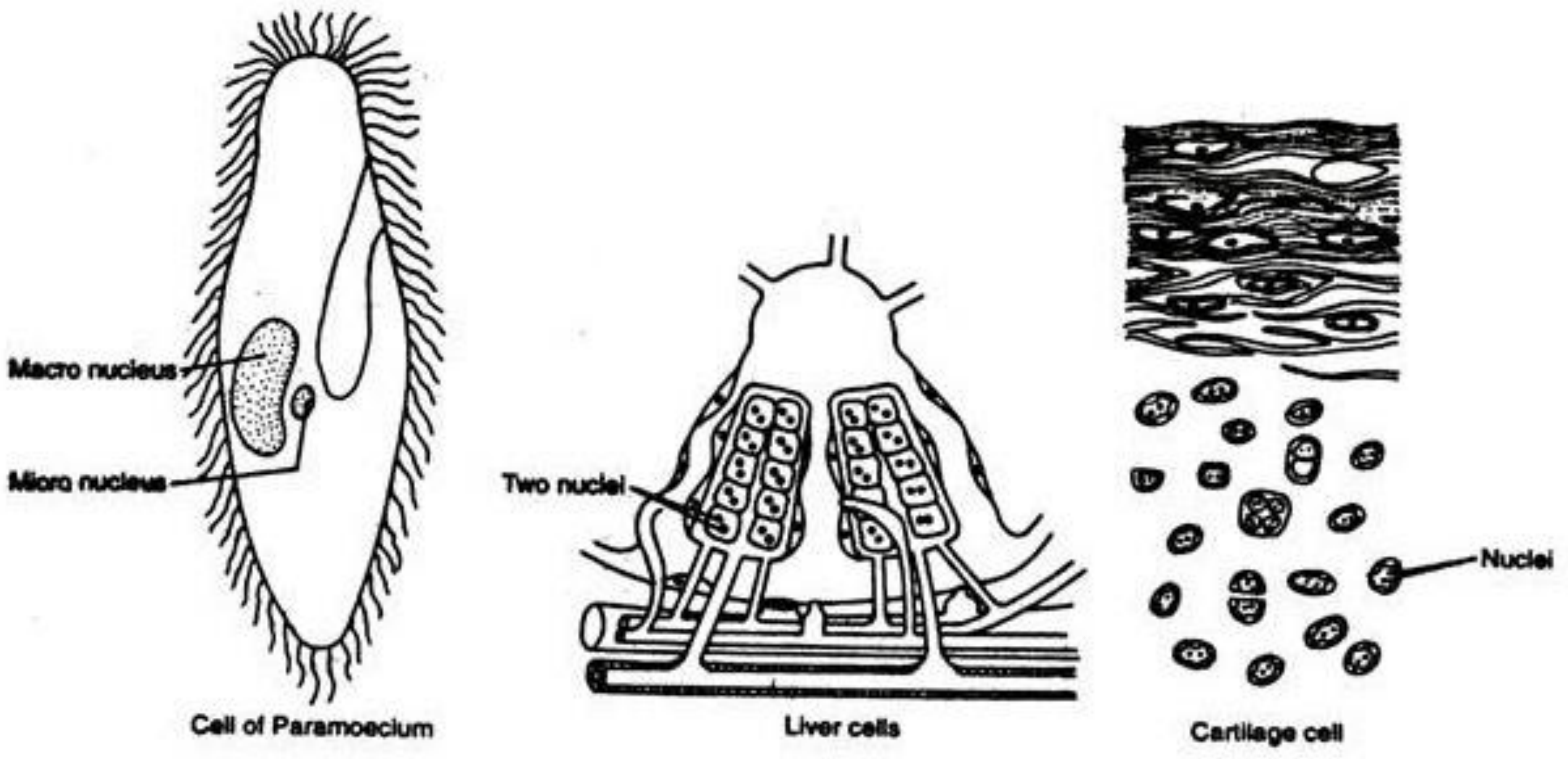
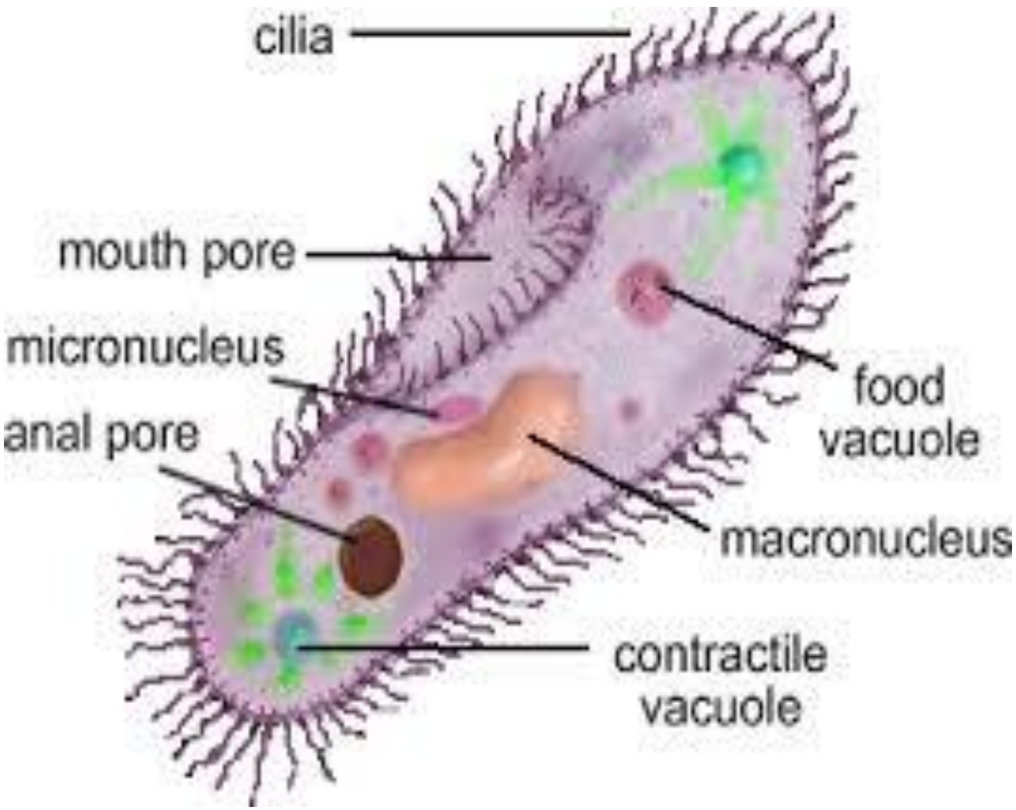
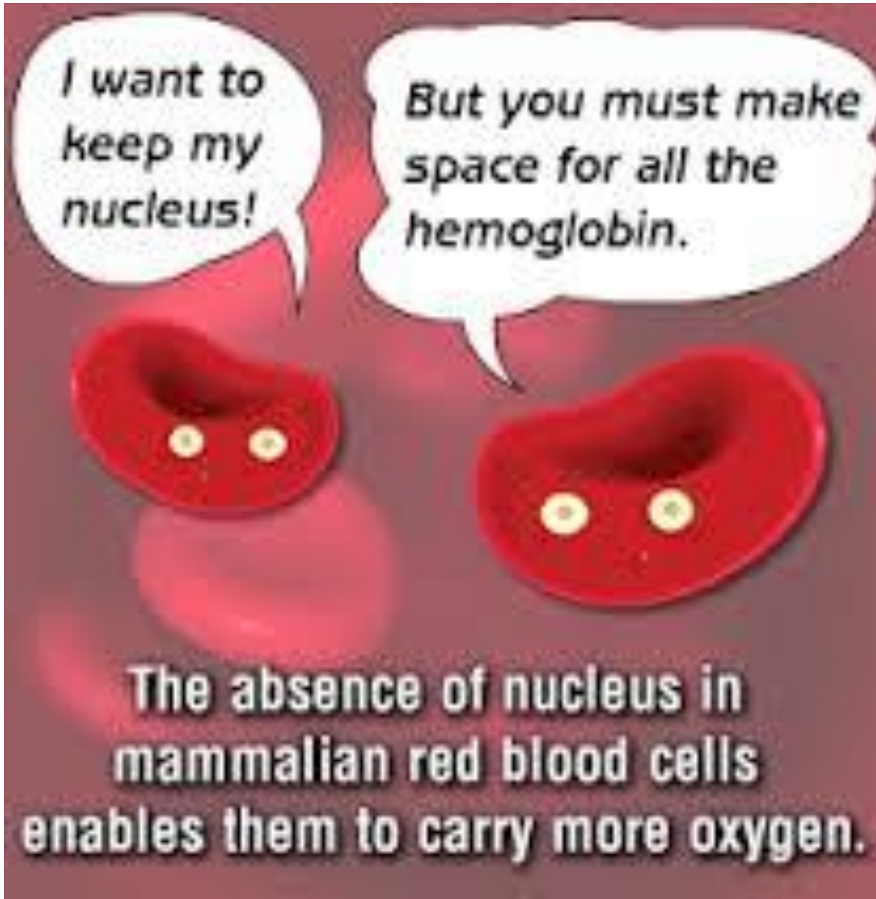


Fig. 9.1: Diagrams showing binucleated cells: (a) Cell of paramoecium; (b) Liver cell and (c) Cartilage cell.

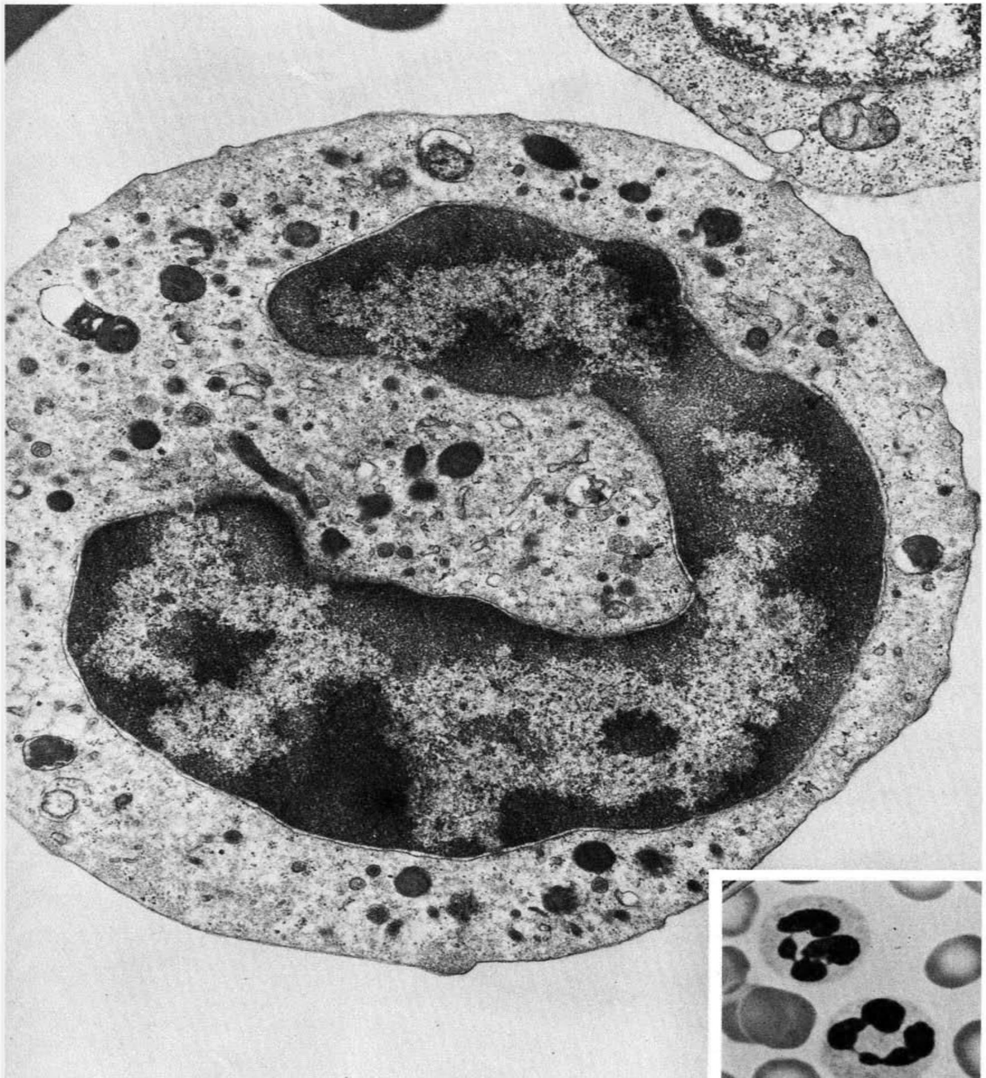

cilia
mouth pore
micronucleus
anal pore
food vacuole
macronucleus
contractile vacuole

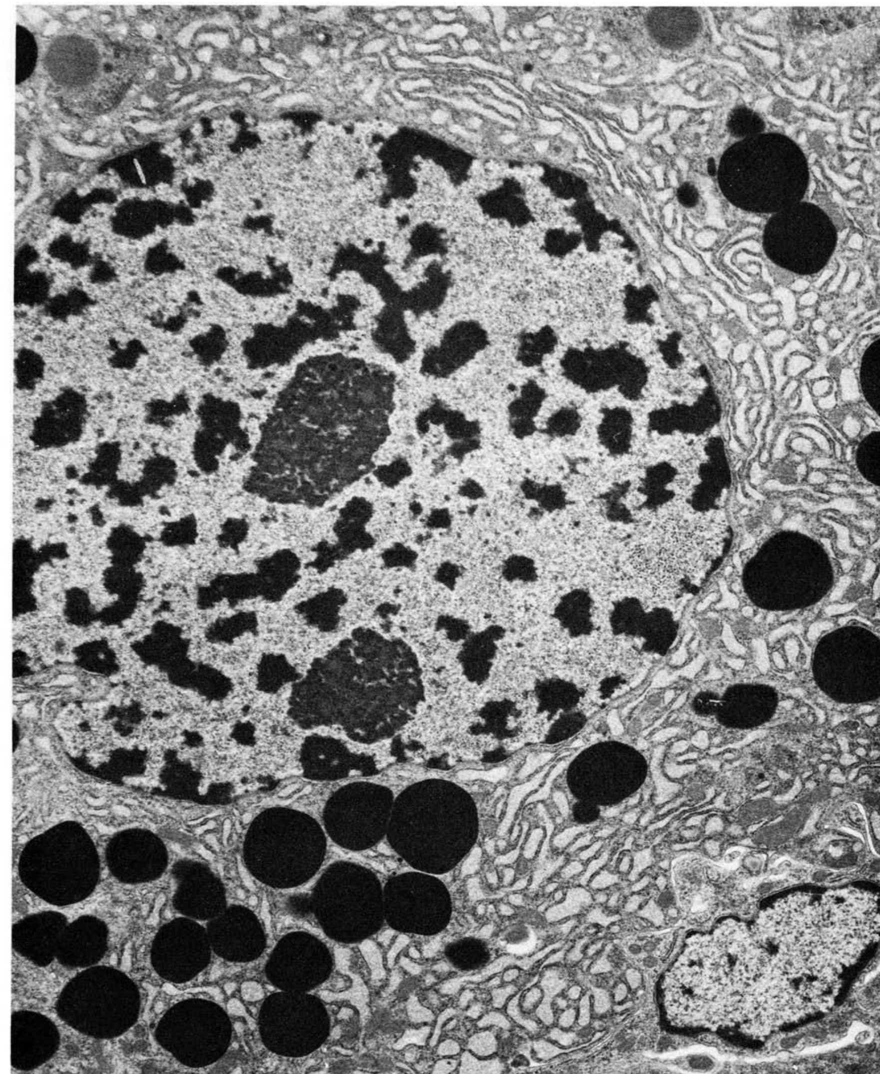

I want to keep my nucleus!
But you must make space for all the hemoglobin.
The absence of nucleus in mammalian red blood cells enables them to carry more oxygen.

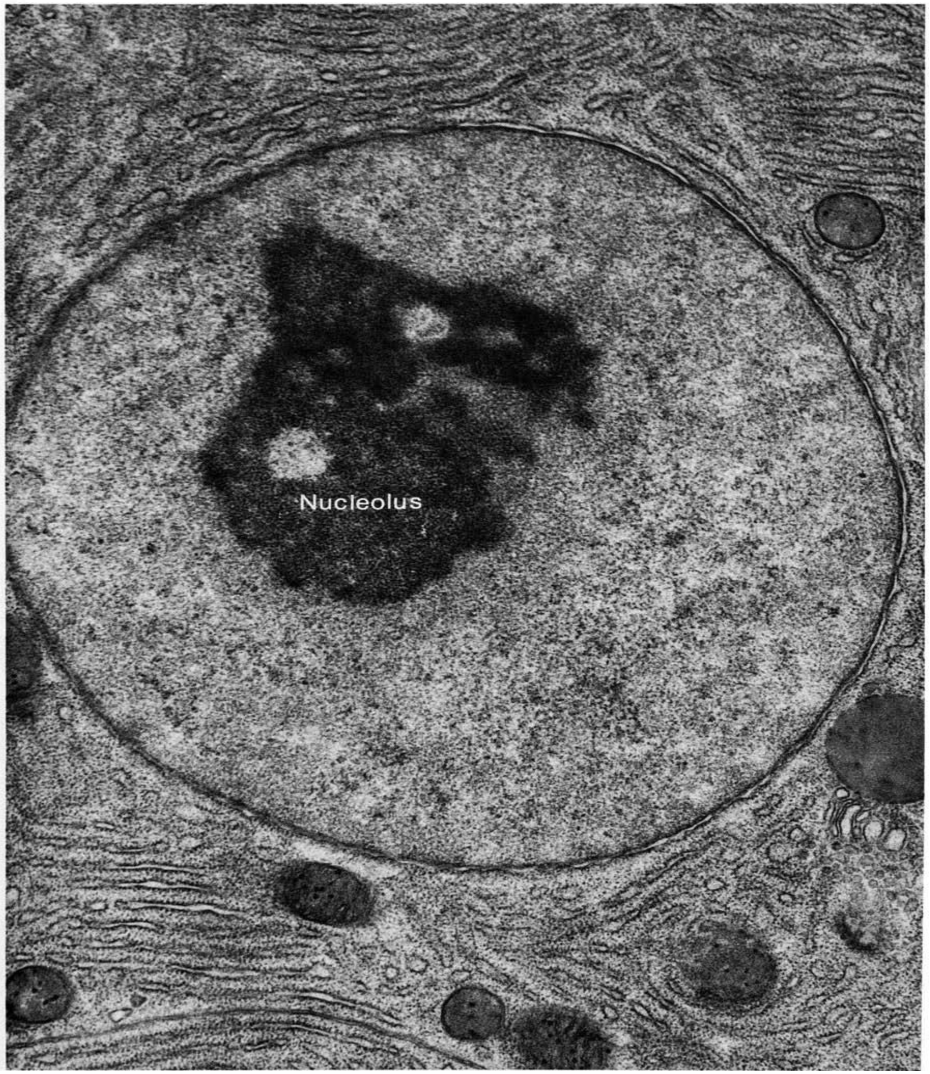
SHAPE OF NUCLEI IN DIFFERENT CELLS

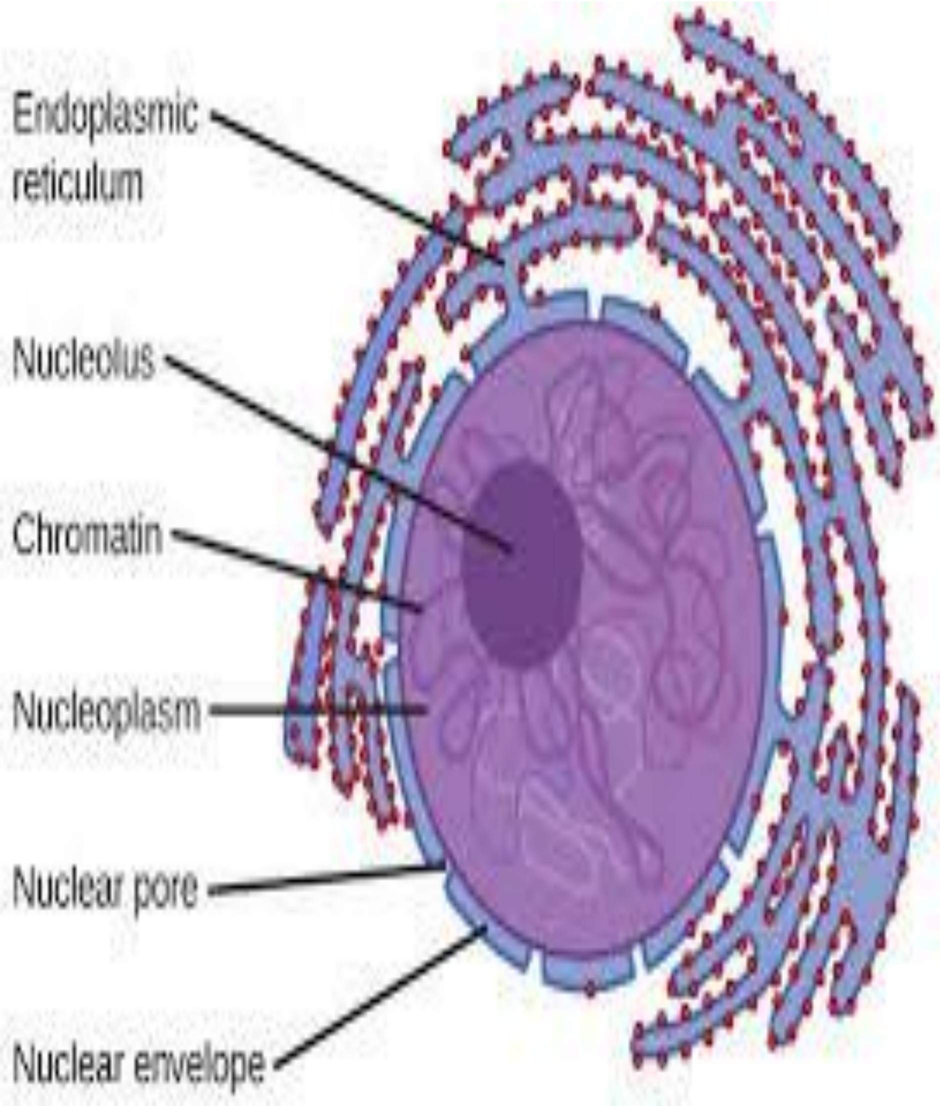
The nucleus may assume an oval, spherical, disc-like, C-shaped, bi or mutli-lobed shape or lens shape depending on the cell under consideration.

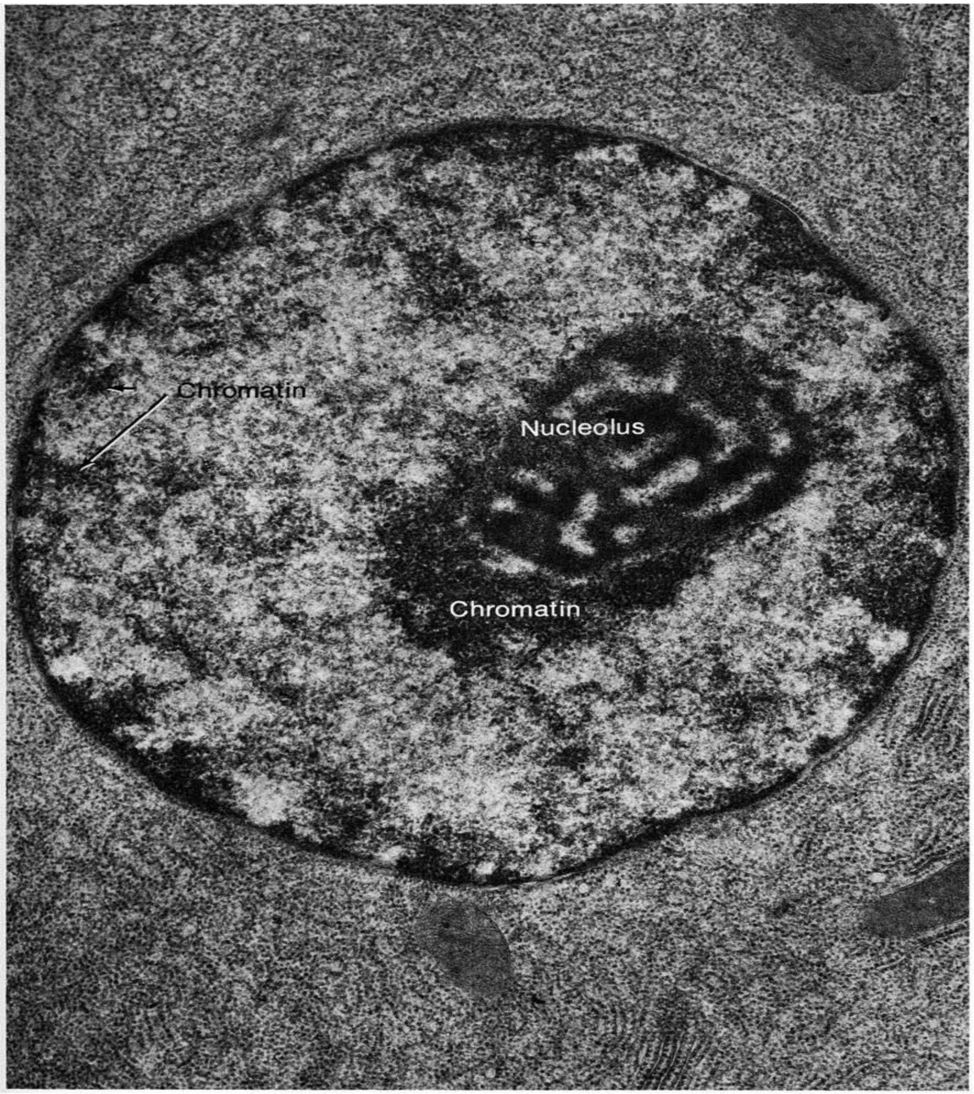
DIFFERENT MICROSCOPIC SHAPES






Nucleolus


Endoplasmic reticulum
Nucleolus
Chromatin
Nucleoplasm
Nuclear pore
Nuclear envelope


Chromatin
Nucleolus
Chromatin

The nucleus consists of the following main parts:

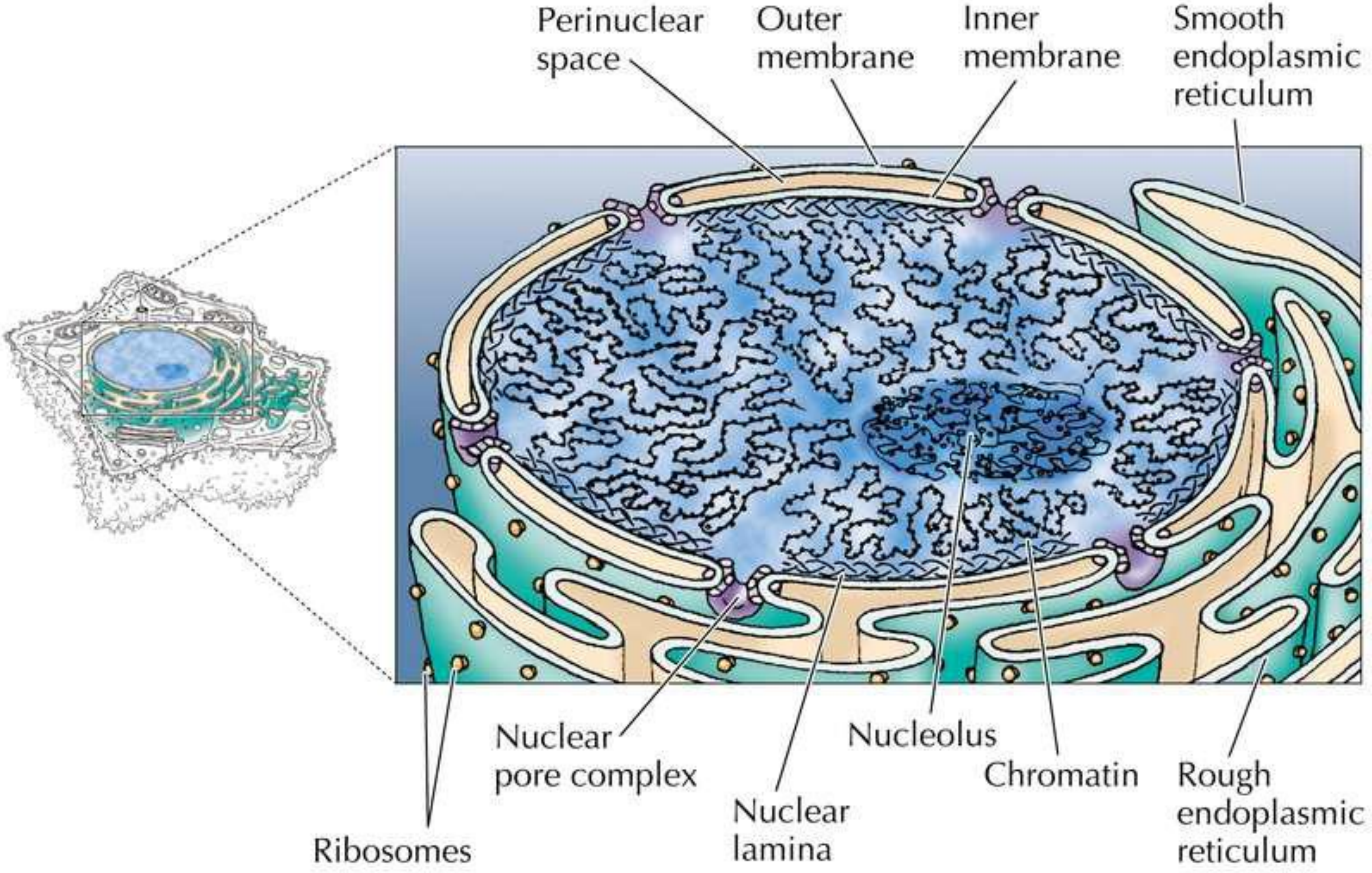
(1) Nucleolemma or nuclear membrane (karyotheca)

(2) Nuclear sap or karyolymph or nucleoplasm

(3) Chromatin network or fibres

(4) Nucleolus

(5) Nuclear Pores



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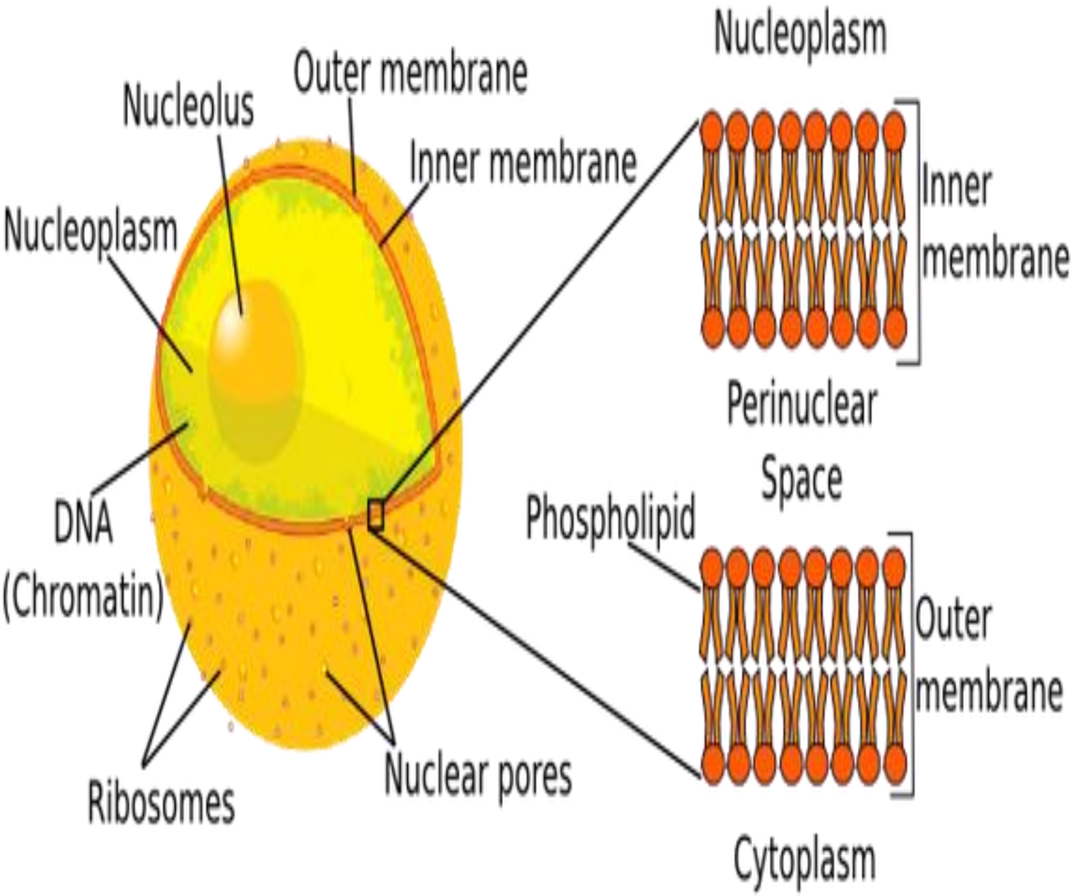
THE NUCLEAR MEMBRANE



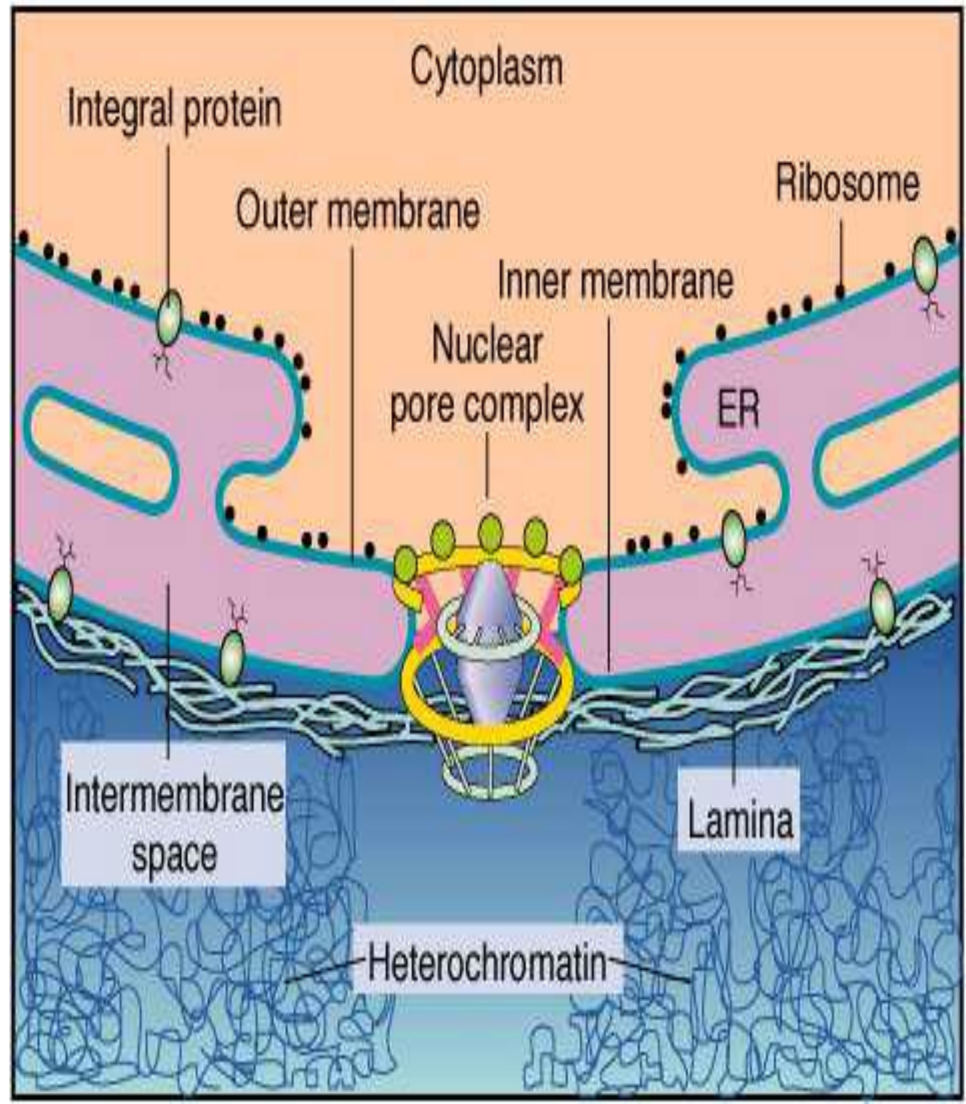
The nucleus is separated from the cytoplasm by a limiting membrane called as karyotheca or nuclear membrane. The Nuclear membrane is a double membrane.

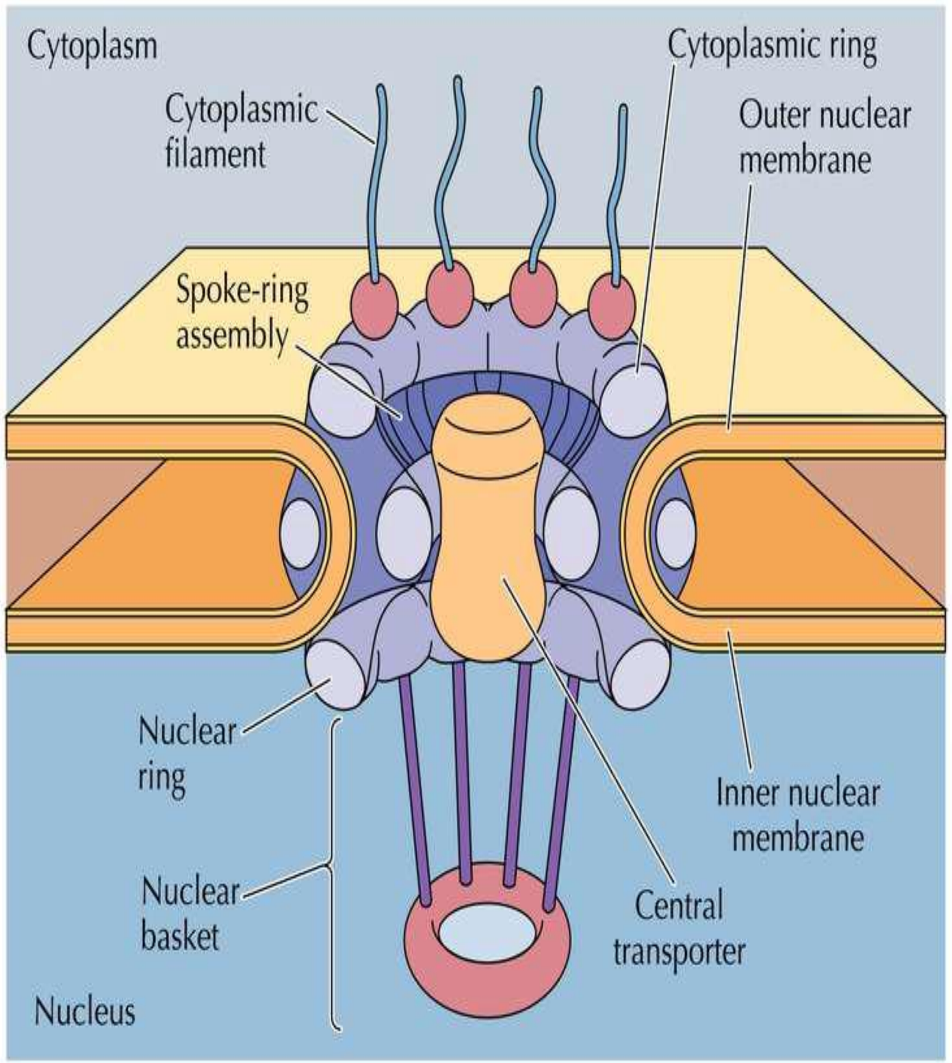
FUNCTION OF NUCLEAR MEMBRANE

This membrane plays an important role for the transport of the material between the nucleus and the cytoplasm. Nuclear envelope regulates nucleocytoplasmic exchanges and coordinates gene action with cytoplasmic activity.

NUCLEAR PORE

The nuclear membrane possesses a number of nuclear pores or annuli, which vary from 40 to 145 per square micro-meter in nuclei of various plants and animals. The nuclear pores are octagonal in shape, their diameter varies from 400-1000 A. The pores and annuli collectively form the pore complex.





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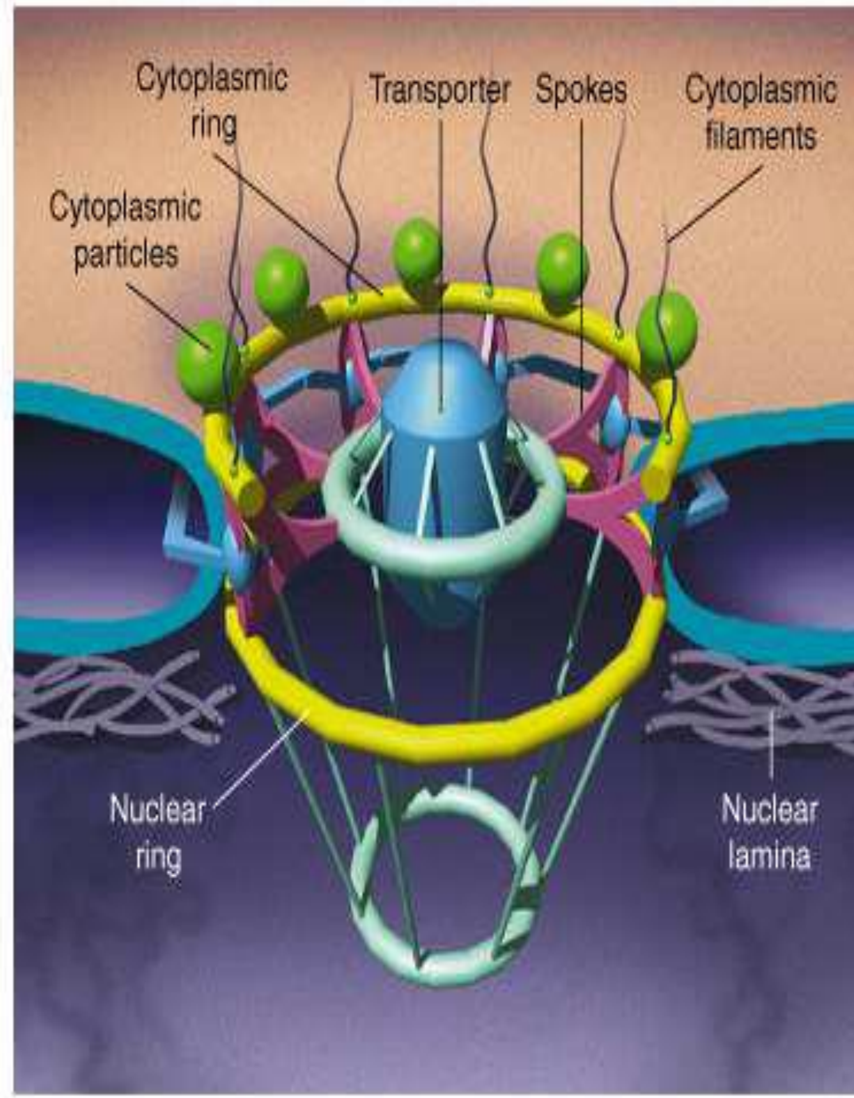

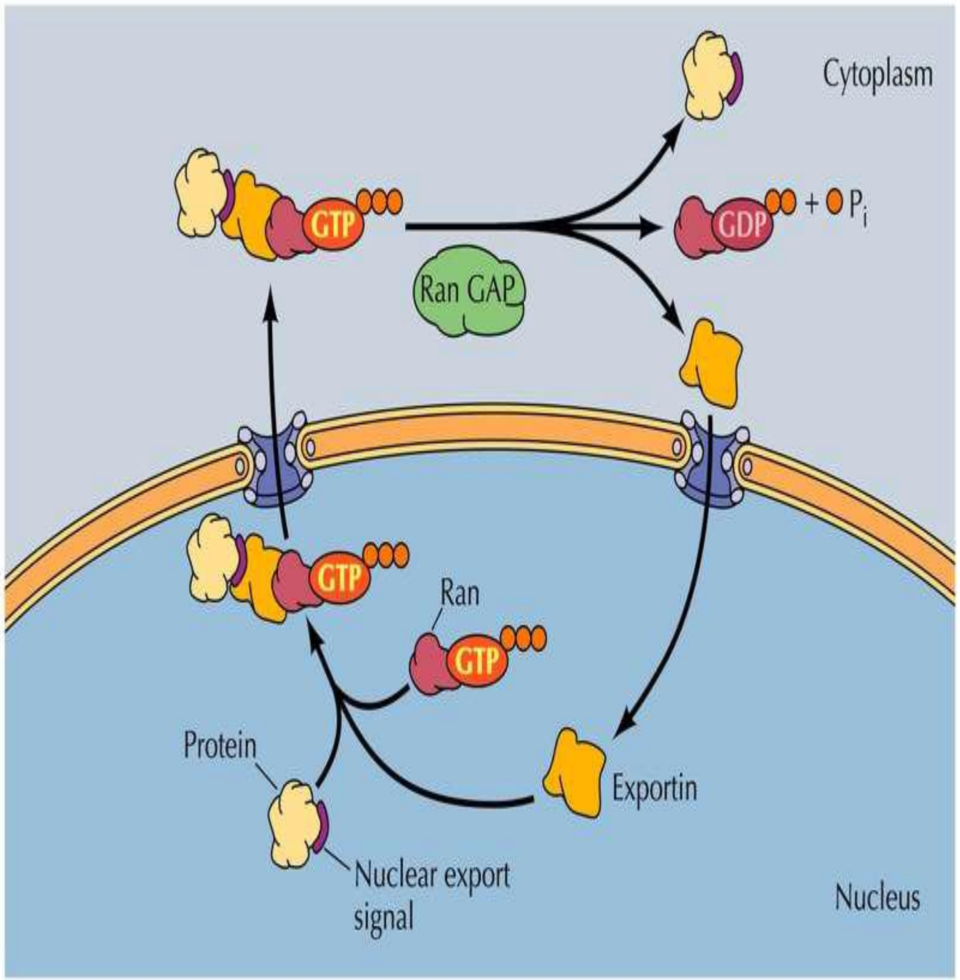

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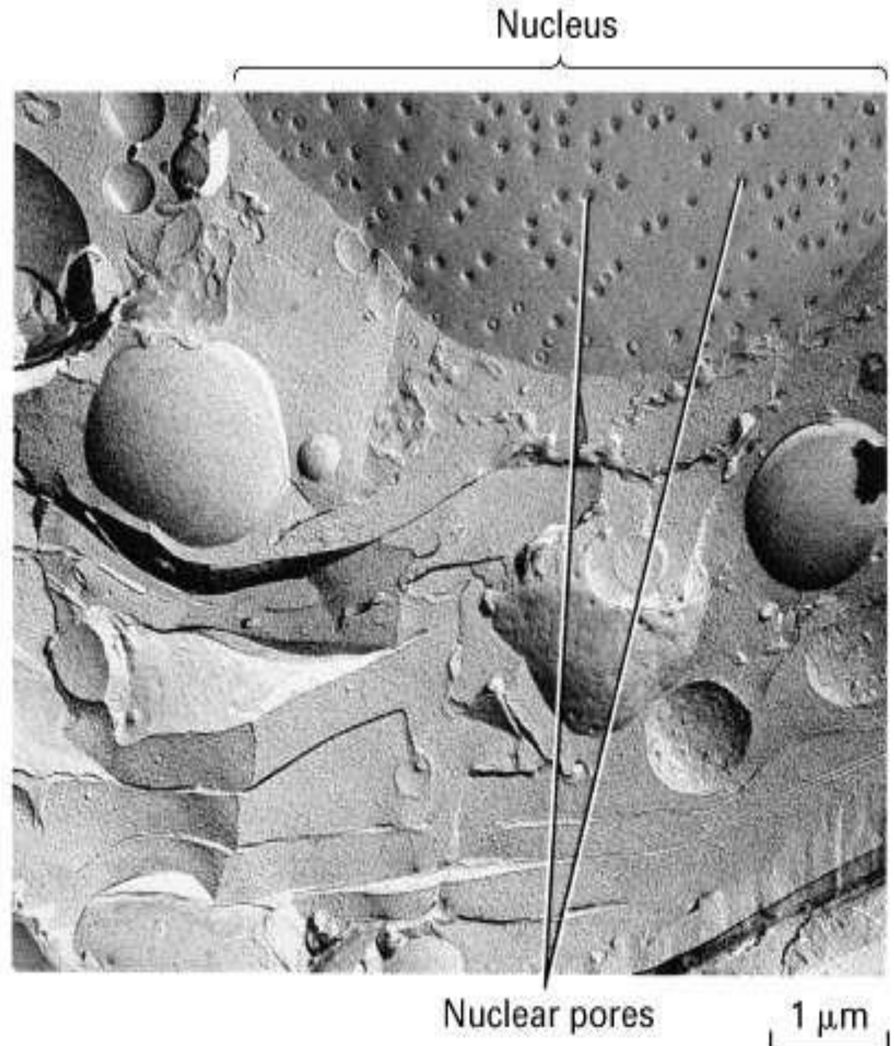
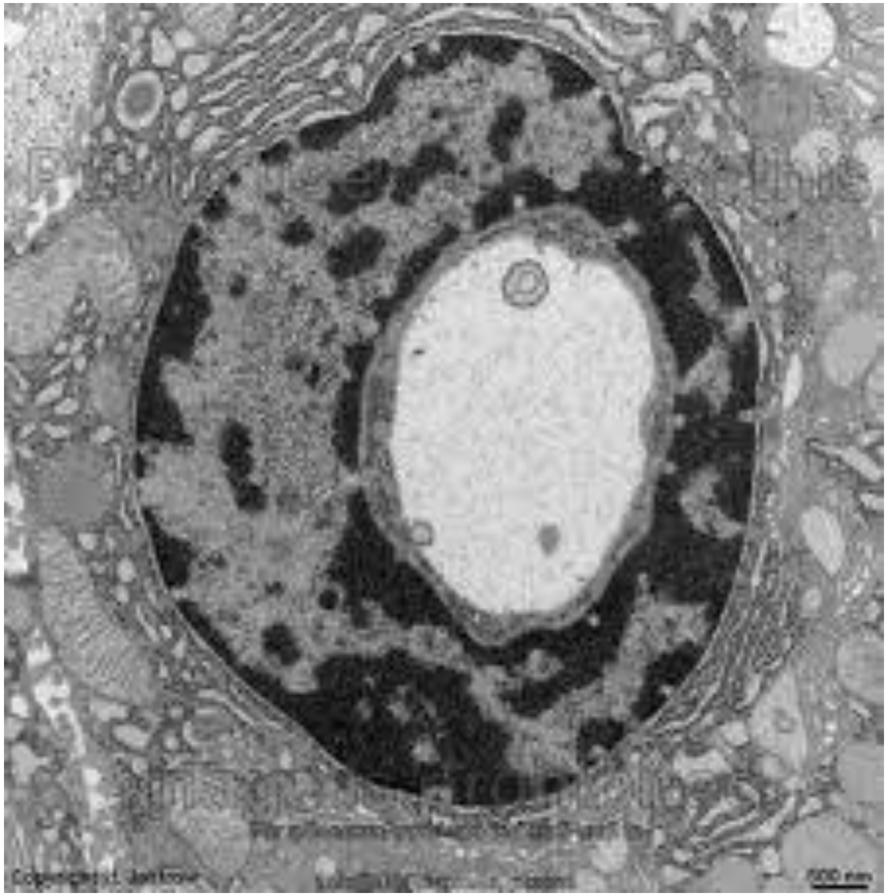


Nuclear sap(Nucleoplasm)

This plasma also termed as karyolymph which is a fluid substance containing many particles and network. Primarily it is composed of proteinous material and is the main site for enzyme activity.

This nuclear sap also shows variable appearance during different stages of cell division. karyolymph also contributes to the formation of the spindle apparatus in plants.



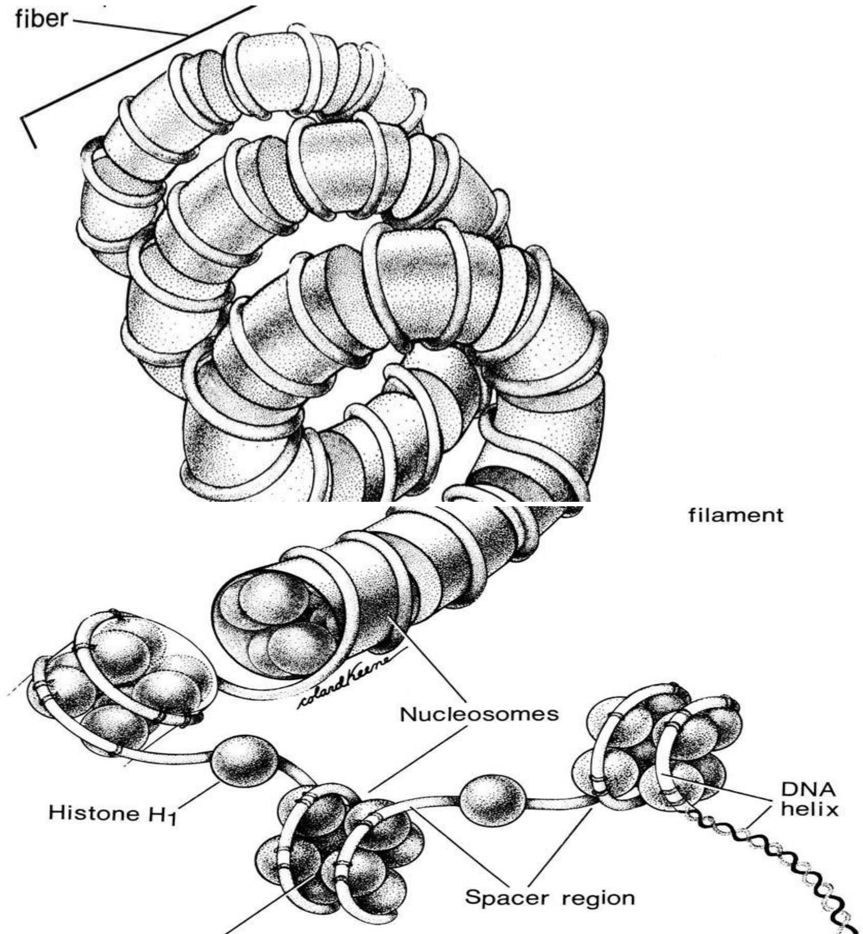
CONSTITUENTS OF THE NUCLEAR SAP

The nuclear sap constitutes different components that support the proper functioning of the nucleus. These include;

1. RNA
2. DNA
3. Proteins of two kinds – histone and non-histone
4. Enzymes

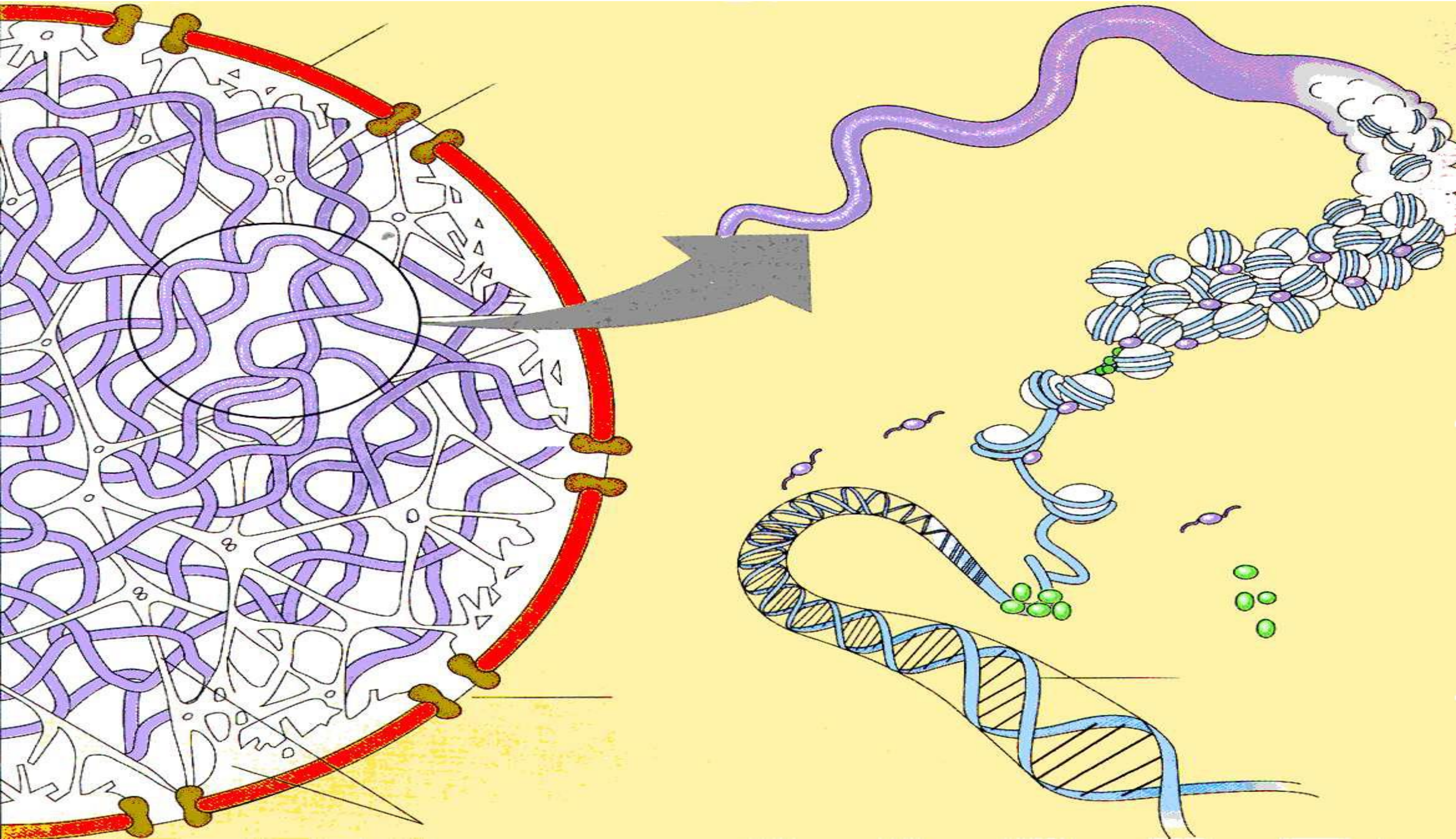
The Nuclear sap supports and organizes the nucleus.

HISTONES



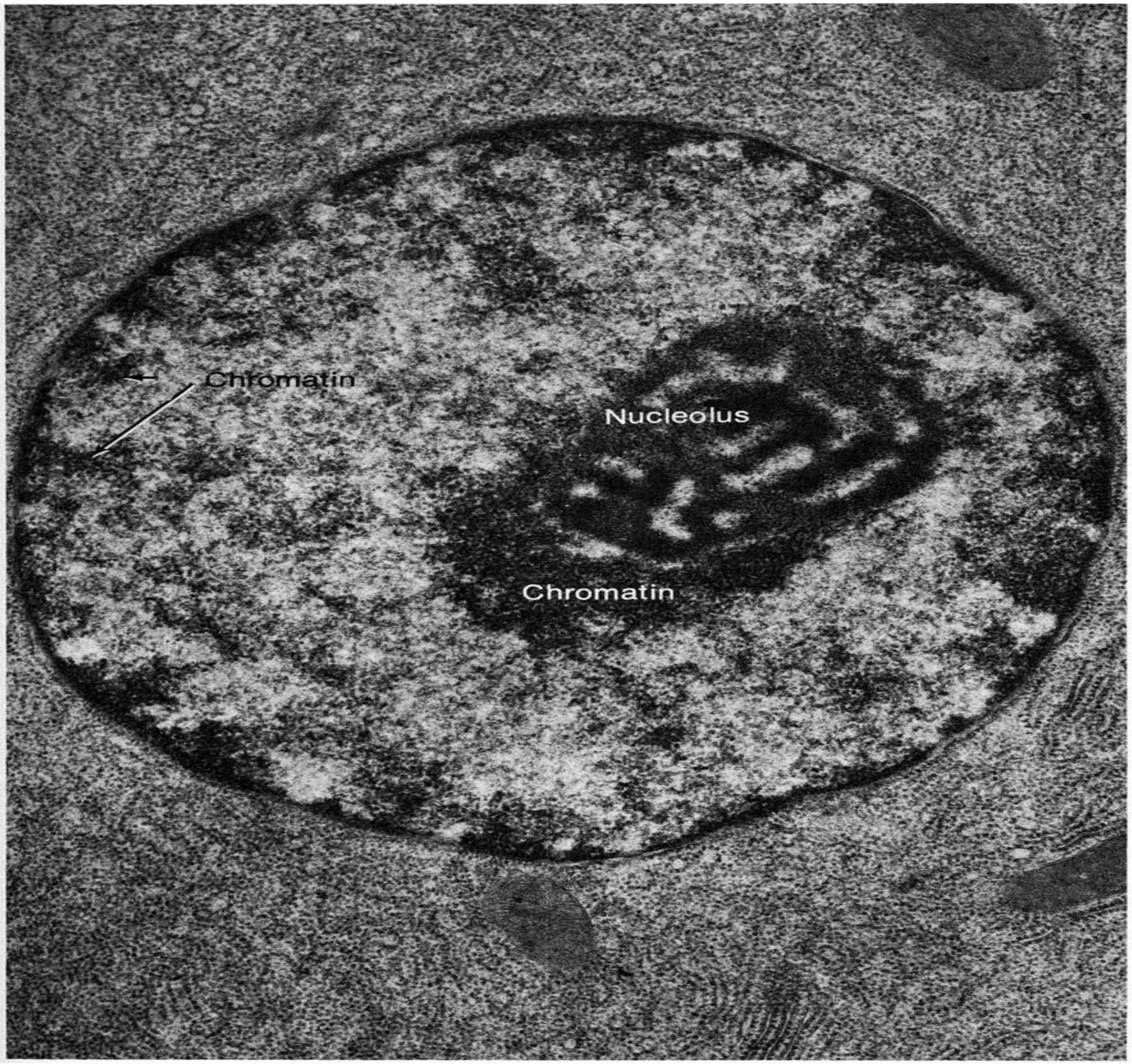
These are small proteins which are basic because they have a high content of the basic amino acids arginine and lysine. Being basic, histones bind tightly to DNA which is acid.

STORAGE OF DNA



CHROMATIN

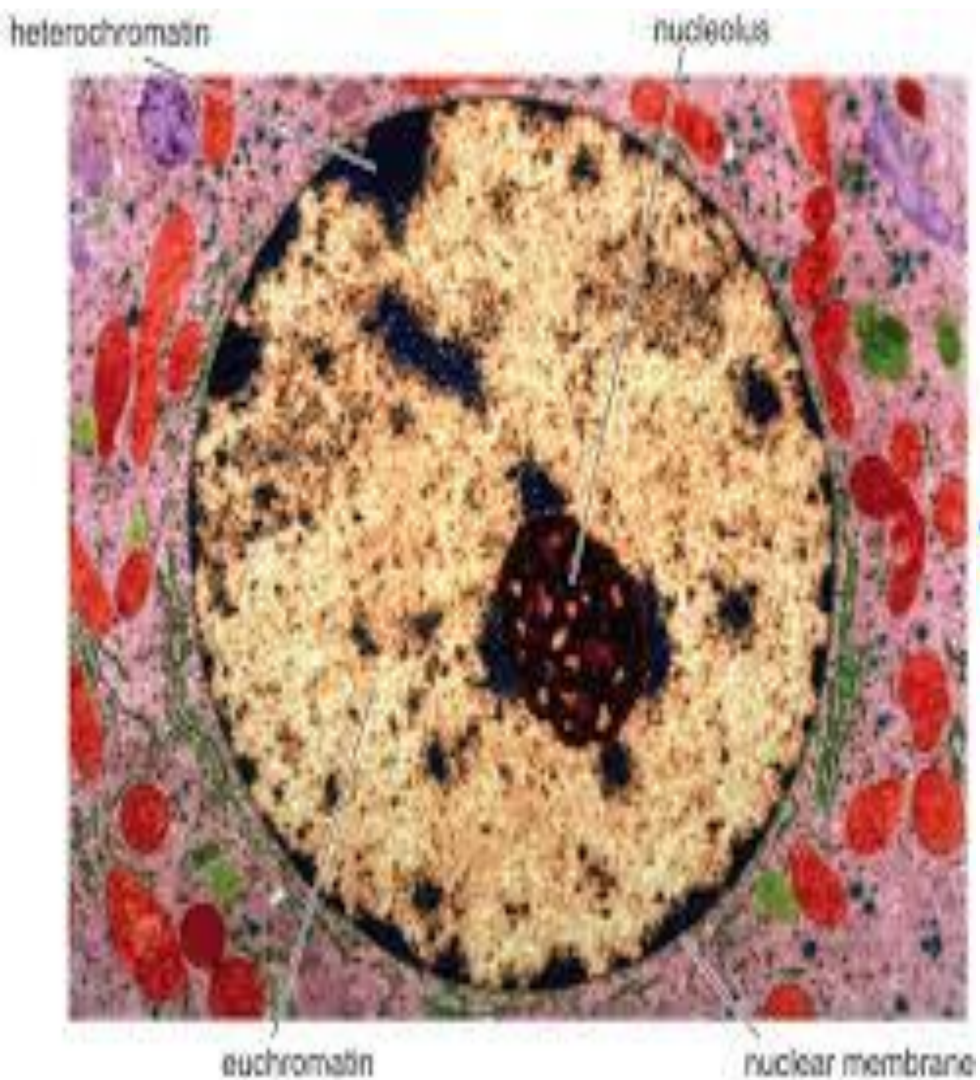
It appears as a viscous, gelatinous substance which contains DNA, RNA, basic proteins called histones, and nonhistone (more acidic) proteins.

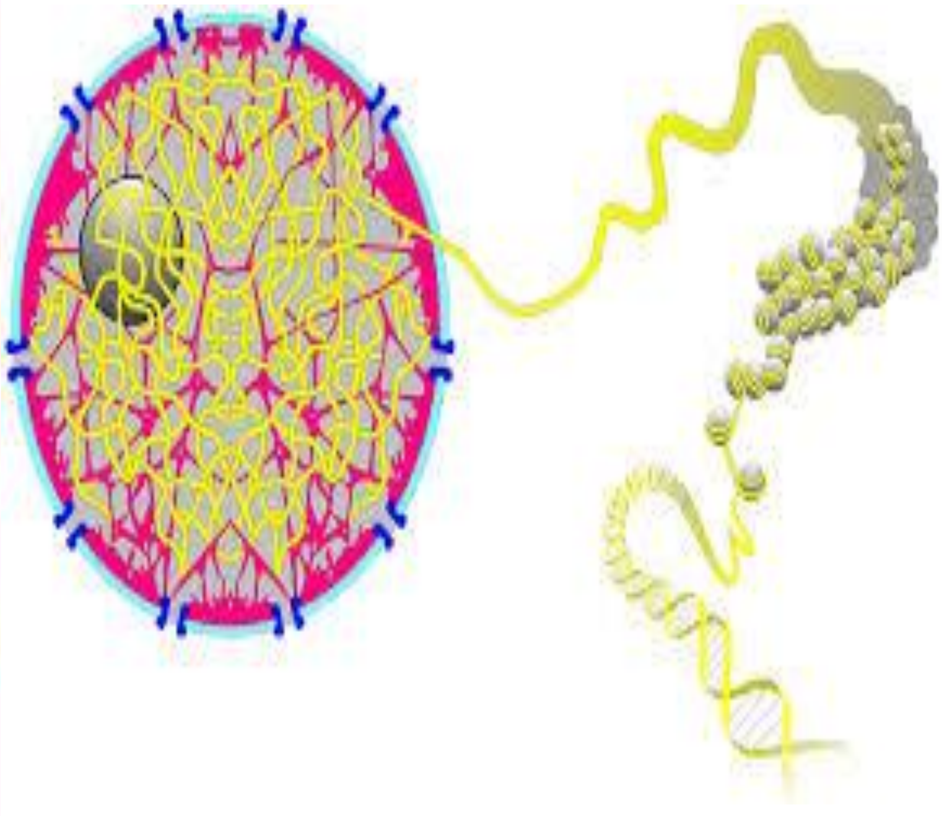


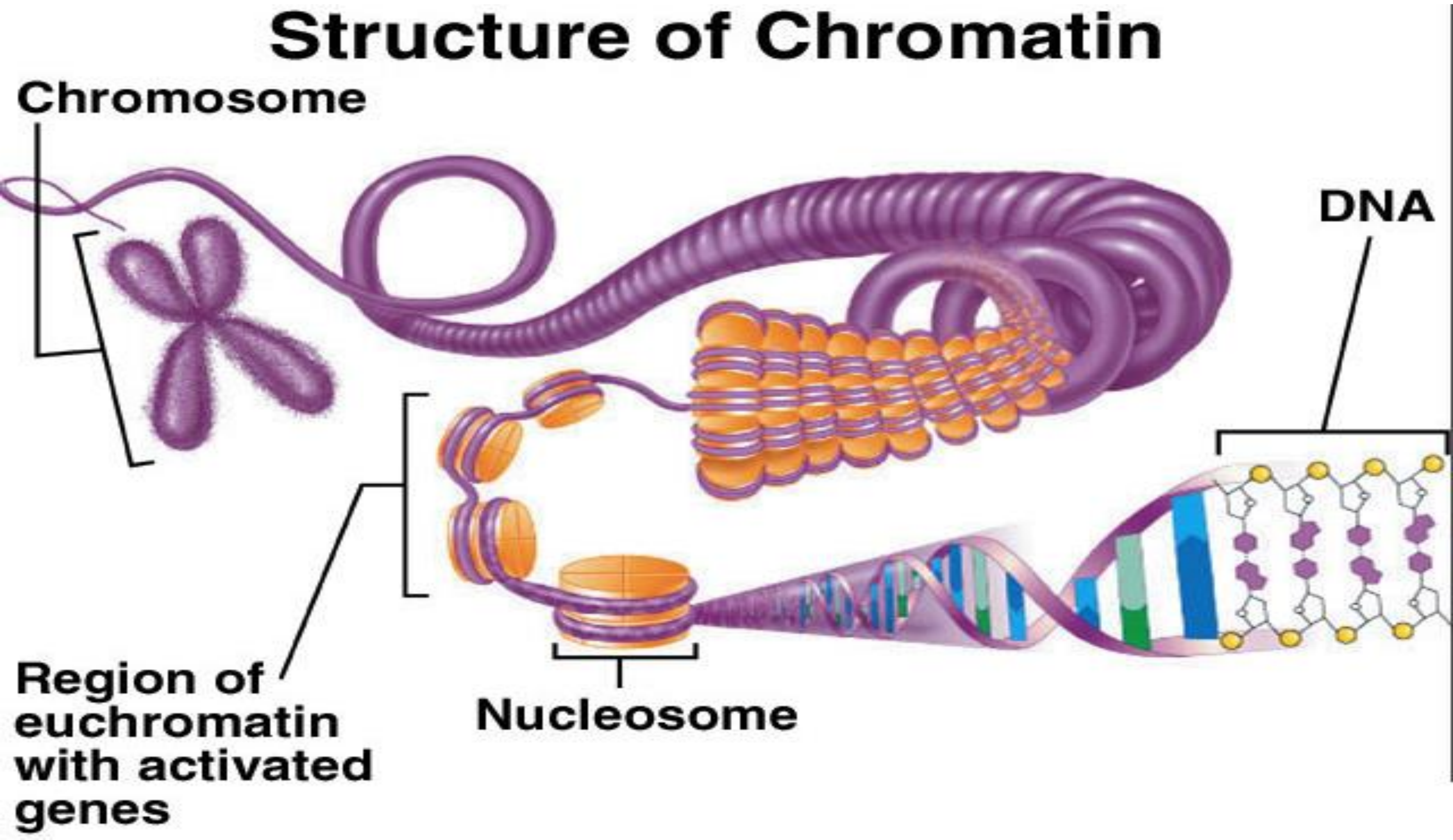
The content of RNA and non-histone protein is variable between different chromatin preparations, but histone and DNA are always present in a fixed ratio about one to one by weight. The nonhistone proteins are very heterogeneous; they vary in different tissues and include RNA and DNA polymerase.

Chromatin Packing

Euchromatin	Heterochromatin
• eu – true	• hetero – different
• loosely packed DNA regions which allows transcription to readily occur	• tightly packed DNA regions with little transcription


heterochromatin
nucleolus
euchromatin
nuclear membrane




Structure of Chromatin
Chromosome
DNA
Region of euchromatin with activated genes
Nucleosome

TYPES OF CHROMATIN

* 1. Hetero-Chromatin
* This is condensed chromatin and is therefore genetically inactive; that is, transcription is not occurring. Heterochromatin is seen associated with the nuclear envelope (peripheral chromatin), with the nucleolus (nucleolar associated chromatin), and scattered throughout the nucleus (chromatin granules).

There are two types of heterochromatin;

- ***Constitutive heterochromatin***, which is permanently inactive (e.g., centromere region of chromosome).

- ***Facultative heterochromatin***, which may have been active in the past and may be so again in the future. It represents inactivated genes. The amount of facultative heterochromatin depends on the cell type and stage of development.

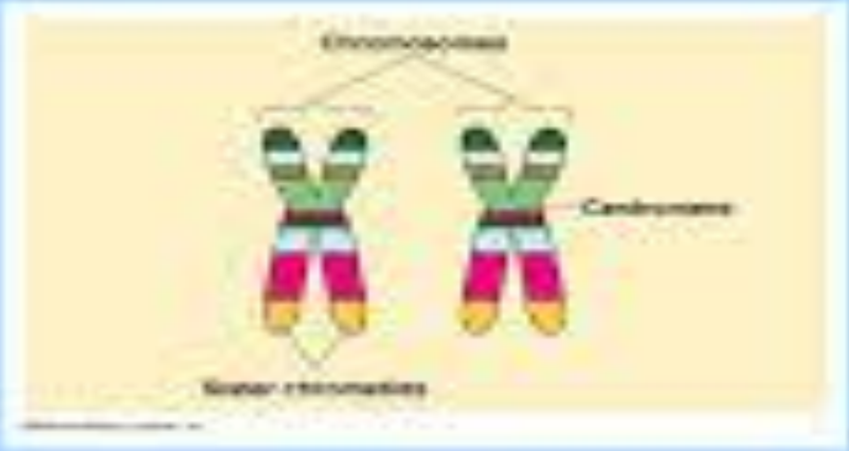
2. Euchromatin

This is **extended chromatin** and is therefore **genetically active**; that is, transcription is occurring. At the EM (electron Microscope) level, euchromatin appears as electronlucent regions interspersed among clumps of electron-dense heterochromatin.

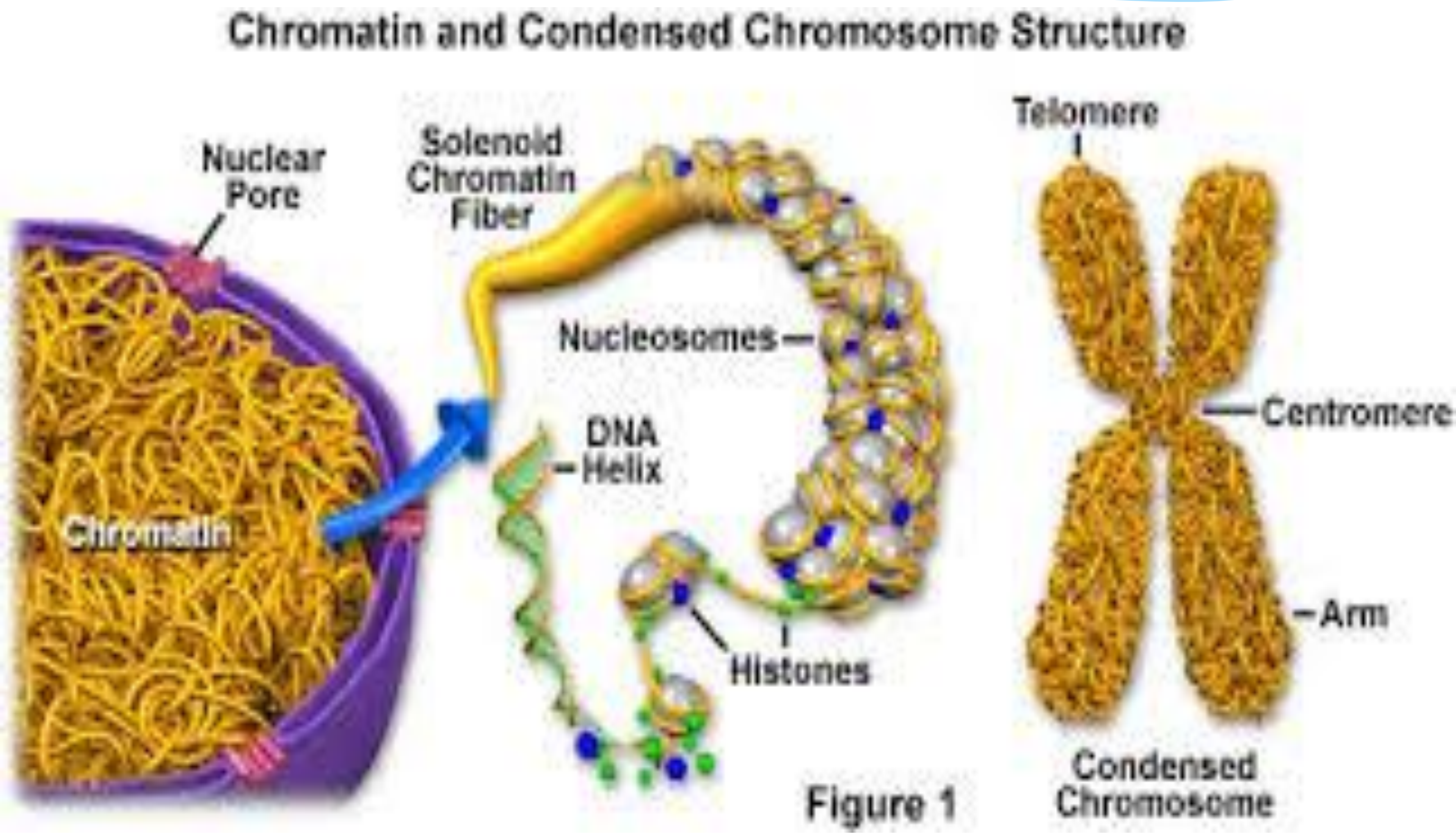
DIFFERENCE BETWEEN CHROMATIN AND CHROMOSOMES

Chromosomes vs. Chromatin

Chromosomes
- Tightly packaged DNA
- Found only during cell division
- DNA is not being used for macromolecule synthesis

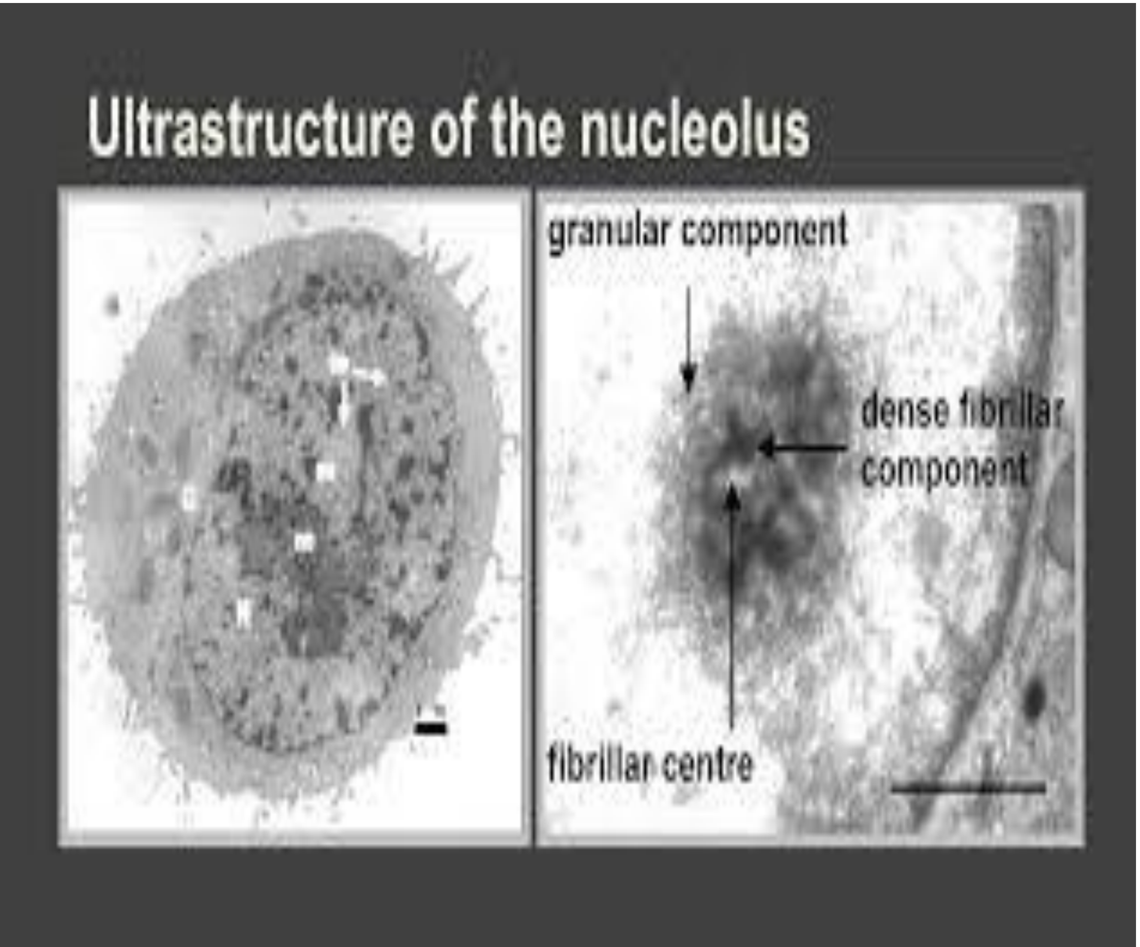


Chromatin
- Unwound DNA
- Found throughout Interphase
- DNA *is* being used for macromolecule synthesis


Chromatin and Condensed Chromosome Structure
Nuclear Pore
Solenoid Chromatin Fiber
Telomere
Nucleosomes
Centromere
DNA Helix
Chromatin
Arm
Histones
Figure 1
Condensed Chromosome

THE NUCLEOLUS

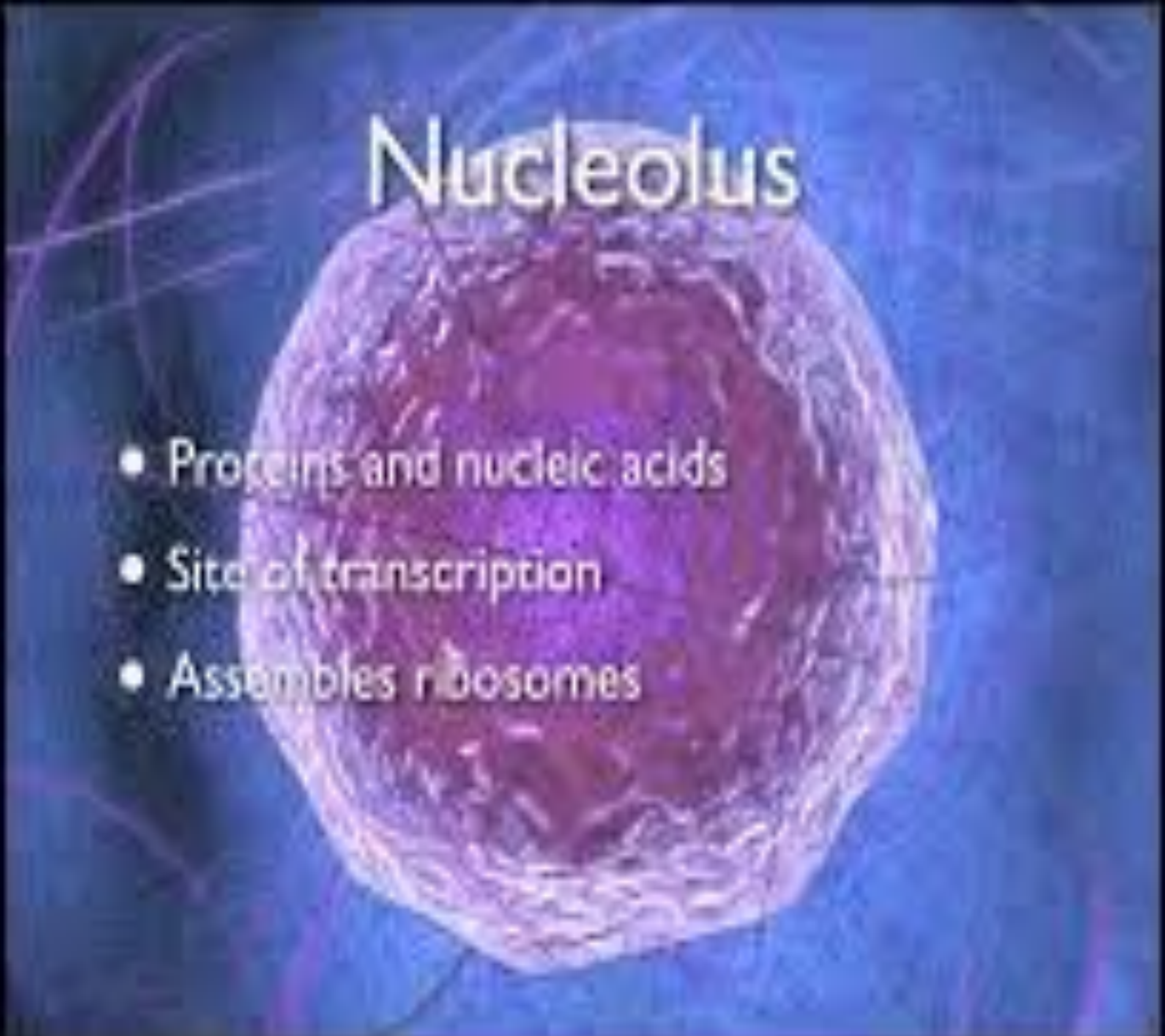
The nucleolus is a none membrane bound separate entity within the nucleus consisting largely of rRNA and protein.



The Nucleolus was first described by Fontana in 1781 (nucleolus meaning 'small nucluns'). Nucleolus has no membrane of its own and is more dense than the surrounding nucleoplasm and hence is distinctly visible.

The nucleolus

- The nucleolus is the main part of the cell that produces the ribosomes that make the proteins.


Nucleolus
Proteins and nucleic acids
Site of transcription
Assembles ribosomes

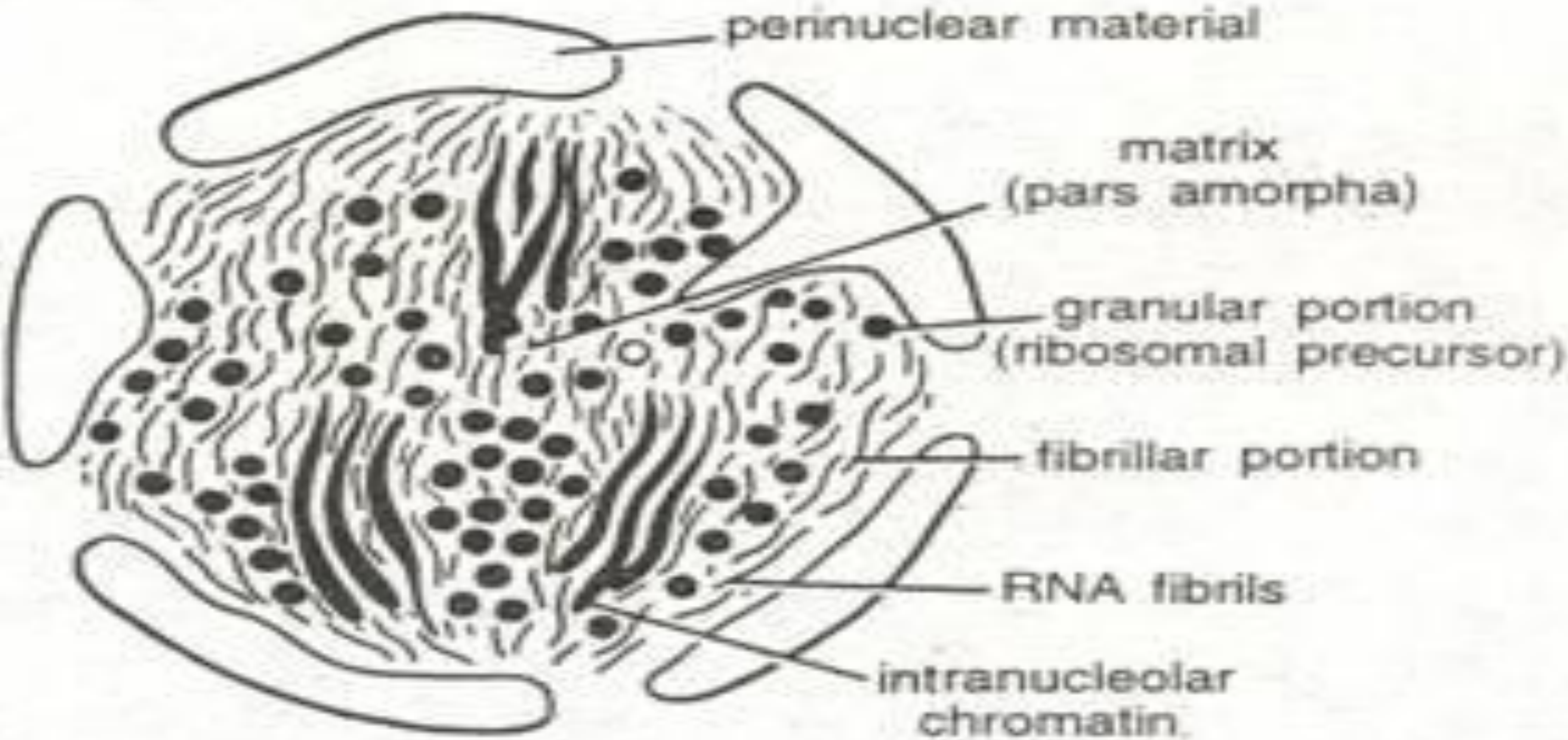


Fig. 7. Ultrastructure of nucleolus.

FUNCTIONS OF NUCLEOLUS

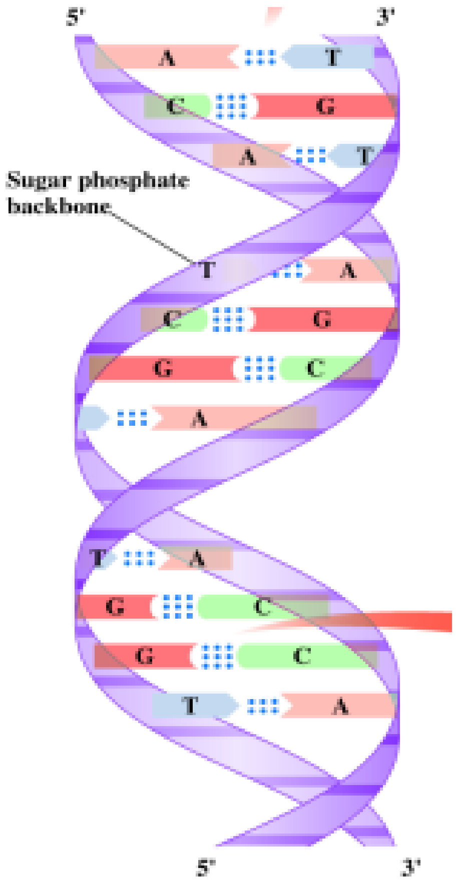
1. **Role in mitosis**
2. **Help in protein synthesis**
3. **As intermediator in the transmission of genetic information**

DEOXYRIBONUCLEIC ACID (DNA)

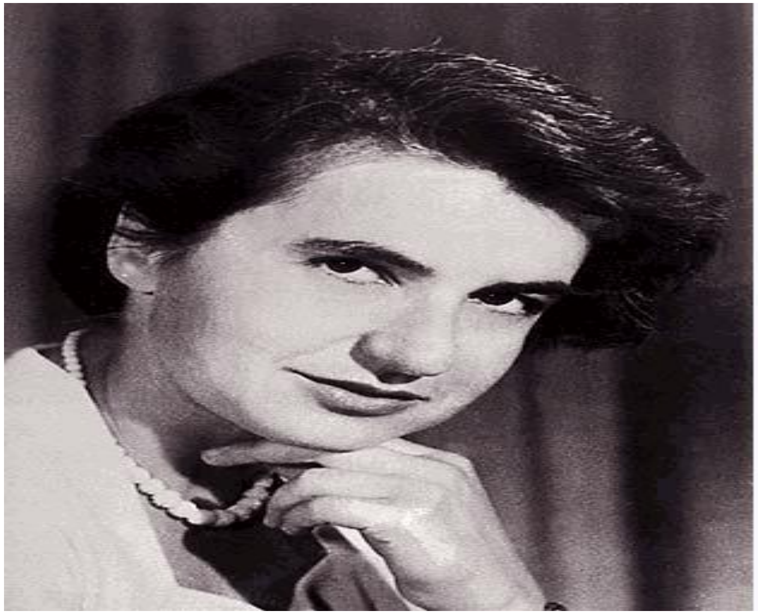
BY: NASSALI GLORIA

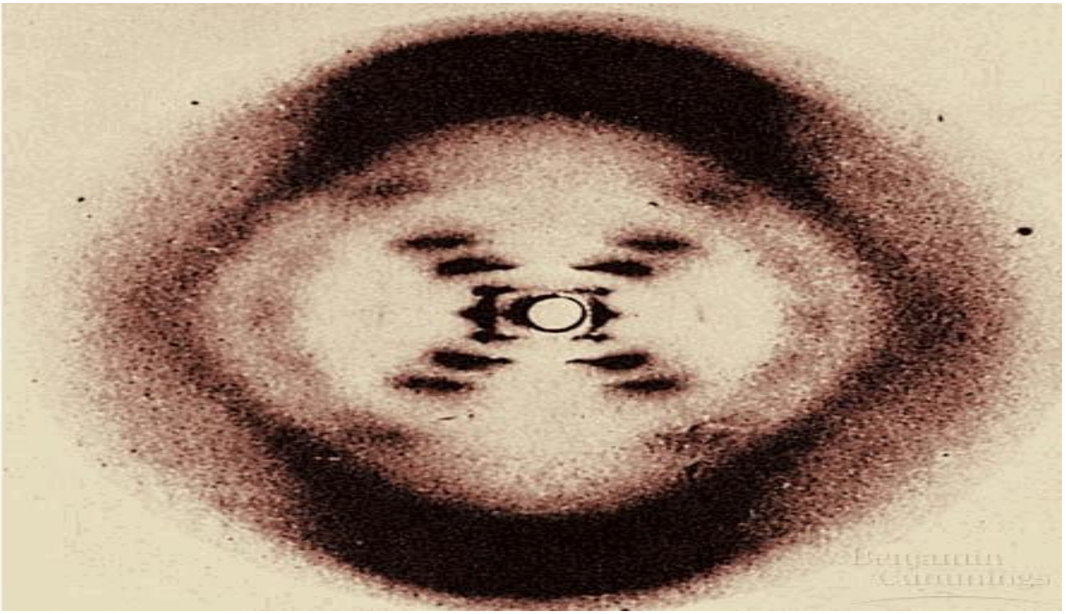
HISTORY

Biologists in 1940s had difficulty in conceiving how DNA could be the genetic material.



Early in 1950s, DNA was examined by X-ray diffraction analysis (a technique for determining the three-dimensional atomic structure of a molecule). The results indicated that DNA was composed of two strands of the polymer wound into a Helix





WATSON AND CRICK – DISCOVERED DNA 1953

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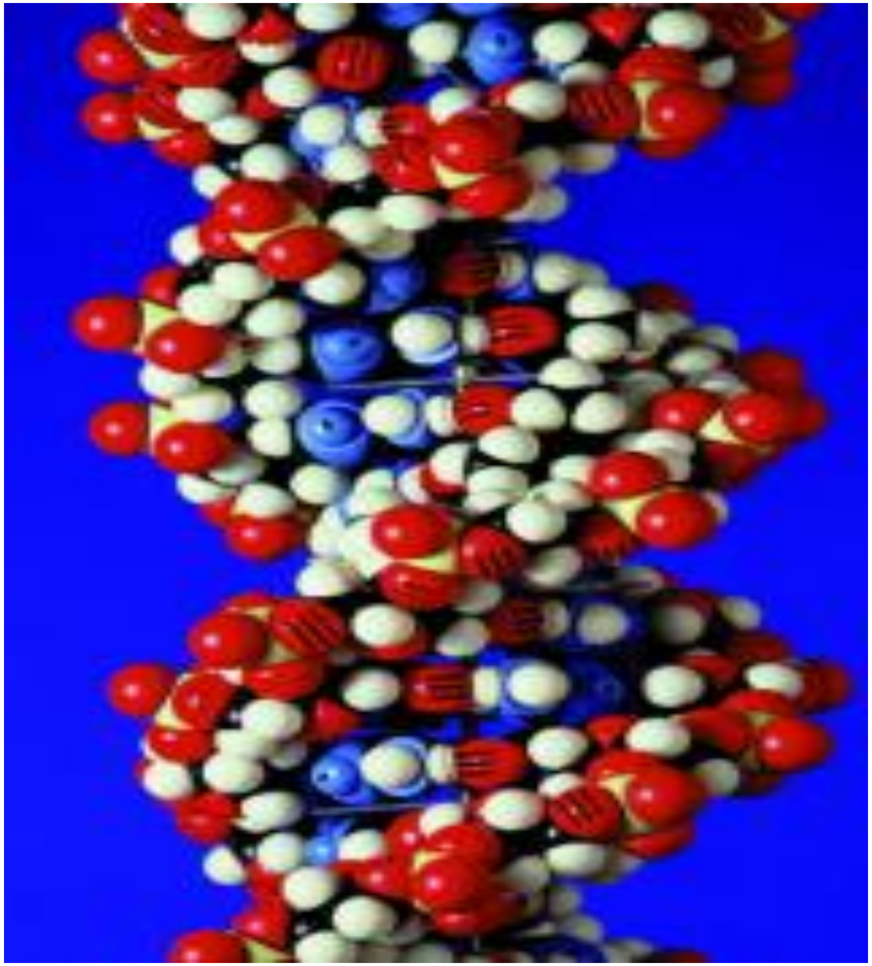
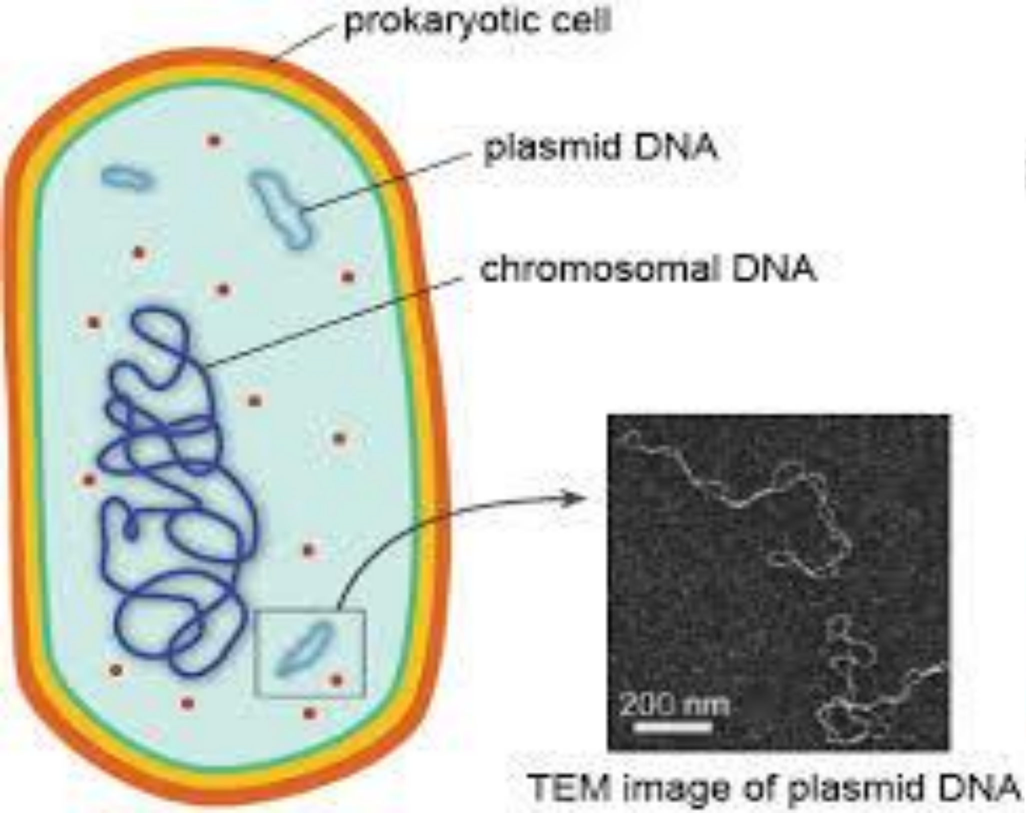
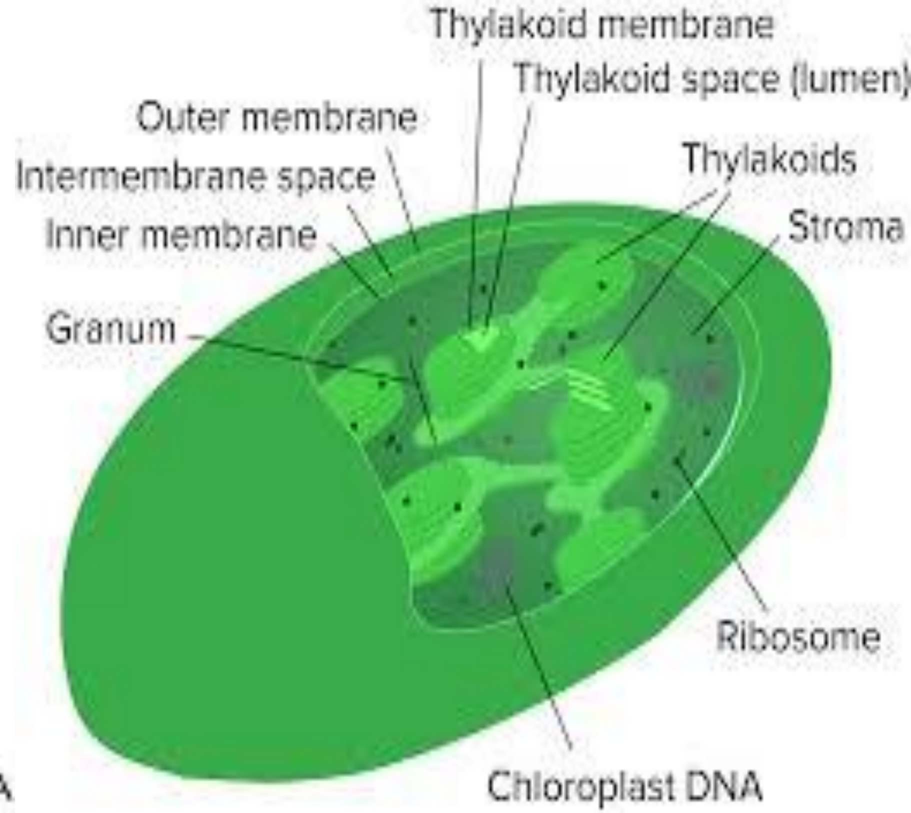
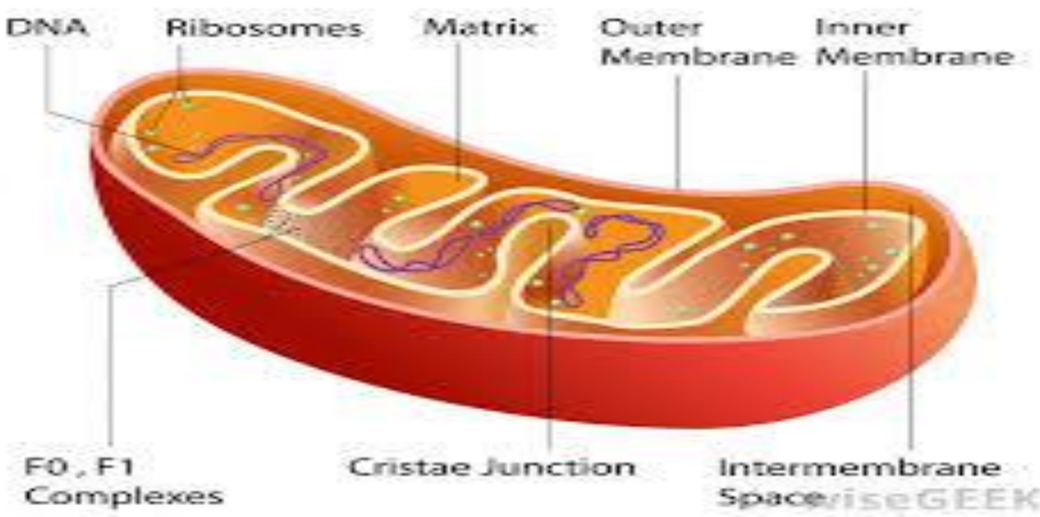


DNA IN EUKARYOTIC AND PROKARYOTIC CELLS

- **In eukaryotic cells, DNA is folded into chromatin**
- **It is found in the membrane bound nucleus.**
- **It is also found in Mitochondria (Mdna) and Chloroplasts.**
- **Prokaryotic lack nuclei.**
- **They contain non chromosomal DNA in form of plasmids.**


prokaryotic cell
plasmid DNA
chromosomal DNA
200 nm
TEM image of plasmid DNA


Thylakoid membrane
Thylakoid space (lumen)
Outer membrane
Thylakoids
Intermembrane space
Stroma
Inner membrane
Granum
Ribosome
Chloroplast DNA

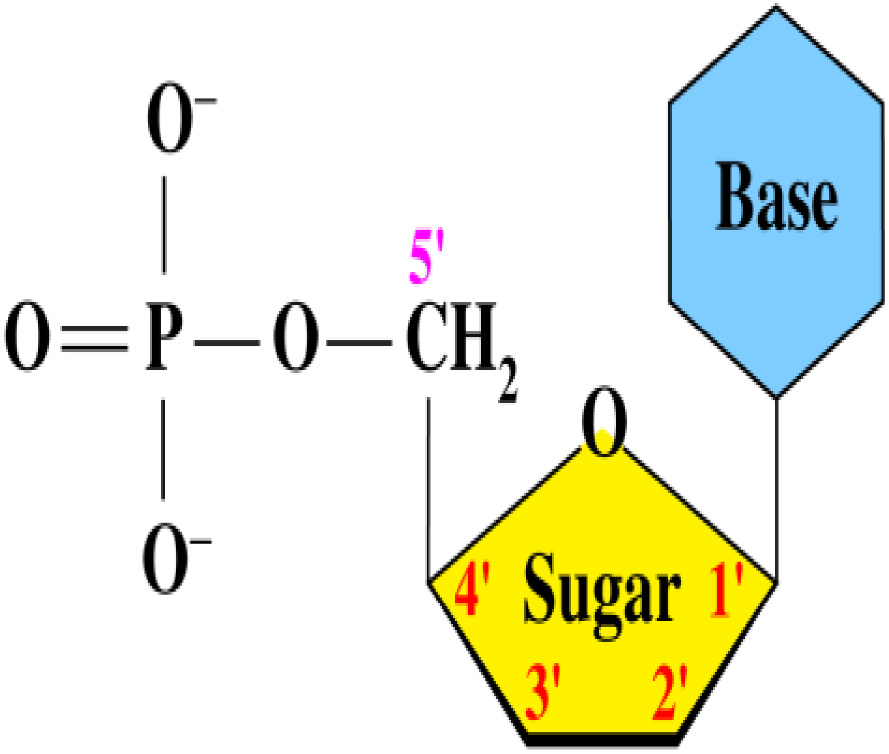

DNA
Ribosomes
Matrix
Outer Membrane
Inner Membrane
F0 , F1 Complexes
Cristae Junction
Intermembrane Space

WHAT IS DNA?

It is a Nucleic Acid that;

- Stores information for cellular growth and reproduction.
- Consists of long chains of monomers called nucleotides.

Thus DNA is a genetic polymer of the cell.



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The nucleic acid DNA consists of monomers called **nucleotides** that consist of a

- pentose sugar (5-Carbon).
- nitrogen-containing base.
- phosphate.

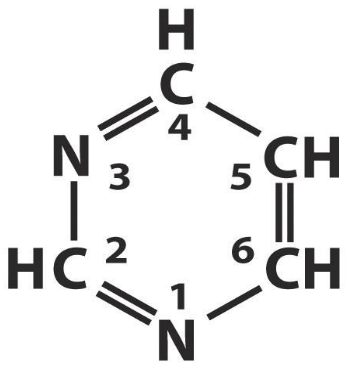
1. Nucleic acids are ***polynucleotides.***

2. Their building blocks are ***nucleotides.***

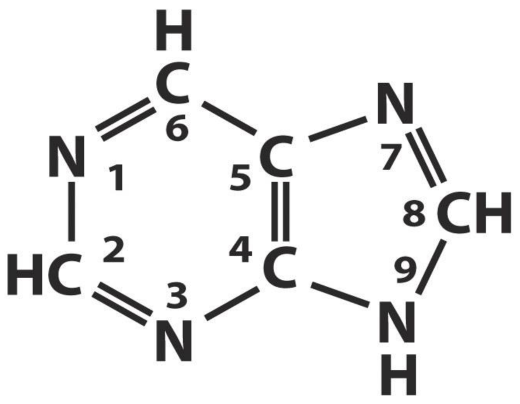
THE NITROGENEOUS BASES

The **nitrogen bases** in DNA are;

- Pyrimidines C, T,
- Purines A and G.



Pyrimidine



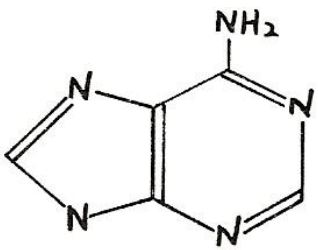
Purine

❑Note:

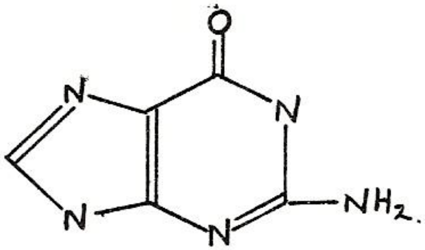
DNA does not contain the pyrimidine base "Uracil – U". It is found in RNA.

PURINES AND PYRIMIDINES - DIFFERENCES

PURINES



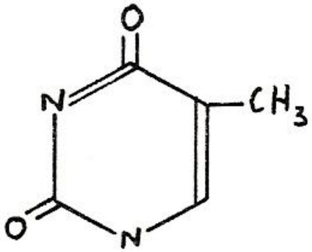
Adenine

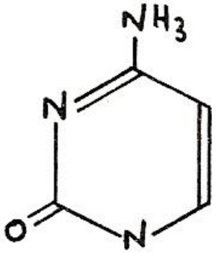


Guanine

PYRIMIDINES

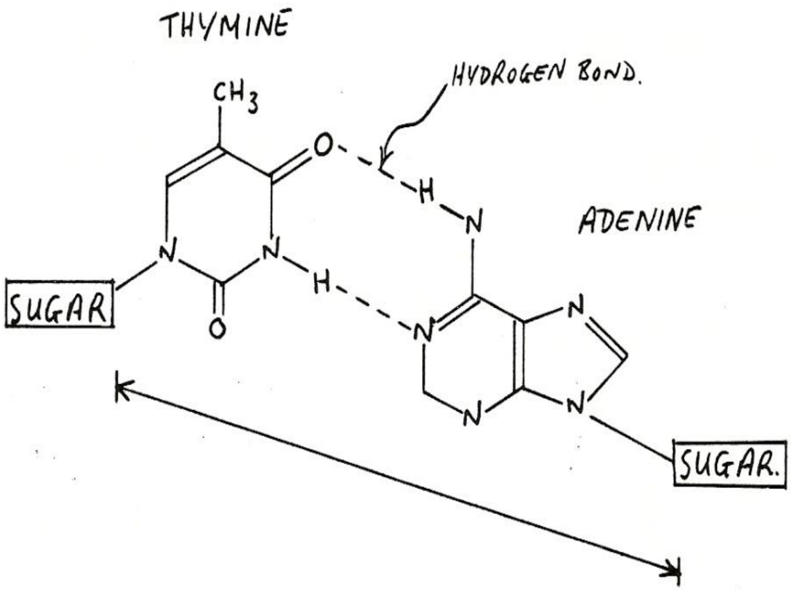
Thymine

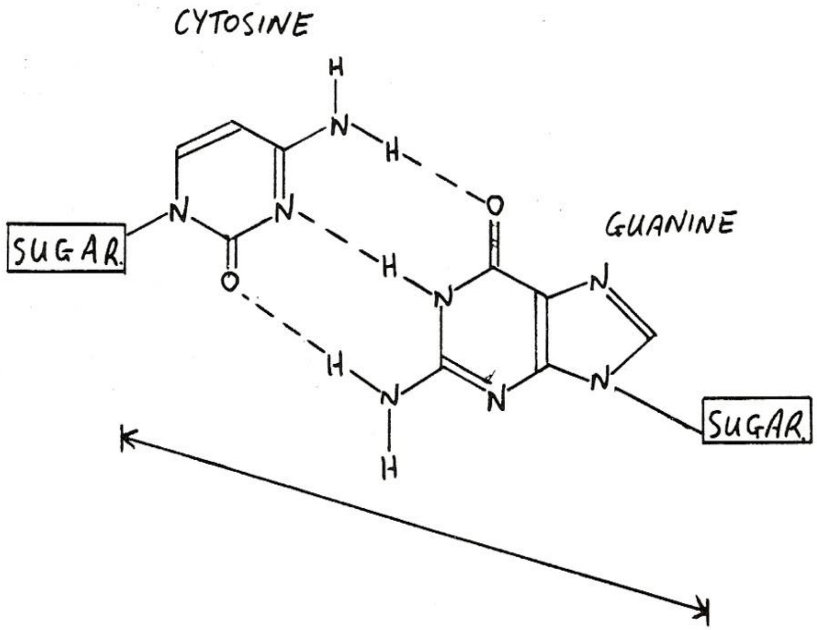




Cytosine

Watson & Crick Base pairing





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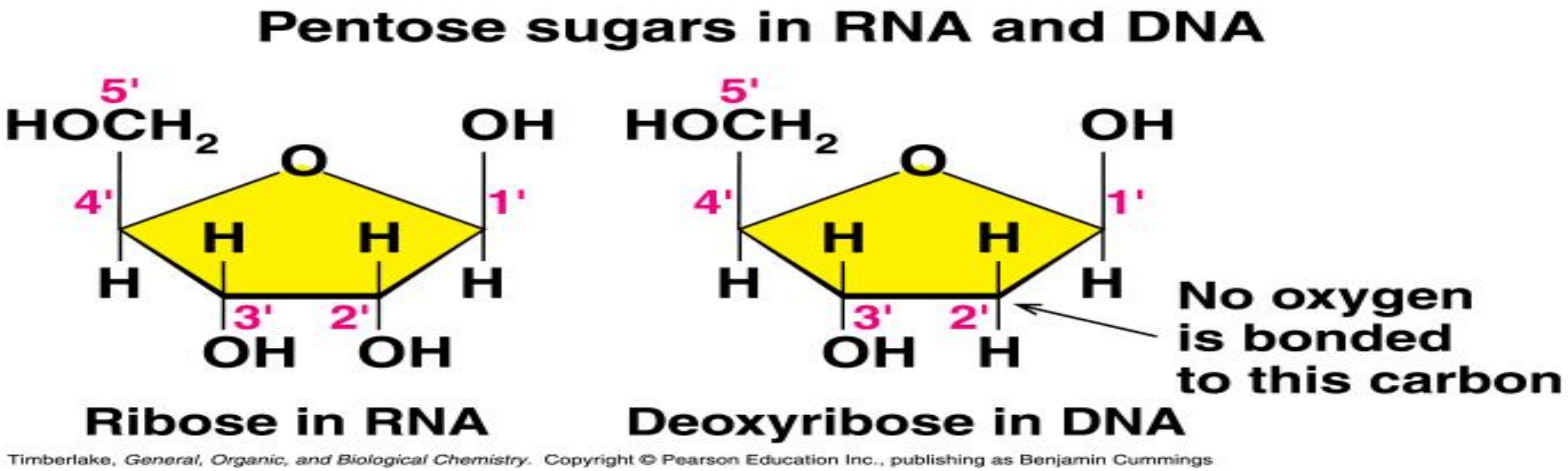
THE PENTOSE SUGAR

The **pentose (five-carbon) sugar**

- Unlike RNA with a **ribose**, DNA is **deoxyribose** with no O atom on carbon 2'.
- The carbon atoms numbered with primes to distinguish them from the atoms in nitrogen bases.



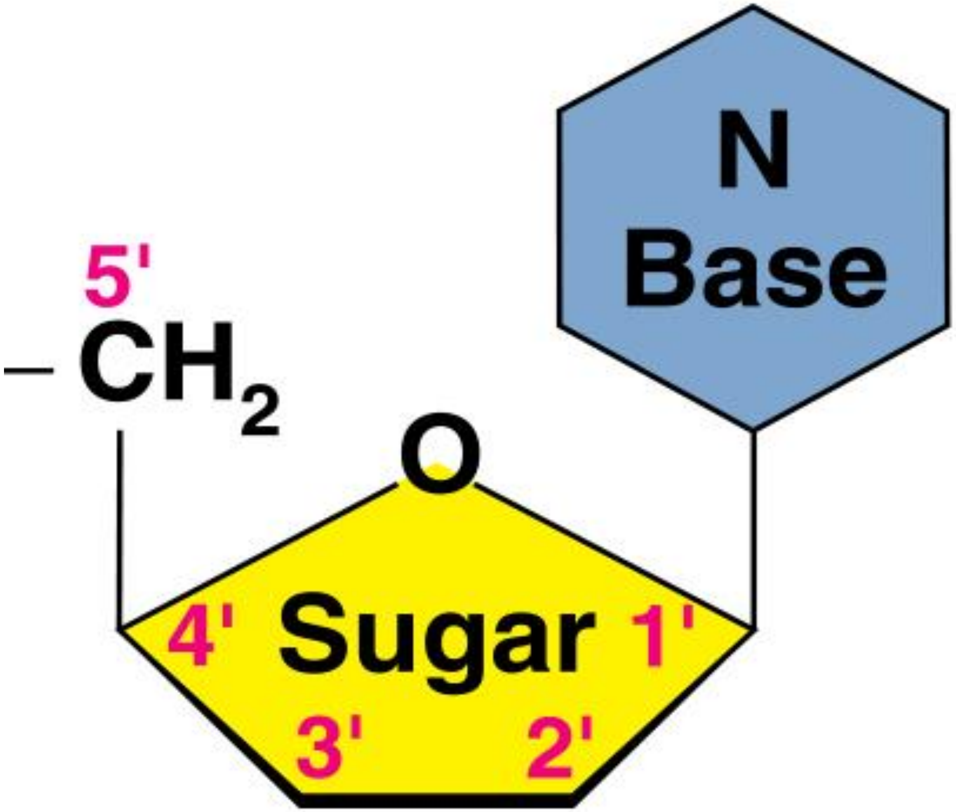
NUCLEOSIDES

A **nucleoside**

- has a nitrogen base linked by a glycosidic bond to C1’ of a sugar (ribose or deoxyribose).
- is named by changing the nitrogen base ending to

–**osine** for purines and

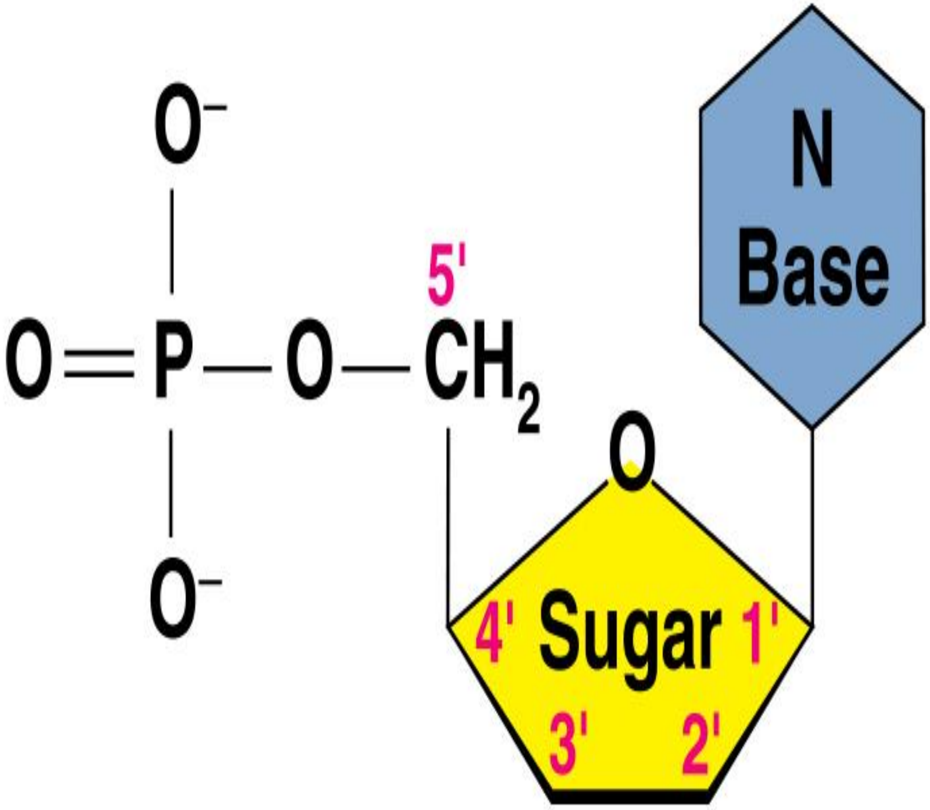
–**idine** for Pyrimidines



Nucleotides

A **nucleotide**

- is a nucleoside that forms a phosphate ester with the C5' –OH group of a sugar (ribose or deoxyribose).
- is named using the name of the nucleoside followed by **5'-monophosphate**.



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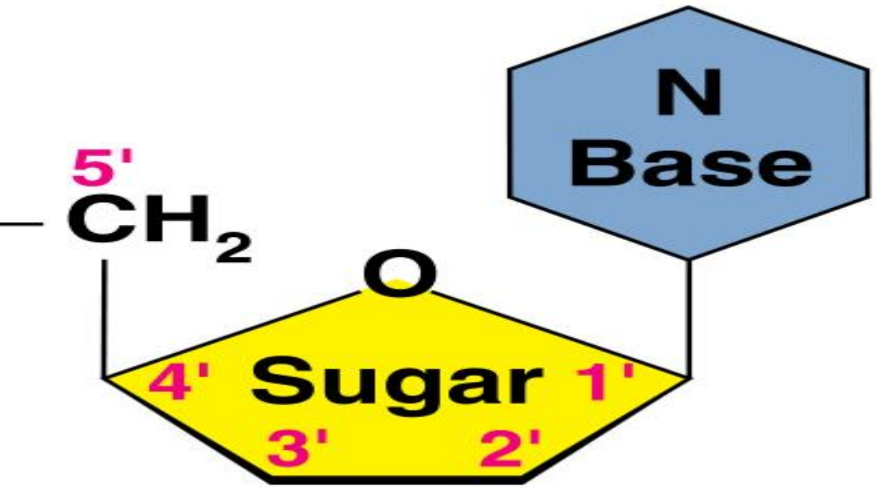
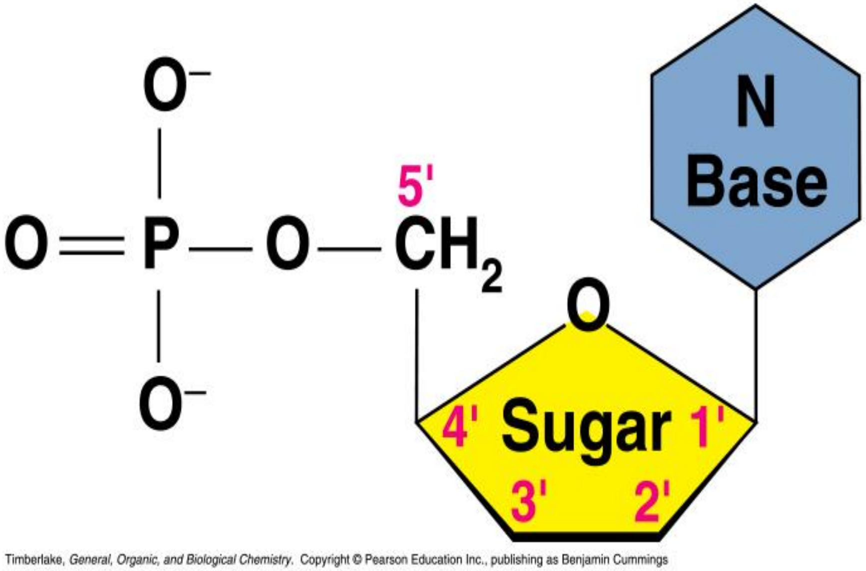
DIFFERENCE BETWEEN NUCLEOSIDE AND NUCLEOTIDE

A Nucleotide is a nucleoside with the attachment of a phosphate group.

NUCLEOSIDE



NUCLEOTIDE



BUILDING BLOCKS OF DNA

WE CAN THEREFORE SAY THAT THE BUILDING BLOCKS OF DNA ARE;

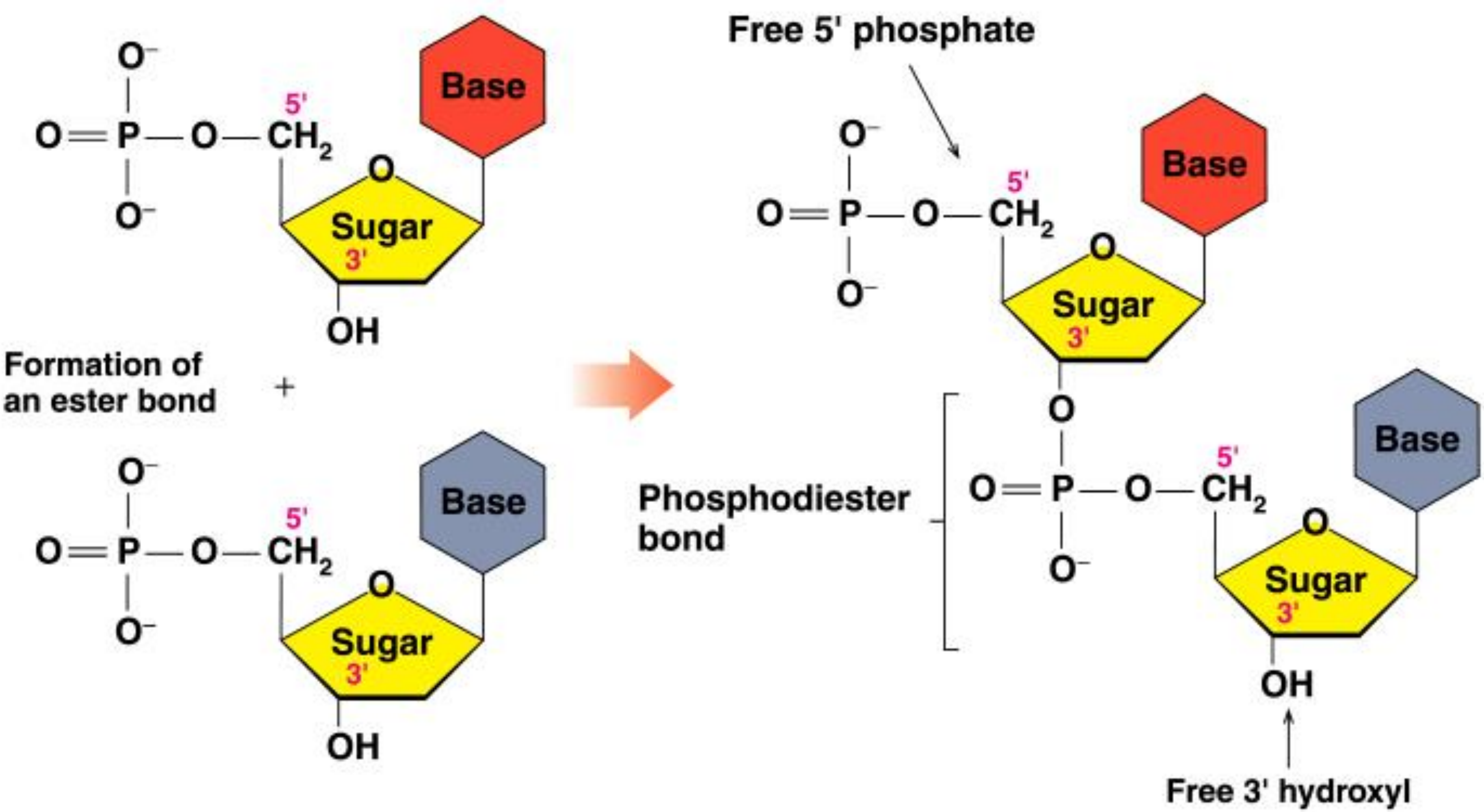
1. Deoxyadenosine
2. Deoxyguanosine
3. Deoxycytodine
4. Deoxythymidine

THE PRIMARY STRUCTURE OF DNA

In the **primary structure** of DNA

- Nucleotides are joined by 3' – 5' **phosphodiester bonds.**

- The 3'-OH group of the sugar in one nucleotide forms an ester bond to the phosphate group on the 5'-carbon of the sugar of the next nucleotide.



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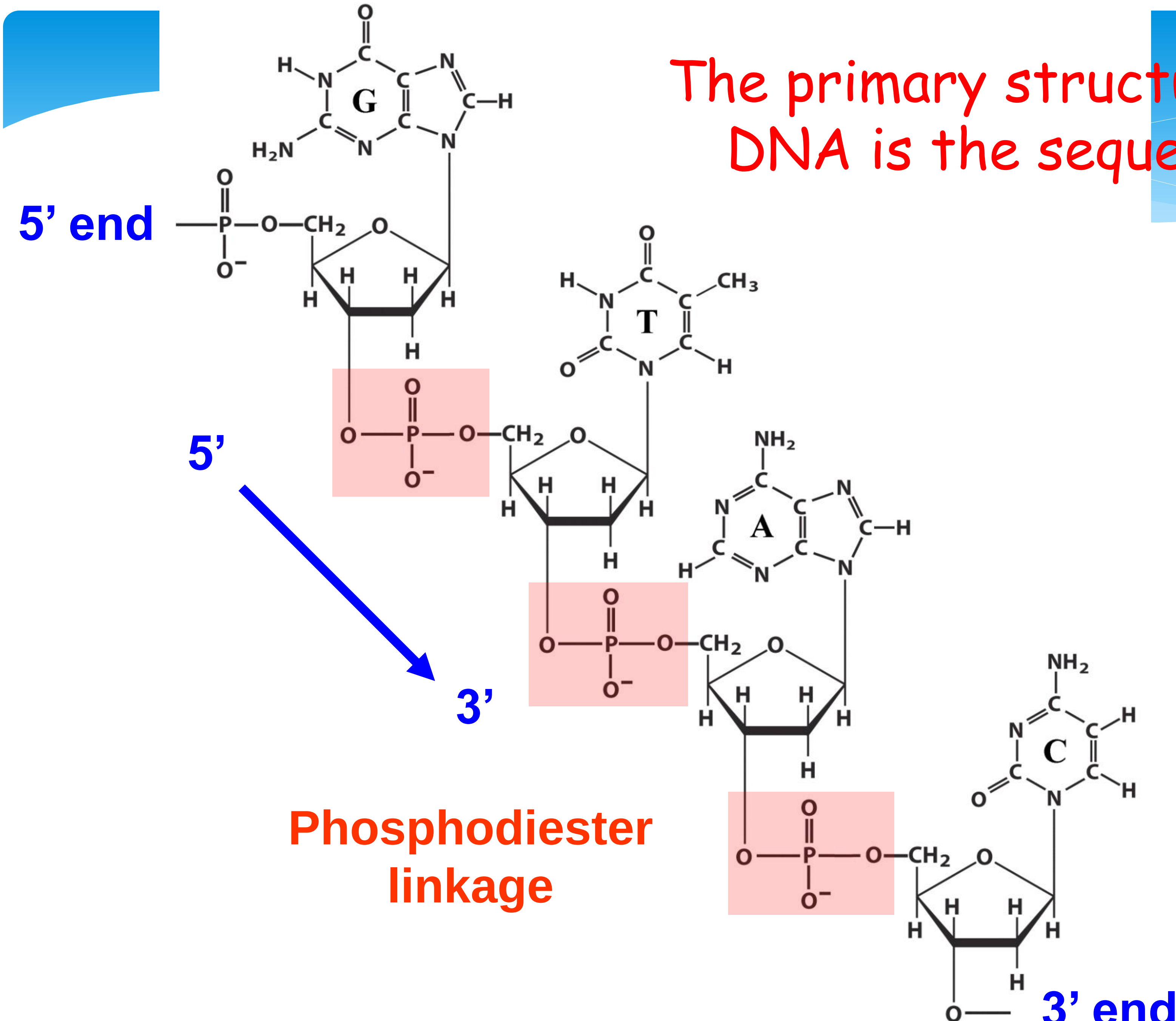
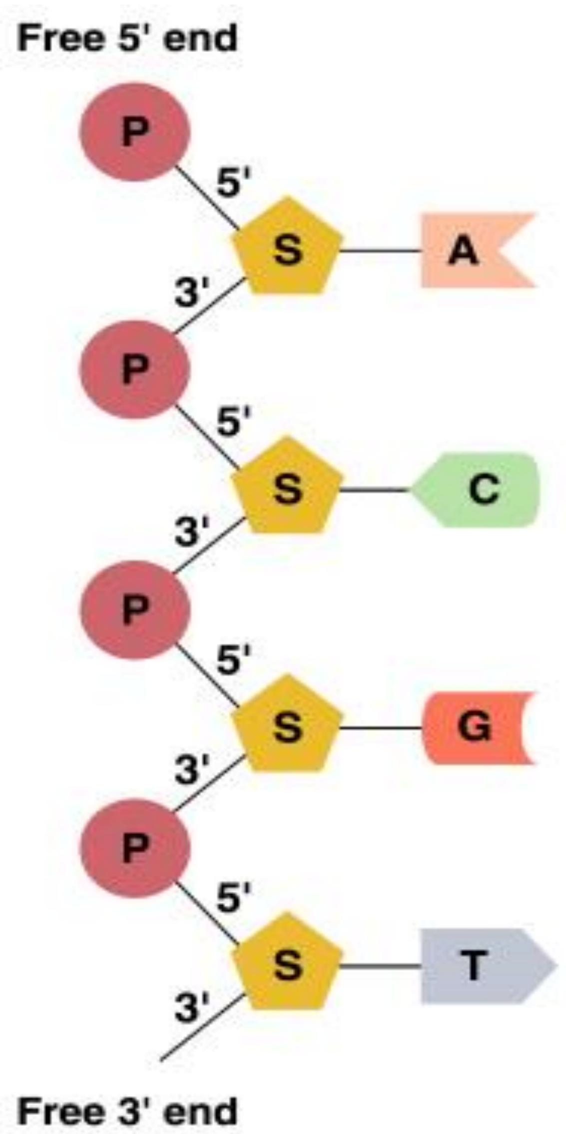
The primary structure of DNA is the sequence





DNA

- has a free 5'-phosphate group at one end and a free 3'-OH group at the other end.

- is read from the free 5'-end using the letters of the bases.

- This example reads

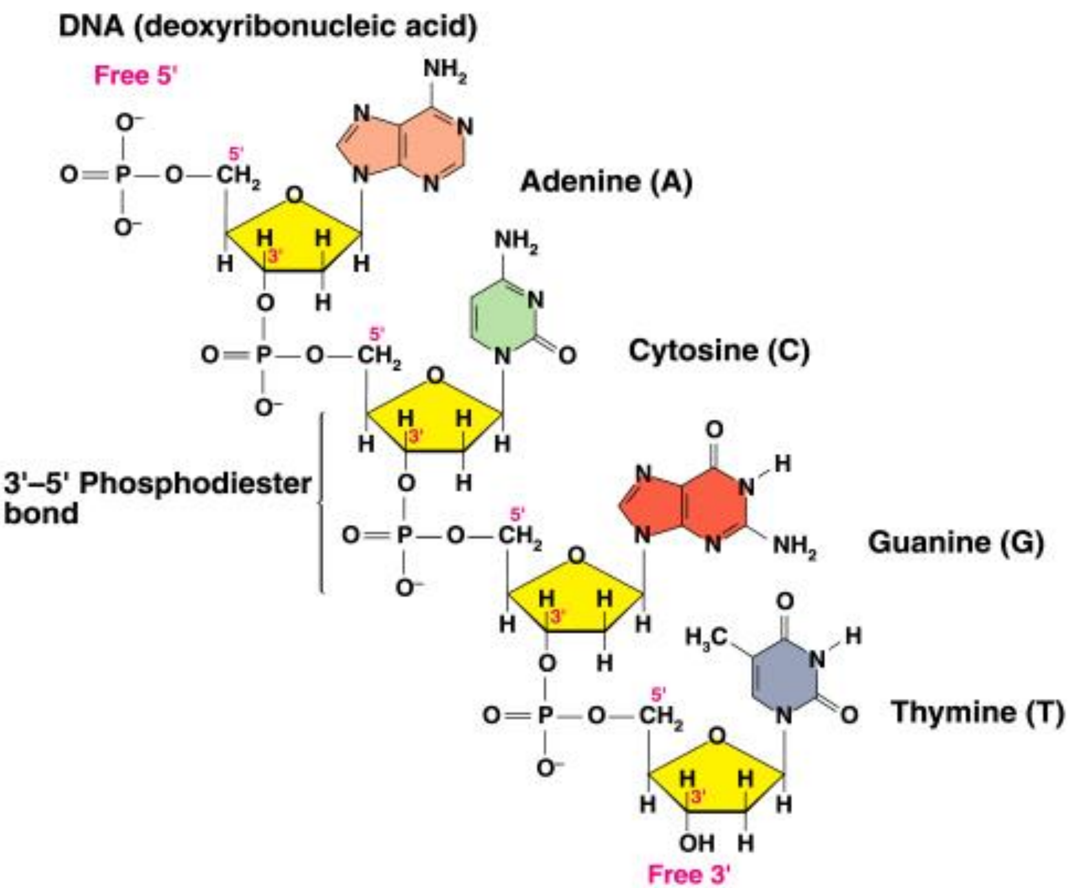
  —A—C—G—T—.

THE DOUBLE HELIX

- When different nucleotides bond the form a single polynucleotide strand.

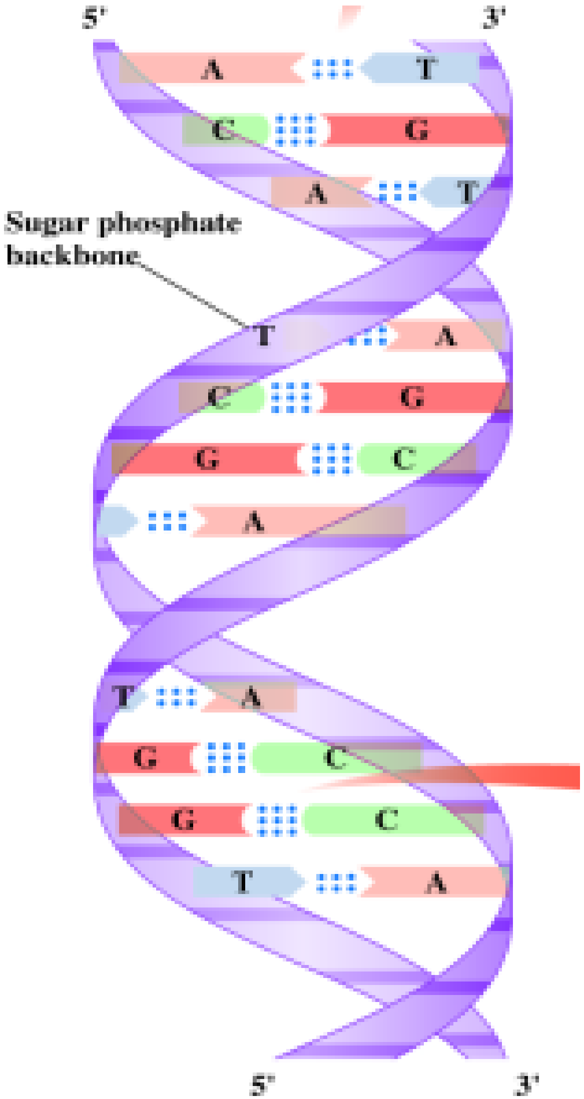
- Then the bases of the two opposite strands form a double strand of DNA by complementarity.

i.e Adenine – Thyamine (A-T) and Guanine – Cytosine (G-C)

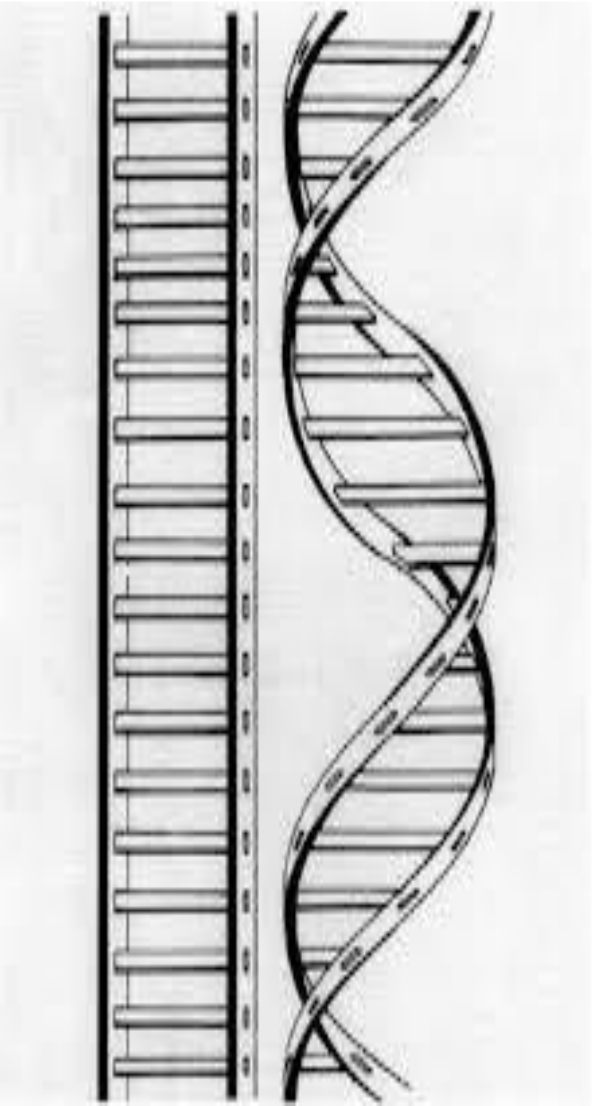


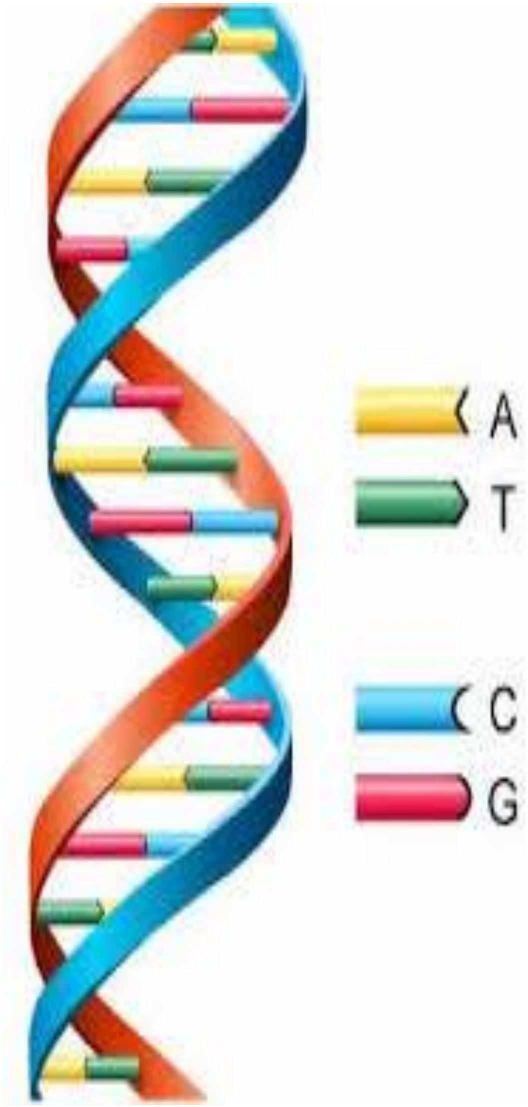
A double helix

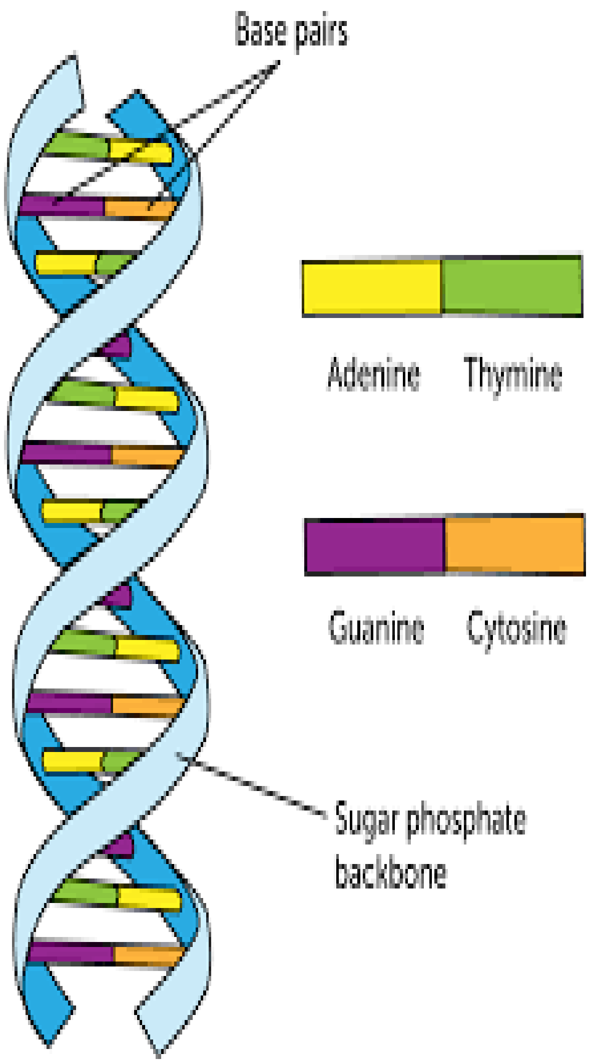
- is the structure of DNA.
- has two strands of nucleotides that wind together.
- is held in place by of two hydrogen bonds that form between the base pairs A-T.
- is held in place by three hydrogen bonds that form between the base pairs G-C.



COMPARED TO TWISTED LADDER OR STAIR CASE





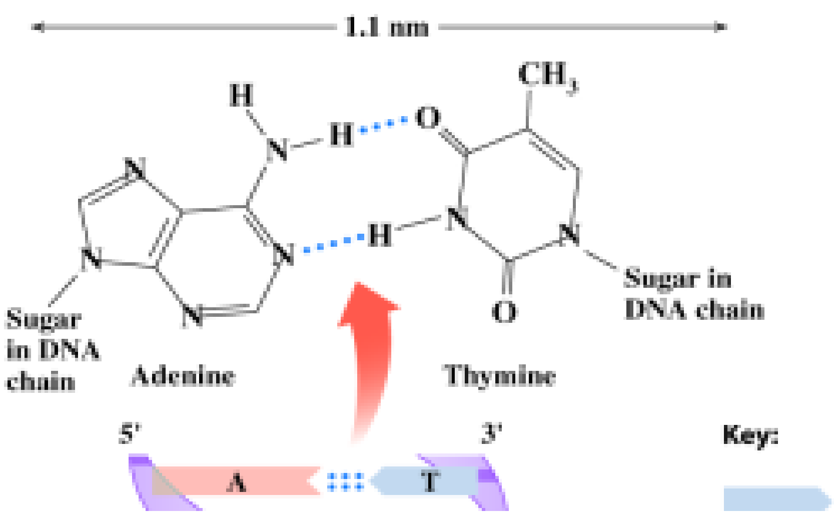

Base pairs
Adenine
Thymine
Guanine
Cytosine
Sugar phosphate
backbone

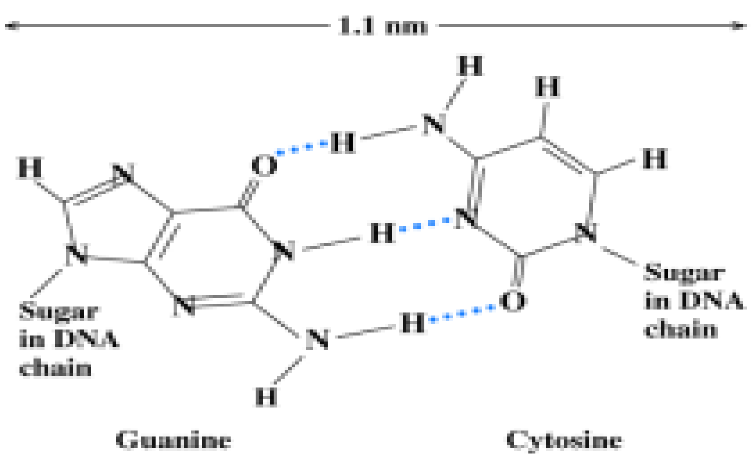
COMPLEMENTARY BASE PAIRING

- DNA contains **complementary base pairs** in which

- Adenine is always linked by two hydrogen bonds with thymine (A–T).

- Guanine is always linked by three hydrogen with Cytosine (G–C).





WHY THE COMPLEMENTALITY?

- The shape and chemical structures of the bases allow hydrogen bonds to form efficiently only between A – T and G – C, because atoms that are able to form hydrogen bonds can be brought close together without distorting the double helix.

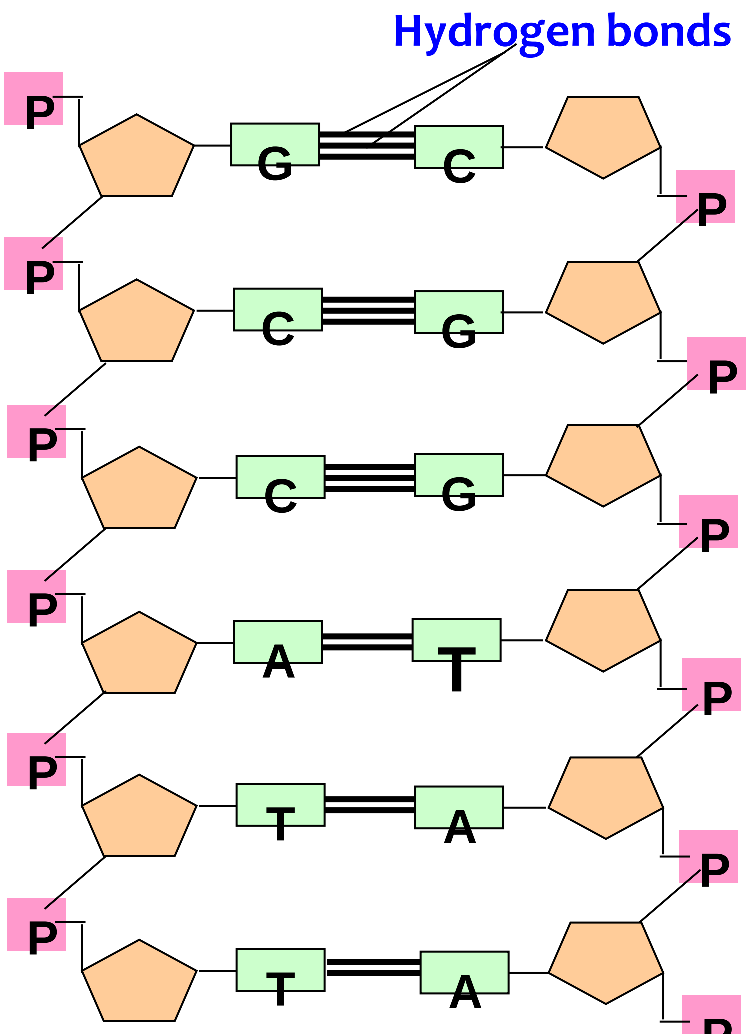
- Two hydrogen bonds form between A – T and three from between G – C. Bases can pair in this way if two nucleotide chains that contain them are ***parallel***.

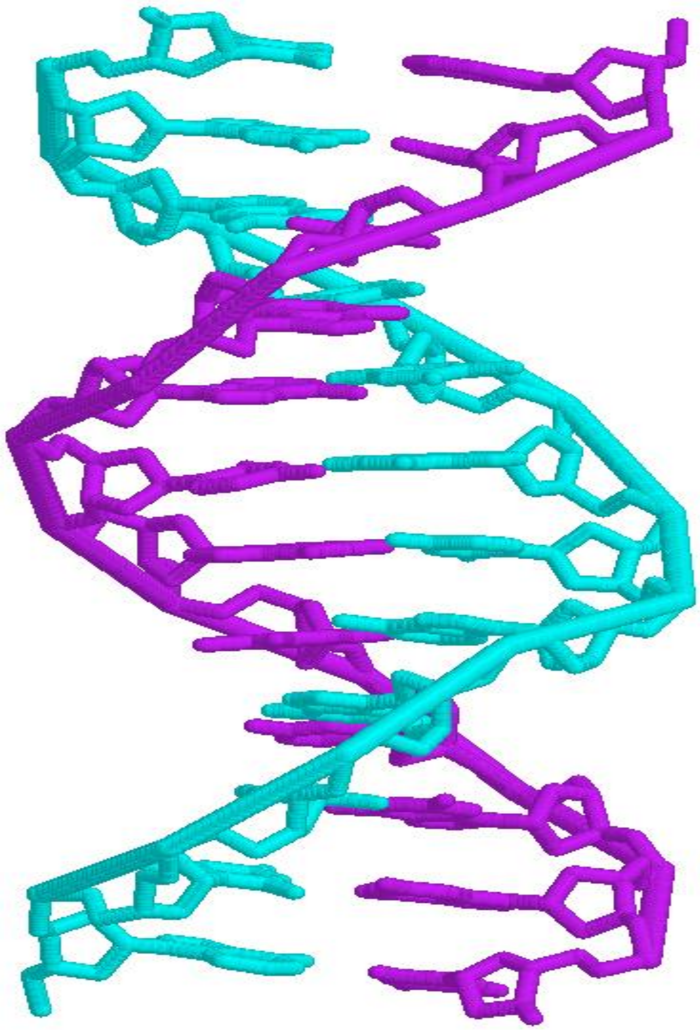
FOR EXAMPLE

Write the complementary base sequence for the matching strand in the following DNA section:

—A—G—T—C—C—A—A—T—C—

’—T—C—A—G—G—T—T—A—G—

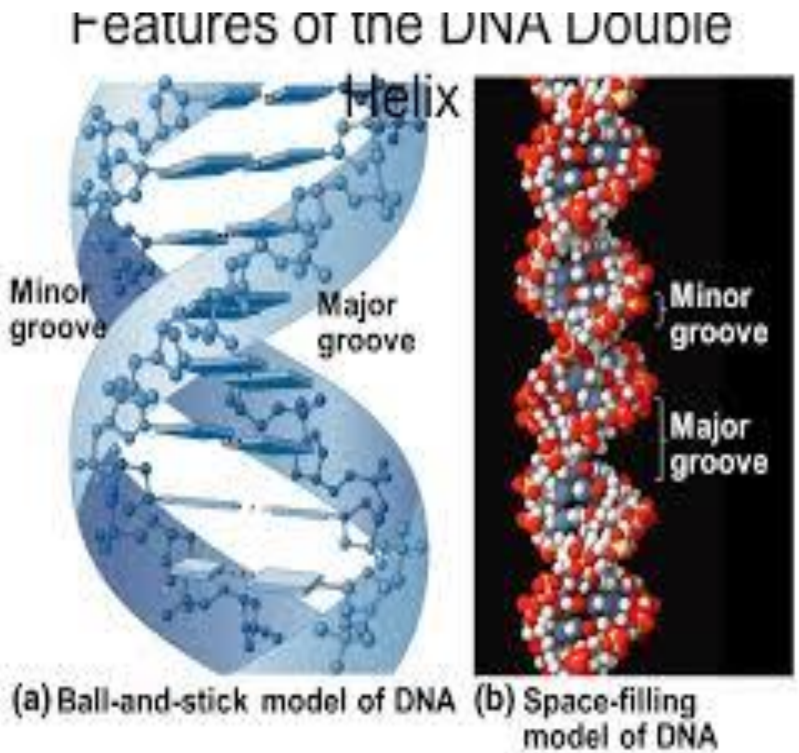

Hydrogen bonds
P
G
C
P
P
C
G
P
P
C
G
P
P
A
T
P
P
T
A
P
P
T
A
P



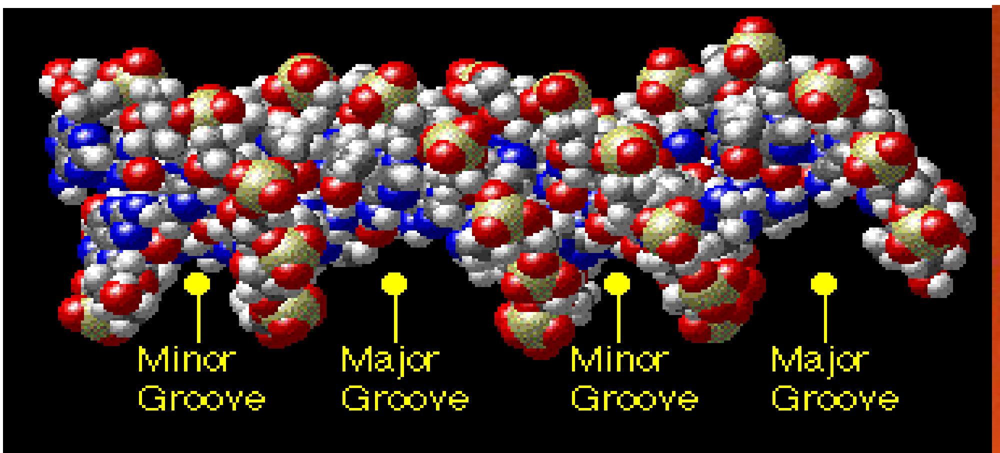
DNA AS A TEMPLATE

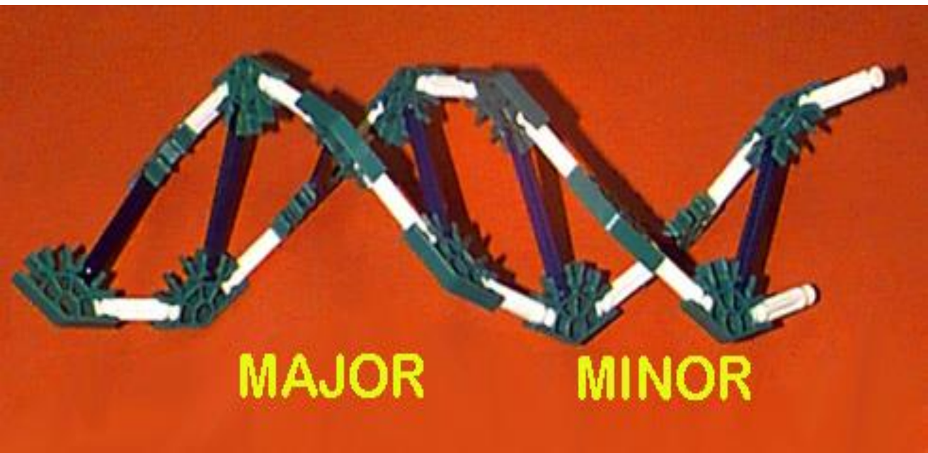
The complementarity between bases enables each strand of DNA to act as template. In this way the double helical DNA can be copied precisely with each parental DNA producing two identical daughter DNA helices.

MAJOR AND MINOR GROOVES

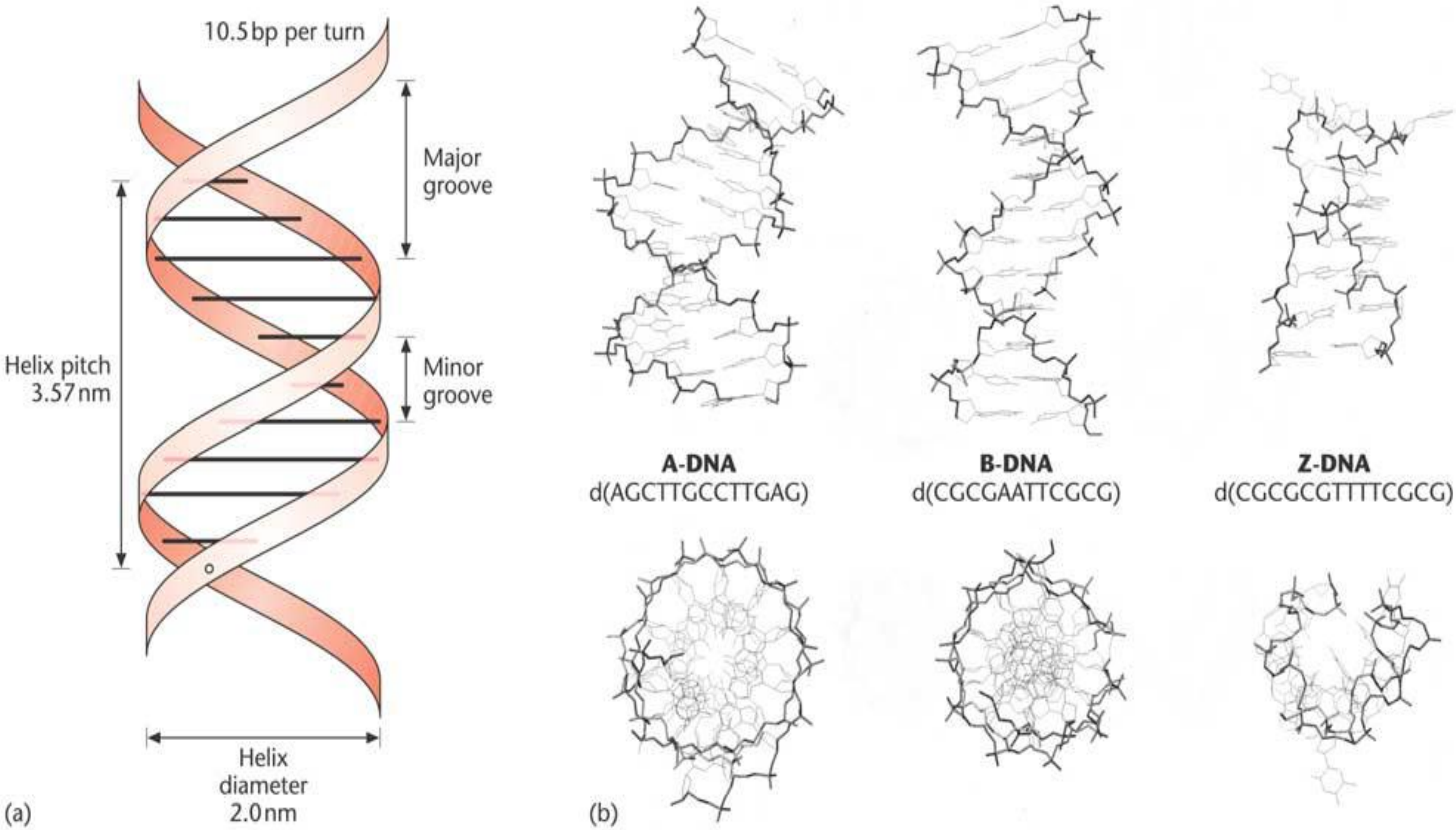


* The major groove occurs where the backbones are far apart, the minor groove occurs where they are close together.



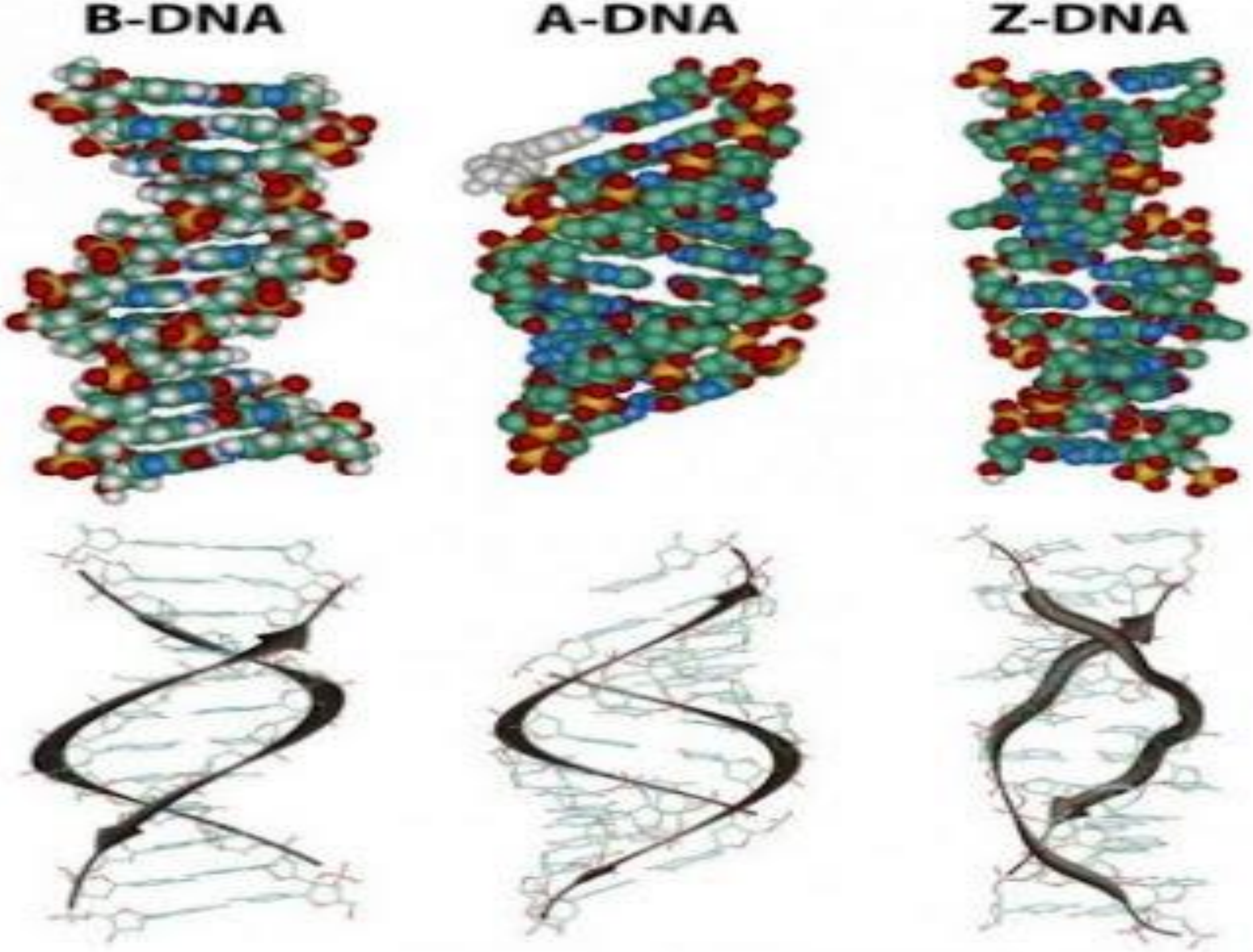


A, B and Z FORMS OF DNA

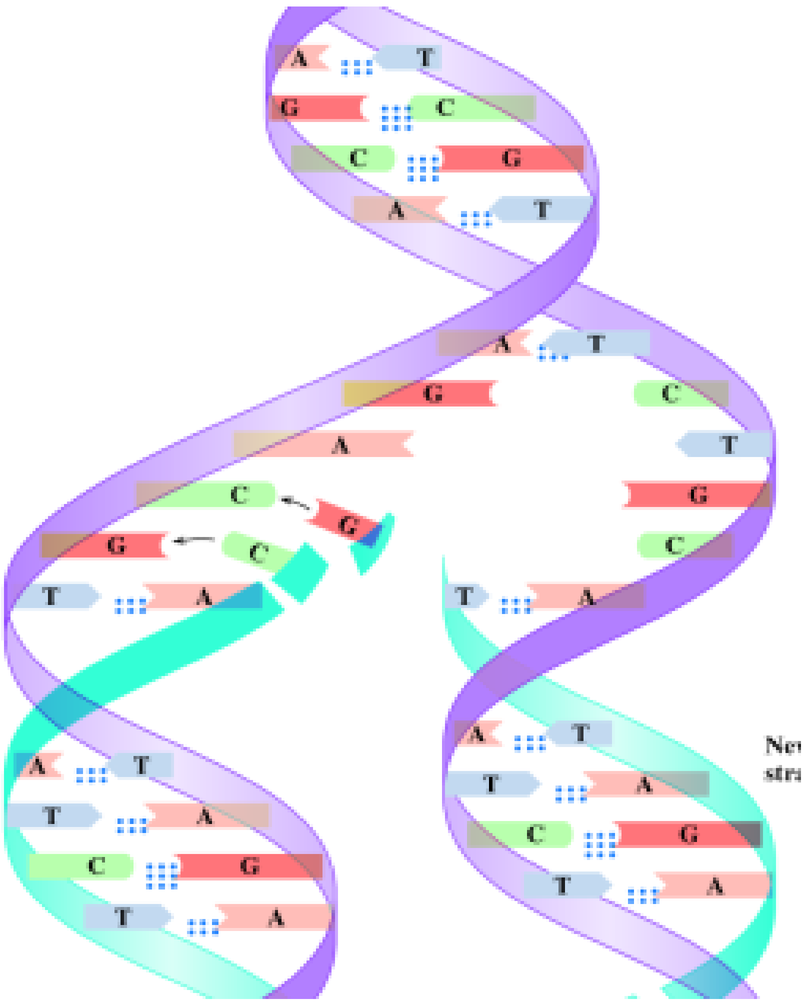


Comparison of different helical parameters for A-, B-, and Z-DNA

Feature	B-DNA	A-DNA	Z-DNA
Type of helix	Right-handed	Right-handed	Left-handed
Helical diameter (nm)	2.37	2.55	1.84
Rise per base pair (nm)	0.34	0.29	0.37
Distance per complete turn (pitch) (nm)	3.4	3.2	4.5
Number of base pairs per complete turn	10	11	12
Topology of major groove	Wide, deep	Narrow, deep	Flat
Topology of minor groove	Narrow, shallow	Broad, shallow	Narrow, deep


B-DNA
A-DNA
Z-DNA

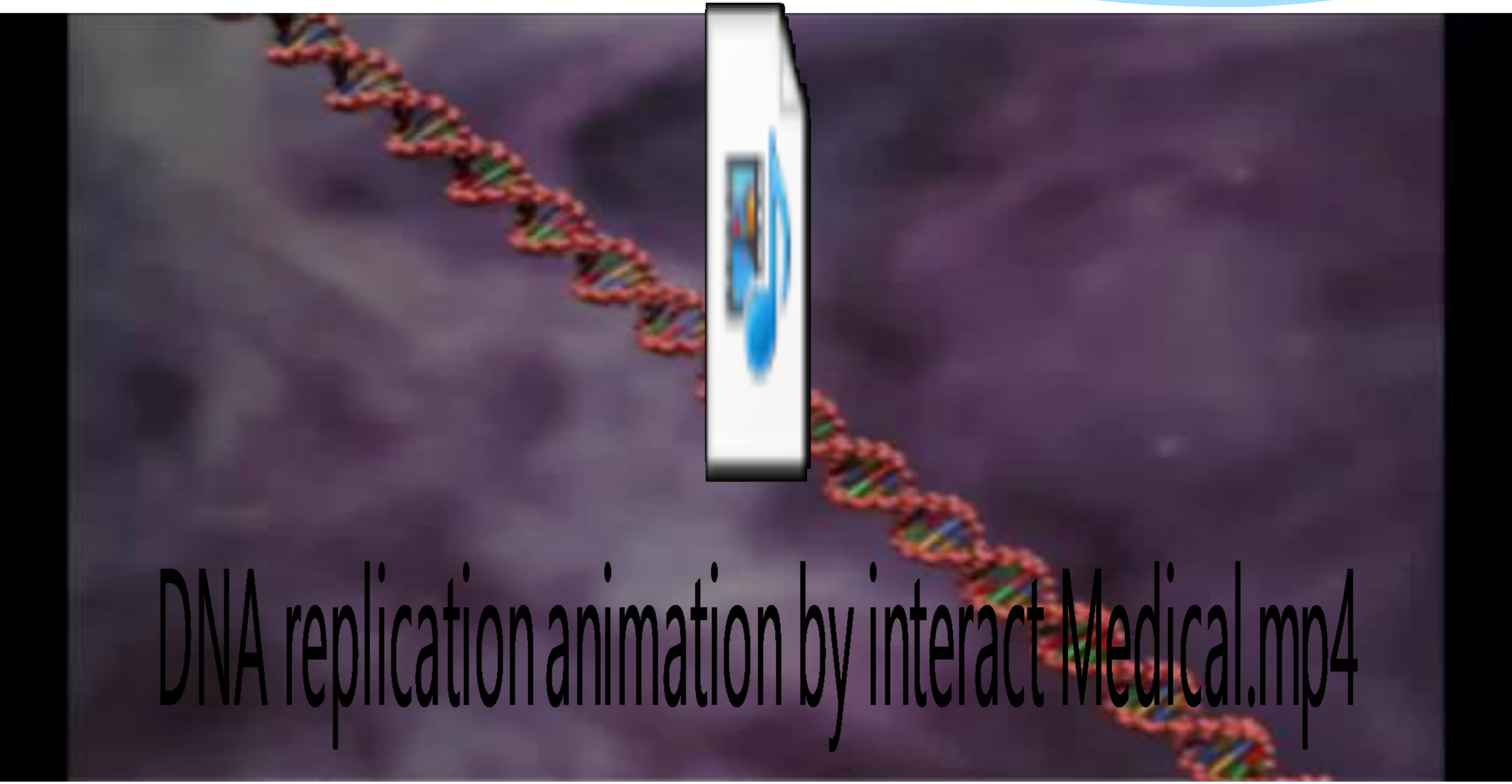
DNA REPLICATION



In **DNA replication**

- genetic information is maintained each time a cell divides.
- the DNA strands unwind.
- each parent strand bonds with new complementary bases.
- two new DNA strands form that are exact copies of the original DNA.

SHORT ILLUSTRATION



ENZYMES INVOLVED IN DNA REPLICATION

1. DNA POLYMERASE – is responsible for catalyzing synthesis of new strands
2. LIGASE – Ligase catalyzes the formation of a phosphodiester bond between the 5' phosphate of one molecule and the 3' OH of another molecule.
3. HELICASE – Works at the replication fork. It 'pulls' apart the DNA helix (melts the DNA).
4. SINGLE-STRANDED DNA BINDING PROTEINS OR SSB – SSB binds to unwound and single stranded template DNA and stabilizes it. It prevents the double helix from zipping up and from becoming tangled.
5. GYRASE (TOPIOSOMERASE II) – Gyrase reduces the resulting torsional (twisting) stress since the advancing replication fork causes the DNA in front of the fork to become more tightly wound.

DENATURATION OF DNA

- The strands of the DNA separate if the pH of the solution is altered and also if the solution is heated.
- Phosphodiester linkages between nucleotides can be cleaved hydrolytically by chemicals or enzymes like deoxyribonucleases in DNA and ribonucleases in RNA.

FUNCTIONS OF DNA

1. Coding for proteins – Acts as a template to form RNA strands (TRANSCRIPTION) for protein synthesis.

2. For DNA replication – When the cell undergoes mitosis, each daughter cell will contain one copy of the replicated DNA.

3. Genetic Code – (or : to hold hereditary material) using the arrangements of base pairs in the DNA.

RIBONUCLEIC ACID (RNA)

BY: MUUNDA MUDENDA

RIBONUCLEIC ACID (RNA)

HISTORY

Nucleic acids were discovered in 1868 by **Friedrich Miescher**, who called the material 'nuclein' since it was found in the nucleus.

RNA is a Nucleic Acid like DNA though differing in some aspects.

Though there are different types of RNA that play different roles it is commonly accepted that the main function of RNA is in the transmission of information from DNA i.e. manufacturing of proteins.

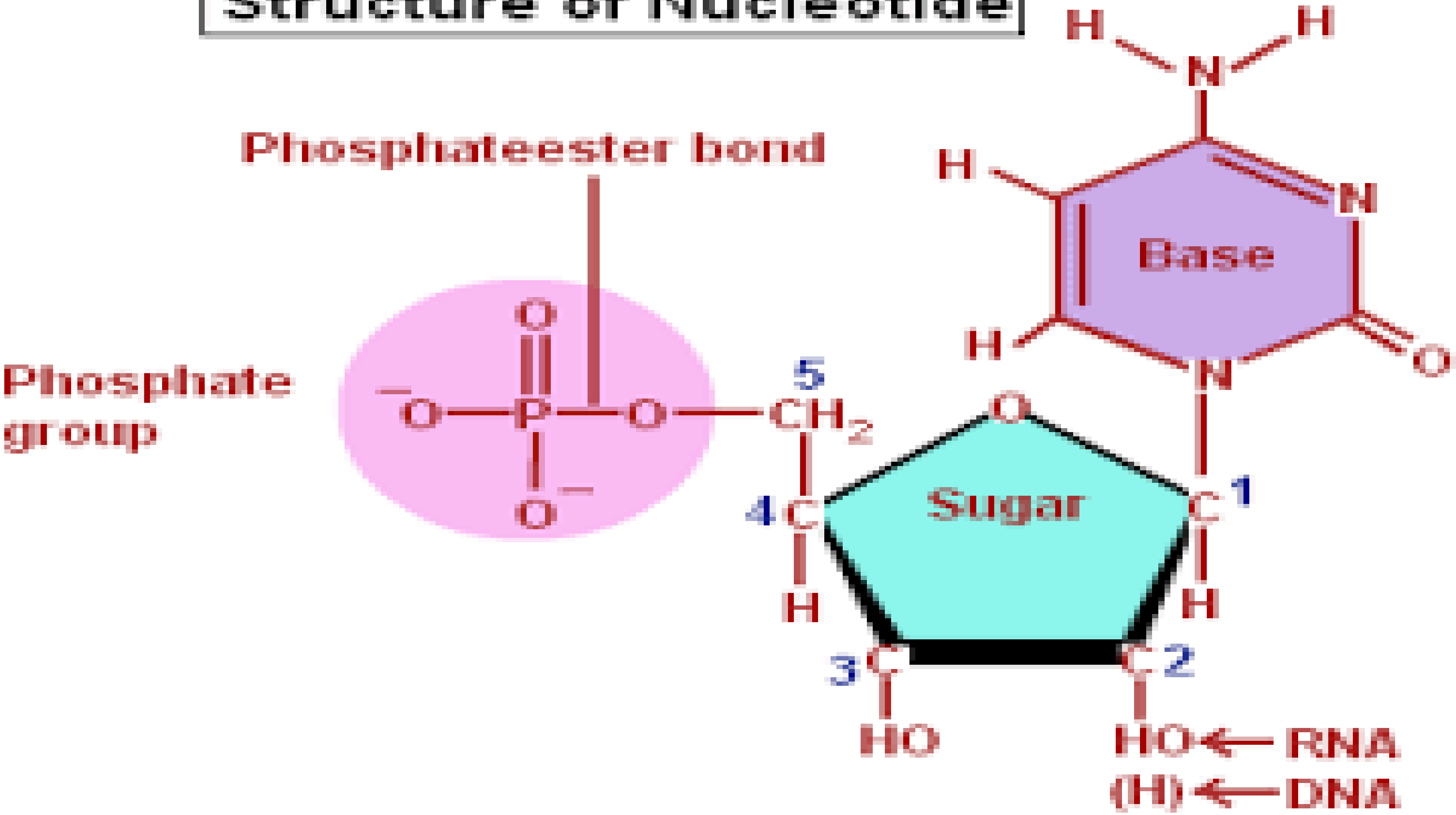
The structure of RNA

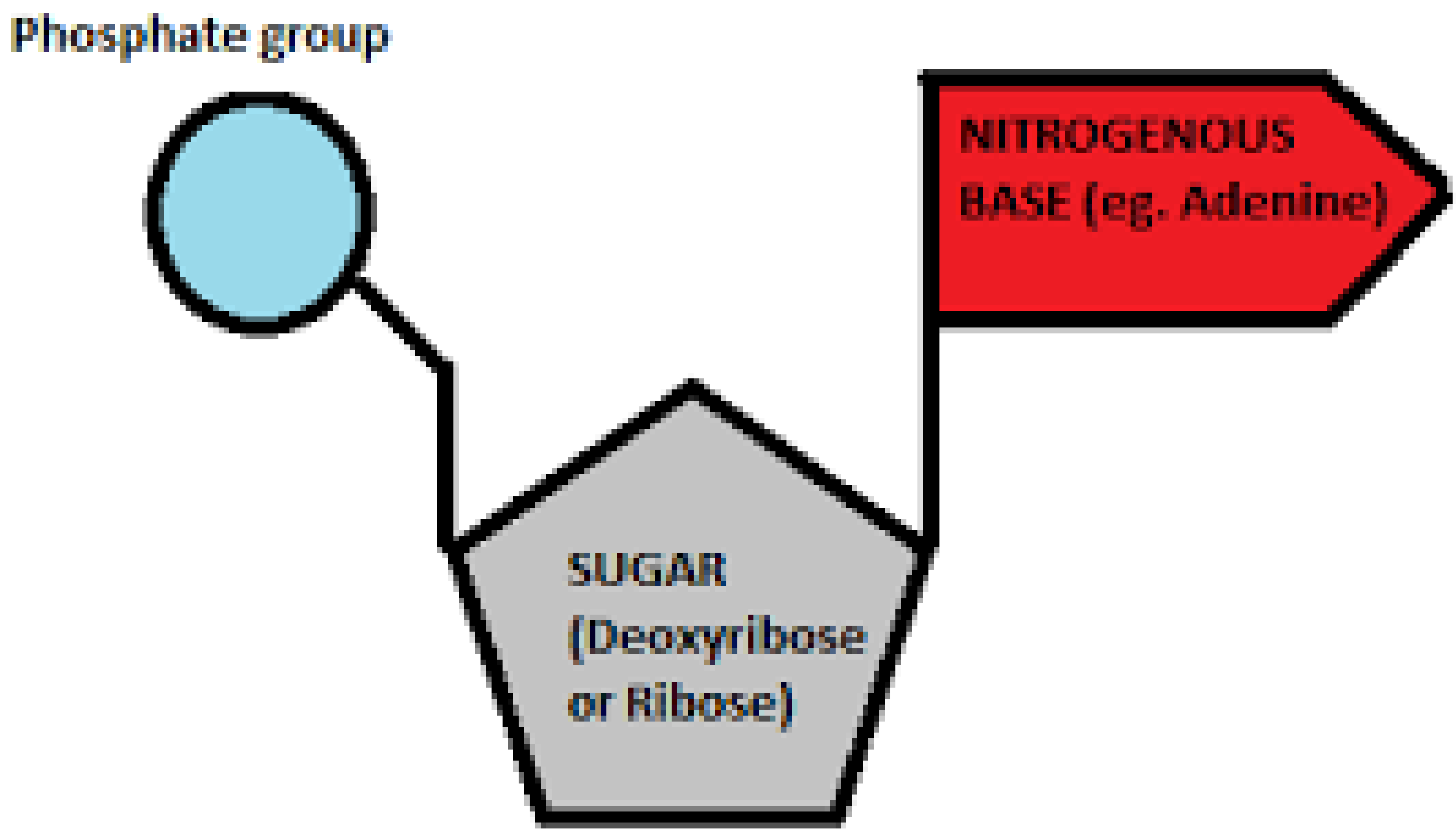
- RNA is a polymer of purine and pyrimidine ribonucleotides linked together by 3'5'-phosphodiester bonds like in DNA .
- The RNA shares many features with DNA the differences exist.
- The sugar in RNA to which phosphates and bases are attached is ribose rather than deoxyribose of DNA.
- Thymine (T) in DNA is replaced by uracil in RNA.
- RNA is single stranded while DNA is double stranded

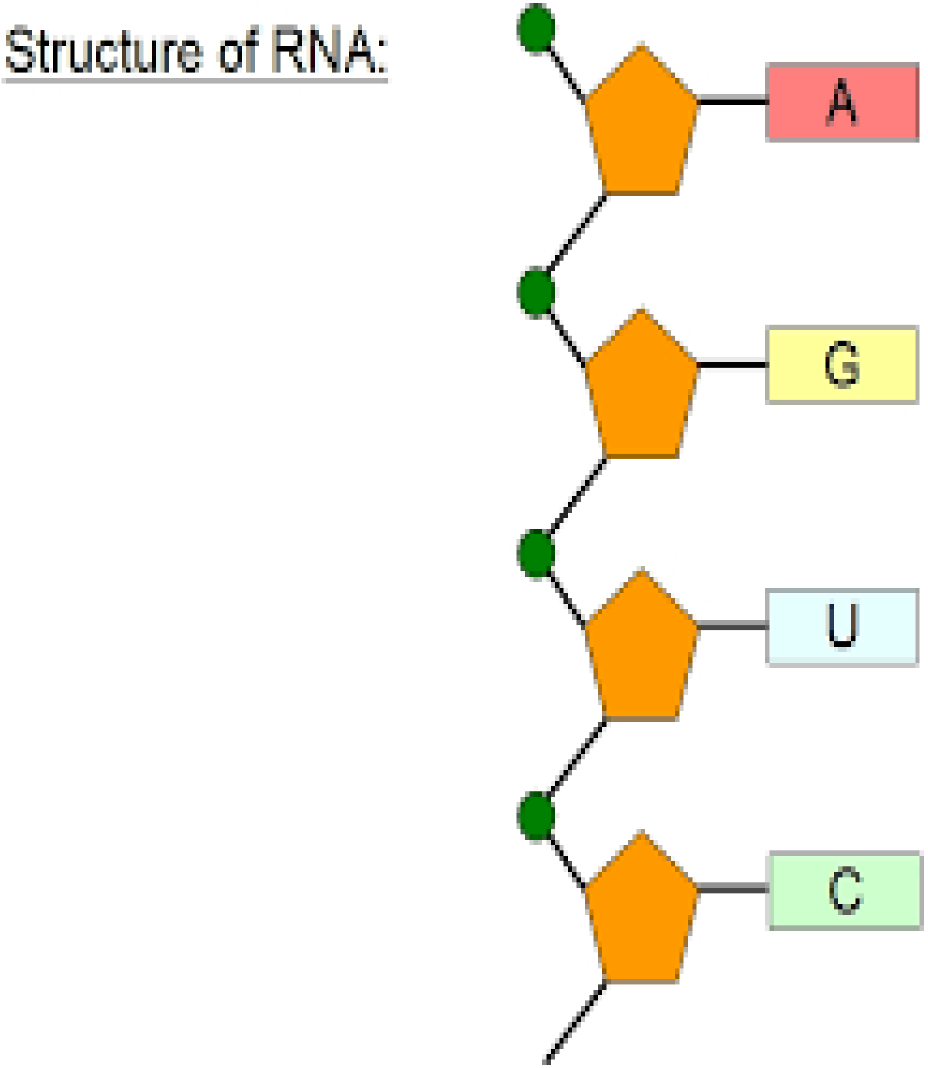
RNA is formed from Nucleotides as well. This means;

1. It has a pentose sugar.
2. Has nitrogenous bases.
3. Has a phosphate group.

Structure of Nucleotide




Phosphate group
NITROGENOUS
BASE (eg. Adenine)
SUGAR
(Deoxyribose
or Ribose)



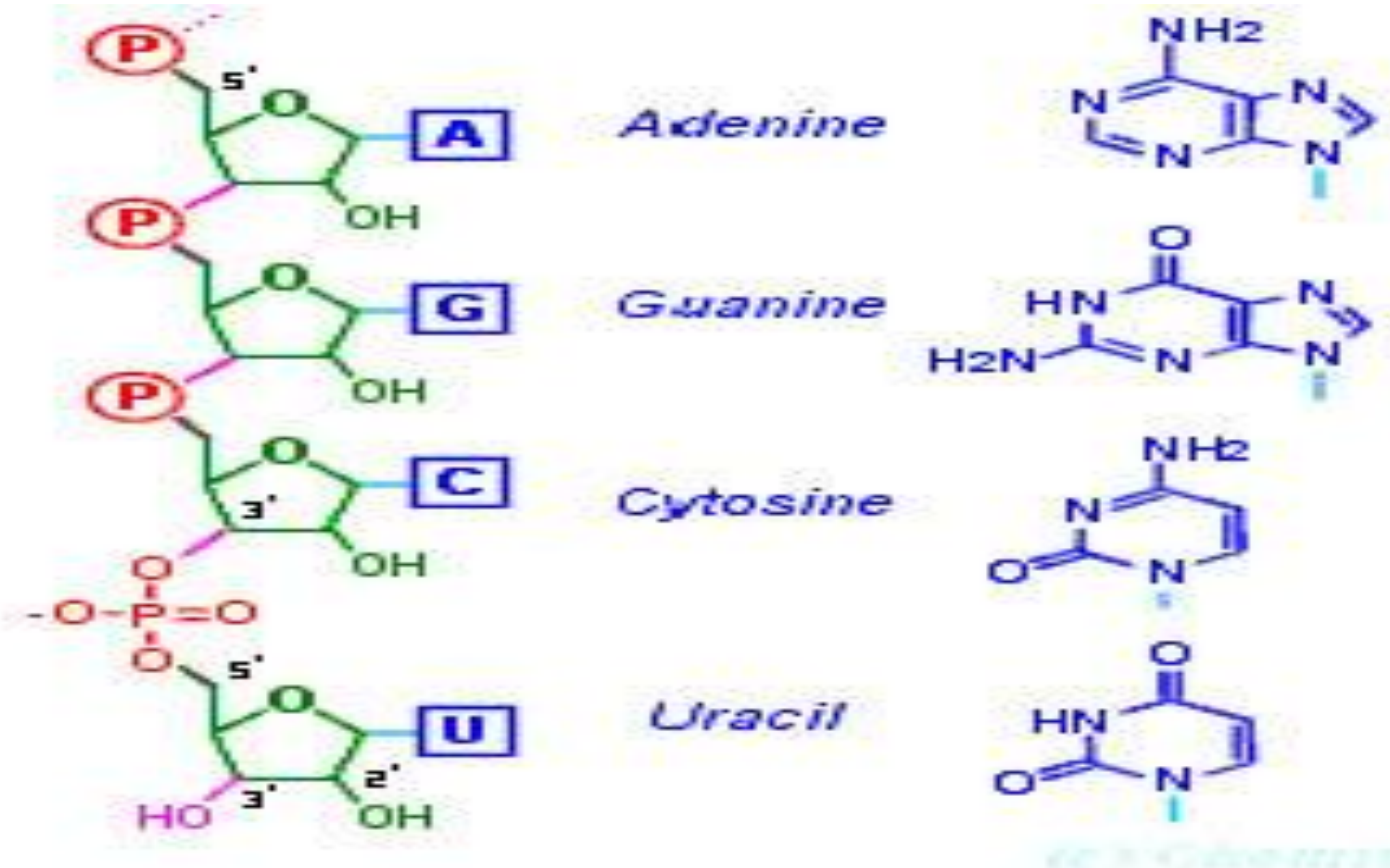
A – Adenine

G – Guanine

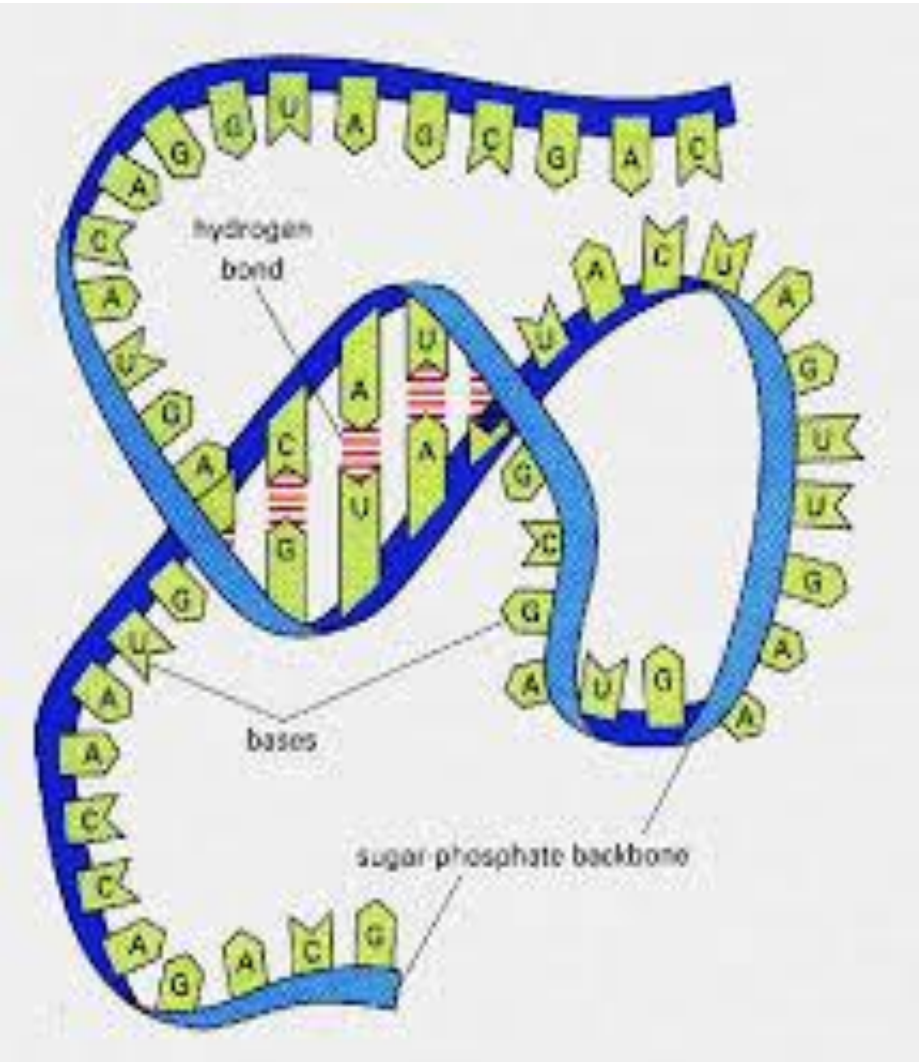
U – Uracil

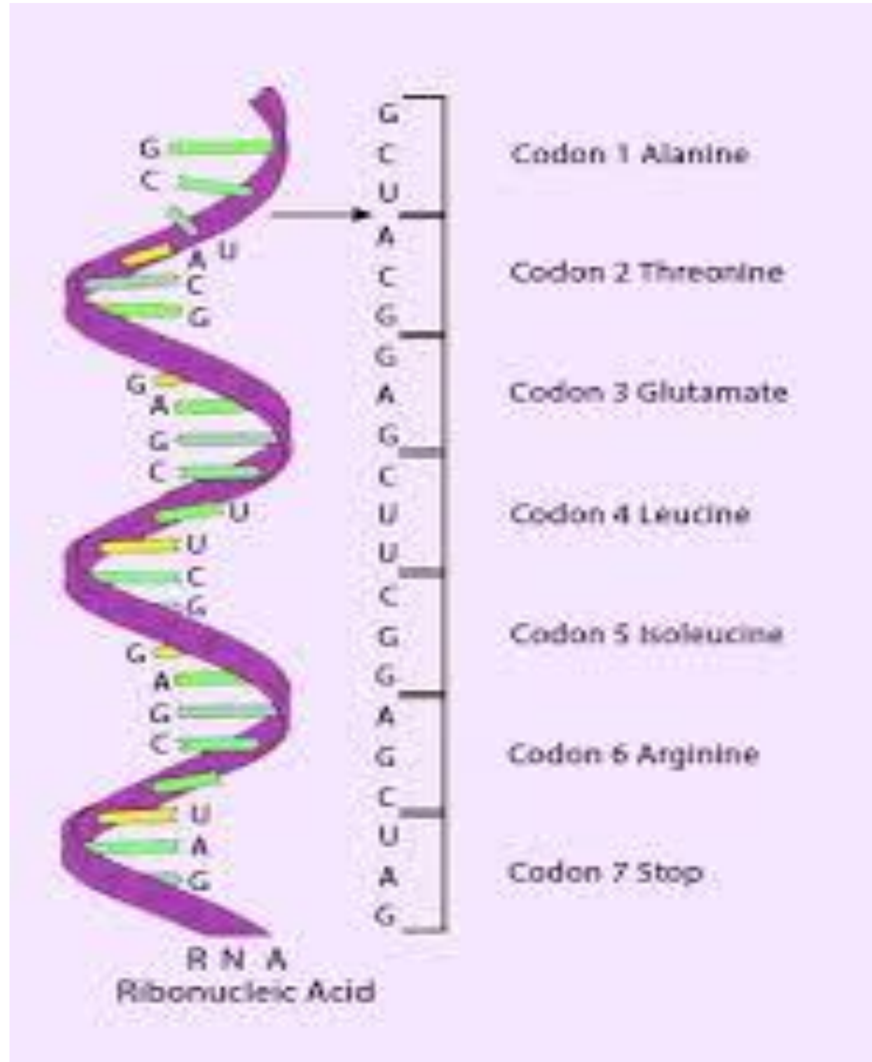
C – Cytosine

STRUCTURE OF RNA

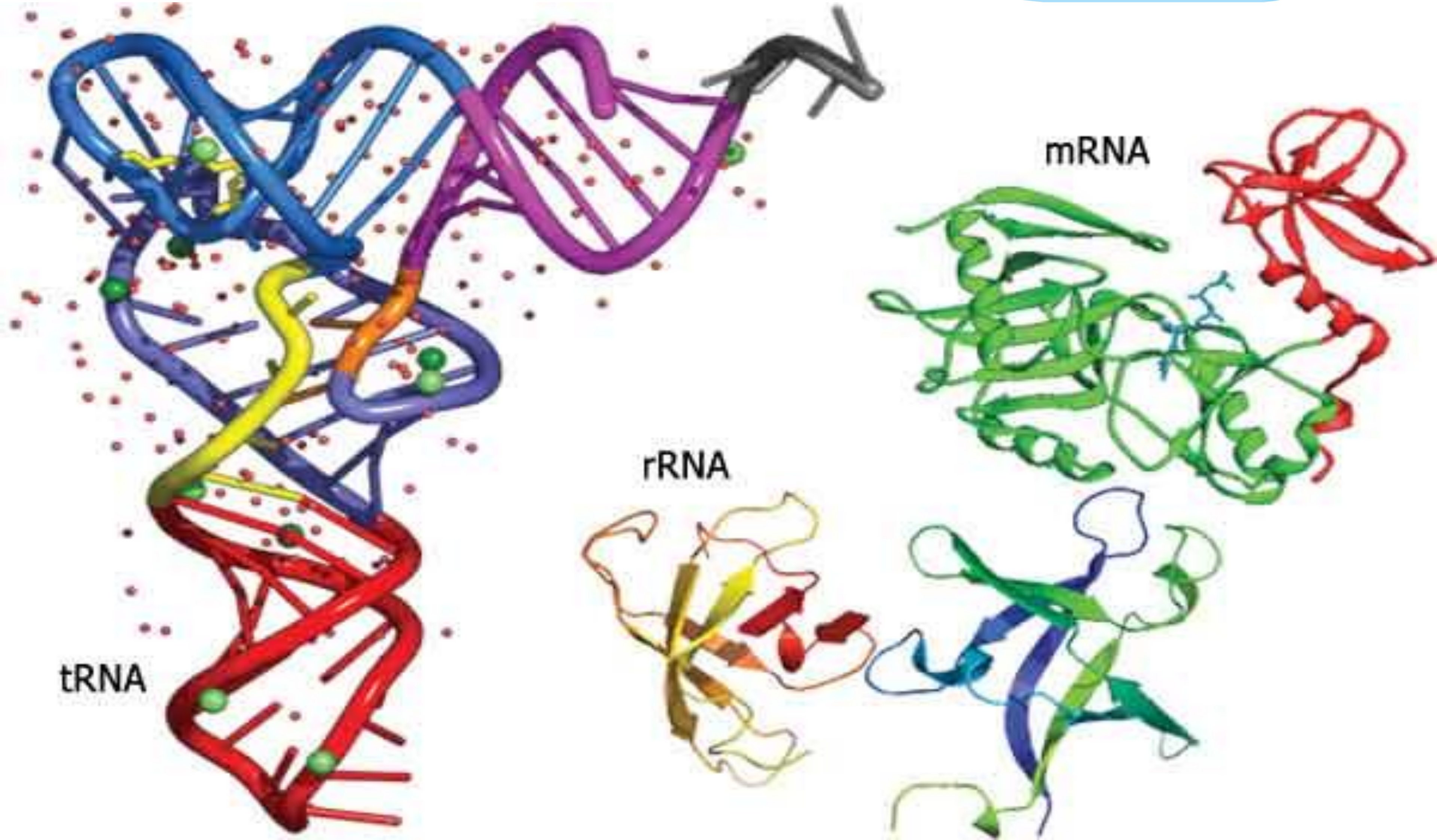


SINGLE STRAND OF RNA





TYPES OF RNA

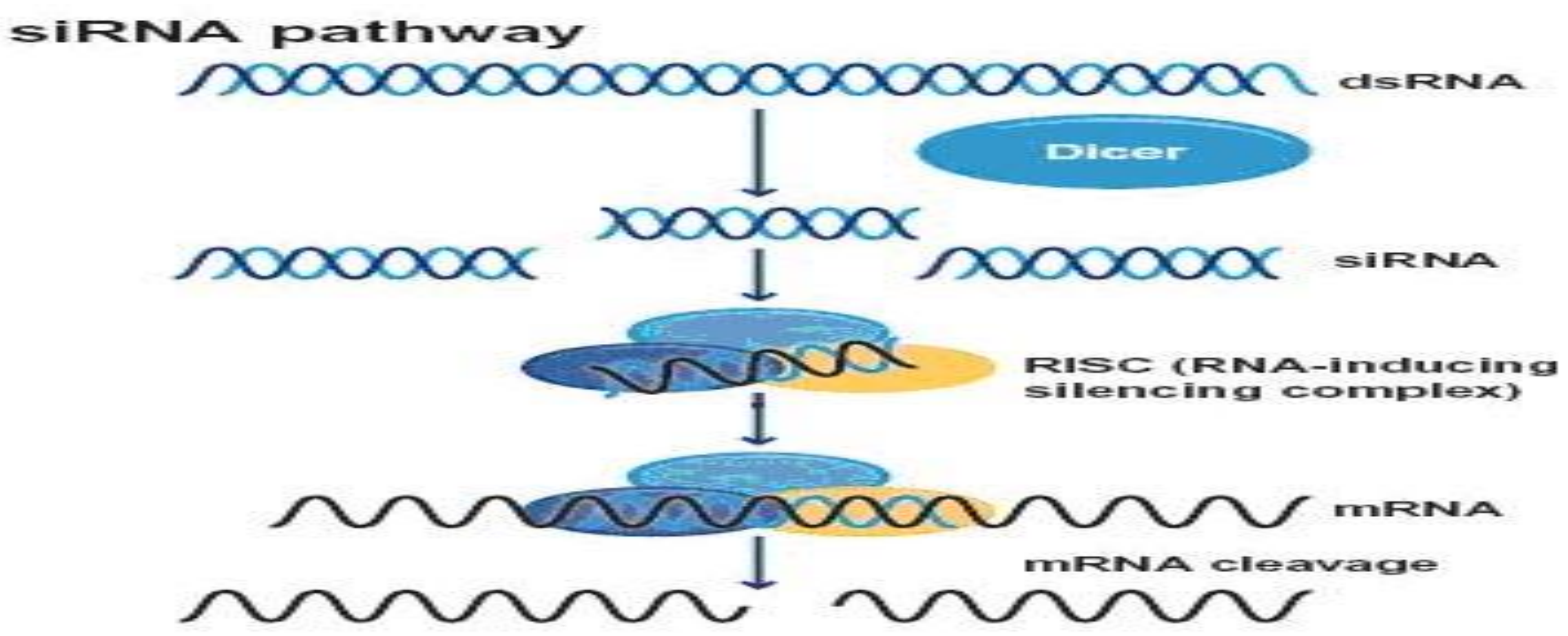


TYPES OF RNA

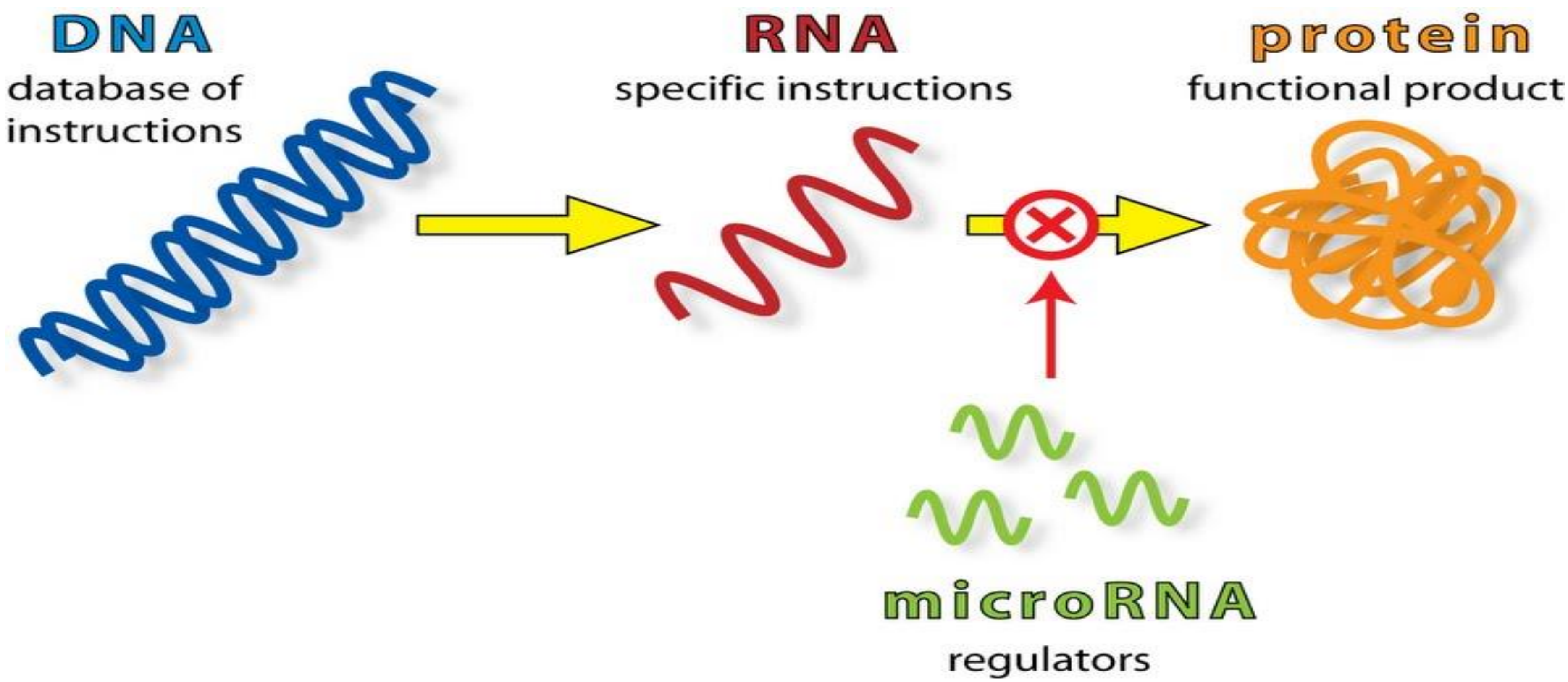
There are many types of RNA but we will focus on the major ones which mRNA, tRNA and rRNA.

OTHER TYPES INCLUDE

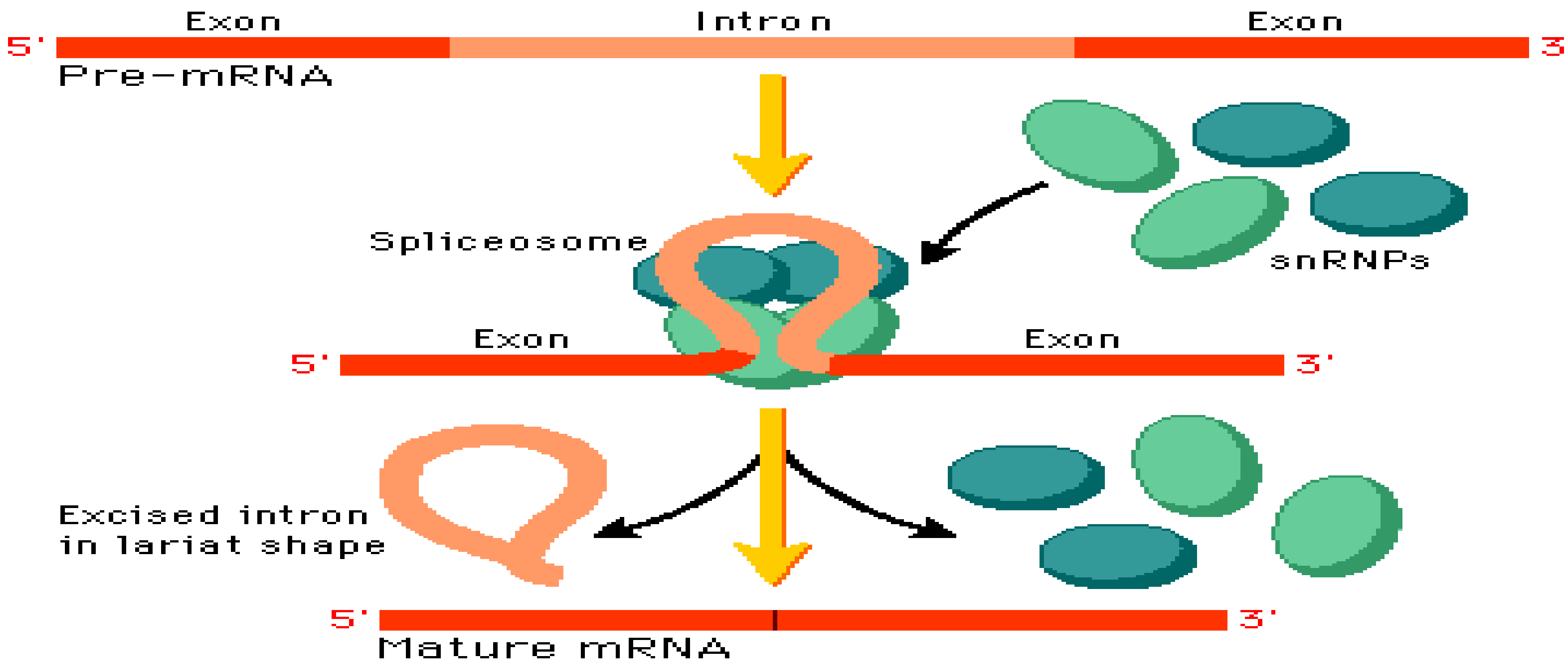
- **snRNA (Small Interfering RNAs) – play significat role in eukayotic mRNA processing by splicing of exons as snRNPs or snurps U1,U2, U4,U5 &U6**



miRNA (Micro RNAs) – play important role in gene silencing by blocking mRNA and preventing translation



* snRNA (Small Nuclear RNAs)) – play significat role in eukayotic mRNA processing by splicing of exons as snRNPs or snurps U1,U2, U4,U5 &U6



tRNA

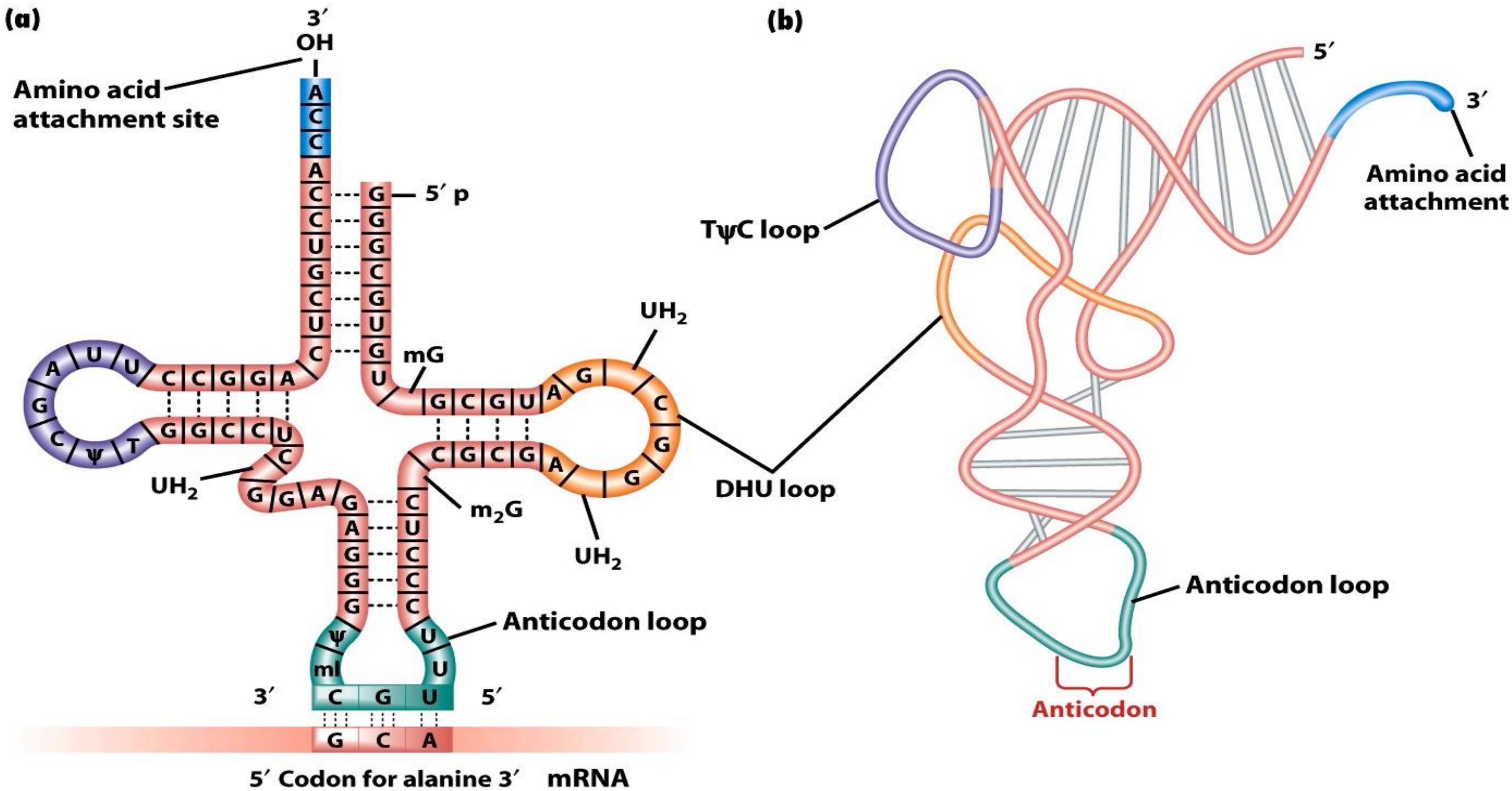


Figure 9-7
Introduction to Genetic Analysis, Ninth Edition
© 2008 W. H. Freeman and Company

ABOUT tRNA

- **Transfer RNA (tRNA) is a small RNA chain of about 80 nucleotides**
- **It transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation**
- **It has sites for amino acid attachment and an anticodon region for codon recognition that binds to a specific sequence on the messenger RNA chain through hydrogen bonding**

mRNA

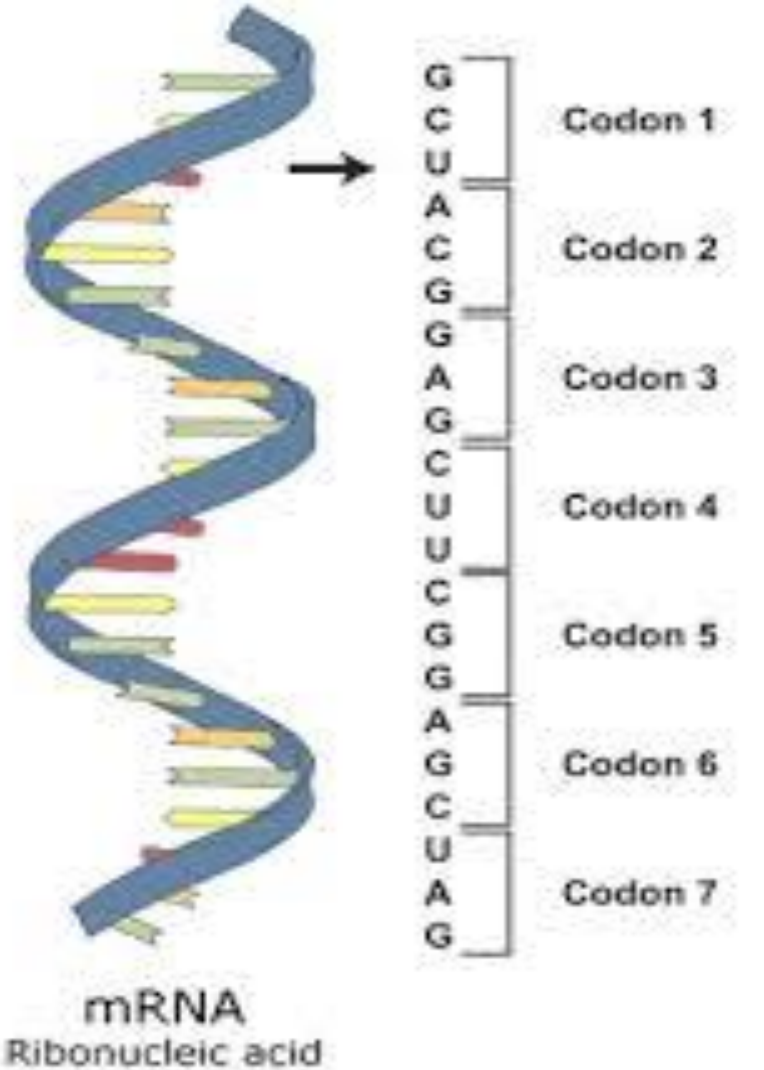
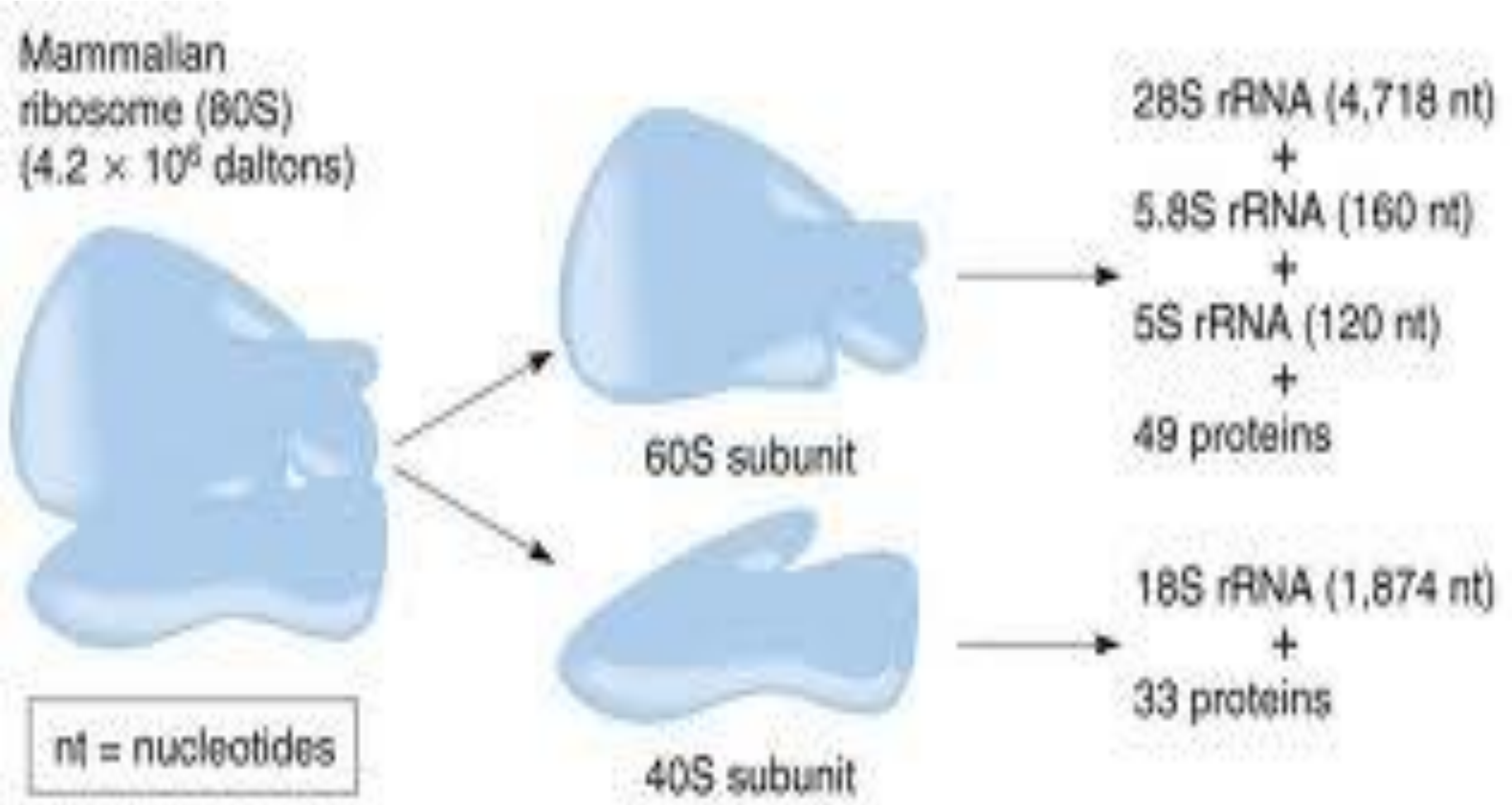


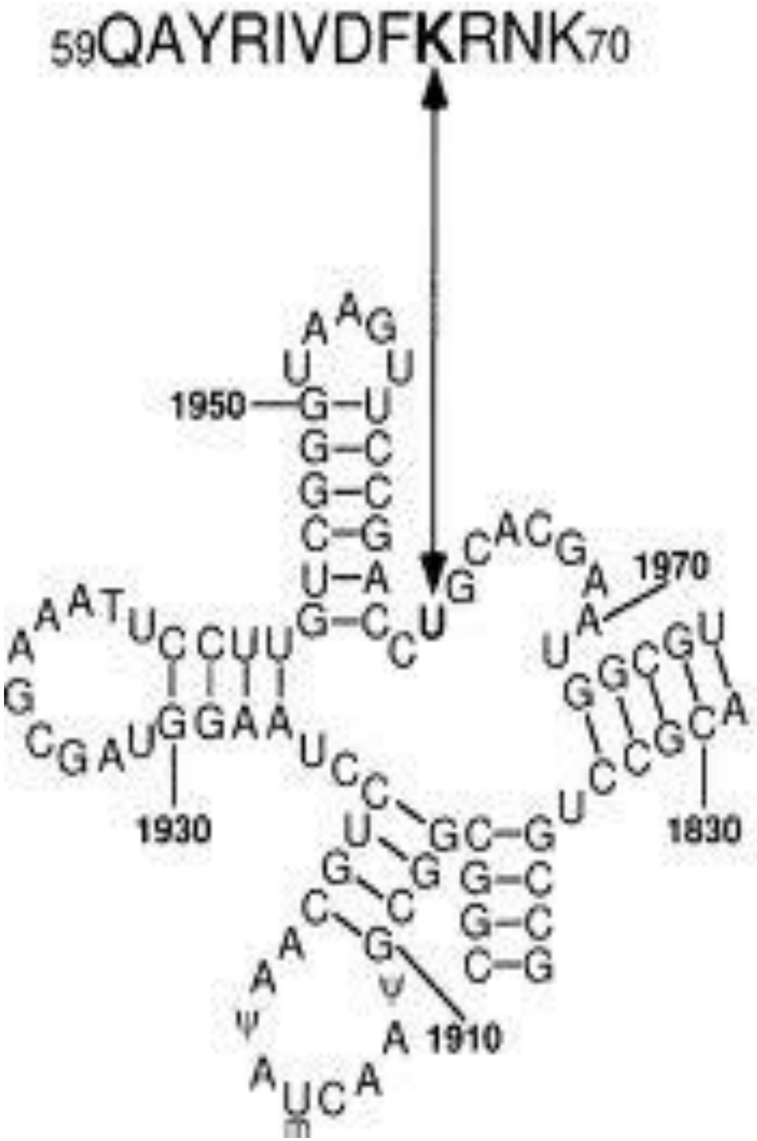
Image adapted from: National Human Genome Research Institute. Talking Glossary of Genetic Terms. Available at: www.genome.gov/Pages/Hyperion/DIR/VIP/Glossary/Illustration/codon.shtml.

- Messenger RNA (mRNA) carries information about a protein sequence to the ribosomes, the protein synthesis factories in the cell
- It is coded so that every three nucleotides (a codon) correspond to one amino acid

rRNA



Mammalian ribosome (80S)
(4.2 × 10⁶ daltons)
60S subunit
40S subunit
28S rRNA (4,718 nt)
+
5.8S rRNA (160 nt)
+
5S rRNA (120 nt)
+
49 proteins
18S rRNA (1,874 nt)
+
33 proteins
nt = nucleotides


59QAYRIVDFKRNK70
1950
1970
1930
1830
1910

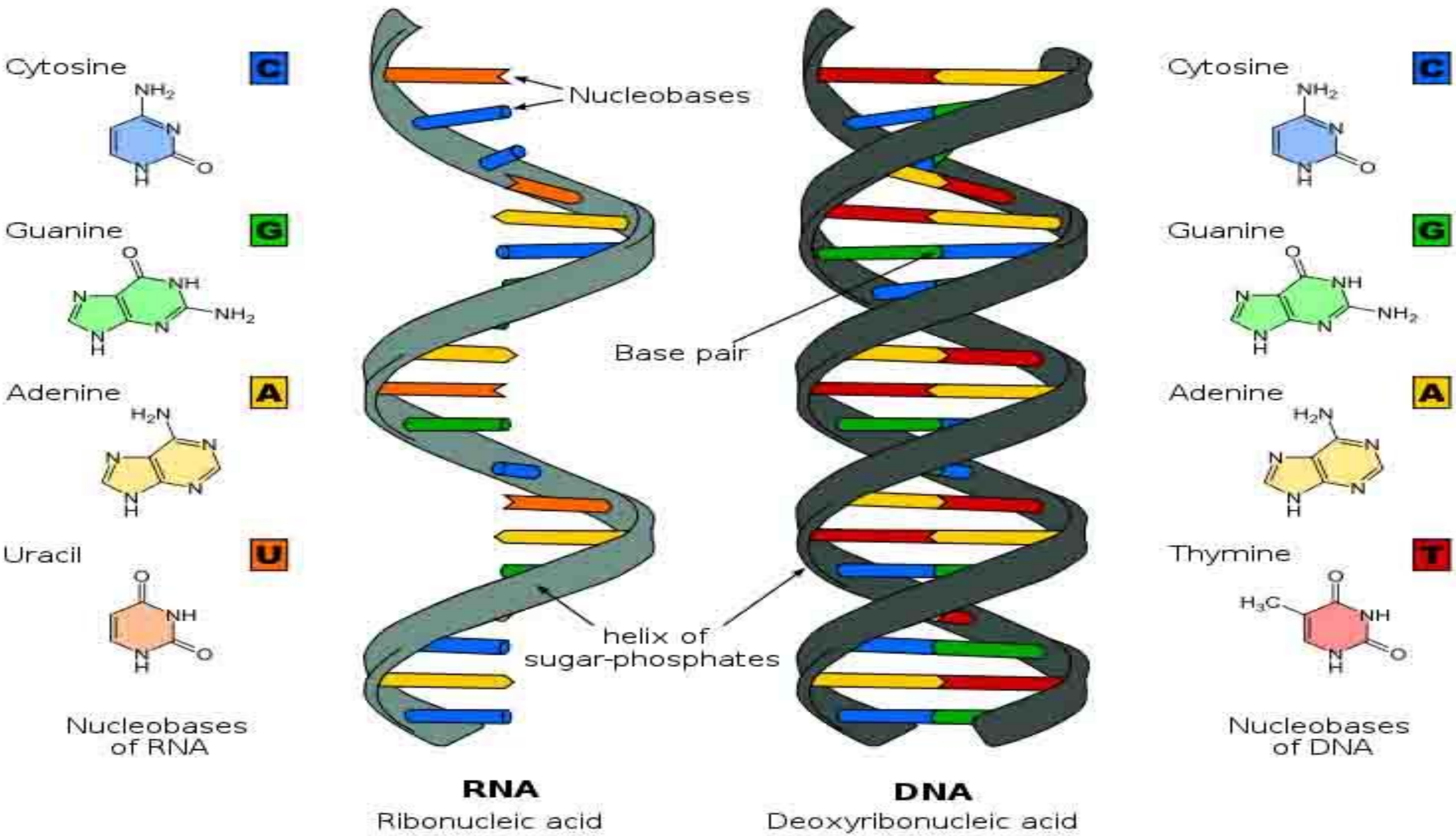
ABOUT rRNA

- **Ribosomal RNA (rRNA) is the catalytic component of the ribosomes**
- **Eukaryotic ribosomes contain four different rRNA molecules: 18S, 5.8S, 28S and 5S rRNA**
- **Three of the rRNA molecules are synthesized in the nucleolus, and one is synthesized elsewhere.**
- **. In the cytoplasm, ribosomal RNA and protein combine to form a nucleoprotein called a ribosome**
- **The ribosome binds mRNA and carries out protein synthesis**
- **Several ribosomes may be attached to a single mRNA at any time.**
- **Nearly all the RNA found in a typical eukaryotic cell is rRNA.**

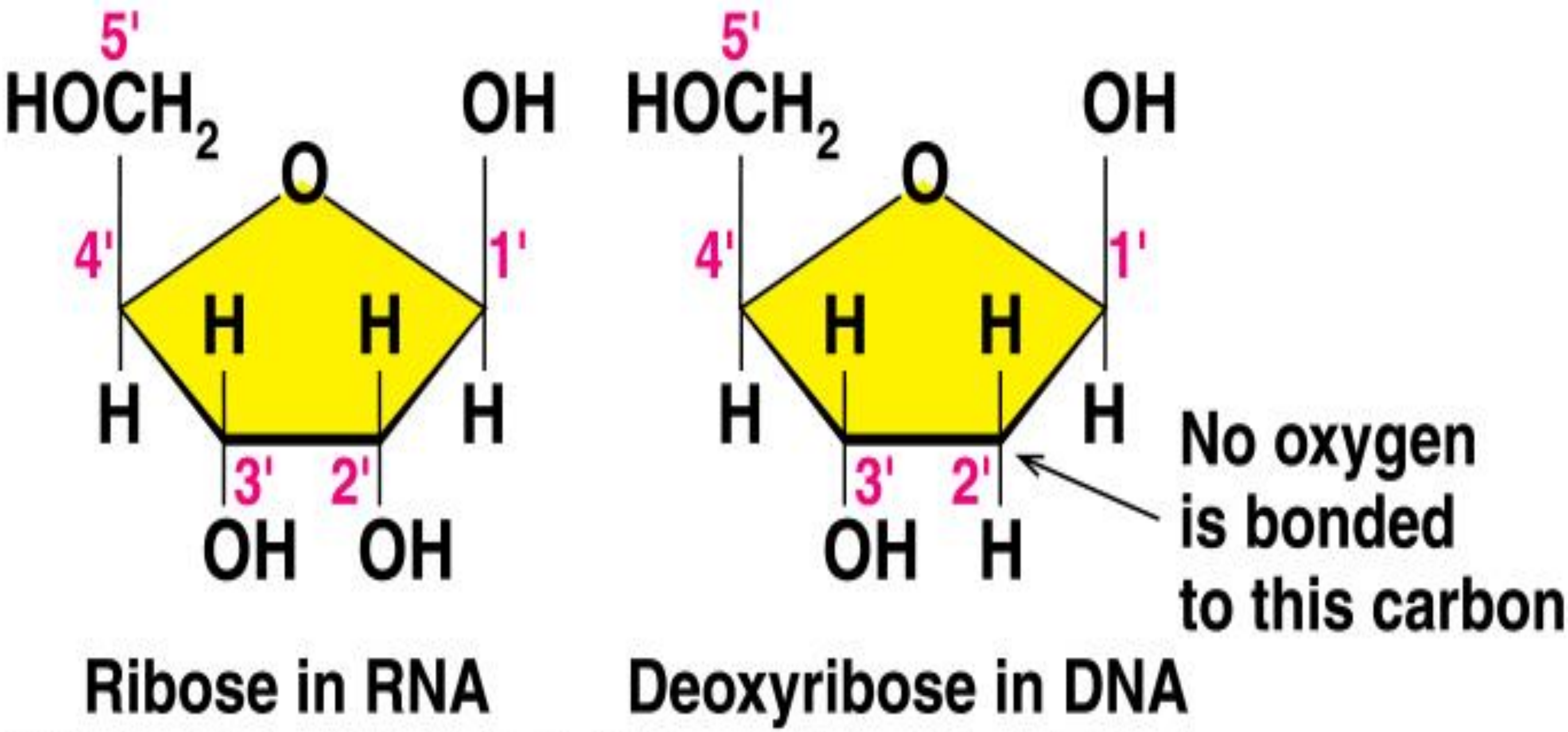
Svedberg units (S),

The sizes of ribosomes and other cell structures are described in terms of Svedberg units (S), which are actually a measure of sedimentation rate in a centrifuge.

DNA Vs. RNA


Cytosine
C
NH_2
N
O
N
H
Guanine
G
O
NH
N
NH_2
N
N
H
Adenine
A
H_2N
N
N
N
N
H
Uracil
U
O
NH
O
N
H
Nucleobases of RNA
Nucleobases
Base pair
helix of sugar-phosphates
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid
Cytosine
C
Guanine
G
Adenine
A
Thymine
T
H_3C
Nucleobases of DNA

Pentose sugars in RNA and DNA



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S.No.	RNA	DNA
1)	Single stranded mainly except when self complementary sequences are there it forms a double stranded structure (Hair pin structure)	Double stranded (Except for certain viral DNA s which are single stranded)
2)	Ribose is the main sugar	The sugar moiety is deoxy ribose
3)	Pyrimidine components differ. Thymine is never found(Except tRNA)	Thymine is always there but uracil is never found
4)	Being single stranded structure- It does not follow Chargaff's rule	It does follow Chargaff's rule. The total purine content in a double stranded DNA is always equal to pyrimidine content.

S.No.	RNA	DNA
5)	RNA can be easily destroyed by alkalies to cyclic diesters of mono nucleotides.	DNA resists alkali action due to the absence of OH group at 2' position
6)	RNA is a relatively a labile molecule, undergoes easy and spontaneous degradation	DNA is a stable molecule. The spontaneous degradation is very 2 slow. The genetic information can be stored for years together without any change.
7)	Mainly cytoplasmic, but also present in nucleus (primary transcript and small nuclear RNA)	Mainly found in nucleus, extra nuclear DNA is found in mitochondria, and plasmids etc
8)	The base content varies from 100- 5000. The size is variable.	Millions of base pairs are there depending upon the organism

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