COURSE CODE: SC202(CHEMISTRY)

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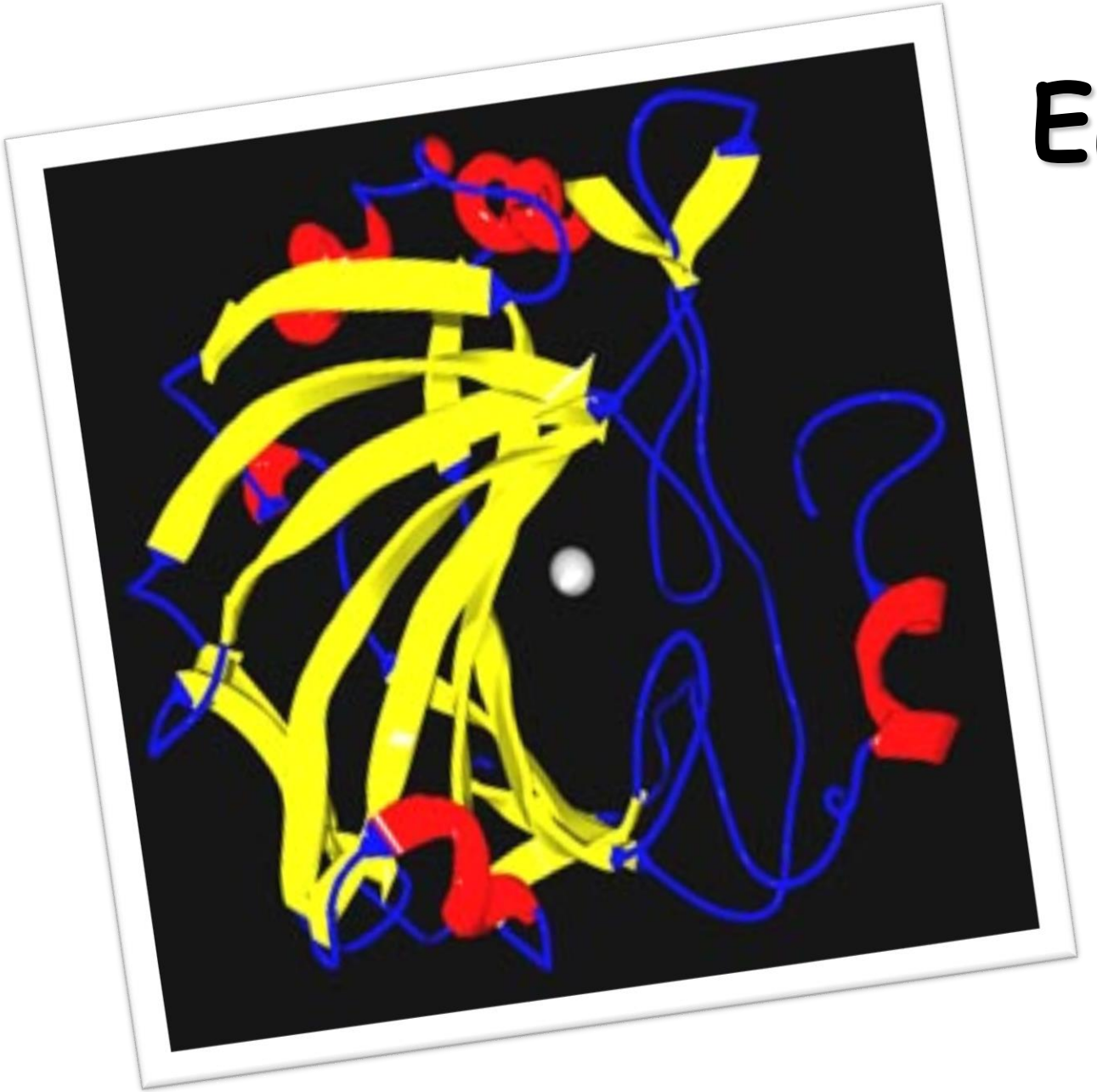
BIOORGANIC CHEMISTRY: ENZYME-NUCLEIC ACID

DEPT. OF SCIENCE AND MATHEMATICS

IIITG, GUWAHATI

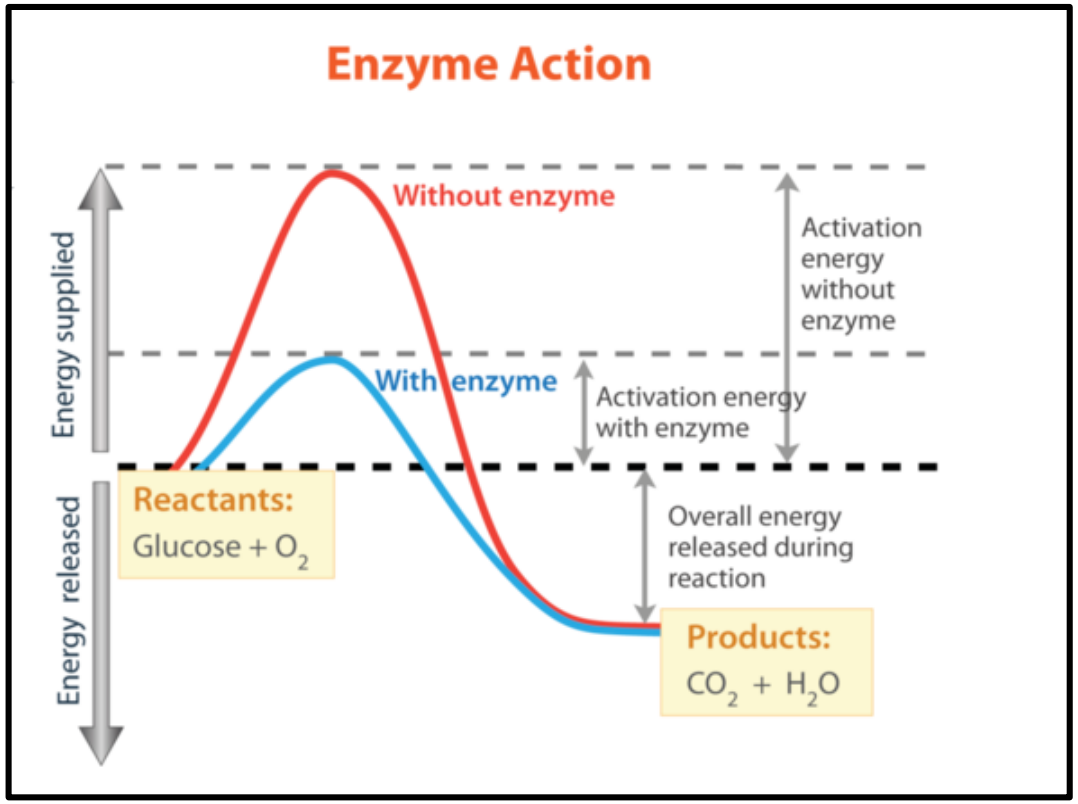
LECTURE DATE: 01.02.2023 & 02.02.2023



Enzymes

What are enzymes?

- Enzymes are biological catalyst.
- A catalyst is a substance that increases the rate of a chemical reaction without being itself changed in the process.
- Enzymes are proteins that increase the rate of reaction by lowering the activation energy.
- Enzymes have unique three-dimensional shape that fit the shape of the reactants known as substrate.



Enzymes are named by **adding the suffix -ase to the name of the substrate** that they modify (i.e., urease and tyrosinase), or the type of reaction they catalyze (dehydrogenase, decarboxylase). There are exceptions also, e.g., pepsin.

Properties of enzyme

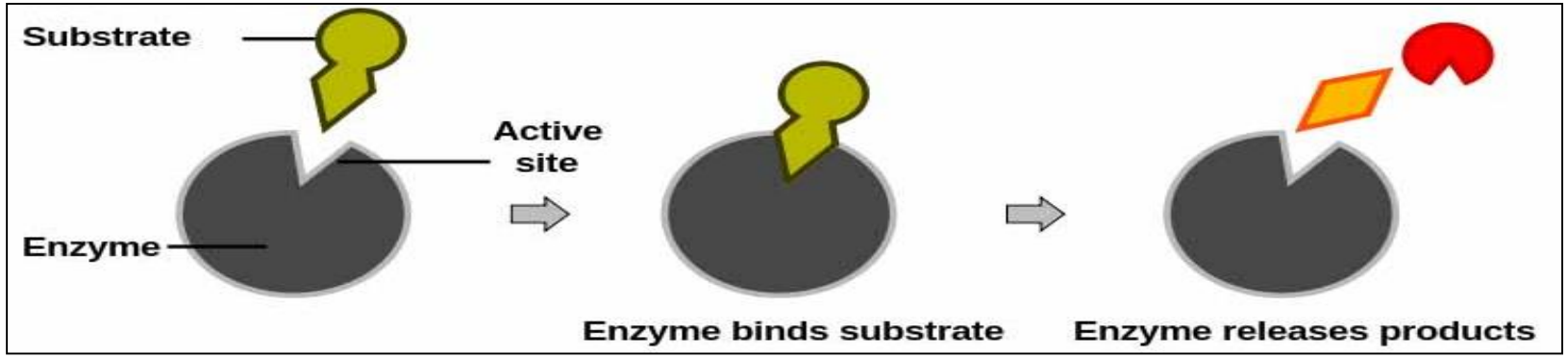
- Colloidal nature: on account of their large size, the enzyme molecule possess extremely low rate of diffusion and form colloidal system in water.
- Catalytic nature: Enzymes increase the rate of a chemical reaction without undergoing any qualitative or quantitative change and producing any side products.
- Catalytic effectiveness: The catalytic power of an enzyme is measured by the **turnover number** which is **the number of substrate molecules converted into product per unit time, when the enzyme is fully saturated with substrate**.

  The value of **turnover number** varies with different enzymes and depends upon the conditions in which the reaction is taking place.

  The efficiency of an enzymatic reaction is 10^{13} to 10^{17} higher than an uncatalysed reaction.
- Specificity: Enzymes are highly specific in nature in choosing the substrate.
- Mild condition: Enzymes need very mild condition e.g., physiological pH, to execute their action.

Active site

- As the substrate molecules are comparatively much smaller than the enzyme molecules, there should be some specific regions or sites on the enzyme for binding with the substrate. Such sites of attachment are variously called as **'active sites'** or **'catalytic sites'** or **'substrate sites'.**
- Active sites has a specific shape due to the tertiary structure of protein, a change in the shape of protein change the structure of active site as well as the function of the enzyme.
- The active site binds the substrate molecules by relatively weak forces.



Cofactors

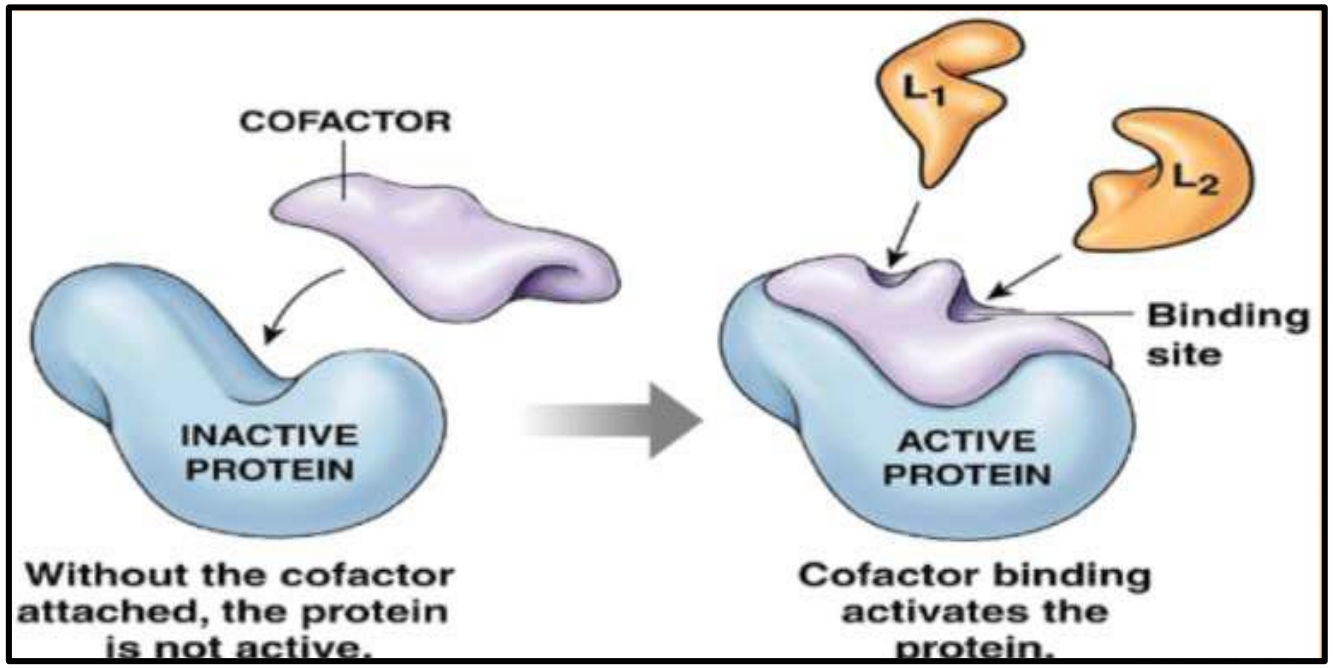
- Cofactor is the non-protein molecule which carries out chemical reactions that can not be performed by standard 20 amino acids.
- Cofactors are of two types:

i) Organic cofactor: These are the organic molecules required for the proper activity of enzymes.

Example: Glycogen phosphorylase requires the small organic molecule pyridoxal phosphate.

ii) Inorganic cofactor: These are the inorganic molecules required for the proper activity of enzymes.

Example: Enzyme carbonic anhydrase requires Zn^{++} for its activity.

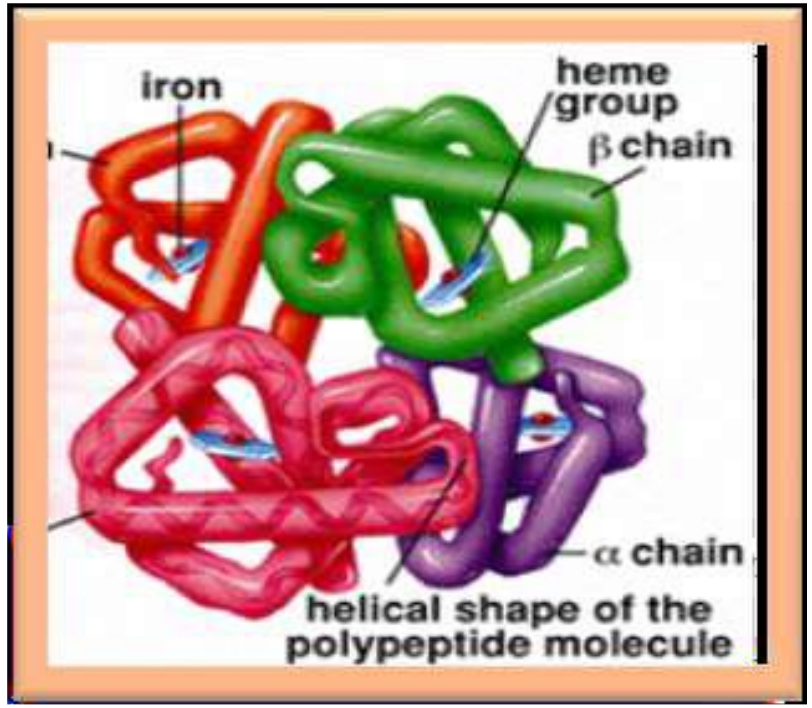


Types of organic cofactor

- A Prosthetic group is a tightly bound organic cofactor

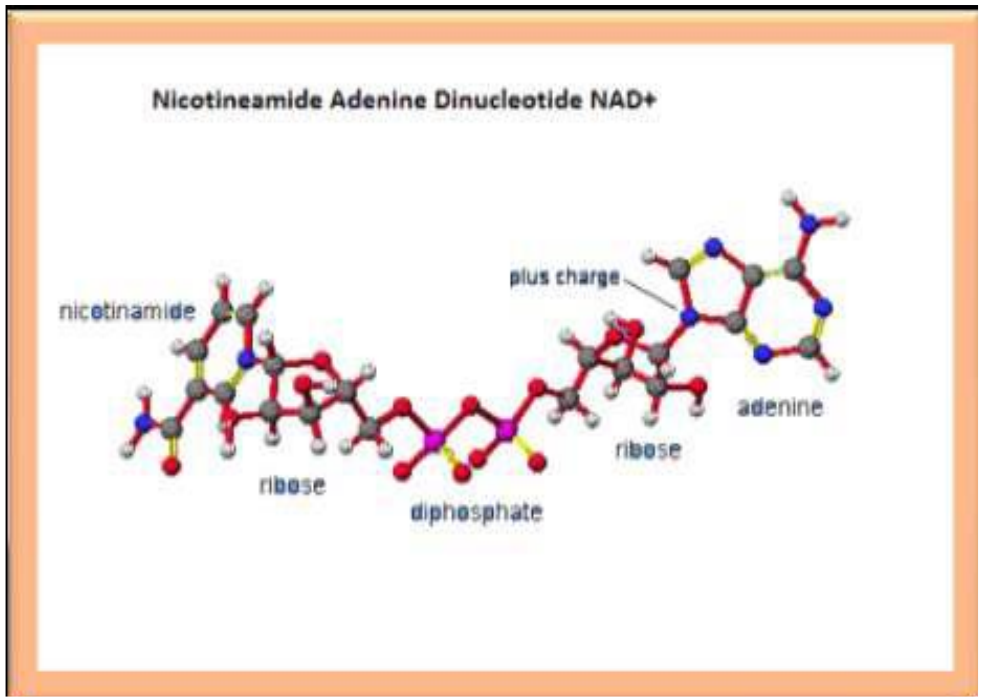
  e.g. Flavins, heme groups and

  biotin.

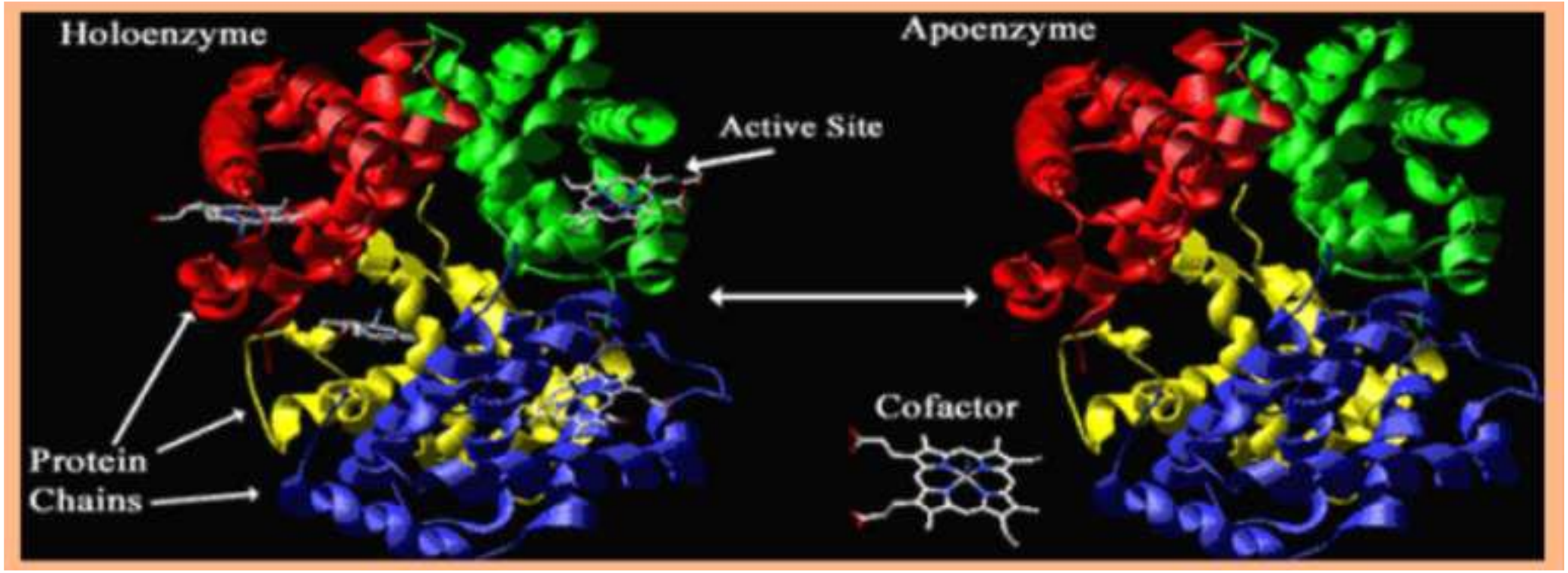


- A coenzyme is loosely bound organic cofactor.

  e.g. NAD^{++}



- An enzyme with its cofactor removed is designated as *apoenzyme*.
- The complete complex of a protein with all necessary small organic molecules, metal ions and other components is termed as *holoenzyme* of *holoprotein*.

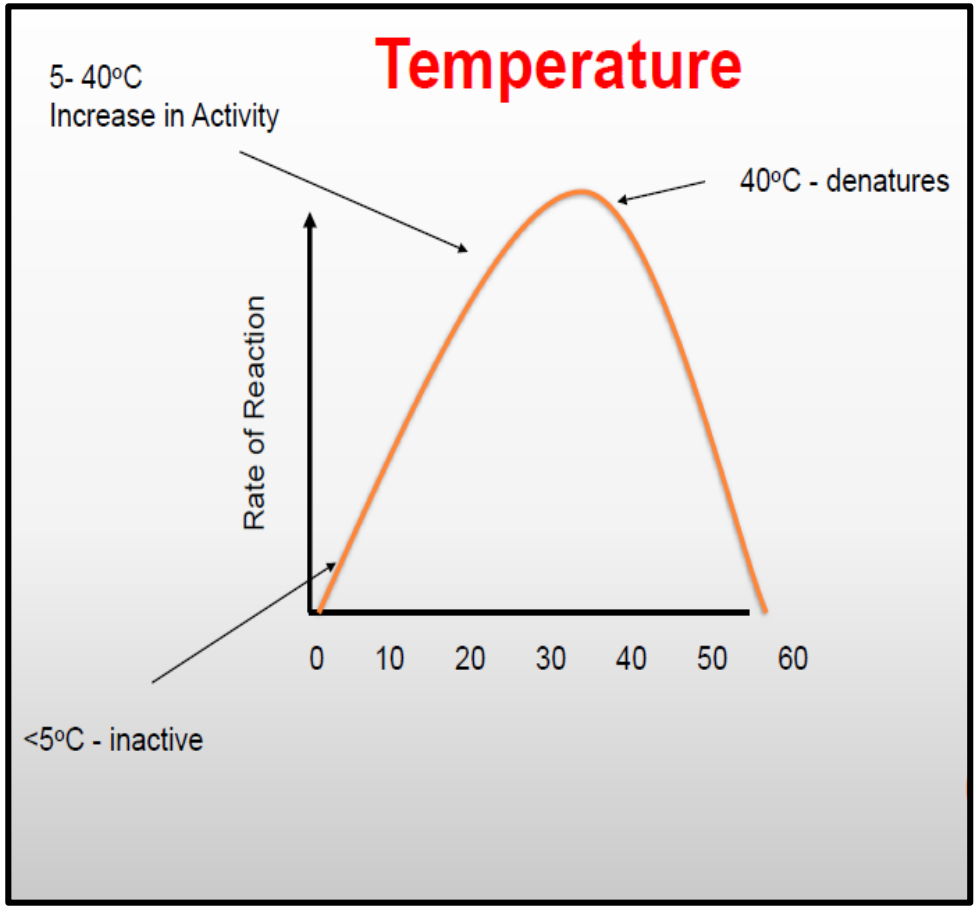


Factors that influence enzymatic activity

- Temperature
- pH
- Substrate concentration
- Inhibitor

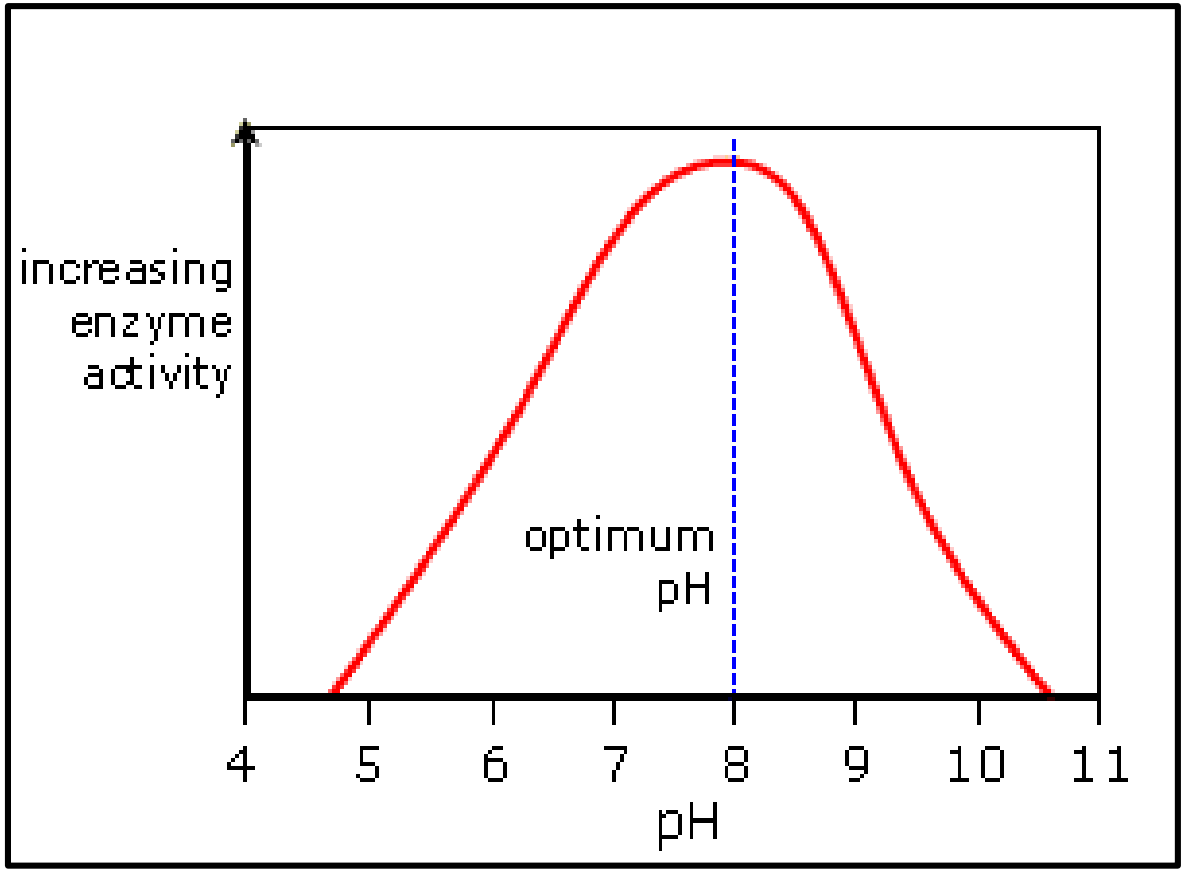
Effect of temperature

- Raising the temperature increases the rate of enzyme-catalyzed reaction by increasing kinetic energy of reacting molecules.
- Enzymes work maximum over a particular temperature known as *optimum temperature*. Enzymes for humans generally exhibit stability up to temperature 35-45°C.
- However some times heat energy can also increase kinetic energy to a point that exceed the energy barrier which results in denaturing of enzymes.



Effect of pH

- Rate of almost all enzyme-catalyzed reactions depends on pH.
- Most enzymes exhibit optimal activity at pH value between *5 and 9.*
- High or low pH value than optimum value will cause ionization of enzyme which result in denaturation of enzyme.



Effects of substrate concentration: Michaelis-Menten model

- Changing the Enzyme and Substrate concentrations affect the rate of reaction of an enzyme-catalysed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so its metabolism.
- Michaelis-Menten Equation:

"It is an equation which describes how reaction velocity varies with substrate concentration."

$$V_o = \frac{V_{max}\,[S]}{K_m + [S]}$$

- Where

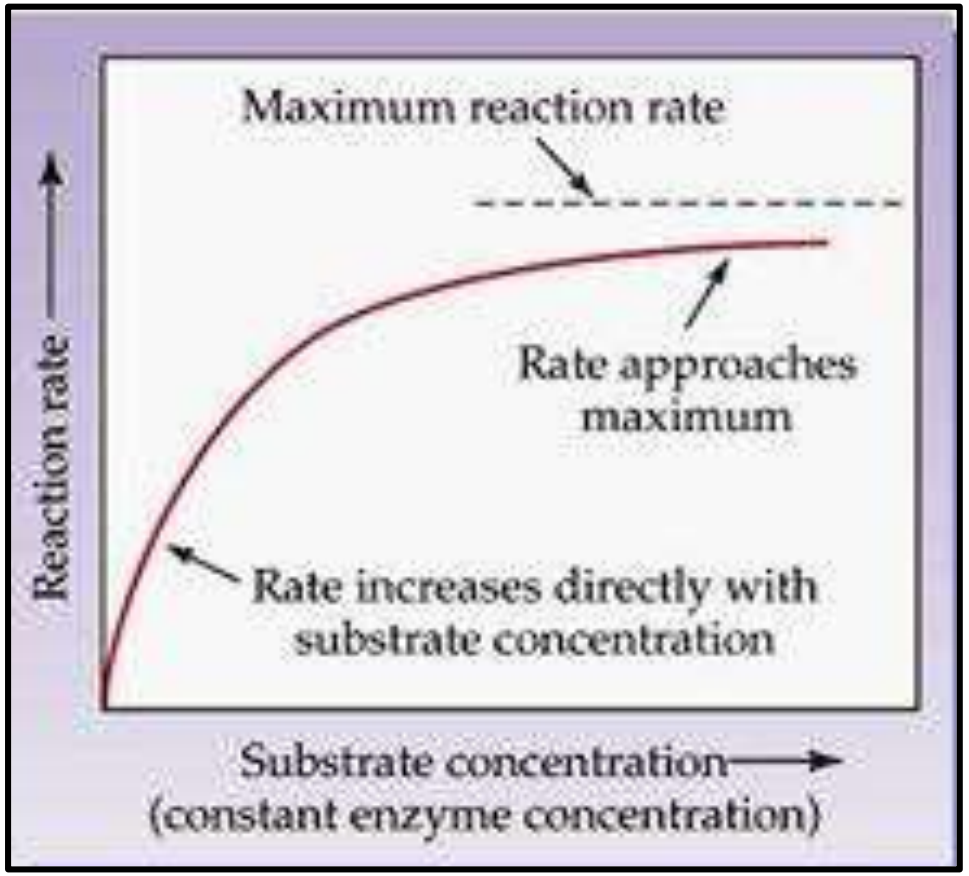
V_o is the initial reaction velocity

V_{max} is the maximum velocity

K_m is the Michaelis constant = $(k_{-1}+k_2)/k_1$, [S] is the substrate concentration.

Assumption for this Michaelis-Menten equation:

- Relative concentrations of E and S
- Steady-State assumptions
- Initial Velocity
- **Increasing Substrate Concentration increases the rate of reaction**. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.
- However, after a certain concentration, any increase will have no effect on the rate of reaction, since *Substrate Concentration will no longer be the limiting factor*. The enzymes will effectively become saturated, and will be working at their maximum possible rate.



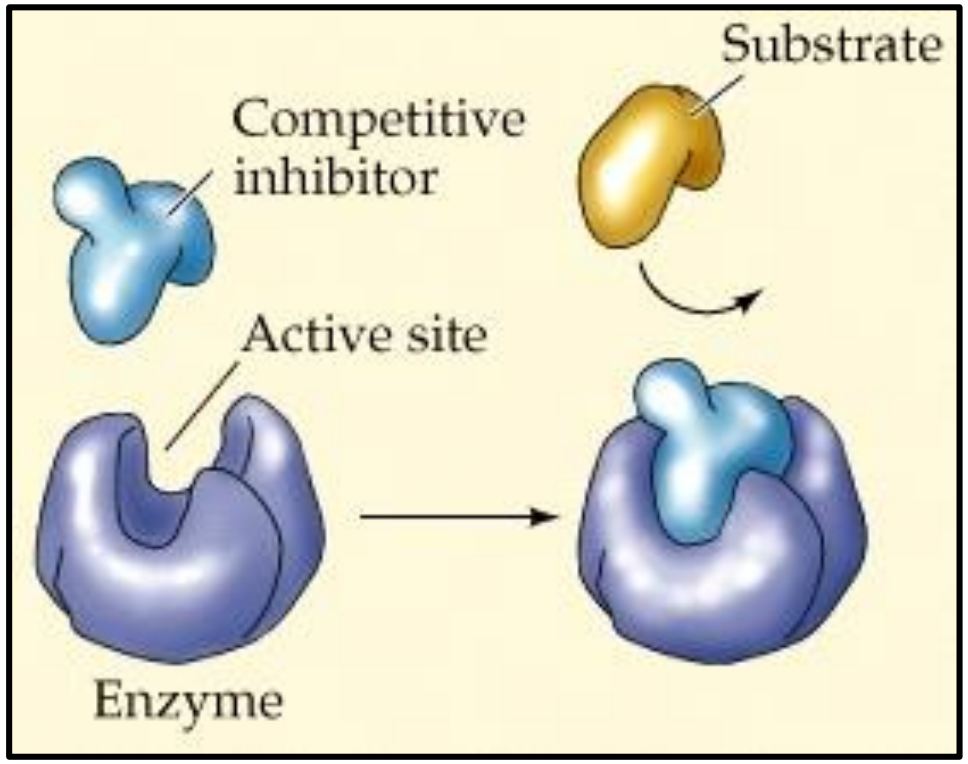
Effect of inhibitor

- Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis.
- There are two types of enzyme inhibition: **reversible and irreversible**
- Reversible inhibition is again divided into three categories. They are: **competitive, non-competitive and mixed inhibition.**

- **Reversible inhibition:** It is an inhibition of enzyme activity in which the inhibiting molecular entity can associate and dissociate from the protein's binding site in a reversible way.

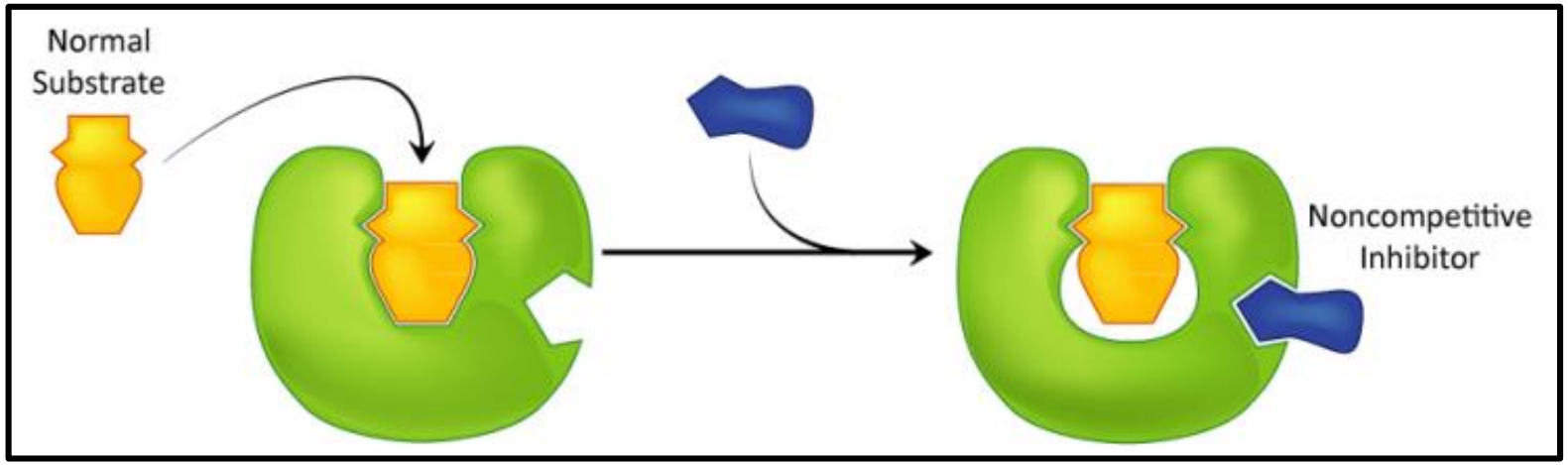
- Competitive inhibition

Competitive inhibition occurs when the substrate and a substance resembling the substrate (inhibitor) are both added to the enzyme. A theory called the "lock-key theory" of enzyme catalysts can be used to explain why inhibition occurs.



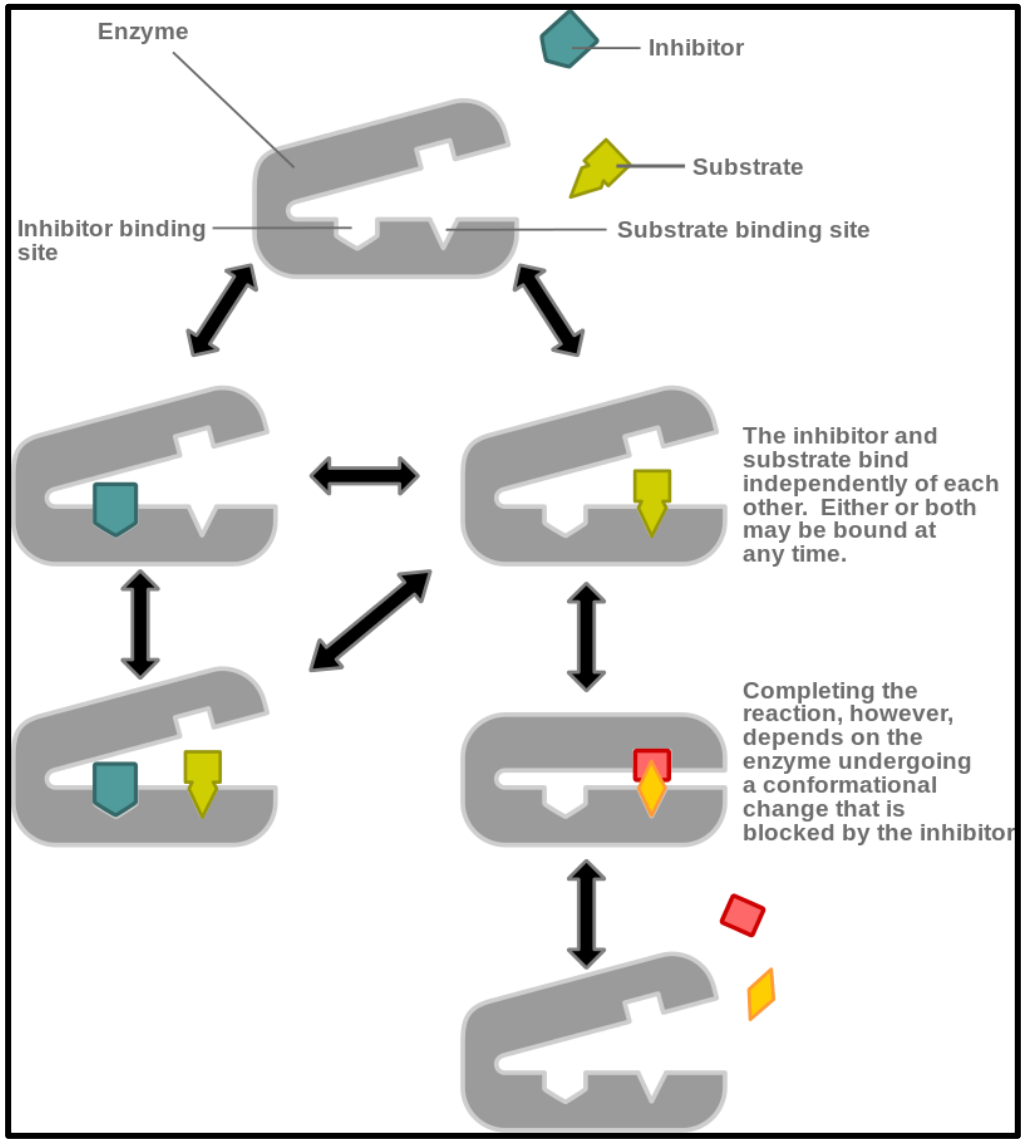
- Non-competitive inhibition

In this type of inhibition, inhibitor does not compete with the substrate for the active site of enzyme instead it binds to another site known as *allosteric* site.

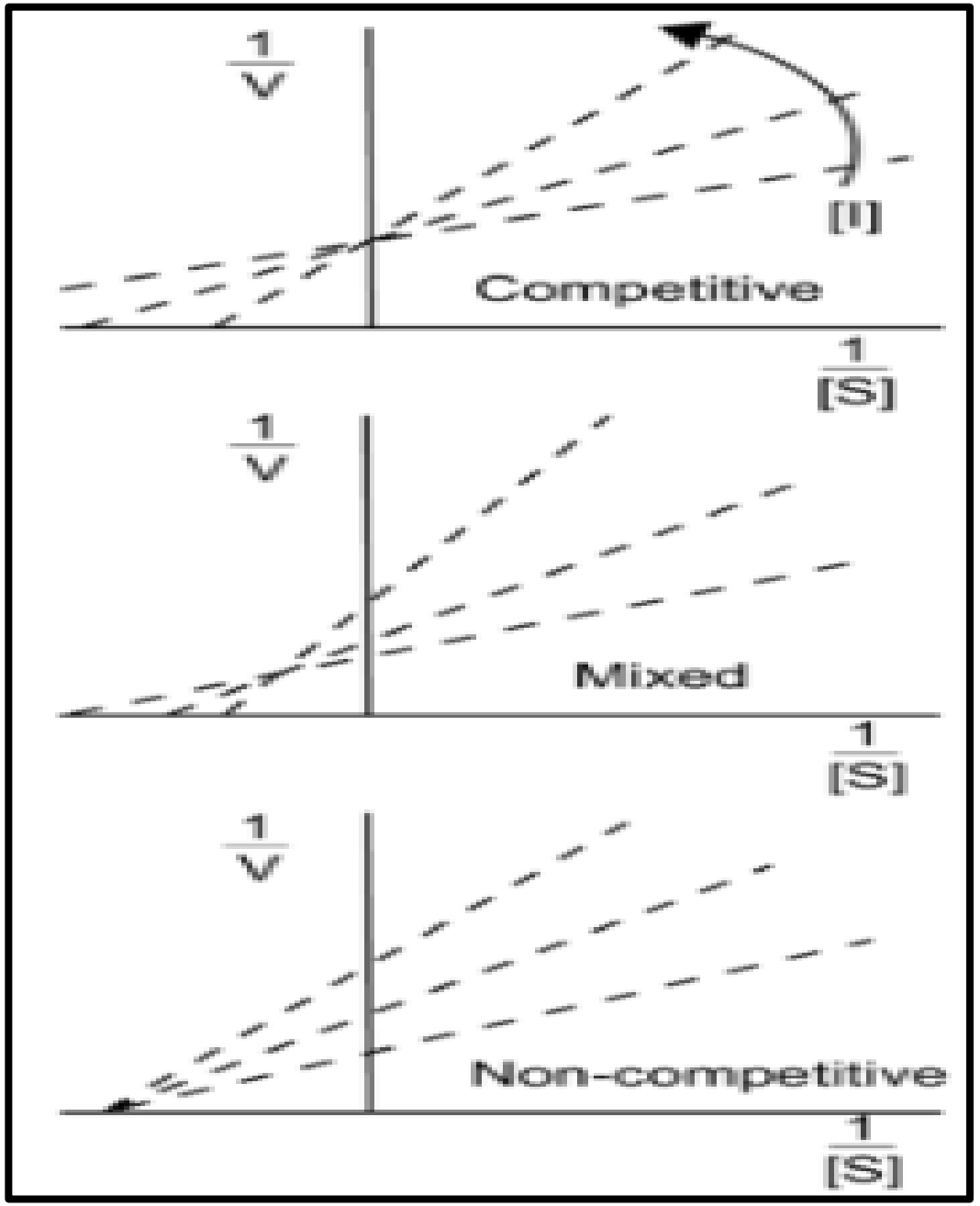


- Mixed inhibition

Mixed inhibition is a type of enzyme inhibition in which the inhibitor may bind to the enzyme whether or not the enzyme has already bound the substrate but has a greater affinity for one state or the other.



Graphs for three kind of inhibition →

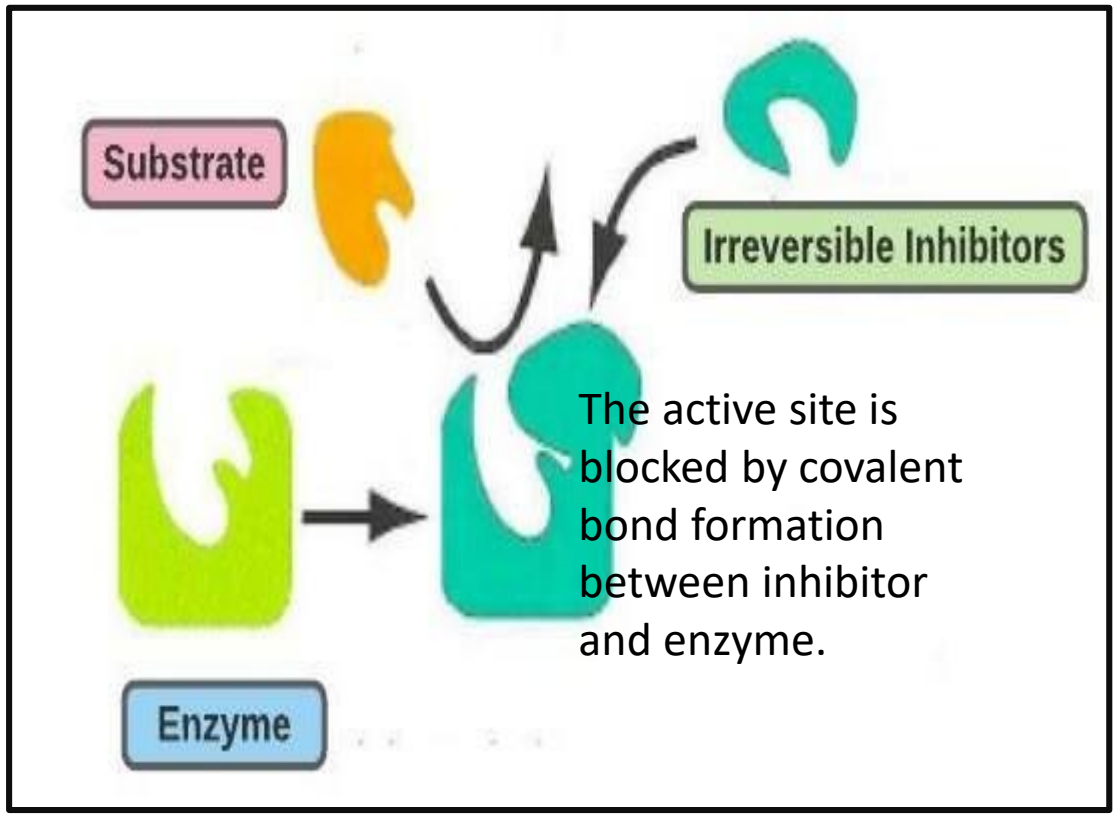


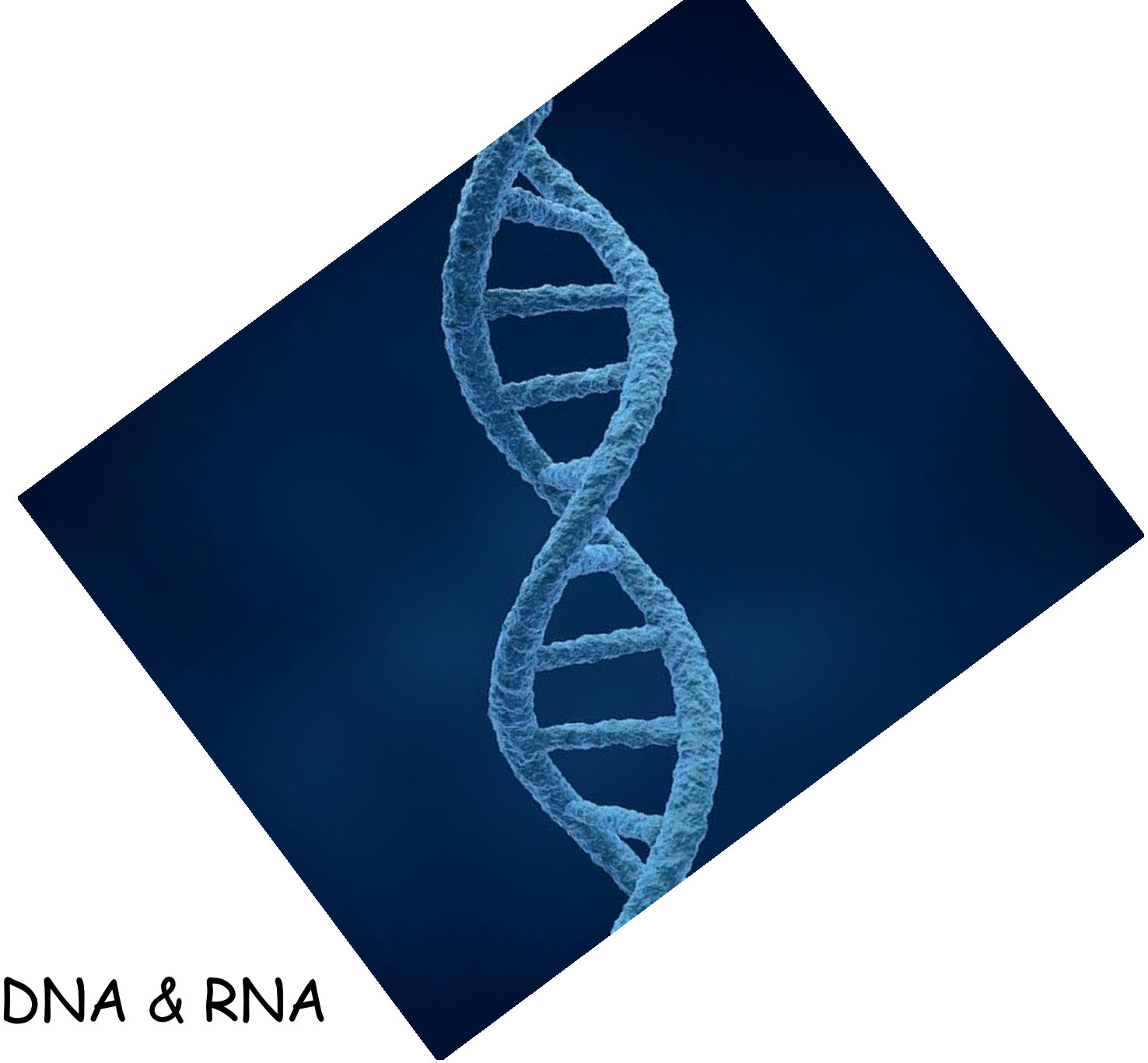
- Irreversible inhibition

➢This type of inhibition involves the *covalent attachment* of the inhibitor to the enzyme.

➢ The *catalytic activity* of enzyme is completely lost.

➢It can only be restored only by synthesizing molecules.



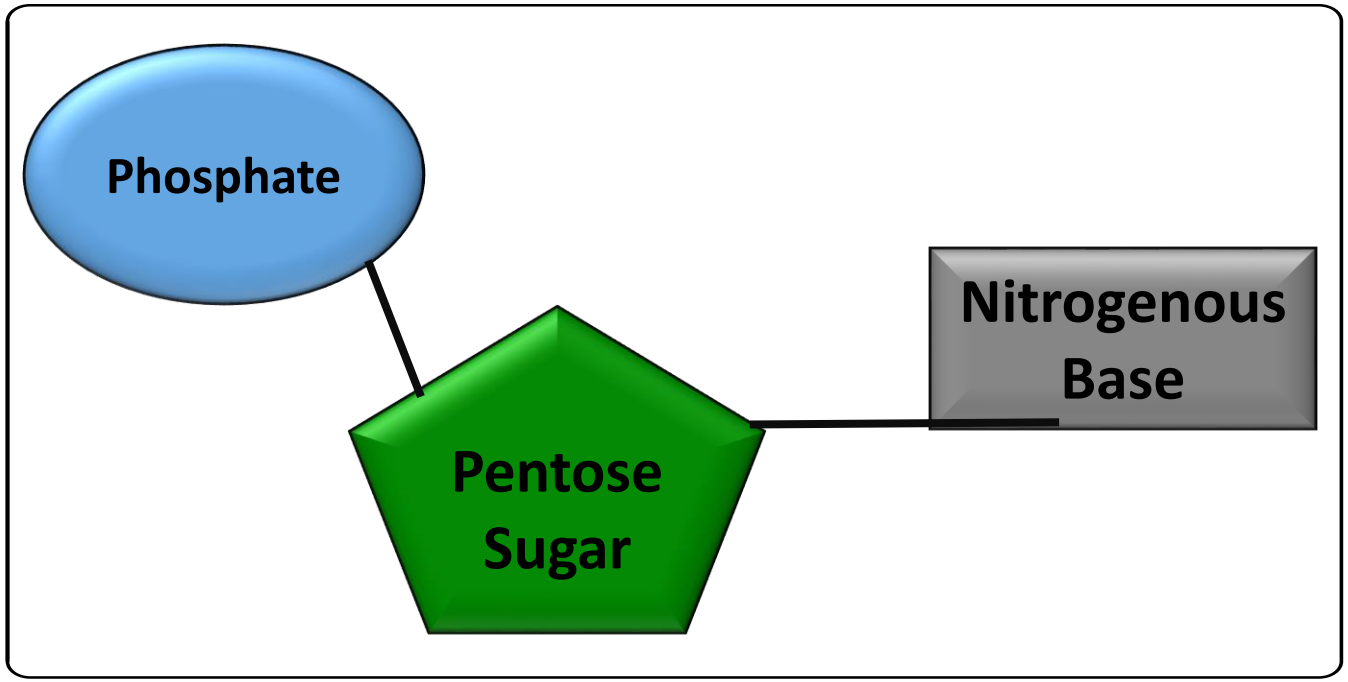


Nucleic Acids-DNA & RNA

Nucleotide

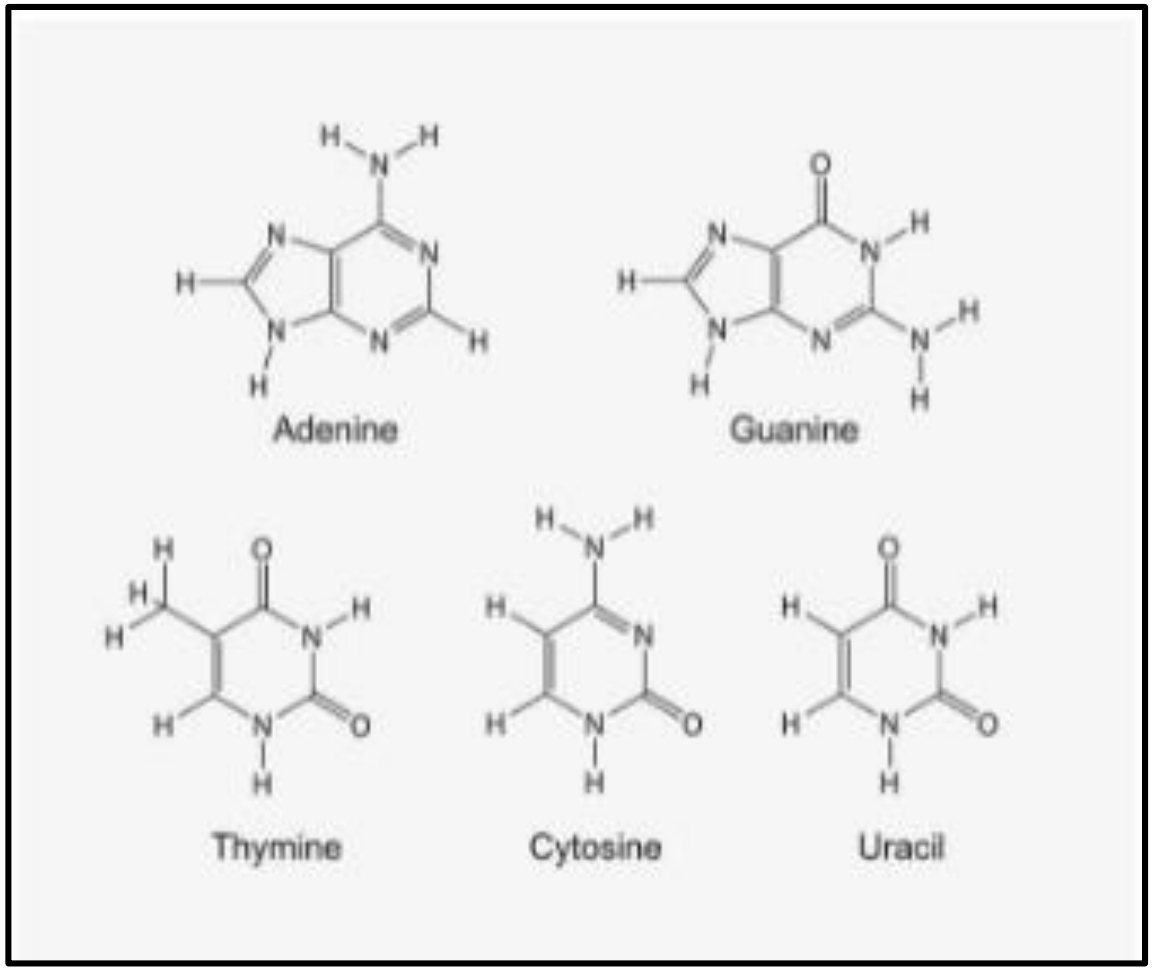
- A molecule of DNA or RNA is made up of millions of monomers called Nucleotides.

- Each nucleotide consists of:
  1. Phosphate group
  2. 5 carbon sugar (ribose in RNA or deoxyribose in DNA)
  3. Nitrogenous base



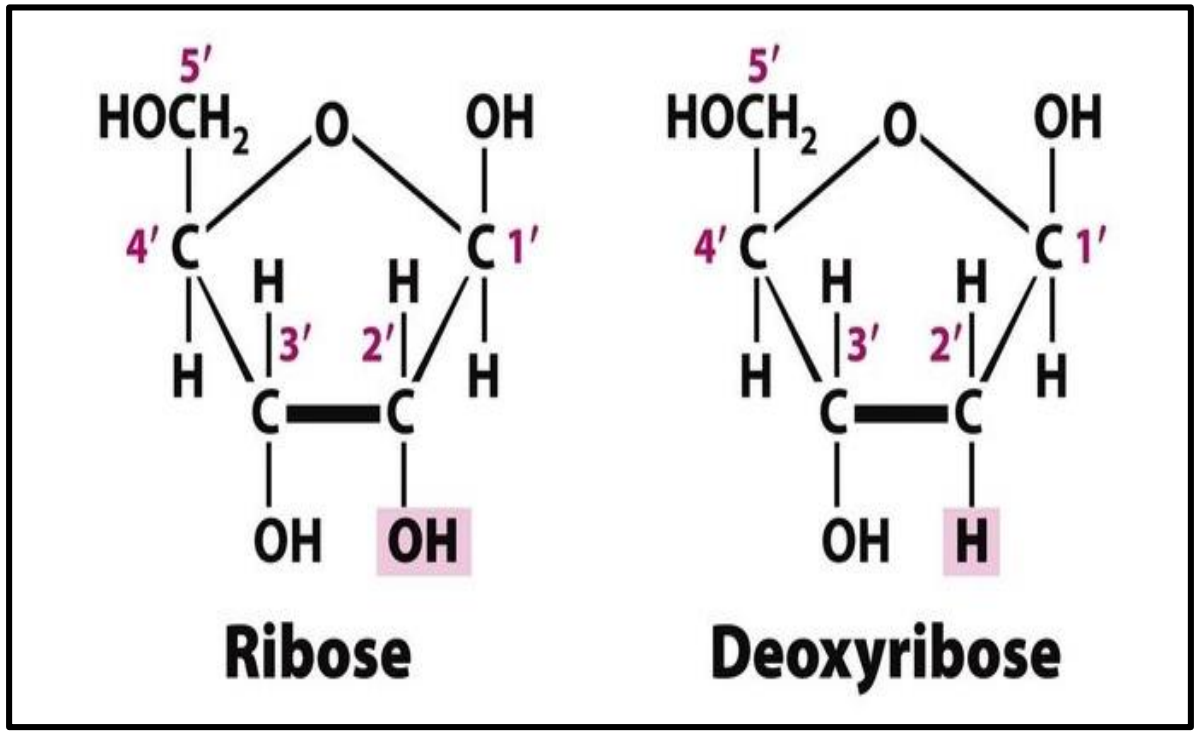
Nitrogen containing bases

- Adenine(A) and Guanine(G) are purine bases.
- Thymine(T) , Cytosine (C) & Uracil (U) are pyrimidine bases.
- In DNA: A, T, C & G are present.
- In RNA: A, U, C & G are present.
- In purine nucleosides, nitrogen-9 of purine ring is linked to carbon-1 of pentose sugar.
- In pyrimidine nucleosides, nitrogen-1 of pyrimidine ring is linked to carbon-1 of pentose sugar.



5 carbon sugar

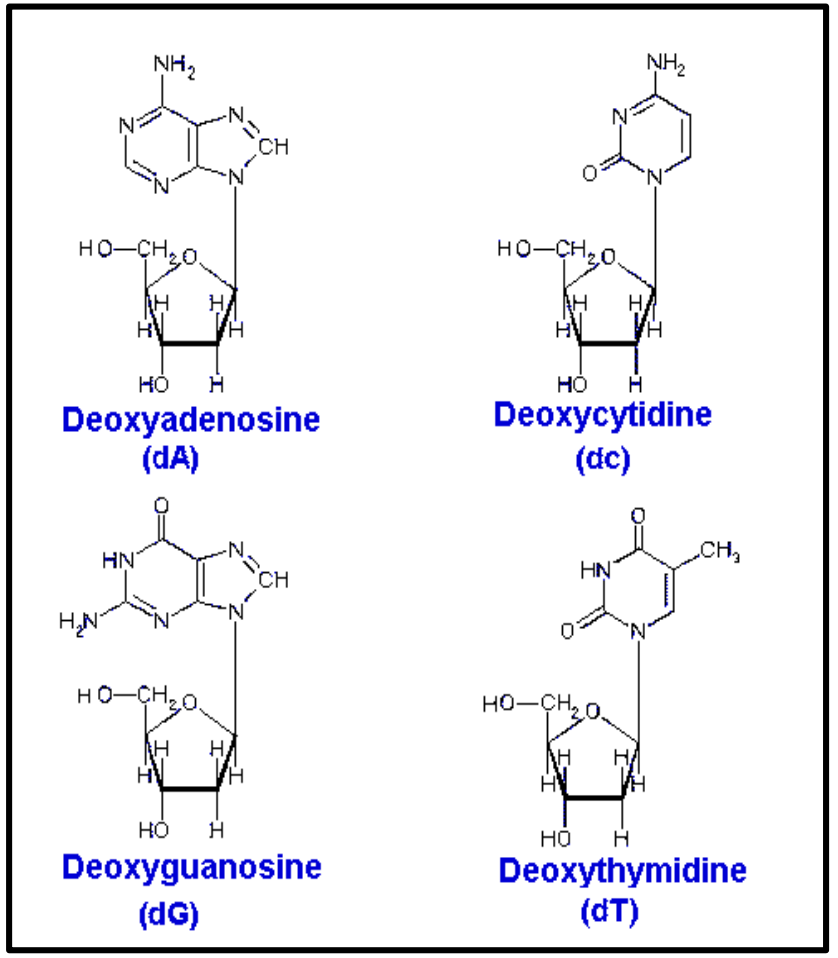
- In DNA, deoxyribose sugar is present; but in RNA, ribose sugar is present.



Nucleosides in DNA

Base	Sugar	Nucleoside
• Adenine (A)	Deoxyribose	Adenosine
• Guanine (G)	Deoxyribose	Guanosine
• Cytosine (C)	Deoxyribose	Cytidine
• Thymine (T)	Deoxyribose	Thymidine

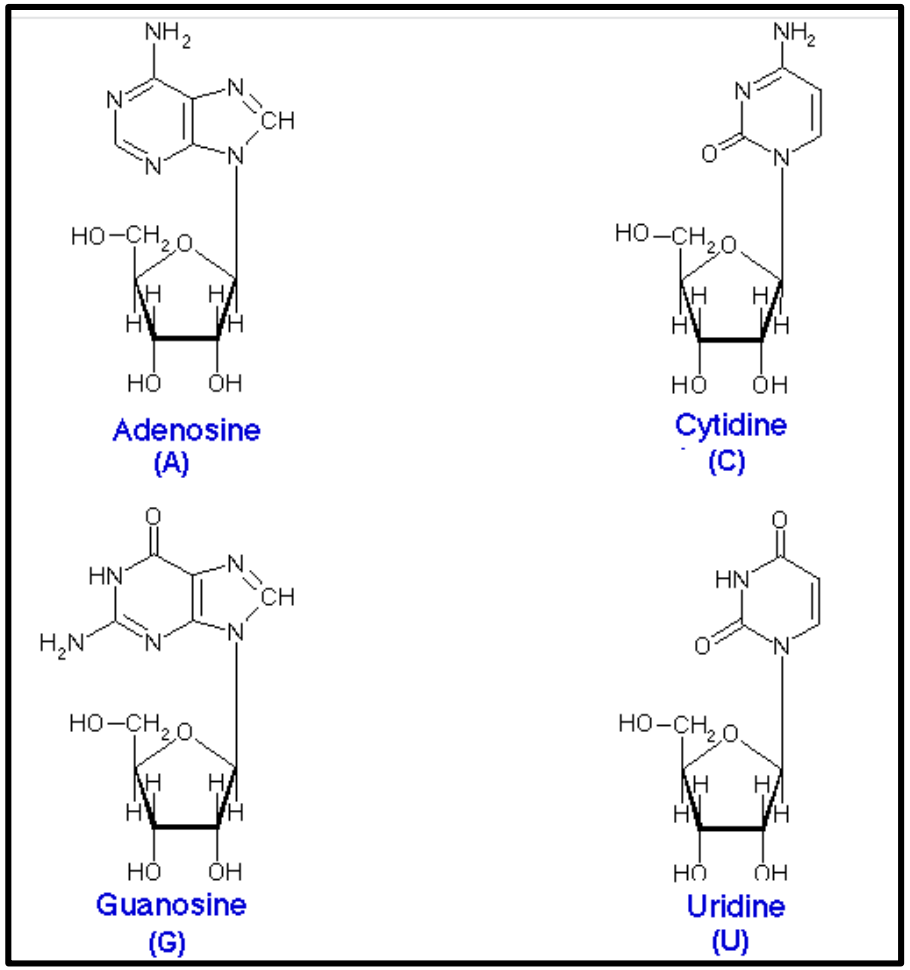
Adenine and Guanine are purine bases;
Cytosine and Thymine are pyrimidine bases.



Nucleosides in RNA

Base	Sugar	Nucleoside
• Adenine (A)	ribose	Adenosine
• Guanine (G)	ribose	Guanosine
• Cytosine (C)	ribose	Cytidine
• Uracil (U)	ribose	Uridine

Adenine and Guanine are purine bases;
Cytosine and Uracil are pyrimidine bases.



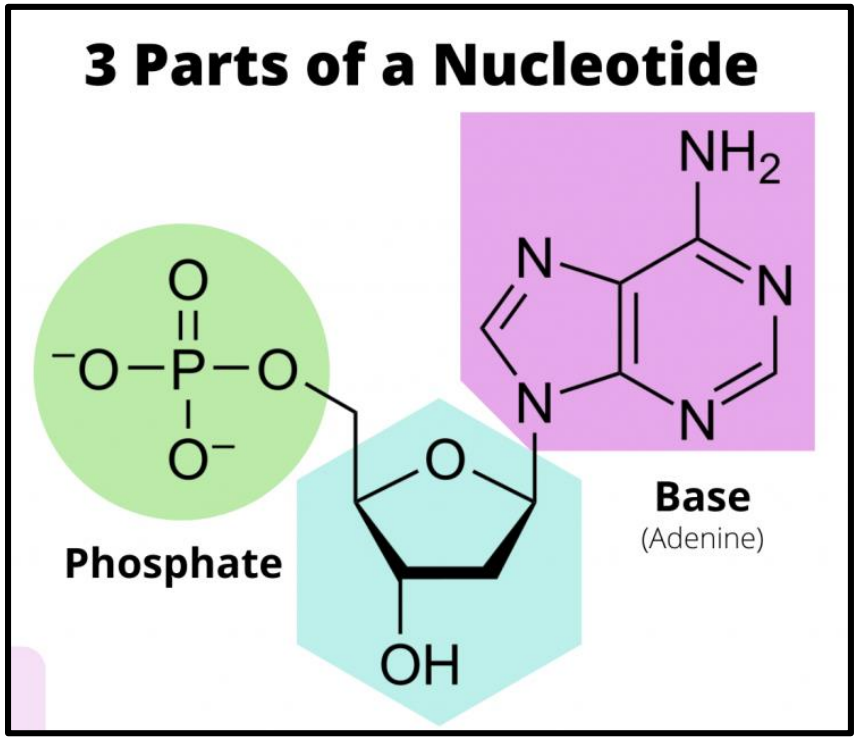
Nucleotides in DNA and RNA

DNA

- **dAMP Deoxyadenosine monophosphate**
- **dGMP Deoxyguanosine monophosphate**
- **dCMP Deoxycytidine monophosphate**
- **dTMP Deoxythymidine monophosphate**

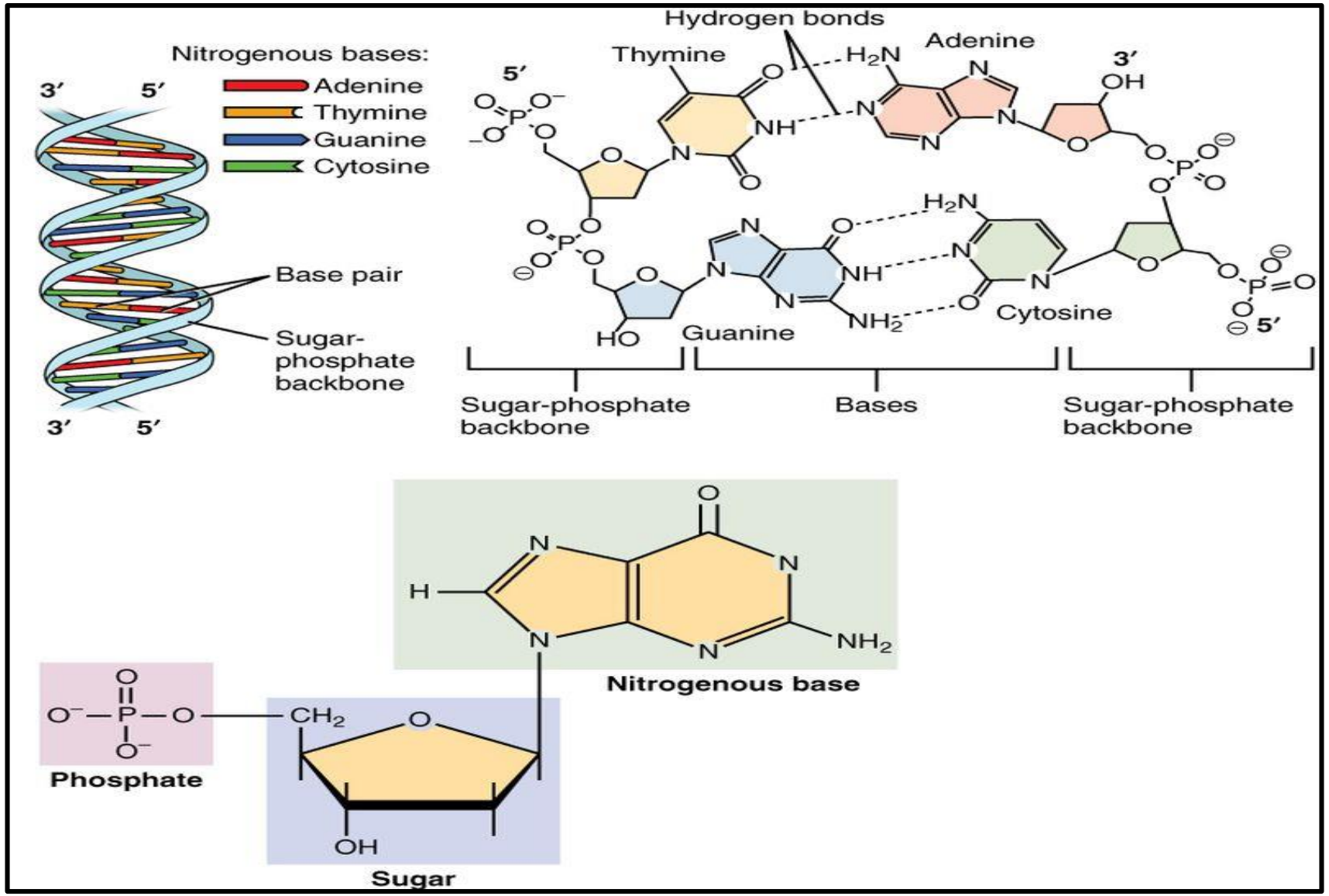
RNA

- **AMP adenosine monophosphate**
- **GMP guanosine monophosphate**
- **CMP cytidine monophosphate**
- **UMP uridine monophosphate**



dAMP

Nucleic acid structure



Structure of DNA

- DNA is a polymer of deoxyribonucleotides.
- Composed of monomeric units namely:

  Deoxyadenylate (dAMP)

  Deoxyguanylate (dGMP)

  Deoxycytidylate (dCMP)

  Deoxythymidylate (dTMP)

- The monomeric units held together by 3',5'-phosphodiester bonds as back bone.
- DNA had equal numbers of adenine & thymine residues and equal number of guanine & cytosine residues.
- Between A and T, there is two H-bonds but between G and C, there exists three H-bonds.

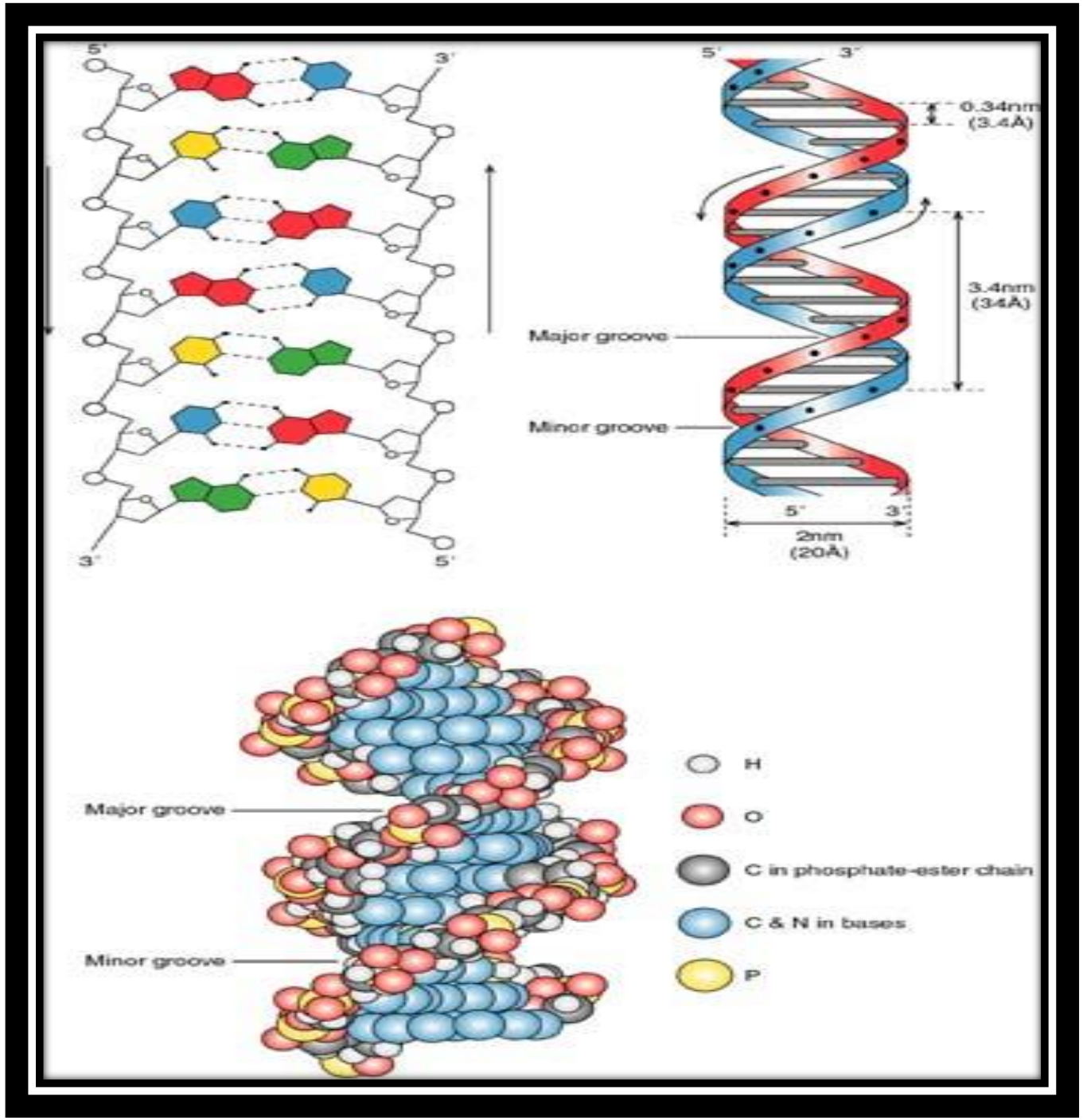
DNA double helix

- Double helical structure was proposed by Watson & Crick in 1953.
- The DNA is a right handed double helix.
- It consists of two polydeoxyribonucleotide chains twisted around each other on a common axis of symmetry.
- The chains are paired in an antiparallel manner, i.e., the 5'-end of one strand is paired with the 3'-end of the other strand.

- The two strands are antiparallel, i.e., one strand runs in the 5 ' to 3 ' direction while the other runs in 3' to 5 ' direction.
- The width (or diameter) of a double helix is 20 A^o (2nm)
- Each turn of helix is 34 A^o (3.4nm) with 10 pairs of nucleotides, each pair placed at a distance of about 3.4 A^o.
- The DNA helix, the hydrophilic deoxyribose-phosphate backbone of each chain is on the outside of the molecule, whereas the hydrophobic bases are stacked inside.

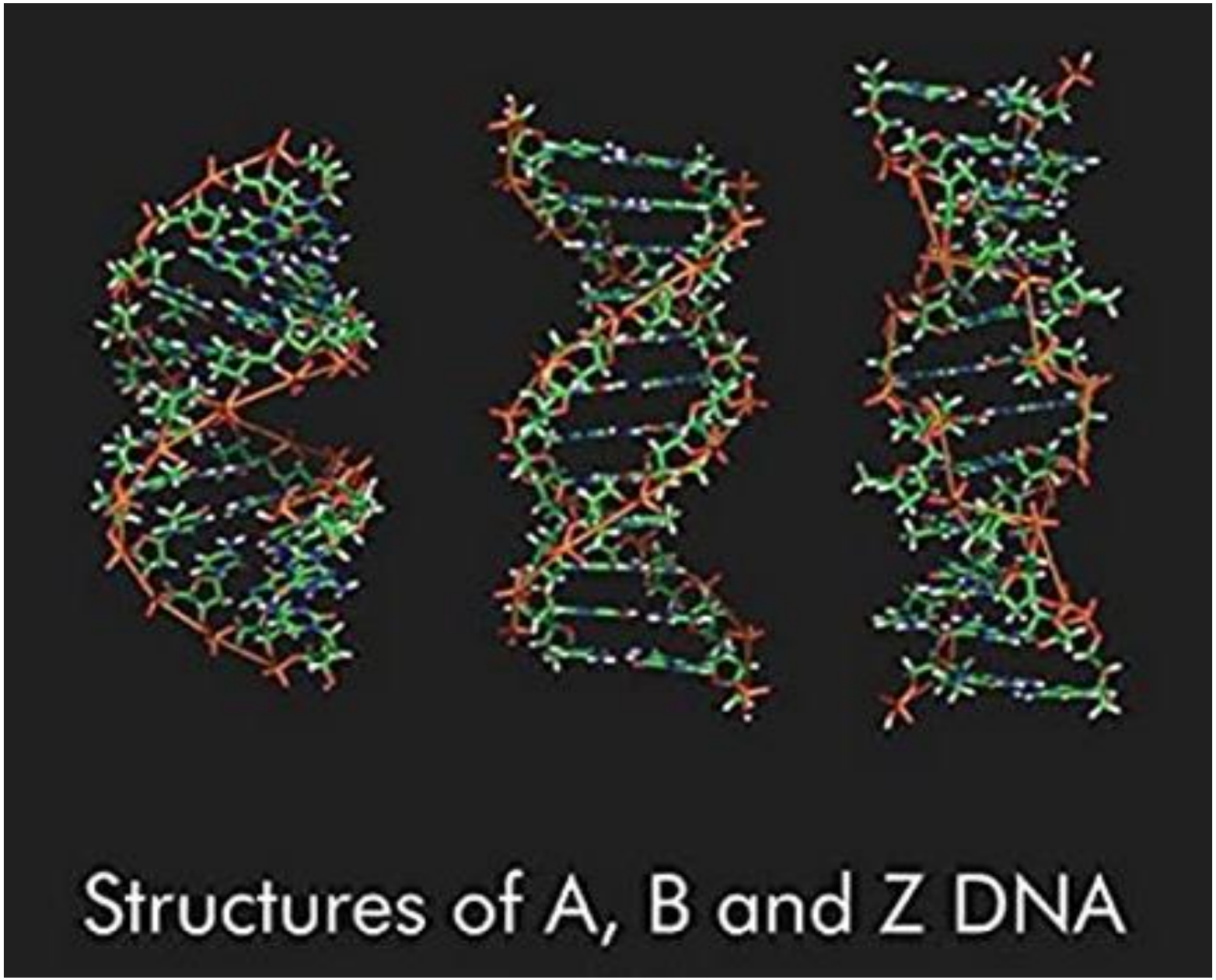
DNA double helix

- The polynucleotide chains are not identical but complementary to each other due to base pairing.
- The two strands are held together by hydrogen bonds.
- The hydrogen bonds are formed between a purine & pyrimidine (two H-bonds between A & T, three H-bonds between C & G).
- The spatial relationship between the two strands in the helix creates a major (wide) groove and a minor (narrow) groove.
- These grooves provide access for the binding of regulatory proteins to their specific recognition sequences along the DNA chain.
- The genetic information resides on one of the two strands known as template strand or sense strand.
- The opposite strand is antisense strand.


5'
3'
3'
5'
5'
3'
0.34nm
(3.4Å)
3.4nm
(34Å)
Major groove
Minor groove
5'
3'
2nm
(20Å)
Major groove
Minor groove
H
O
C in phosphate-ester chain
C & N in bases
P

Conformation of DNA double helix

- The double helical structure of DNA exists in 6 forms A,B,C,D,E and Z form.
- Among these B, A & Z forms are important.
- B-form is most predominant form under physiological conditions.
- A-from is also right-handed helix, contains 11 base pairs, there is a tilting of the base pairs by 20^{o} away from the central axis.
- Z-form is a left –handed helix and contains 12 base pairs per turn.
- The polynucleotide strands of DNA move in a somewhat zig-zag fashion, hence called as Z-DNA.


Structures of A, B and Z DNA

Complementary strands

- The two strands of DNA are not identical but two strands are complementary to each other.
- The complementarity results from base pairing.
- Adenine pairs with thymine through two hydrogen bonds.
- Guanine pairs with cytosine through three hydrogen bonds.
- G-C base pairs are more stable than A-T base pairs.
- It also accounts for how each DNA strand acts as a template for the synthesis of its complementary strand during DNA replication.

Structure of RNA

- RNA is a polyribonucleotide.
- It is single stranded polynucleotide.
- Phosphodiester bond links the nucleotides.
- Bond formation occurs between 3-OH group of one pentose sugar & 5-OH group of another pentose sugar of ribonucleotide.
- Nucleotides found in RNA are:

  AMP

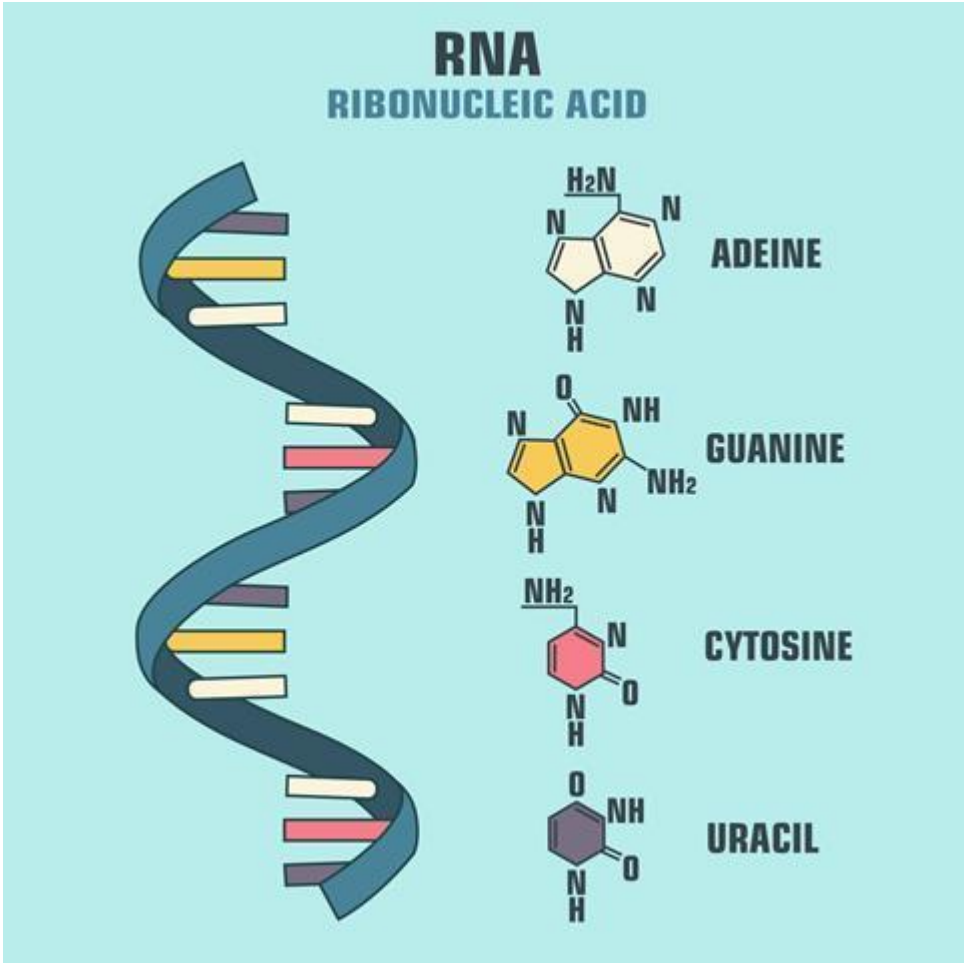
  GMP

  CMP

  UMP
- Thymine base is absent in RNA.

Structure of RNA

- It undergoes alkali hydrolysis.
- Alkali can hydrolyse RNA to 2’,3’-cyclic diesters.
- This is due to presence of -OH group at 2’position.



Types of RNA

- Three major types:
  - Messenger RNA: 5-10%
  - Transfer RNA: 10-20%
  - Ribosomal RNA: 50-80%
- RNAs are synthesized from DNA.
- RNA is involved in protein synthesis.

- Messenger RNA:
  It is also known as ***mRNA***
  It carries genetic information from DNA for protein synthesis.

- Transfer RNA:
  Transfer RNA, also known as ***tRNA*** (soluble RNA) and contains 71-80 nucleotides.
  It is required for the transfer of specific amino acids to the site of protein synthesis.
  It contains many unusual bases & nucleosides.

- Ribosomal RNA:

  Ribosomal RNA, also known as *r*RNA, is found in ribosomes.

  It is composed of two major nucleoprotein complexes: 60s subunit & 40s subunit.

  Its main function is protein biosynthesis.

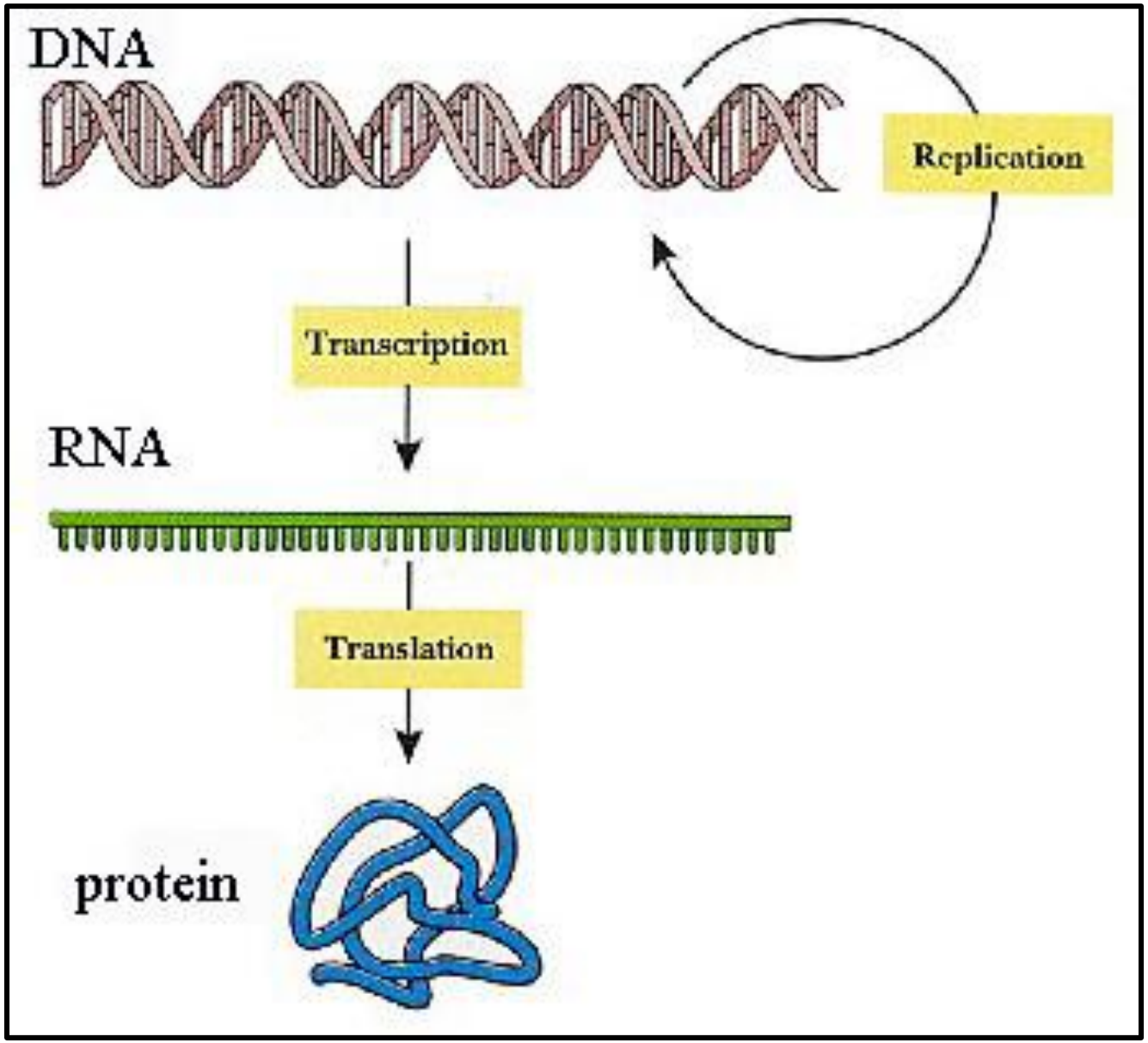
Nucleic acid and protein synthesis

- The following three processes are involved In duplication, transfer and use of genetic information.

1) Replication: The process by which a replica or identical copy of DNA is made when a cell divides.

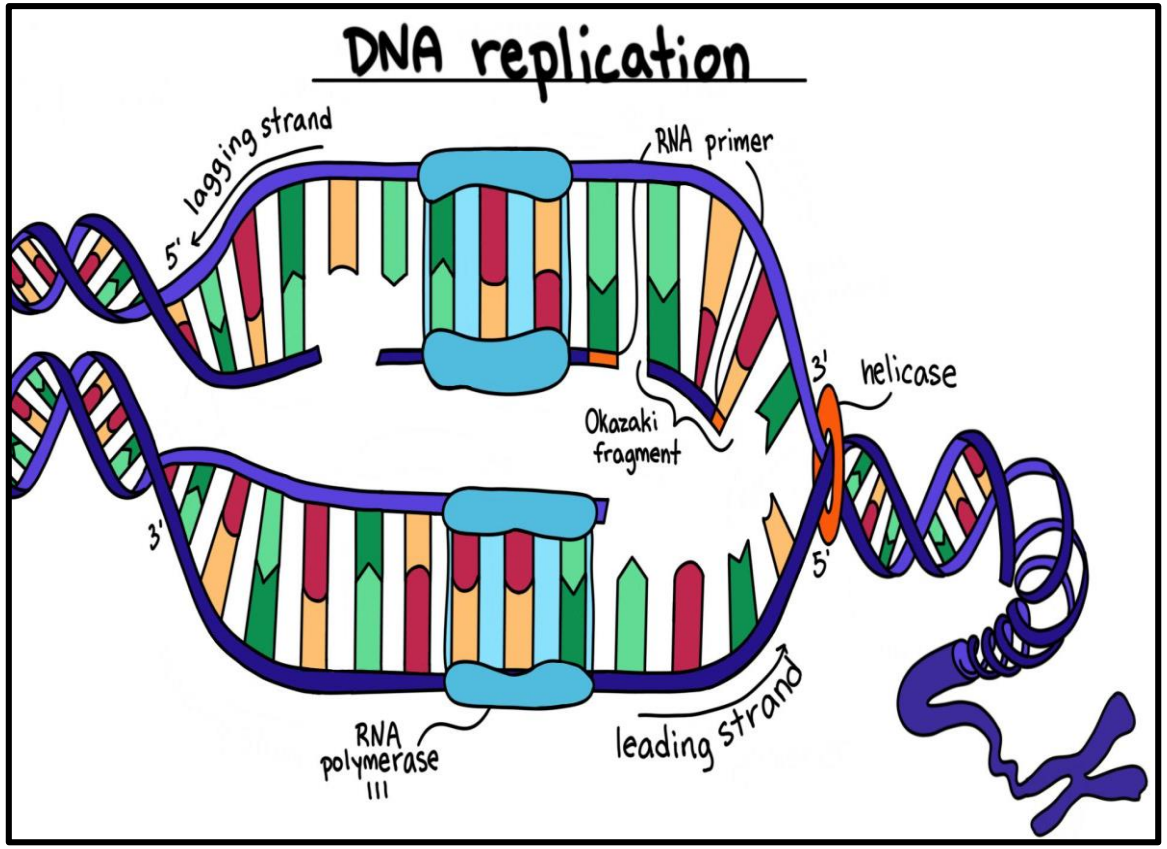
2) Transcription: The process by which the genetic messages contained in DNA are read and copied.

***3) Translation*:** The process by which the genetic messages carried by RNA are decoded and used to build proteins.


DNA
Replication
Transcription
RNA
Translation
protein

Replication

- DNA in the chromosomes replicates itself in every cell division.
- It maintains correct genetic information.
- The two strands of DNA unwind.
- Each strand acts like a template.
- New bases pair with their complementary bases.
- Two double helices form that are copies of original DNA.



Transcription

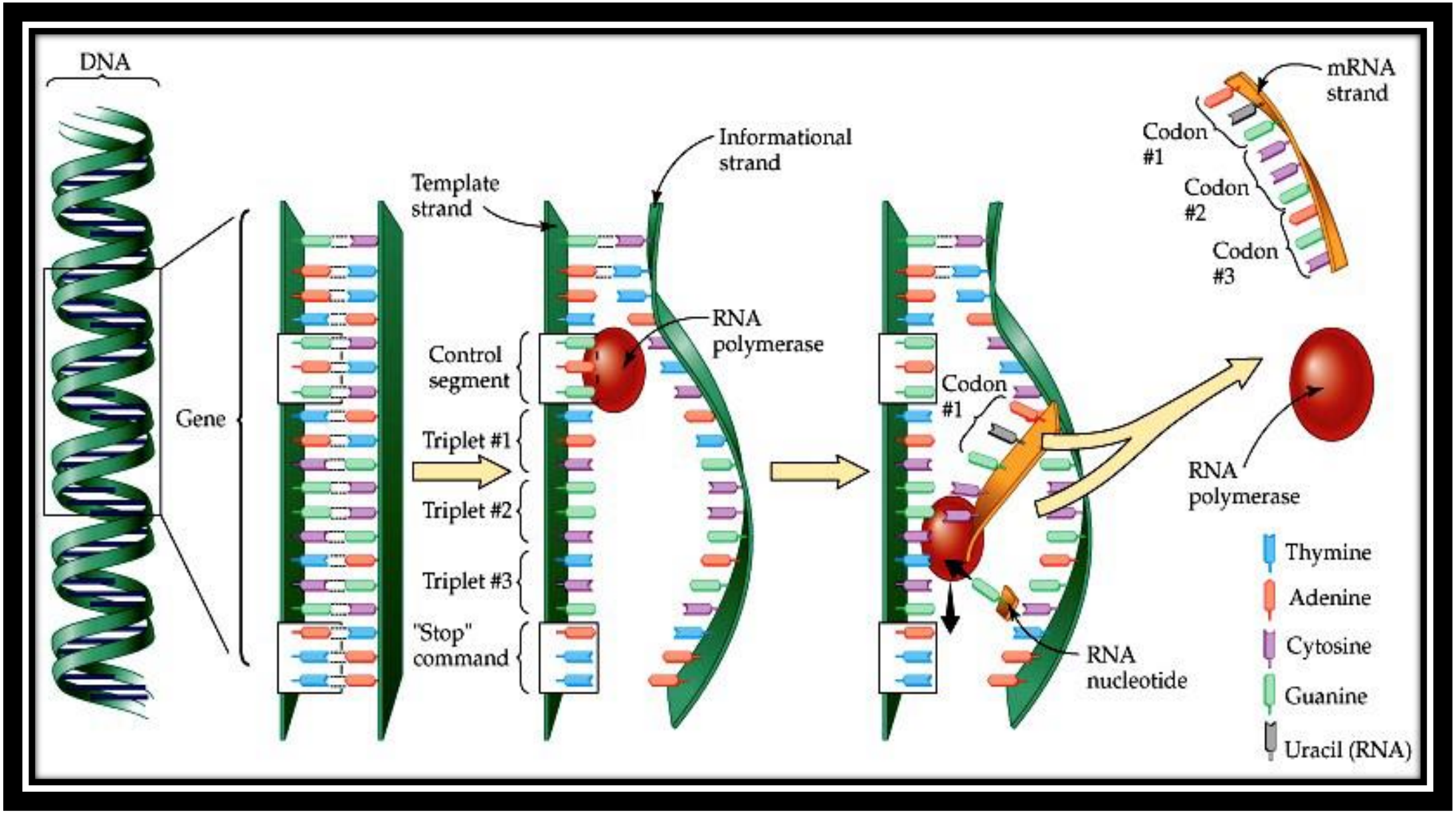
- The first step in using the information stored in DNA to produce proteins is transcription - using DNA as a template to make RNA.
- It is controlled by interactions of promoter and enhancers.
- Several different types of RNAs are produced including mRNA, tRNA and rRNA.
- Transcription involves 4 steps:

  INITIATION

  ELONGATION

  TERMINATION

  PROCESSING


DNA
Gene
Template strand
Informational strand
RNA polymerase
Control segment
Triplet #1
Triplet #2
Triplet #3
"Stop" command
mRNA strand
Codon #1
Codon #2
Codon #3
Codon #1
RNA polymerase
RNA nucleotide
Thymine
Adenine
Cytosine
Guanine
Uracil (RNA)

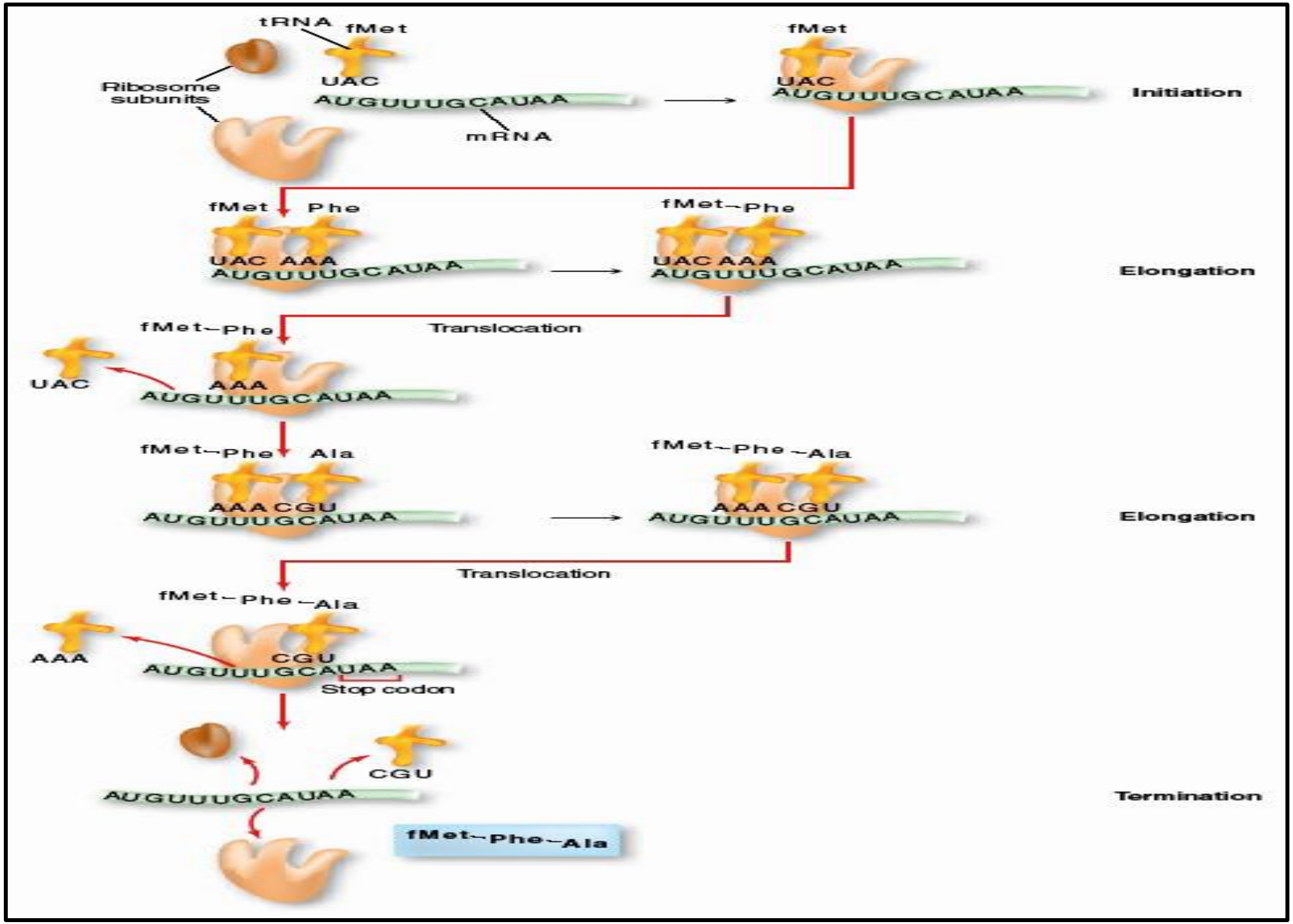
Translation

- The synthesis of proteins occur at ribosomes, which are outside the nucleus and within the cytoplasm of cells.
- The mRNA connects with the ribosome; and the amino acids attached to *transfer RNA* are delivered one by one.
- **Protein synthesis or translation, takes place in *three* steps:**

  1. Initiation- a ribosome, mRNA and tRNA come together to form a complex.

  2. Elongation- amino acids are joined to the growing polypeptide chain.

  3. Termination- the protein gets synthesized and the ribosome-mRNA-tRNA complex dissociates.


tRNA
fMet
Ribosome subunits
UAC
AUGUUUGCAUAA
mRNA
fMet
UAC
AUGUUUGCAUAA
Initiation
fMet
Phe
UAC AAA
AUGUUUGCAUAA
fMet–Phe
UAC AAA
AUGUUUGCAUAA
Elongation
Translocation
fMet–Phe
UAC
AAA
AUGUUUGCAUAA
fMet–Phe
Ala
AAA CGU
AUGUUUGCAUAA
fMet–Phe–Ala
AAA CGU
AUGUUUGCAUAA
Elongation
Translocation
fMet–Phe–Ala
AAA
CGU
AUGUUUGCAUAA
Stop codon
CGU
AUGUUUGCAUAA
Termination
fMet–Phe–Ala

Genetic code

- The ribonucleotide sequence in a mRNA chain is like a coded sentence that specifies the order in which amino acid residues should be joined to form a protein.
- Each word or **codon** in the mRNA sentence is a series of ***three*** ribonucleotides that code for specific amino acid.
- For example, the series uracil-uracil-guanine (UUG) on an mRNA chain is a codon directing incorporation of the amino acid leucine into a growing protein chain.

Reference

- Principle of biochemistry by Lehninger
- www. Slideshare.net
- www. google.com