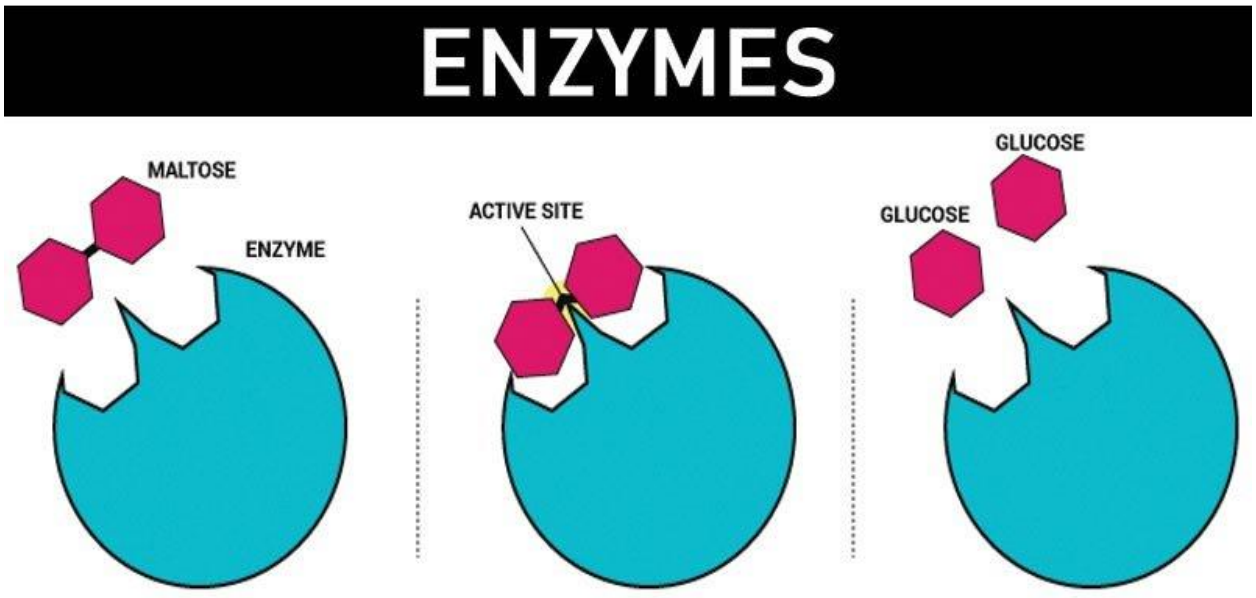
Enzymes- Properties, Classification and Significance

- Enzymes are the biological macromolecules which speed up the rate of biochemical reactions without undergoing any change. They are also called as biological catalysts.
- An enzyme is a highly selective catalyst that greatly accelerates both the rate and specificity of metabolic reactions.



Properties of Enzymes

- Nearly all enzymes are proteins, although a few catalytically active RNA molecules have been identified.
- Enzyme catalyzed reactions usually take place under relatively mild conditions (temperatures well below 100◦C, atmospheric pressure and neutral pH) as compared with the corresponding chemical reactions.
- Enzymes are catalysts that increase the rate of a chemical reaction without being changed themselves in the process.
- Enzymes are highly specific with respect to the substrates on which they act and the products that they form.
- Enzyme activity can be regulated, varying in response to the concentration of substrates or other molecules.

- They function under strict conditions of temperature and pH in the body.

Coenzymes and prosthetic groups

- Many enzymes require the presence of small, non-protein units or cofactors to carry out their particular reaction.
- Cofactors may be either one or more inorganic ions, such as Zn^{2+} or Fe^{2+} or a complex organic molecule called a coenzyme.
- A metal or coenzyme that is covalently attached to the enzyme is called a prostheticgroup (heme in hemoglobin).
- Some coenzymes, such as NAD^{+}, are bound and released by the enzyme during its catalytic cycle and in effect function as co-substrates. Many coenzymes are derived from vitamin precursors.

Holo enzymes and Apo enzymes

A complete catalytically-active enzyme together with its coenzyme or metal ion is called a holoenzyme.
The protein part of the enzyme on its own without its cofactor is termed an apoenzyme.

Isoenzymes

- Isoenzymes are different forms of an enzyme which catalyze the same reaction, but which exhibit different physical or kinetic properties, such as isoelectric point, pH optimum, substrate affinity or effect of inhibitors.
- Different isoenzyme forms of a given enzyme are usually derived from different genes and often occur in different tissues of the body.
- An example of an enzyme which has different isoenzyme forms is lactate dehydrogenase (LDH) which catalyzes the reversible conversion of pyruvate into lactate in the presence of the coenzyme NADH.
- LDH is a tetramer of two different types of subunits, called H and M, which have small differences in amino acid sequence. The two subunits can combine randomly with each other, forming five isoenzymes that have the compositions H_4, H_3M, H_2M_2, HM_3 and M_4. The five isoenzymes can be resolved electrophoretically.

Active site of Enzymes

- The active site of an enzyme is the region that binds the substrate and converts it into product.
- It is usually a relatively small part of the whole enzyme molecule and is a three-dimensional entity formed by amino acid residues that can lie far apart in the linear polypeptide chain.
- The active site is often a cleft or crevice on the surface of the enzyme that forms a predominantly nonpolar environment which enhances the binding of the substrate.
- The substrate(s) is bound in the active site by multiple weak forces (electrostatic interactions, hydrogen bonds, van der Waals bonds, hydrophobic interactions; and in some cases by reversible covalent bonds.

Substrate Specificity of Enzymes

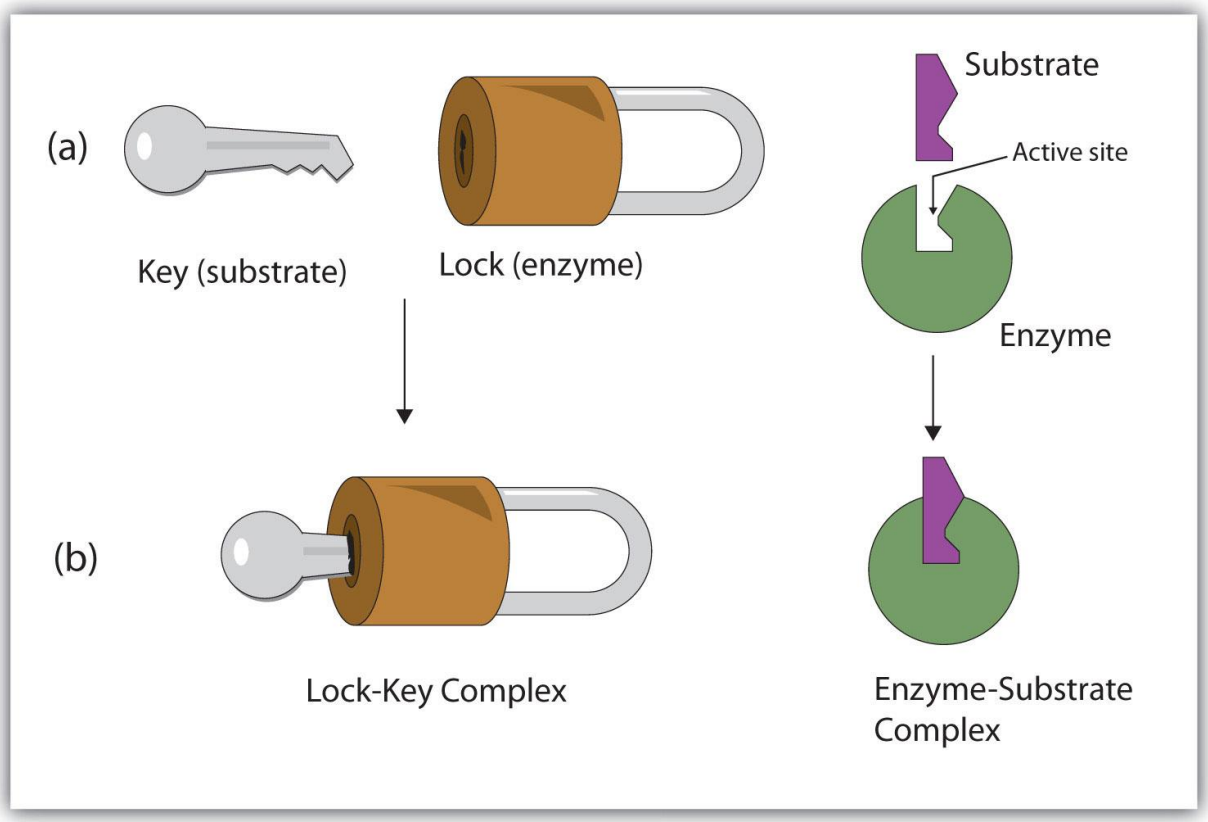
- The properties and spatial arrangement of the amino acid residues forming the active site of an enzyme will determine which molecules can bind and be substrates for that enzyme.
- Substrate specificity is often determined by changes in relatively few amino acids in the active site.
- This is clearly seen in the three digestive enzymes trypsin, chymotrypsin and elastase**.**

Mechanism of Action of Enzymes

- The substrate(s) is bound in the active site by multiple weak forces which result into the enzyme-substrate complex.
- Once bound active residues within the active site of the enzyme act on the substrate molecule to transform it first into the transition state complex and then into product, which is released.
- The enzyme is now free to bind another molecule of substrate and begin its catalytic cycle again.

The Substrate-Enzyme Binding

Originally two models were proposed to explain how an enzyme binds its substrate.



The Lock and Key Model

- In the lock-and-key model proposed was proposed by Emil Fischer in 1894.
- According to the model, the shape of the substrate and the active site of the enzyme are thought to fit together like a key into its lock.
- The two shapes are considered as rigid and fixed, and perfectly complement each other when brought together in the right alignment.

The Induced Fit Model

- In the induced-fit model was proposed by Daniel E. Koshland, Jr., in 1958.
- It states that the binding of substrate induces a conformational change in the active site of the enzyme.
- In addition, the enzyme may distort the substrate, forcing it into a conformation similar to that of the transition state.

- For example, the binding of glucose to hexokinase induces a conformational change in the structure of the enzyme such that the active site assumes a shape that is complementary to the substrate (glucose) only after it has bound to the enzyme.

The reality is that different enzymes show features of both models, with some complementarity and some conformational change.

Nomenclature of Enzymes

- Many enzymes are named by adding the suffix '-ase' to the name of their substrate.

Example. Urease is the enzyme that catalyzes the hydrolysis of urea, and fructose-1,6-bisphosphatase hydrolyzes fructose-1,6-bisphosphate.

- However, other enzymes, such as trypsin and chymotrypsin, have names that do not denote their substrate.
- Some enzymes have several alternative names.
- To rationalize enzyme names, a system of enzyme nomenclature has been internationally agreed.
- This system places all enzymes into one of six major classes based on the type of reaction catalyzed. Each enzyme is then uniquely identified with a four-digit classification number.

Example: Trypsin has the Enzyme Commission (EC) number 3.4.21.4, where

1. the first number (3) denotes that it is a hydrolase
2. the second number (4) that it is a protease that hydrolyzes peptide bonds
3. the third number (21) that it is a serine protease with a critical serine
4. residue at the active site, and
5. the fourth number (4) indicates that it was the fourth enzyme to be assigned to this class.

- For comparison, chymotrypsin has the EC number 3.4.21.1, and elastase 3.4.21.36.

Classification of Enzymes

1. **Oxidoreductases**

- Catalyze oxidation-reduction reactions where electrons are transferred.
- These electrons are usually in the form of hydride ions or hydrogen atoms.

- The most common name used is a dehydrogenase and sometimes reductase is used.
- An oxidase is referred to when the oxygen atom is the acceptor.

2. **Transferases**

- Catalyze group transfer reactions.
- The transfer occurs from one molecule that will be the donor to another molecule that will be the acceptor.
- Most of the time, the donor is a cofactor that is charged with the group about to be transferred.
- Example: Hexokinase used in glycolysis.

3. **Hydrolases**

- Catalyze reactions that involve hydrolysis.
- It usually involves the transfer of functional groups to water.
- When the hydrolase acts on amide, glycosyl, peptide, ester, or other bonds, they not only catalyze the hydrolytic removal of a group from the substrate but also a transfer of the group to an acceptor compound
- For example: Chymotrypsin.

4. **Lyases**

- Catalyze reactions where functional groups are added to break double bonds in molecules or the reverse where double bonds are formed by the removal of functional groups.
- For example: Fructose bisphosphate aldolase used in converting fructose 1,6-bisphospate to G3P and DHAP by cutting C-C bond.

5. **Isomerases**

- Catalyze reactions that transfer functional groups within a molecule so that isomeric forms are produced.
- These enzymes allow for structural or geometric changes within a compound.
- For example: phosphoglucose isomerase for converting glucose 6-phosphate to fructose 6-phosphate. Moving chemical group inside same substrate.

6. **Ligases**

- They are involved in catalysis where two substrates are ligated and the formation of carbon-carbon, carbon-sulfide, carbon-nitrogen, and carbon-oxygen bonds due to condensation reactions.
- These reactions are coupled to the cleavage of ATP.

Significance of Enzymes

1. In the absence of an enzyme, biochemical reactions hardly proceed at all, whereas in its presence the rate can be increased up to 10^7-fold. Thus, they are crucial for normal metabolism of living systems.
2. Besides in the body, extracted and purified enzymes have many applications.

- Medical applications of enzymes include:

1. To treat enzyme related disorders.
2. To assist in metabolism
3. To assist in drug delivery.
4. To diagnose & detect diseases.
5. In manufacture of medicines.

- Industrial applications of enzymes include:

1. Amylase, lactases, cellulases are enzymes used to break complex sugars into simple sugars.
2. Pectinase like enzymes which act on hard pectin is used in fruit juice manufacture.
3. Lipase enzymes act on lipids to break them in fatty acids and glycerol. Lipases are used to remove stains of grease, oils, butter.
4. Enzymes are used in detergents and washing soaps.
5. Protease enzymes are used to remove stains of protein nature like blood, sweat etc.

Action and Nature of Enzymes

Once substrate (S) binds to this active site, they form a complex (intermediate-ES) which then produces the product (P) and the enzyme (E). The substrate which gets attached to the enzyme has a specific structure and that can only fit in a particular enzyme. Hence, by providing a surface for the substrate, an enzyme slows down the activation energy of the reaction. The intermediate state where the substrate binds to the enzyme is called the transition state. By breaking and making the bonds, the substrate binds to the enzyme (remains unchanged), which converts into the product and later splits into product and enzyme. The free enzymes then bind to other substrates and the catalytic cycle continues until the reaction completes.

The enzyme action basically happens in two steps:

Step1: Combining of enzyme and the reactant/substrate.

E+S → [ES]

Step 2: Disintegration of the complex molecule to give the product.

[ES]→E+P

Thus, the whole catalyst action of enzymes is summarized as:

E + S → [ES] → [EP] → E + P

Biological Catalysts

Catalysts are the substances which play a significant role in the chemical reaction. Catalysis is the phenomenon by which the rate of a chemical reaction is altered/ enhanced without changing themselves. During a chemical reaction, a catalyst remains unchanged, both in terms of quantity and chemical properties. An enzyme is one such catalyst which is commonly known as the biological catalyst. Enzymes present in the living organisms enhance the rate of reactions which take place within the body.

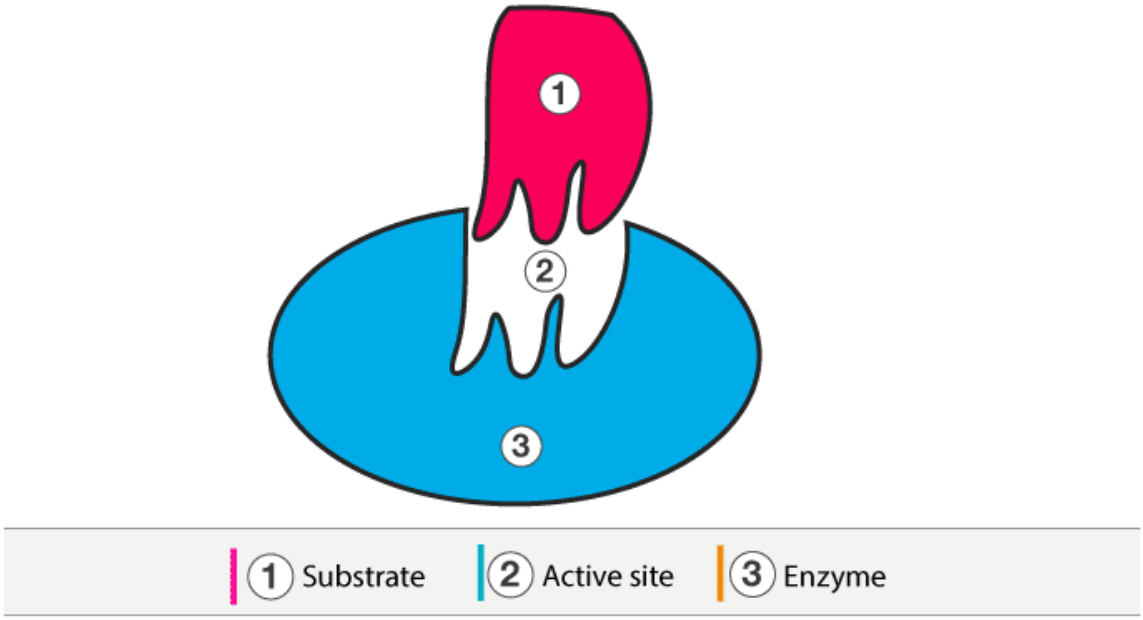
Biological catalysts, enzymes, are extremely specific that catalyze a single chemical reaction or some closely associated reactions. An enzyme's exact structure and its active site decide an enzyme's specificity. Substrate molecules attach themselves at the active site of an enzyme. Initially, substrates associate themselves by noncovalent interactions to the enzymes which include ionic, hydrogen bonds and hydrophobic interactions. Enzymes reduce the reactions and activation energy to progress towards equilibrium quicker than the reactions that are not catalyzed. Both eukaryotic and prokaryotic cells usually make use of allosteric regulation to respond to fluctuations in the state inside the cells.

The nature of enzyme action and factors affecting the enzyme activity are discussed below.

Factors Affecting Enzyme Activity

The conditions of the reaction have a great impact on the activity of the enzymes. Enzymes are particular about the optimum conditions provided for the reactions such as temperature, pH, alteration in substrate concentration, etc.

ENZYMES: ACTION AND FACTORS AFFECTING



Typically, enzyme activities are accelerated with increasing temperatures. As enzymes are functional in cells, the feasible conditions for nearly all enzymes are temperatures that are moderate. At higher temperatures, given a specific point, there is a drastic decrease in the activity with the denaturation of enzymes. In diluted solutions, purified enzymes denature quickly compared to enzymes in crude extracts. Denaturation of enzymes can also take place when enzymes are incubated for long durations. More appropriate is to utilize a shorter time duration when it comes to incubation time to gauge the starting velocities of such enzyme reactions.

The International Union of Biochemistry suggests the standard assay temperature to be 30 °C. Almost all enzymes are extremely sensitive to pH change. Just some enzymes feasibly operate with pH above 9 and below 5. Most enzymes have their pH – optimum near to neutrality. Any alteration of pH causes the ionic state of amino acid residues to change in the whole protein and in the active site. The modifications in the ionic state can modify catalysis and substrate binding. The preference of substrate concentration is critical as at lower concentrations, the rate is driven by concentration, however, at high concentrations, the rate does not depend on any increase in the concentration of the substrate.

Active site

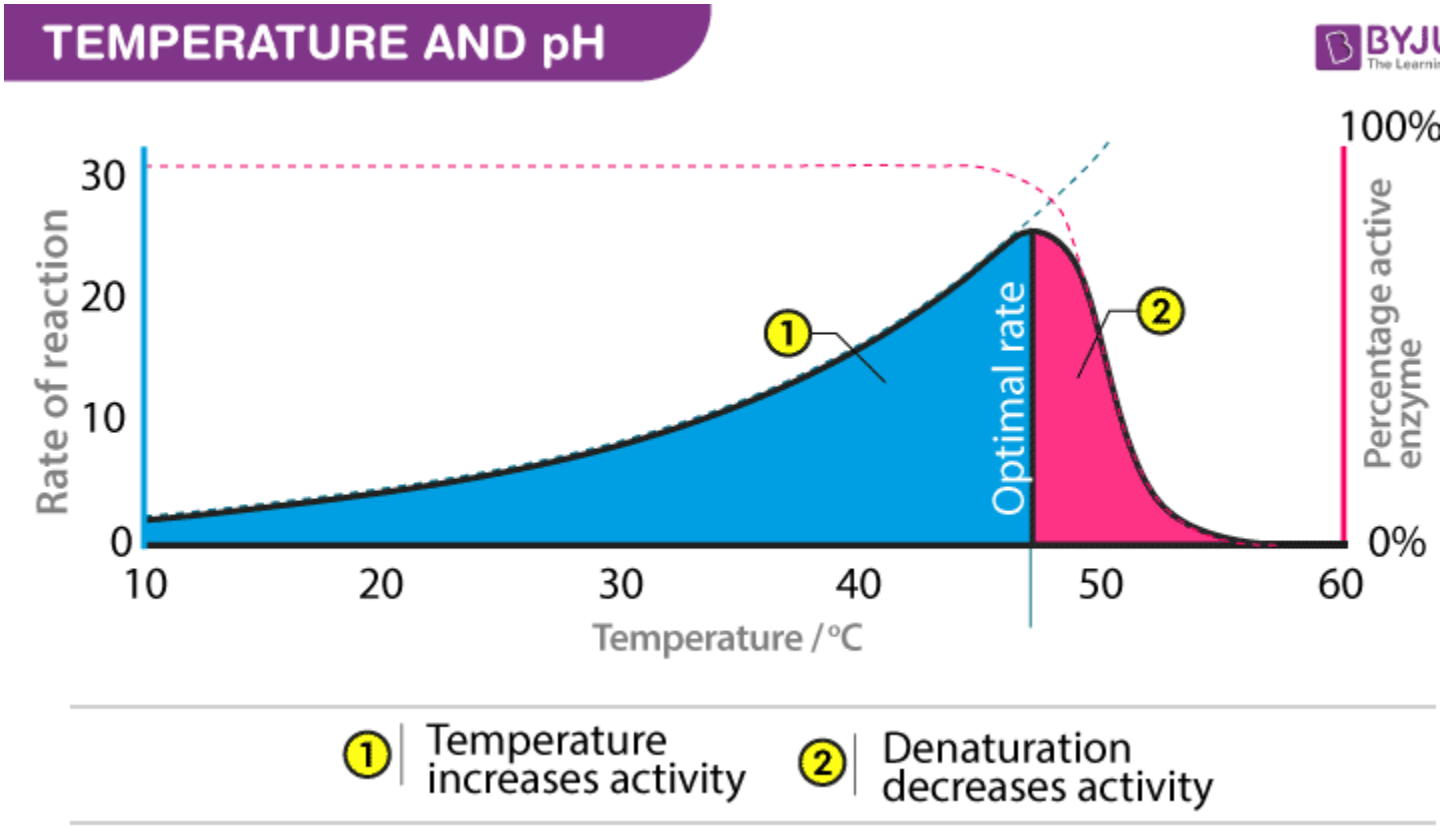
Enzymatic catalysis depends upon the activity of amino acid side chains assembled in the active centre. Enzymes bind the substrate into a region of the active site in an intermediate conformation.

Often, the active site is a cleft or a pocket produced by the amino acids which take part in catalysis and substrate binding. Amino acids forming an enzyme's active site is not contiguous to the other along the sequence of primary amino acid. The active site amino acids are assembled to the cluster in the right conformation by the 3-dimensional folding of the primary amino acid sequence. The most frequent active site amino acid residues out of the 20 amino acids forming the protein are polar amino acids, aspartate, cysteine, glutamate, histidine, Serine, and lysine. Typically, only 2-3

essential amino acid residues are involved directly in the bond causing the formation of the product. Glutamate, Aspartate, and histidine are the amino acid residues which also serve as a proton acceptor or donor.

Temperature and pH

Enzymes require an optimum temperature and pH for their action. The temperature or pH at which a compound shows its maximum activity is called optimum temperature or optimum pH, respectively. As mentioned earlier, enzymes are protein compounds. A temperature or pH more than optimum may alter the molecular structure of the enzymes. Generally, an optimum pH for enzymes is considered to be ranging between 5 and 7.



- Optimum T°
- The greatest number of molecular collisions
- human enzymes = 35°- 40°C
- body temp = 37°C
- Heat: increase beyond optimum T°
- The increased energy level of molecule disrupts bonds in enzyme & between enzyme & substrate H, ionic = weak bonds
- Denaturation = lose 3D shape (3° structure)
- Cold: decrease T°
- Molecules move slower decrease collisions between enzyme & substrate

Concentration and Type of Substrate

Enzymes have a saturation point, i.e., once all the enzymes added are occupied by the substrate molecules, its activity will be ceased. When the reaction begins, the velocity of enzyme action keeps on increasing on further addition of substrate. However, at a saturation point where substrate molecules are more in number than the free enzyme, the velocity remains the same.

The type of substrate is another factor that affects the enzyme action. The chemicals that bind to the active site of the enzyme can inhibit the activity of the enzyme and such substrate is called an inhibitor. Competitive inhibitors are chemicals that compete with the specific substrate of the enzyme for the active site. They structurally resemble the specific substrate of the enzyme and bind to the enzyme and inhibit the enzymatic activity. This concept is used for treating bacterial infectious diseases.

Salt concentration

Changes in salinity: Adds or removes cations (+) & anions (–)

- Disrupts bonds, disrupts the 3D shape
- Disrupts attractions between charged amino acids
- Affect 2° & 3° structure
- Denatures protein
- Enzymes intolerant of extreme salinity
- The Dead Sea is called dead for a reason

- **Activation Energy:**
- Most of the chemical reactions do not start automatically because the reactant molecules have an energy barrier to become reactive.

- **The energy barrier may be on account of:**
- (i) Mutual repulsion due to presence of electrons over their surfaces,

- (ii) Solvation or holding of reactants in solution form by hydrogen bonds,

- (iii) Reaction sites of the reactive molecules being small, precise collisions do not occur.

- Therefore, an external supply of energy is needed for the start of the chemical reaction. It is called activation energy. Activation

energy increases the kinetic energy of the system and brings about forceful collisions between the reactants. The requirements of activation energy is quite high. For example, acidic hydrolysis of sucrose requires 32000 cal/ mole of energy.

- As already noted about 1000 chemical reactions are taking place in a cell at any time. Activation energy required for such a large number of reactions cannot be provided by living systems. Enzymes lower the activation energy required for a reaction (Fig. 9.32).

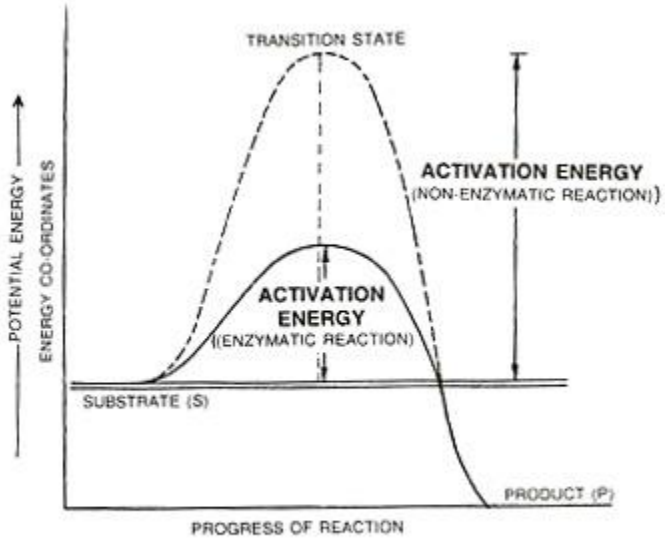


Fig. 9.32. Lowering of activation energy by enzyme in the energy relations of a chemical reaction.

- For example, in the presence of enzyme sucrase or invertase, hydrolysis of sucrose requires an activation energy of 9000 cal/mole (instead of 32,000 cal/ mole).

- **This is achieved by four ways:**
- (i) De-solvation or taking the reactants out of solution state,

- (ii) Establishing weak bonds between reactants and enzyme. It releases energy called bond energy,

- (iii) Bringing the reactant molecules closer in the region of active sites of enzymes,

- (iv) Development of strain in the bonds of the reactants by electrophilic and nucleophilic attack,
- (v) Formation of unstable intermediate structural states collectively called transition state. During the transition state the substrate bonds are broken and new bonds are established that transform the substrate molecules into products,
- (vi) In exothermic reactions, the energy content of the products is lower than that of substrate (Fig. 9.32).
- It is higher in case of endothermic reactions. However, whether the reaction is endothermic or exothermic, energy is required for pushing the substrate molecules into transition state. The difference in the energy level of substrate (S) and transition state is the activation energy required to start the reaction.

Modes of Enzyme Action:

There are two view points by which enzymes are supposed to bring about chemical reaction.

i. Lock and Key Hypothesis:

It was put forward by Emil Fischer in 1894. According to this hypothesis, both enzyme and substrate molecules have specific geometrical shapes. 'In the region of active sites the surface configuration of the enzyme is such as to allow the particular substrate molecules to be held over it. The active sites also contain special groups having —NH_2, —COOH, —SH for establishing contact with the substrate molecules.

The contact is such that the substrate molecules or reactants come together causing the chemical change. It is similar to the system or lock and key. Just as a lock can be opened by its specific key, a substrate molecule can be acted upon by a particular enzyme. This also explains the specificity of enzyme action.

After coming in contact with the active site of the enzyme, the substrate molecules or reactants form a complex called enzyme-substrate complex. In the complexed state the molecules of the substrate undergo chemical change.

The products remain attached to the enzyme for some time so that an enzyme-product complex is also formed. However, the products are soon released (Fig. 9.34) and the freed enzyme is able to bind more substrate molecules.

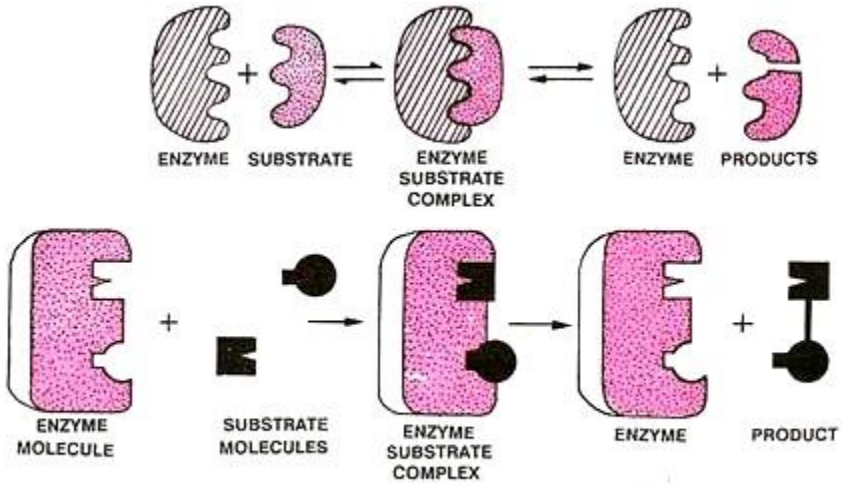


Fig. 9.33. Lock and key theory of enzyme action.
Upper Series – Breakdown Reaction Lower Series – Biosynthetic Reaction

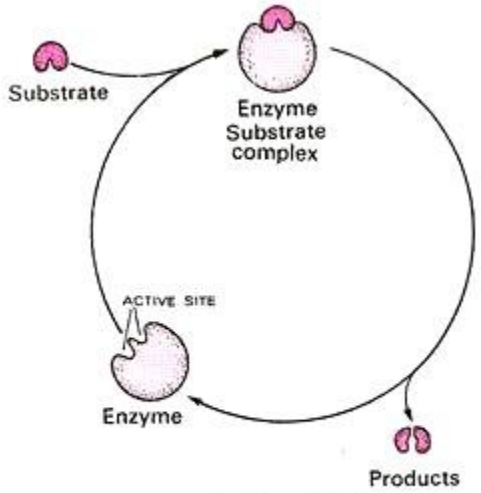


Fig. 9.34. The method by which the same enzyme molecule can be used again and again.

Enzyme + Substrate ⇋ Enzyme – Substrate Complex

Enzyme – Substrate Complex ⇋ Enzyme – Products Complex

Enzyme – Products Complex ⇋ Enzyme + Products

Thus we see that the chemical reactants do not cause any alteration in the composition or physiology of the enzyme. The same enzyme molecule can be used again and again (Fig. 9.35). Hence, enzymes are required in very small concentrations.

Evidences:
1. Blow and Steitz (1970) have found the formation of complex between the enzyme chymotrypsin and its substrate.

2. Keilen and Maun have observed that the absorption spectra of the same enzyme are different in the free state and in the presence of the substrate.

3. The theory explains how a small concentration of enzyme can act upon a large amount of the substrate.

4. Lock and key theory explains how the enzyme remains unaffected at the end of chemical reaction.

5. It is able to predict the increase in the rate of chemical reaction on the addition of more enzyme or substrate.

6. The theory explains how a substance having a structure similar to the substrate can work as competitive inhibitor.

ii. Induced-Fit Theory (Fig. 9.35):
It is modification of lock and key hypothesis which was proposed by Koshland in 1959. According to this theory the active site of the enzyme contains two groups, buttressing and catalytic. The buttressing group is meant for supporting the substrate. The catalytic group is able to weaken the bonds of reactants by electrophilic and nucleophilic forces.

The two groups are normally at a distance. As soon as the substrate comes in contact with the buttressing group, the active site of the

enzyme undergoes conformational changes so as to bring the catalytic group opposite the substrate bonds to be broken.

Catalytic group helps in bringing about chemical reaction. The substrate is converted into product. The product is unable to hold on the buttressing site due to change in its structure and bonds. Buttressing group reverts to its original position. The product is released.

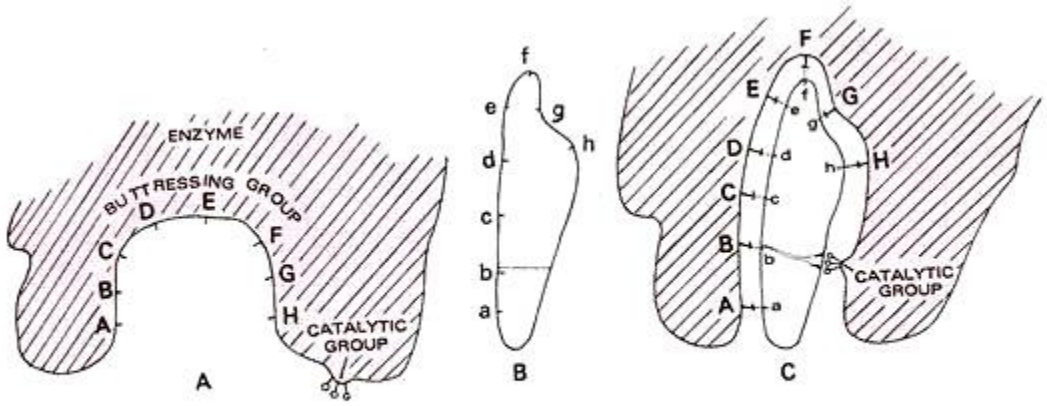


Fig. 9.35. Induced-fit theory of enzyme action. A, active site of enzyme. B, substrate molecule. C, enzyme-substrate complex with conformational changes so as to bring the catalytic group against the substrate bonds to be broken.

Inhibition of Enzyme Action:

Reduction or stoppage of enzyme activity due to presence of adverse conditions or chemicals is called enzyme inhibition. It is of several types. Inhibition can be classified into two (a) Reversible and irreversible (b) Competitive and non-competitive.

Reversible inhibition is that inhibition which can be overcome by withdrawal of the inhibitor because the effect of the latter is of temporary nature due to blocking of active site or binding to linkages required for maintenance of active site. Dilution and dialysis reduces or eliminates the effect of reversible inhibition. Irreversible inhibition is of permanent nature as the enzyme conformation is harmed.

Denaturation of enzyme is an example of irreversible inhibition. Heavy metals (e.g., Ag^{+}, Hg^{2+}, As^{+}) and iodoacetic acid cause irrevers-

ible inhibition by combining with —SH groups and destroying protein structure. Dilution and dialysis have little effect once irreversible inhibition has set in.
Competitive inhibition is caused by swamping of the active sites by a chemical which is similar in structure to the substrate but does not undergo chemical change. Competitive inhibition is usually reversible.
Non-competitive inhibition is caused by alteration of conformation of the enzyme by a chemical that binds to a site other than the active site. It may be reversible or irreversible.

Four common types of enzymes inhibition are as follows:
i. Protein Denaturation:
Enzyme activity is dependent upon the maintenance of tertiary structure of the protein moiety. The latter is destroyed by several factors like heat, high energy radiations and salts of heavy metals.

ii. Competitive inhibition:
It is the inhibition of enzyme activity by the presence of a chemical that competes with the substrate for binding to the active site of the enzyme. The inhibitor chemical is also called substrate analogue or competitive inhibitor.

It resembles the substrate in structure and gets bound up to the active site of the enzyme without getting transformed by the latter (Fig. 9.37). As a result, the enzyme cannot participate in catalytic change of the substrate. This is similar to the jamming of a lock by a key similar to original one.

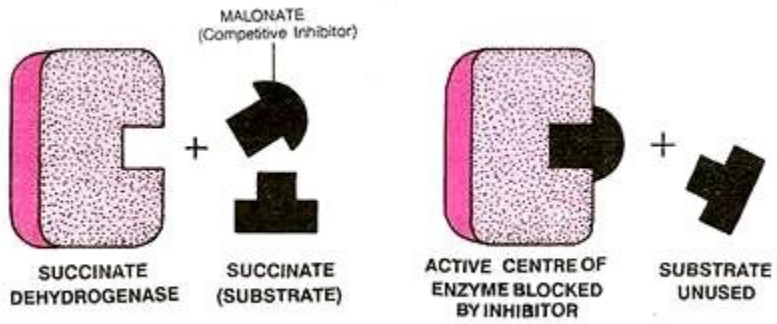
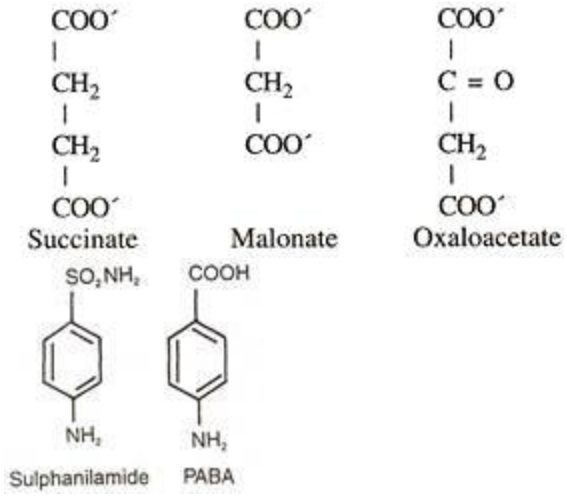


Fig. 9.37. Competitive inhibition of enzyme action.

Equilibrium constant for inhibitor binding is called K_i. A high K_i reduces enzyme activity while a low K_i allows enzyme activity to continue though at a reduced rate. Classical example of competitive inhibition is reduction of activity of succinate dehydrogenase by malonate, oxaloacetate and other anions which resemble succinate in their structure.



Competitive inhibition is usually reversible since the addition of more substrate tends to reduce the effect of the inhibitor.

The inhibition is important in that:
(i) It gives evidence for lock and key hypothesis of enzyme action,

(ii) Substrate analogues are not metabolized by enzymes,

(iii) Control of bacterial pathogens has been effected through competitive inhibition.

Sulpha drugs (e.g., sulphanilamide) inhibit the synthesis of folic acid in bacteria by competing with p-amino benzoic acid (PABA) for the active site of enzyme. Preformed folic acid is obtained by animal cells. Therefore, sulpha drugs do not harm them.

iii. Non-competitive Inhibition:

It is an irreversible inhibition of enzyme activity by the presence of a substance that has no structural similarity with the substrate. It is of two types, reversible and irreversible.

The irreversible non-competitive inhibitor destroys or combines irreversibility with a functional group of enzyme that is essential for its catalytic function. Cyanide inhibits the activity of cytochrome oxidase by combining with its metallic ions.

It has no structural similarity with the substrate of the enzyme, namely cytochrome c. Cytochrome oxidase is a respiratory enzyme. In its inhibition, the animal is unable to perform the respiration properly and gets killed. Di-isopropyl fluorophosphates (DFP, a nerve gas) prevents impulse transfer by combining irreversibly with amino acid serine of acetylcholine esterase.

It also poisons a number of other enzymes like trypsin, chymotrypsin, phosphoglucomutase, elastase, etc. lodoacetamide inhibits enzymes having sulphahydryl (—SH) or imidazole group.

iv. Allosteric Modulation or Feed Back Inhibition:
It is a type of reversible inhibition found in allosteric enzymes. The inhibitor is non-competitive and is usually a low molecular intermediate or product of a metabolic pathway having a chain of reactions involving a number of enzymes. It is, therefore, also called end product or feedback inhibition.

The inhibitor is also called modulator. Modulator is a substance that attaches with an allosteric enzyme at a site other than catalytic one but influences the latter, either inhibiting or activating the same. An example of feed back or allosteric inhibition is stoppage of activity of enzyme hexokinase (glucokinase) by glucose-6-phosphate, the product of reaction catalysed by it (Fig. 9.38).

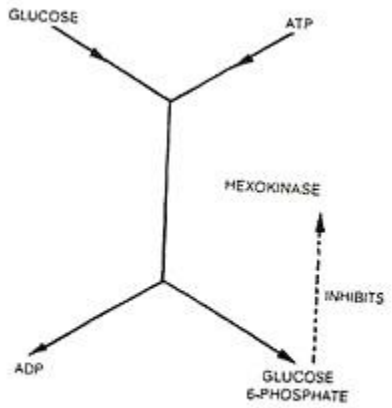


Fig. 9.38. Feedback or allosteric inhibition of hexokinase.

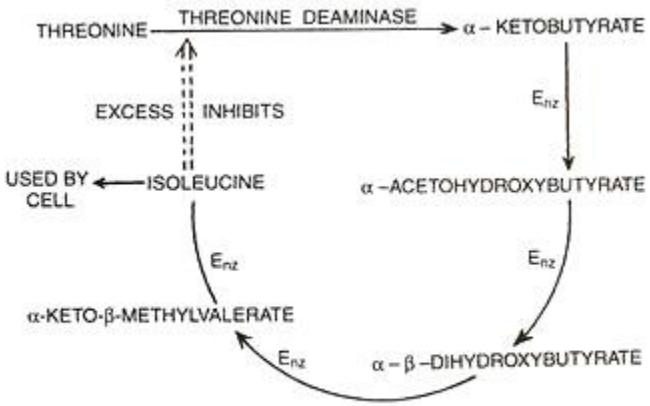


Fig. 9.39. Feedback or end product in case of isoleucine.

Another example is inhibition of threonine deaminase by isoleucine (Fig. 9.3). Amino acid isoleucine is formed in bacterium Escherichia coli in a 5-step reaction from threonine. Each step requires a separate enzyme. When isoleucine accumulates beyond a threshold value, its further production stops.

Isoleucine added to the medium of bacterium also stops its internal production showing that its excess prevents some step of the reaction. The latter was found out to be enzyme threonine deaminase which is involved in the first step of the reaction (threonine to a-ketobutyrate).

Importance:
(i) It has a regulatory role on enzyme activity,

(ii) Enzyme inhibitors have been used in the study of metabolic pathways,

(iii) Some inhibitors are used in controlling pathogenic activity, e.g., sulpha drugs,

(iv) Use of inhibitors have shown the mechanism of enzyme action.

Regulation of Enzyme:
Biochemical reaction studies have shown that the pace of a chemical reaction in a biological system is maintained by the activities of the enzymes. Enzymes are rather unstable molecules and are synthesized

and degraded simultaneously. Their activities may be regulated either through their synthesis or by modifying the existing enzyme molecules.

The activities of enzyme molecules are regulated by several ways which are the following:

I. Allosteric Regulation:

Allosteric regulation is a fine mechanism of controlling a reaction through the enzyme activity. Some enzymes (called allosteric enzymes), show sigmoidal curve between the substrate concentration and the activity. The activity of these enzymes is modified by several metabolites. The effect of different concentrations of 'activator' and 'inhibitor' on these enzymes is also sigmoid.

These effector molecules have a structure different from the substrate molecules. In most of the cases, allosteric inhibitors are the end products of the reaction; inhibiting the first enzyme in the series.

Thus, this kind of inhibition is called feedback inhibition, end product inhibition or retro-inhibition. The allosteric activators arc normally one of the substrates or cofactors of the enzyme. The effect of the allosteric 'inhibitor' or 'activator' on the enzyme is reversible.

When they are withdrawn, the enzyme resumes the original activity:

i. Allosteric Inhibition:

Inhibition of threonine deaminase by isoleucine is an example of allosteric inhibition. Threonine deaminase deaminates threonine to α-ketrobutyrate. The final product of the reaction is isoleucine.

Whenever the accumulation of isoleucine occurs, conversion of threonine to α-ketobutyrate and consequently formation of other intermediaries in the biosynthesis of isoleucine is stopped. When isolcucine is used up, threonine deaminase is reactivated and reactions for the biosynthesis of isoleucine start again.

ii. Allosteric Activation:

Activation of glycogen synthetase by glucose-6-phosphate is an example of allosteric activation. Another example of allosteric regulation (of both inhibitory and activating type) is observed during Pasteur effect. Pasteur effect is the inhibition of glycolysis and fermentation by oxygen. The molecular basis of this effect is the allosteric inhibition of enzyme phosphofructokinase by ATP and citrate and its activation by AMP.

Like many others, this kind of regulation is also of adaptive significance. As the level of AMP increases due to increased use of ATP in the cell, glycolysis is increased by the activation of phosphofructokinase with the result of more formation of ATP. When ATP level exceeds normal requirement of the cell, inhibition of glycolysis occurs through the same enzyme phosphofructokinase and ATP synthesis is stopped.

iii. Mechanism of Allosteric Regulation:

Regarding the mechanism of allosteric regulation, it is proposed that allosteric enzymes have two active centers; one for the substrate and the other for effector. These two sites lie either on same or on two different subunits. Binding of a effector molecule to one type of subunit changes the structure of the enzyme molecule in such a way that binding of the substrate to the other subunit is affected.

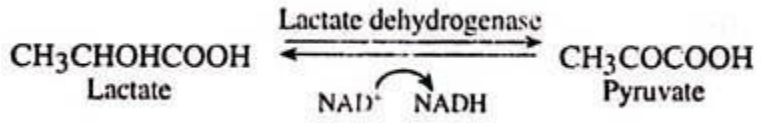
To explain the mechanism, an example of allosteric regulation of aspartate transcarbamylase may be cited. Asparate transcarbamylase contains two types of subunits. These two types of subuntis may be split apart by treatment with mercurials; with one type retaining the ability to bind with the substrate, whereas the other to recognize the inhibitor.

When these two species of subunits are together (active enzyme molecule), binding of the inhibitor to one type of subunit changes the structure of other subunits in such a way that the binding of the substrate is inhibited. When the subunits containing binding sites for the inhibitor are removed enzyme is not affected by the inhibitor.

Further, it gives a typical Michaelis-Menten curve with the substrate concentration. Similarly, the binding of activator may change the molecular structure in such a way that the binding of substrate is facilitated.

II. Isozyme Formation:

Another phenomenon that controls cellular metabolism is the formation of isozymes (isoenzymes). Isozymes are different physical forms of the same enzyme performing the same general function at different rates. They differ to some extent in their amino acid composition also, so that they may be separated by electrophoresis. Lactate dehydrogenase is a classic example of isozyme formation. It catalyses the oxidation of lactate to pyruvate with the help of NAD^+.



Lactate dehydrogenase enzyme is a tetramer composed of two distinct types (H and M types) of subunits.

Depending upon the relative number of two types of subunits, lactate dehydrogenase forms 5 isozymes as follows:

LD_1 = HHHH
LD_2 = HHHM
LD_3 = HHMM
LD_4=HMMM
LD_5 = MMMM

The molecular weight of the enzyme is 13,500 but when it is treated with urea or guanidine hydrochloride, it dissociates into subunits each having a molecular weight of about 35,000. The regulation of different isozymes is different. LD_1 (HHHH) type of lactate dehydrogenase is found in the heart muscles.

This species is most active at low pyruvate concentration and is inhibited by high concentrations of pyruvate. LD_5 (MMMM) type of enzyme is found in skeletal muscle cells and it remains active at high pyruvate concentrations.

Another example of isozyme formation is that of aspartokinase. This enzyme catalyzes the reaction between aspartic acid and ATP to form aspartyl phosphate. Amino acids lysine, methionine, and threonine are final products of the reaction.

The enzyme aspartokinase exists in three forms—aspartokinase I, aspartokinase II and aspartokinase III. Aspartokinase I is inhibited by threonine and III by lysine. Aspartokinase II is insensitive to any of these amino acids. Thus, when any one of these amino acids accumulates, the synthesis of the other is affected very little.

III. Multienzyme System:

Some enzymes exist not as individuals but as aggregates of several enzymes and coenzymes. This they do to channel the metabolities in a pathway efficiently. In an aggregate, each component is arranged in a way that the product of one enzyme becomes the substrate for the other and so on.

An example of enzyme aggregation is that of pyruvic acid dehydrogenase of E. coli. This complex consists of three enzymes-pyruvate decarboxylase, dihydrolipoic dehydrogenase and lipoyl reductase transacetylase. The coenzyme associated with the complex are thiamine pyrophosphate (TPP) and flavin adenine dinucleotide (FAD). A schematic diagram of pyruvate dehydrogenase complex is given in Fig. 27.13.

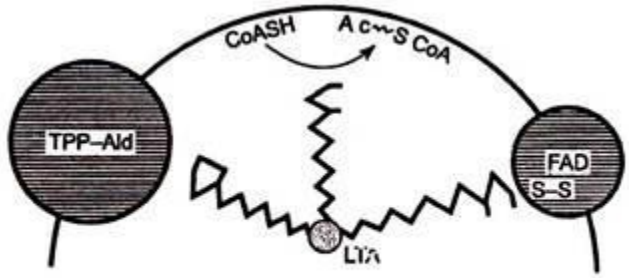


FIG. 27.13. A schematic representation of pyruvate dehydrogenase complex system.

The stepwise reactions catalyzed by this complex may be written as follows:

Pyruvate + thiamine pyrophosphate → α-hydroxythyl thiamine + pyrophosphate + CO_2
α -hydroxyethyl thiamine + lipoate → Thiamine pyrophosphate + acetyl dihydrolipoate

Acetyl dihydrolipoate + CoASH → Acetyl CoA + dihydrolipoate

Dihydrolipoate + NAD^+/FAD^+ → Lipoate + $NADH/FADH_2$

IV. Regulation by Adenylate Energy Charge:

The importance of adenosine phosphates in metabolic processes has been well recognised for living systems. The adenylate energy charge is the measure of total pool of adenosine phosphates in the form of ATP, ADP and AMP. D.D. Atkinson (1969) defines adenylate energy charge as follows.

$$\text{Adenylate energy charge} = \frac{[ATP] + 1/2\,[ADP]}{[AMP] + [ADP] + [ATP]}$$

In most systems, an increase in adenylate energy charge in the physiological range results in stimulation of regulatory enzymes. Although it is a well-known phenomenon in animals and microorganisms, some instances have been recorded from plants also.

It has been shown that the adenylate energy charge affects the activity of pyrophosphomevalonate decarboxylase, which is the key enzyme in the biosynthesis of kaurene from mevalonate. An increase in enzyme activity is observed between adenylate energy charge of 0.8 and 1.0.

Factors Influencing Enzyme Activities:

i. Temperature:

An enzyme is active within a narrow range of temperature. The temperature at which an enzyme shows its highest activity is called optimum temperature (Fig. 9.29). It generally corresponds to the body temperature of warm blooded animals, e.g., 37°C in human beings. Enzyme activity decreases above and below this temperature.

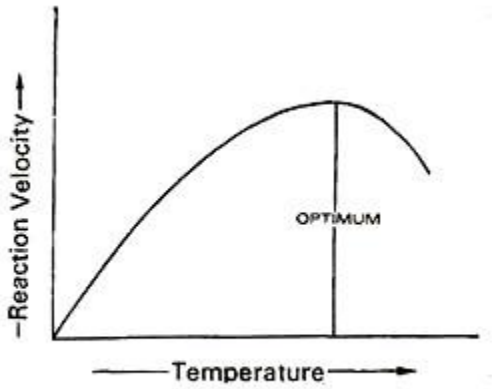


Fig. 9.29. Relation between temperature and enzyme controlled reaction velocity.

Enzyme becomes inactive below minimum temperature and beyond maximum temperature. Low temperature preserves the enzymes in the inactive state. Therefore, it is used in preservation of foods inside cold storages.

Low temperature present inside cold storages prevents spoilage of food by two methods:
(i) Inactivity of enzymes present inside food article and

(ii) Non-activity of microbes because their enzymes also become inactive at low temperature.

High temperature destroys enzymes by causing their denaturation. This occurs at 50°C or so. In between the minimum and maximum temperatures, the reaction velocity doubles for every rise in 10°C (general rule of thumb). A time factor appears beyond optimum temperature. Here there is a rise in velocity for a short time followed by a sharp fall.

As opposed to warm blooded or homoeothermal animals (mammals, birds), there are cold blooded or poikilothermal animals (reptiles, amphibians, fishes, invertebrates) whose body temperature rises or falls with that of environmental temperature.

These animals cannot live in very hot or very cold environment as enzyme functioning will be impaired. Because of this reason, frog

seeks moist shady environment during summer and lies in an inactive form (hibernation) in the deeper layers of the soil during winter.

ii. Optimum pH:
Every enzyme has an optimum pH when it is most effective. A rise or fall in pH reduces enzyme activity by changing the degree of ionisation of its side chains.

A change in pH may also start reverse reaction. Fumarase catalyses fumarate → malate at 6.2 pH and reverse at 7.5 pH. Most of the intracellular enzymes function near neutral pH with the exception of several digestive enzymes which work either in acidic range of pH or alkaline, e.g., 2.0 pH for pepsin, 8.5 for trypsin.

iii. Enzyme Concentration:
The rate of a biochemical reaction rises with the increase in enzyme concentration up to a point called limiting or saturation point (Fig. 9.30). Beyond this, increase in enzyme concentration has little effect.

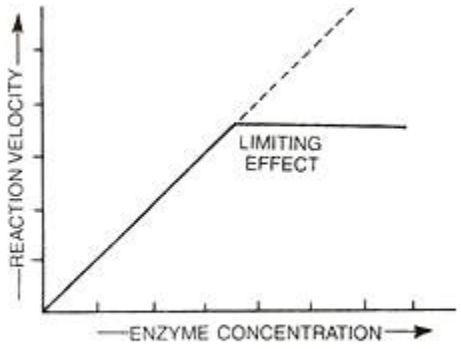


Fig. 9.30. Effect of enzyme concentration on the rate of biochemical reaction.

iv. Product Concentration:
If the products are allowed to remain in the area of the reaction, the rate of forward reaction will fall. Reverse reaction can also start.

v. Activators:
They increase activity of enzymes (e.g., chloride for salivary amylase), function as cofactors (e.g., K^+, Mn^{2+}) and convert pro-enzymes to enzyme state. HCl of digestive juice changes pro-enzyme pepsinogen

to enzyme pepsin. Pepsin also possesses autocatalytic property as it can also change pepsinogen to pepsin state.

vi. Protein Poisons:

Cyanides, azides, iodoacetate, and salts of heavy metals destroy tertiary structure of enzymes by either combining with cofactor or a group of apoenzyme (—SH group, —COOH).

vii. Radiation Energy:

High energy radiations break hydrogen bonds, ionic bonds, and other weak linkages to destroy enzyme structure.

viii. Substrate Concentration:

Increase in substrate concentration increases the rate of reaction.

The enhanced rate is due to two factors:

(a) Occupation of more and more active sites by the substrate molecules;

(b) Higher number of collisions between substrate molecules.

The rise in velocity is quite high in the beginning but it decreases progressively with the increase in substrate concentration. If a graph is plotted for substrate concentration versus reaction velocity, it appears as a hyperbolic curve.

A stage is reached where velocity is maximum. It does not increase further by increasing the substrate concentration. At this stage the enzyme molecules become fully saturated and no active site is left free to bind additional substrate molecules. This saturation effect is shown by all enzymes. Because of this Victor Henri (1903) proposed the formation of enzyme-substrate complex as an essential step in enzyme catalysis.

Michaelis Constant (Michaelis Menten Constant, Km). It is a mathematical derivation or constant which indicates the substrate concentration at which the chemical reaction catalysed by an enzyme attains half its maximum velocity (Fig. 9.31).

Constant was given forth by Leoner Michaelis and Mand Menten (1913). Km or Michaelis Menten constant generally lies between 10^{-1} to 10^{-6} M. Km indicates affinity of the enzyme for its substrate. A high Km indicates low affinity while a low Km shows strong affinity. If an enzyme acts on more than one substrate it shows different Km values for them. Thus enzyme protease acts on large number of proteins. Its Km value will differ from protein to protein.

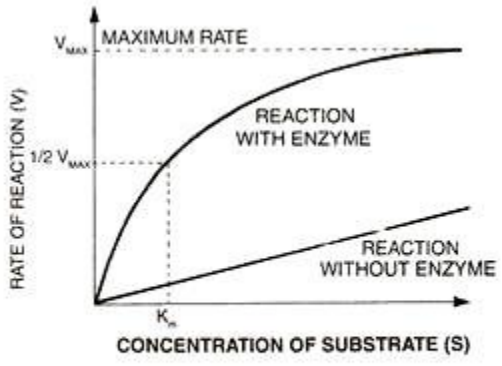


Fig. 9.31. Effect of substrate concentration on reaction velocity with or without enzyme, calculation of maximum velocity and Km value.

Allosteric enzymes do not show a typical Michaelis Menten constant or behaviour. The classical hyperbolic curve is replaced by a sigmoid saturation curve.

Mutation and types of mutation

Mutation

- Mutation is the change in sequence of nucleotide of DNA.
- Change in sequence of nucleotide brings sudden change in morphological characteristics of an organism. If such change are heritable, then it is called as mutation.
- So, mutation is defined as any heritable change in the sequence of nucleotide of DNA.

Organism with mutation is called **mutant** while the organism without mutation is **wild type**.

Types of Mutation

Based on change in genotype and phenotype, mutation are of two types

1. Point mutation
2. Frameshift mutation

1. Point mutation

- It occurs as a result of replacement of one nucleotide by other in specific nucleotide sequence of gene. Point mutation brings little phenotypic change as compared to frameshift mutation.

Point mutation are two types based on the base pair substitution

i) Transition:

- It is the point mutation occur by substitution of one purine by another purine or one pyrimidine by another pyrimidine.

ii) Transversion:

- It is the point mutation occur by substitution of purine by pyrimidine and vice versa.

Based on transcriptional property point mutation are of **three types.**

i) Silent mutation

ii) Missense mutation

iii) Non-sense mutation

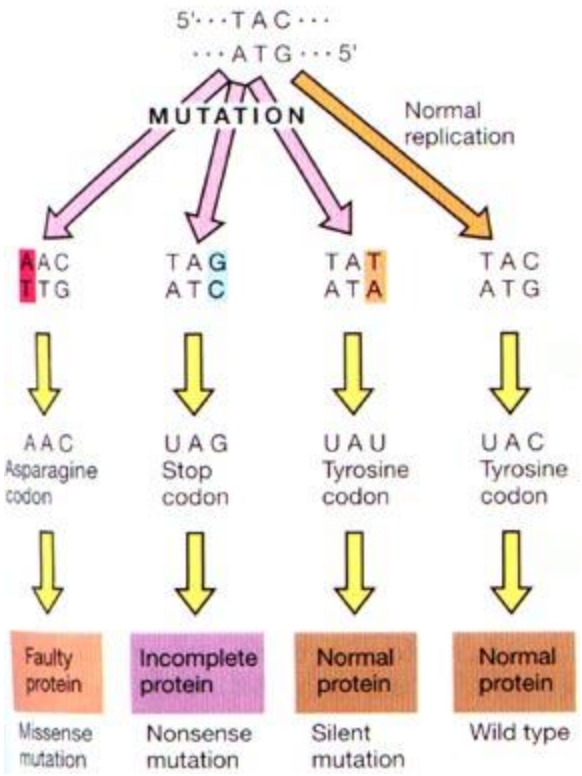


Figure: types of point mutation

i) Silent mutation:

- It is also known as neutral mutation.
- It is the mutation in which mutated codon codes same amino acids as the original codon. Since the aminoacid is same as original one, it does not effects the structure and composition of protein.
- Silent mutation causes phenotype of bacteria remain similar to that of wild type.

ii) Missense mutation:

- **I**n this mutation mutated codon codes different amino acid (other than original). Since new aminoacid coded by mutated codon is altered, the protein formed from it is also altered. Such protein can be less active or completely inactive.
- If altered aminoacids lie on active site of protein then such protein become completely non-functional.
- The missense mutation causes phenotypic change in organism.

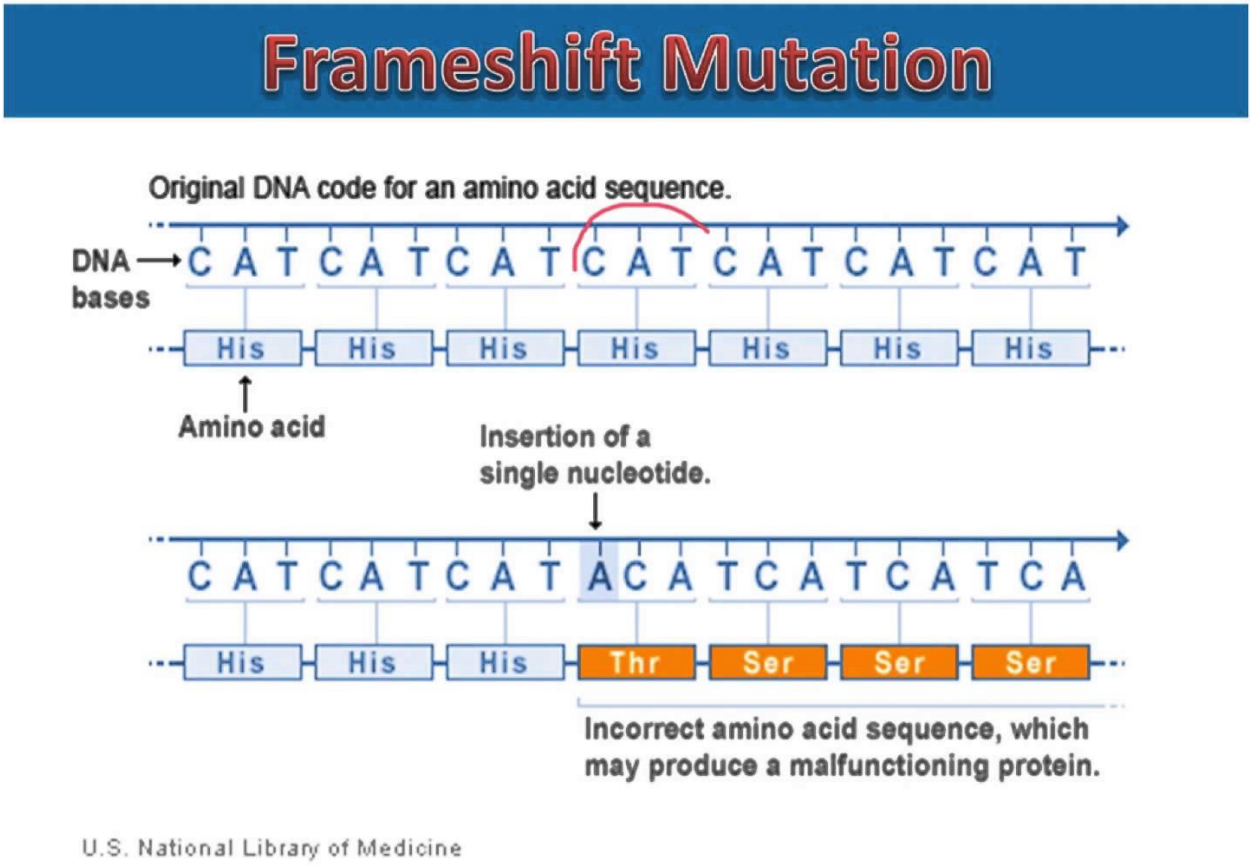
iii) Non sense mutation:

- Mutation in which altered codon is stop codon or chain terminating codon, such mutation is called non-sense mutation.
- Non sense mutation causes incomplete synthesis. Such incomplete protein is always non-functional.

- Non-sense mutation bring greatest change in phenotype of an organism.

2. Frameshift mutation

- It occurs as a result of addition or deletion of nucleotide in the sequence of DNA. Addition or deletion of nucleotide causes shift of the reading frame of mRNA.
- In a mRNA each codon is represented by three bases without punctuation and insertion or deletion of a nucleotide changes the entire frame. So frame shift mutation bring greater phenotypic change than point mutation.
- Insertion or deletion of one or two base pair of nucleotide causes shift in frame. However, insertion or deletion of three base pair adds or remove a whole codon, this results in addition of removal of single amino acid from polypeptide chain.
-



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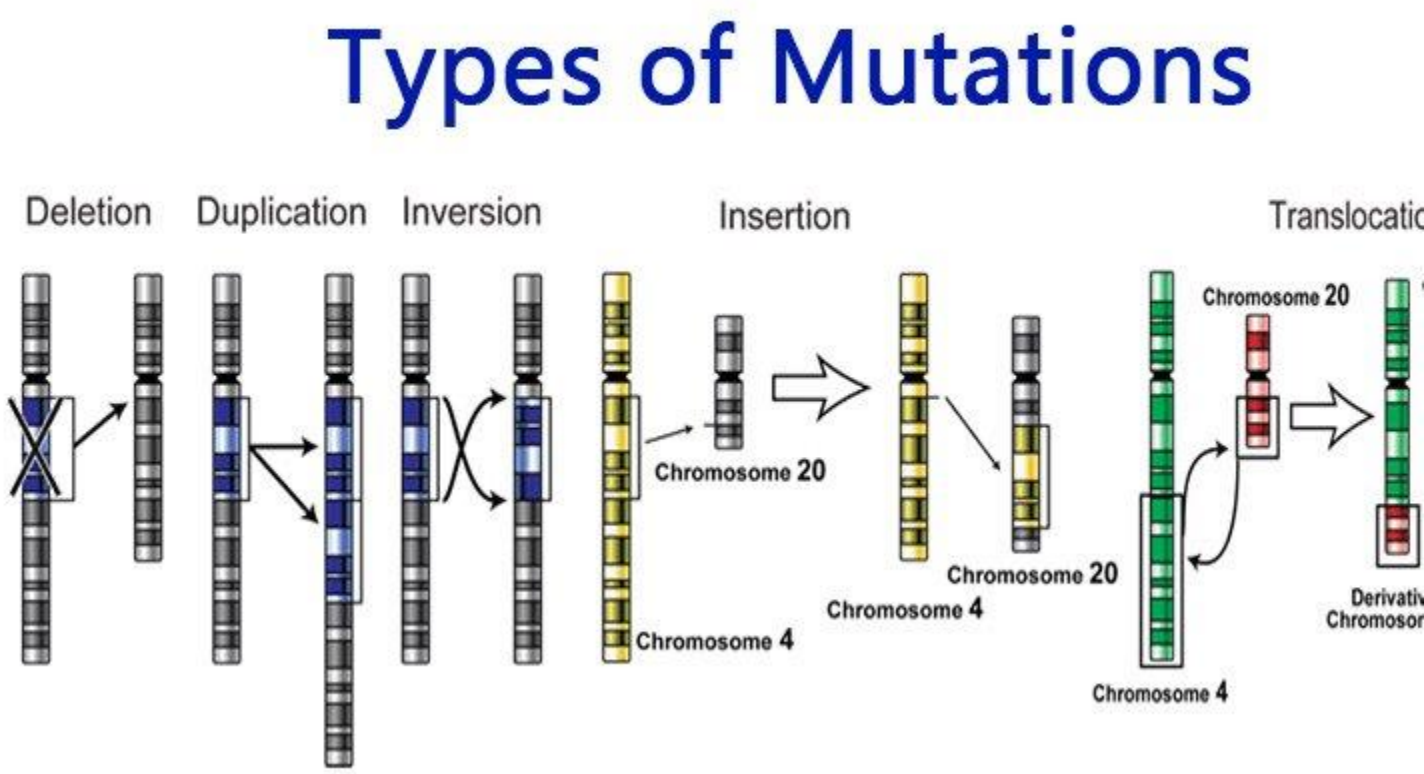
Types of Mutations

- The mutation is a process that produces a gene or chromosome that differs from the wild type (arbitrary standard for what "normal" is for an organism).
- The mutation may result due to changes either on the gene or the chromosome itself. Thus, broadly mutation maybe:

1. **Gene mutation** where the allele of a gene changes.
2. **Chromosome mutation** where segments of chromosomes, whole chromosomes, or entire sets of chromosomes change.



Types of Mutations
There are various schemes for classification of different kind of mutations. Depending on:

A. The Type of Cell Involved

1. **Somatic mutations**
- Mutations that are in the somatic tissues of the body.
- Mutations are not transmitted to progeny.
- The extent of the phenotypic effect depends upon whether the mutation is dominant or recessive (dominant mutations generally have a greater effect).
- The extent of the phenotypic effect depends upon whether it occurs early or late in development (early arising mutations have a greater effect).

2. **Germinal mutations**
- Mutations that are in the germ tissues of the body.

- Mutations may be transmitted to progeny
- Dominant mutations are seen in first generation after the mutation occurs
- If a female gamete containing an X-linked mutation is fertilized, the males will show the mutant phenotype
- Recessive mutations will only be seen upon the chance mating with an individual carrying the recessive allele too; thus, the recessive mutation may remain hidden for many generations

B. Mode of Origin

(1) Spontaneous mutations

The spontaneous mutations occur suddenly in the nature and their origin is unknown. They are also called "background mutation" and have been reported in many organisms such as, Oenothera, maize, bread molds, microorganisms (bacteria and viruses), Drosophila, mice, man, etc.

(2) Induced mutations

Besides naturally occurring spontaneous mutations, the mutations can be induced artificially in the living organisms by exposing them to abnormal environment such as radiation, certain physical conditions (i.e., temperature) and chemicals.

C. Direction of Mutation

According to their mode of direction following types of mutations have been recognised:

1. Forward mutations

In an organism when mutations create a change from wild type to abnormal phenotype, then that type of mutations are known as forward mutations. Most mutations are forward type.

2. Reverse or back mutations

The forward mutations are often corrected by error correcting mechanism, so that an abnormal phenotype changes into wild type phenotype.

D. Size and Quality

According to size following two types of mutations have been recognized:

1. Point mutation

When heritable alterations occur in a very small segment of DNA molecule, i.e., a single nucleotide or nucleotide pair, then this type of mutations are called "point mutations". The point mutations may occur due to following types of subnucleotide change in the DNA and RNA.

– Deletion mutations. The point mutation which is caused due to loss or deletion of some portion (single nucleotide pair) in a triplet codon of a cistron or gene is called deletion mutation.

– Insertion or addition mutation. The point mutations which occur due to addition of one or more extra nucleotides to a gene or cistron are called insertion mutations.

The mutations which arise from the insertion or deletion of individual nucleotides and cause the rest of the message downstream of the mutation to be read out of phase, are called **frameshift mutations.**

– Substitution mutation. A point mutation in which a nucleotide of a triplet is replaced by another nucleotide, is called substitution mutation.

2. Multiple mutations or gross mutations.

When changes involving more than one nucleotide pair, or entire gene, then such mutations are called gross mutations. The gross mutations occur due to rearrangements of genes within the genome. It may be:

1. The rearrangement of genes may occur within a gene. Two mutations within the same functional gene can produce different effects depending on gene whether they occur in the cis or trans position.
2. The rearrangement of gene may occur in number of genes per chromosome. If the numbers of gene replicas are non-equivalent on the homologous chromosomes, they may cause different types of phenotypic effects over the organisms.
3. Due to movement of a gene locus new type of phenotypes may be created, especially when the gene is relocated near heterochromatin. The movement of gene loci may take place due to following method:

(i) Translocation. Movement of a gene may take place to a non-homologous chromosome and this is known as translocation.

(ii) Inversion. The movement of a gene within the same chromosome is called inversion.

E. Phenotypic Effects

1. **Morphological mutations** are mutations that affect the outwardly visible properties of an organism (i.e. curly ears in cats)
2. **Lethal mutations** are mutations that affect the viability of the organism (i.e. Manx cat).
3. **Conditional mutations** are mutations in which the mutant allele causes the mutant phenotype only in certain environments (called the restrictive condition).

In the permissive condition, the phenotype is no longer mutant.
Example. Siamese cat – mutant allele causes albino phenotype at the restrictive temperature of most of the cat body but not at the permissive temperature in the extremities where the body temperatures is lower.

4. **Biochemical mutations** are mutations that may not be visible or affect a specific morphological characteristic but may have a general affect on the ability to grow or proliferate.

For example, the bacterium Escherichia coli does not require the amino acid tryptophan for growth because they can synthesize tryptophan. However, there are E. coli mutants that have mutations in the trp genes. These mutants are auxotrophic for tryptophan, and tryptophan must be added to the nutrient medium for growth.

F. Magnitude of Phenotypic Effect

According to their phenotypic effects following kinds of mutations may occur:

1. **Dominant mutations**

The mutations which have dominant phenotypic expression are called dominant mutations. For example, in man the mutation disease aniridia (absence of iris of eyes) occurs due to a dominant mutant gene.

2. **Recessive mutations**

Most types of mutations are recessive in nature and so they are not expressed phenotypically immediately. The phenotypic effects of mutations of a recessive gene is seen only after one or more generations, when the mutant gene is able to recombine with another similar recessive gene.

3. **Isoalleles**

Some mutations alter the phenotype of an organism so slightly that they can be detected only by special techniques. Mutant genes that give slightly modified

phenotypes are called isoalleles. They produce identical phenotypes in homozygous or heterozygous combinations.

G. Loss of Function or Gain of Function

1. **Loss of function mutation**

Loss of function mutation is also called inactivating mutations, result in the gene product having less or no function (being partially or wholly inactivated).

2. **Gain of function mutations**

The gain of function mutations also called activating mutations, change the gene product such that its effect gets stronger (enhanced activation) or even is superseded by a different and abnormal function.

H. Type of Chromosome Involved

According to the types of chromosomes, the mutations may be of following two kinds:

1. **Autosomal mutations.** This type of mutation occurs in autosomal chromosomes.
2. **Sex chromosomal mutations.** This type of mutation occurs in sex chromosomes.

Nucleic Acids

These are important organic substances found in nucleus and cytoplasm. They control the important biosynthetic activities of the cell and carry hereditary information from generation to generation.

Thus, nucleic acids are macromolecules of the utmost biological importance.

They are associated with the chromosomes and transmit various information to cytoplasm.

All the hereditary (genetic) information of the cell (i.e., all the information necessary to reproduce and maintain a new organism) is stored in coded form in molecules of DNA. DNA is replicated and distributed to daughter cells during cell division, and in this way all the hereditary information accumulated over billions of years of evolution is passed from cell to cell and from one generation of an organism to another.

With the aid of RNA, this information is expressed as specific patterns of protein synthesis. These nucleic acids are of two types: (i) deoxyribonucleic acid (DNA) and (ii) ribonucleic acid (RNA). DNA is the major store of genetic information. This information is transmitted by transcription into RNA molecules, proteins are then synthesized in a process involving translation of the RNA.

DNA → transcription RNA→ translation Protein

In higher cells DNA is localized mainly in the nucleus as part of the chromosomes. A small amount of DNA is present in the cytoplasm and contained within mitochondria and chloroplasts. RNA is found both in the nucleus, where it is synthesized, and in the cytoplasm, where the synthesis of proteins takes place.

Nucleic acids consist of a sugar (pentose), nitrogenous bases (purines and pyrimidines), and phosphoric acid. A nucleic acid molecule is a linear polymer in which nucleotides are linked together by means of phosphodiester 'bridges' or bonds.

These bonds link the 3′ carbon in the pentose of one nucleotide to the 5′ carbon in the pentose of the adjacent nucleotide. Thus the backbone of a nucleic acid consists of alternating phosphates and pentoses. The nitrogenous bases are attached to the sugars of this backbone.

Nucleic acids are basophilic, i.e., stain readily with basic dyes. After a mild hydrolysis the nucleic acids are decomposed into nucleotides.

[I] Deoxyribonucleic acid (DNA):

It forms about 9% part of nucleus as found by spectrophotometric analysis. Chemically it consists of mainly three components: phosphoric acid, sugar, and bases.

1. Phosphoric acid:

It may occur also as phosphate and forms the backbone of DNA molecule along with sugar molecule. It links the nucleotides by joining the deoxyribose (pentoss sugar) of two adjacent nucleotides with an ester-phosphate bond. These bonds connect carbon 3′ in one nucleotide with carbon 5′ in next. This acid is a channel for the chemical energy used by the molecule.

2. Pentoses:

These are of two types: ribose in RNA and deoxyribose in DNA. DNA has one oxygen atom less than that of RNA. The pentose sugar in nucleic acids is always ribose-, in RNA it is D-ribose and in DNA, it is deoxyribose. It is always the OH on C-l carbon which is the point of attachment of the base.

This linked to the 1-nitrogen atom in case of pyrimidines and to 9-nitrogen atom in purines. Both deoxyribose and ribose (pentose sugars of nucleic acids) have a pentagonal ring with five carbons,

among which two (i.e., 3′ and 5′) are attached to phosphoric acid and three (Γ) to the base.

3. Bases:

These may be of two types:

(a) Purines and

(b) Pyrimidines.

(a) Purines:

These are characterised by the presence of two fused benzene rings. They may be adenine (A) and guanine (G). RNA contains uracil (U) instead of thymine. The combination of base plus a pentose, minus the phosphate, forms a nucleoside. For example, adenine is a purine base; adenosine (adenine + ribose) is the corresponding nucleoside, i.e., deoxyadenosine and deoxyguanosine.

(b) Pyrimidines:

These are characterized by the occurrence of single benzene ring. They are thymine (T) and cytosine (C).

Nucleotides:

Nucleotides are phosphate esters of nucleosides, purine or pyrimidine bases linked to sugars. In the nucleotides, the 3-nitrogen of the pyrimidine bases or the 9-N of the purine bases is attached to the 1-carbon atom of the sugar, and the phosphoric acid residue is attached to the 5′ carbon atom of the sugar.

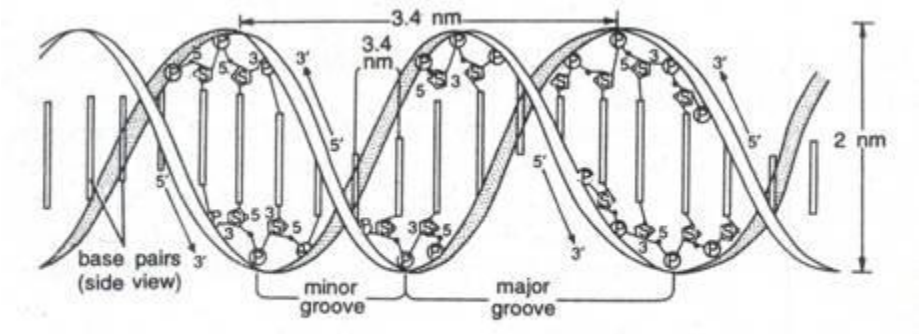


Fig. 1. Double-helical model of DNA.

Thus nucleotides of purines are deoxyriboadenylic acid and deoxyriboguanylic acid, and of pyrimidines are deoxyribothymidylic acid and deoxyribocytidylic acid.

[II] DNA:

Purines and pyrimidines are weak bases. Adjacent nucleotides of the nucleic acids are connected by a linkage between the phosphoric acid residue of one nucleotide and the 3′ carbon atom of the sugar on the next nucleotide. Both the bases and sugars have an approximately planar structure. In polynucleotides the planes are oriented with respect to one another at an angle of 70° to 75°.

In addition to four common bases, a number of unusual bases are found is .DNA. DNA of animal origin contains trace amounts of 5-methy- lcytosine, while large amounts of this base are found in DNA of plant origin. Similarly, 6-methyl aminopurine is found in DNA from bacteria and viruses.

Cytosine in DNA of T-even bacteriophages of E.-coli is replaced by 5-hydroxymethylcytosine, to which glucose or other sugars may be linked at the hydroxyl group. In some viral DNA's, the rare base 5-hydroxy— methyluracil substitutes for thymine.

[III] DNA base composition:

DNA in living organisms is found as a linear molecules of extremely high molecular weight. For example, in E-coli it is a single circular DNA molecule weighing about 2.7×10^9 daltons and its length is 1.4 mm. In higher organisms the amount of DNA may be several thousand times larger. For example, in a single human diploid cell its total length when fully extended is 1.7 meters.

All the genetic information of a living organism is stored in its linear sequence of the four bases. Therefore, a four letter alphabet (A, T, G, C) must code for the primary structure (i.e., number and sequence of 20 amino acids) of all proteins. The base composition vary from one

species to another, but in all cases amount of adenine is equal to amount of thymine (A=T).

Similarly amount of cytosine is equal to guanine (C=G). Consequently, total quantity of purines equals the total quantity of pyrimidines (i.e., A+G=C+T). On the other hand, AT/GC ratio varies considerably between species.

[IV] DNA is double helix:

On the basis of X-ray diffraction data of Wilkins and Franklin, Watson and Crick (1953) proposed a model for DNA structure. It is composed of two right-handed helical polynucleotide chains that form a double helix around the same central axis. The two strands are antiparallel, meaning that their 3′, 5′ phosphodiester links run in opposite directions. The bases are stacked inside the helix in a plane perpendicular to the helical axis.

The two strands are held together by hydrogen bonds present between pairs of bases. Since there is a fixed distance between two pentose sugars in the opposite strands, only certain base pairs can fit into the structure.

As shown in figure 5 two hydrogen bonds are formed between A and T, three are formed between C and G, therefore a CG pair is more stable than AT pair. In addition to hydrogen bonds, hydrophobic interactions established between the stacked bases are important in maintaining the double helical structure.

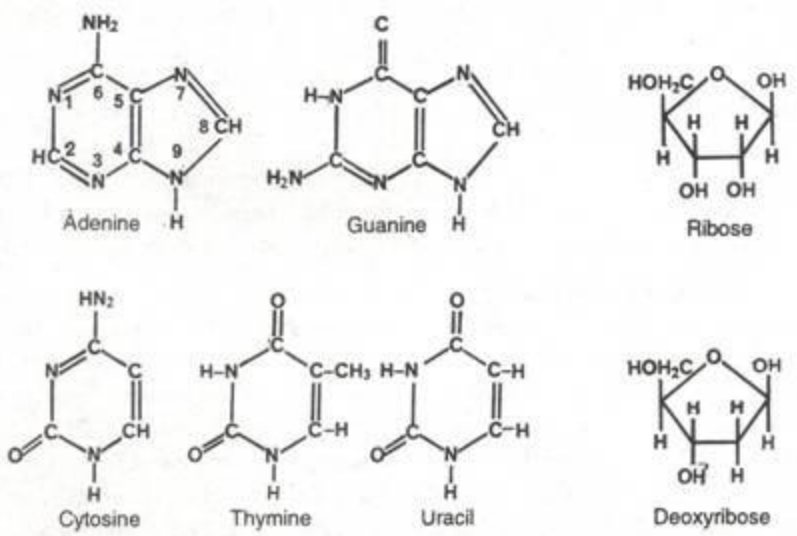


Fig. 2. Chemical formulae of bases and sugars.

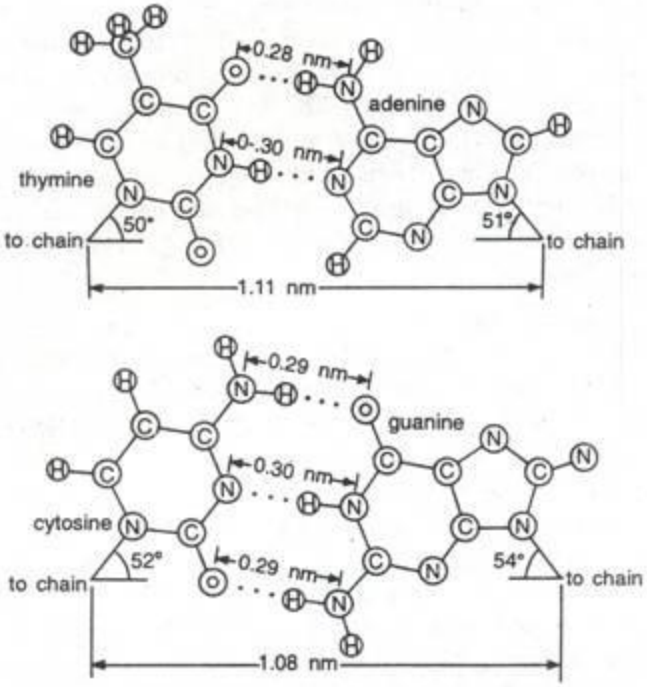


Fig. 3. Two base pairs in DNA. Complimentary bases are T=A and C ≡ G linked with hydrogen bonds.

The axial sequence of bases along one polynucleotide chain may vary considerably, but on the other chain the sequence must be complementary, as given below —

First chain	5′	T	G	C	T	G	A	C	G	T	3′
		||	|||	|||	||	|||	||	|||	|||	||	
	3′	A	C	G	A	C	T	G	C	A	5′

Because of this property, order of bases on one chain, the other chain is complimentary. During duplication the two chains dissociate and each one serves as a template for the synthesis of a new complementary chain.

[V] Separation of DNA strands:

DNA double helix is preserved by weak interactions (i.e., hydrogen bonds and hydrophobic interactions between stacked bases); two strands may be separated by heating or alkaline pH. This separation is called melting or denaturation of DNA. The melting point depends on AT/GC ratio. Breakage of GC pairs needs higher temperature to that of AT pairs.

If DNA is cooled slowly after denaturation, double helical conformation will be restored. This process is called renaturation or annealing and this is the base-pairing properties of nucleotides.

DNA renaturation can be used to estimate the size (number of nucleotides) of the genome of a given organism. A large genome (e.g., calf) take more time to reanneal than a small genome (e.g., E. coli). This is because the individual sequences take longer time to find the correct partners.

Single stranded DNA will also anneal to complimentary RNA, resulting in a hybrid molecule in which one strand is DNA and the other is RNA. Molecular hybridization is a very powerful method for characterizing RNAs since RNA; molecule will hybridize only to DNA from which it was transcribed.

[VI] Ribonucleic acid (RNA):

RNA is present in considerable amounts in the nucleolus and is also found in small amounts on chromosomes. The major part of the cells RNA is in the cytoplasmic ribosomes. A small amount of RNA is also present in mitochondria and chloroplasts.

Transfer RNA and mRNA are present in solution in the cytoplasmic matrix unless affixed to the ribosomes. The RNA content of nucleus and cytoplasm varies with activity cycles of the cell. The cytoplasmic RNA increases in quantity during cell growth preceding mitosis and is partitioned equally between the daughter cells.

RNA accumulates in both nucleus (especially in nucleolus) and cytoplasm during high metabolic activity or growth, as in regenerating nerve cells, active neurons, gland cells, cells infected with virus and tumor cells. Actively metabolizing yeast cells contain a large amount of RNA, but starved yeast cells have little RNA. Infact, starved cells in general show RNA depletion.

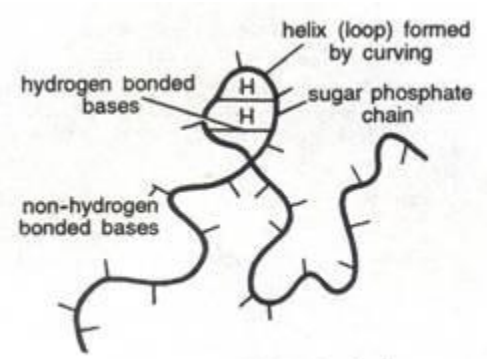


Fig. 4. Sructure of RNA showing loop.

RNA also varies with other physiological conditions such as lack of oxygen and presence of metabolic poisons. RNA is labile in dividing cells and also in active cells that are not dividing.

[VII] Structure of RNA:

RNA is a long-chain molecule built up of repeating nucleotide units linked by 3′ to 5′ phosphate diester bonds. Sugar component of RNA is ribose and three out of four bases, adenine, guanine and cytosine are the same as in DNA, and the fourth base is uracil in place of thymine of DNA, Uracil has one methyl group less.

Nucleotides:

RNA nucleotides are formed from pentose sugar ribose, phosphoric acid and either adenine guanine, cytosine or uracil (U). Nucleotides are regarded as phosphorylated derivatives of nucleosides.

Nucleosides are combinations of a nitrogenous base and a pentose sugar without an attached phosphate group.

A nucleotide unit consists of a molecule of sugar, a base and a phosphoric acid. A single nucleic acid contains a large number of nucleotide units consisting of high molecular weight (about 8,000,000).

Nucleotides are the monomeric units of the nucleic acid macromolecule. The nucleotides result from the covalent bonding of a phosphate and a heterocyclic base to the pentose. Within the nucleotide, the combination of a base with the pentose forms a nucleoside.

For example, adenine is a purine base; adenosine (adenine+ ribose) is the corresponding nucleoside, and adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are nucleotides. Nucleotides, thus constitute the building blocks of nucleic acids and they are also used to store and transfer chemical energy.

Polynucleotide:

Nucleotides are joined together to form a polynucleotide chain by a covalent linkage between the phosphoric acid residue of one nucleotide and 3′ carbon of the sugar on the next nucleotide. This linkage is often called a 3′, 5′ phosphodiester bond, because the phosphate is esterified to two OH groups, one attached to the 3′ carbon and one attached to the 5′ carbon.

The backbone of a polynucleotide chain thus consists of alternating sugar and phosphate units.

The sequence of nucleotides in DNA and RNA is the key to their genetic functions, just as the sequence of amino acids determines the biological activity of a particular protein. Even though both DNA and RNA are usually composed of only four different nucleotides, the number of possible sequences of nucleotides is enormous in a large polymer.

RNA usually exists as a single-stranded polynucleotide chain and have no regular helical configuration. The linear chain is thought to be folded in many ways, with certain nucleotides pairing off and forming short double-stranded regions.

[VIII] Kinds of ribonucleic acid:

The ribonucleic acids are of three types —

1. Messenger RNA:

This ribonucleic acid is of nuclear origin and conveys genetic information from DNA in the nucleus to the ribosomes in the cytoplasm, where amino acids become grouped to form proteins.

2. Transfer or adapter RNA:

It is another important type of ribonucleic acid which is present in the cytoplasm, helping there in protein synthesis. It has been recently found that t-RNA originates from nucleus near the nucleolar region.

3. Ribosomal RNA:

This ribonucleic acid is the major component of cytoplasmic particles called ribosomes. Ribosomal RNA comprises up to 80% of the cellular RNA of Escherichia coli. It is the site of amino acids union.

For detailed structure see chapter-Protein synthesis.

[IX] Significance of nucleic acids:

Deoxyribonucleic acids and ribonucleic acids are the key centres which control all the metabolic activities of cell and in turn the whole organism.

(1) If there occurs any deficiency in the DNA amount, nucleus loses its capacity to support adenosine triphosphate (ATP) synthesis.

(2) Nucleus also becomes inefficient to incorporate amino acids into proteins.

(3) Besides, DNA is the main genetic material constituting genes and chromosomes which carry hereditary information from generation to generation. DNA helps in the RNA synthesis in the cell. If the loops of amphibian oocytic chromosome (lamp brush) are exposed to actinomycin (which has the property to fuse with DNA and thereby causing decrease in DNA amount), RNA synthesis is inhibited.

(4) Recently, McConnell and Cameron (1968) have produced the evidence that RNA amount increases the intelligence and learning capacity of men.

Molecular arrangement of components in nucleic acids:

In deoxyribonucleic acid (DNA), nucleotides are arranged in the form of helixes or chains spirally coiling around each other. According to Watson and Crick (1962), DNA consists of two helixes coiled about each other. The chain of each helix is made of sugar and phosphate group.

These two helixes are interconnected by the bases through hydrogen bonds. Generally one purine becomes attached with one pyrimidine to form base connection between the chain. Thus, adenine along with thymine, and cytosine along with guanine becomes connected with the sugar molecule of chain alternately.

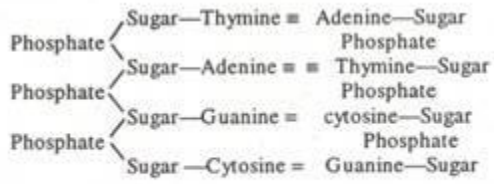


Fig. 5. Molecular arrangement of components in DNA.

The direction of one helix is opposite to the other. In DNA one helix serves as a template for the formation of complementary helix, i.e., adenine in one helix forms the thymine in new helix and similarly cytosine effects the formation of guanine in new helix.

In ribonucleic acid (RNA), the nucleotides are not arranged in double helical model but for the most part RNA exists as a single strand. Sometimes it may form also smaller helices in some parts due to folding and convolutions. These secondary helical structures in RNA are regular according to recent research. In the formation of helix, bases become hydrogen-bonded like DNA except uracil substitutes thymine.

Genetic Material

Properties of Genetic Material:

A living cell is composed of several inorganic and organic components. Among them, one will obviously act as genetic material responsible for controlling hereditary characters. Identification of this genetic material remained controversial for a long time.

Now if any component is to be genetic material, it must fulfil a number of basic properties:

i. Genotypic function or replication or auto-synthesis.

ii. Phenotype function or expression or hetero catalysis.

iii. Mutation.

The first property states that the genetic material must be capable of storing hereditary information and replicate with high efficiency in successive cell generations forming the basis for transmission of hereditary characteristics it controls.

The second property is a fundamental one involved in gene action which through a series of chemical reactions results in the ultimate expression of the characteristics within the organism. The third property states that the genetic material does undergo occasional heritable changes called mutation.

It creates variations among the organisms besides recombination. Variations, on the other hand, are the important source of raw materials for evolution.

Besides the above-mentioned important properties of genetic material, the gene substance also shows the following additional properties:

a. To control the innumerable diversities in the characteristics of organism available in nature, the genetic material must show a very wide diversity in form.

b. Since phenotype character is the final expression of a chain of reactions initiated at the gene level, obviously the genetic material must be a chemically unique entity.

Before 1900 several biologists proposed that hereditary material must be in the chromosome of the cell nucleus. In 1903, Sutton and Bovery postulated that genes were located in chromosome. In eukaryotic system, chromosomes are made of mainly protein and nucleic acid (DNA and RNA) and one of them obviously constitute the genetic material.

But which one would be the most suitable candidate for the position of genetic material remained controversial for a long time.

Early molecular biologists have assigned the properties of genetic material to the chromosomal proteins because they found nucleic acid too simple to carry genetic information. Besides this, nucleic acid is made of monotonous chemical components like sugar, phosphate and base.

On the other hand, protein showed a highly complex structure composed of a variety of amino acid. So a wide range of diversities is possible in protein structure to fulfill the diversity required in the genetic material for controlling the countless diversities in the characteristic of organism.

The controversy about the assignment of gene substance either to chromosomal protein or to nucleic acid, existed up to 1950 when finally it was unanimously accepted that the genetic information resides in the nucleic acids rather than in proteins.

More specifically, several elegant experiments showed that DNA is the genetic material of most microorganisms and higher organisms. Later on, RNA was found to be the genetic material of some viruses where DNA is absent.

Evidence of Genetic Material:

The concept that DNA or RNA is the genetic material of most organisms has been developed and supported by following evidence:

i. Direct evidence;

ii. Indirect evidence.

i. Direct Evidence:

(a) Transformation in Pneumococcus:

The first direct evidence showing that the genetic material is DNA rather than protein or RNA was published by O. T. Avery, C. M. Macleod and M. McCarthy in 1944. They discovered that the substance of the cell responsible for the phenomenon of transformation in the bacterium Diplococcus pneumoniae is DNA.

Transformation is the mode of exchange or transfer of genetic information (recombination) from one strain of bacterium to another strain of bacterium without involving any direct contact between them. The process of transformation was first discovered by Frederick Griffith in 1928.

This was called as Griffith's effect. The experiment of Griffith demonstrated transformation but he could not recognise the transforming principle.

Different strains of Pneumococci shows the genetic variability that can be recognised by existence of different phenotypes. Griffith initially conducted his experiment on two strains of pneumococci which were phenotypically distinct.

When they are grown artificially on nutrient agar medium, they form two types of colonies:

(1) Smooth and

(2) Rough.

The cells of strains forming smooth (S) colonies have a smooth glittering appearance due to presence of strain-specific polysaccharides (a polymer of glucose and glucuronic acid) capsule. Such strains are able to produce pneumonia and are called virulent.

The polysaccharide capsule is required for virulence since it protects the bacterial cell against phagocytosis by leucocytes. But the cells of stain lack this capsule and they produce dull rough (R) colonies. Such stains are termed as avirulent since they cannot produce pneumonia.

Therefore smooth (S) and rough (R) phenotypic characteristic are directly related to the presence or absence of the capsule and this trait is known to be genetically determined.

Both S and R forms occur in several subtypes and are designated as S I, S II, S III, etc. and R I, R II, R III, etc., respectively, on the basis of antigen properties of the polysaccharides present in their capsule. This property ultimately depends on the genotype of the cell.

The experiments of Griffith (Fig. 12.1) are briefly described below stepwise on the basis of his observation:

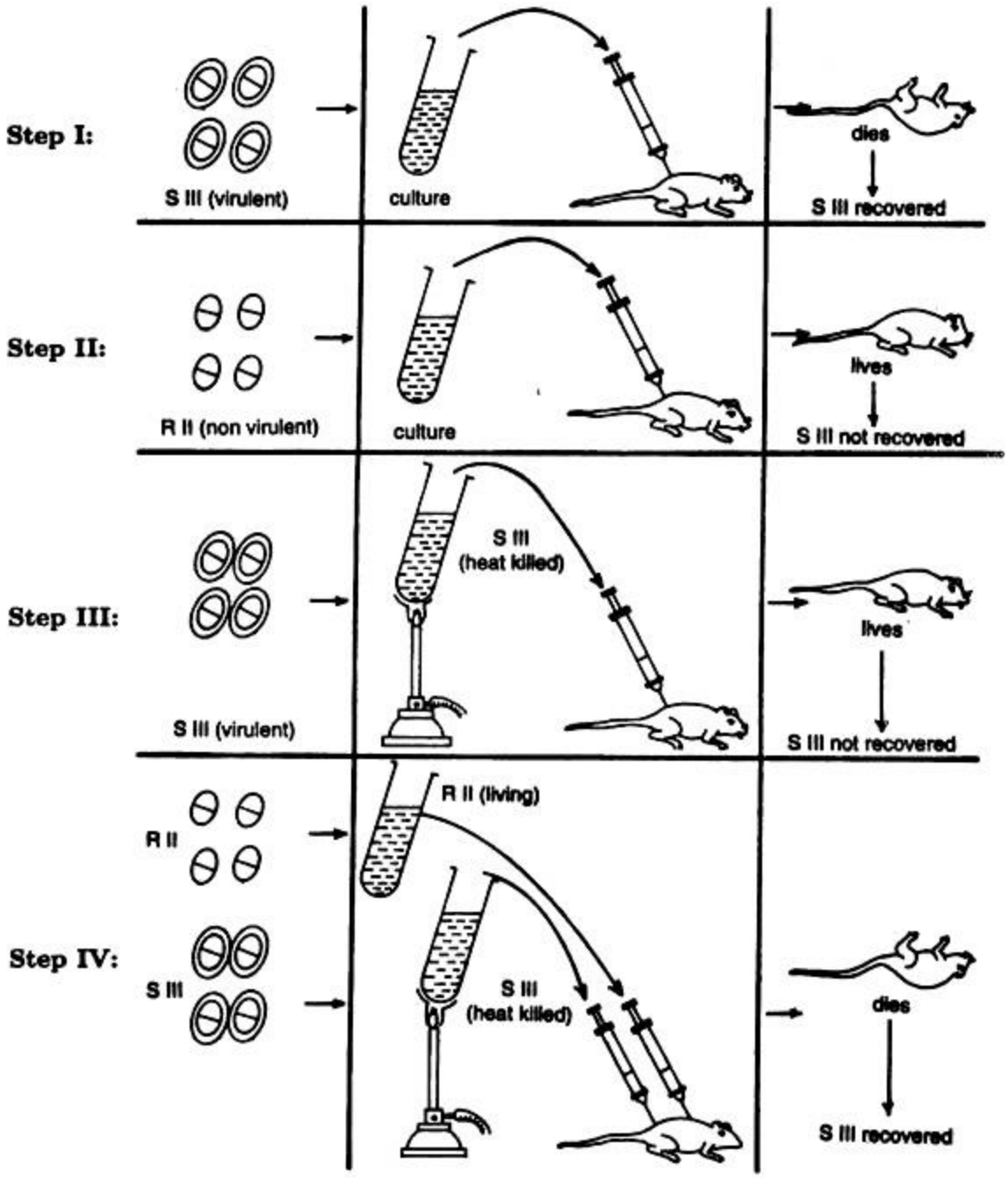


Fig. 12.1: Griffith's experiment.

Step I:

Griffith injected live cells of the virulent type III S into mice, all the mice died due to pneumonia and live type III S cells were recovered from the serum of blood of the dead bodies of mice.

Step II:
When live cells of the avirulent type II R were injected into a separate group of mice, none of the mice died and live type II R were isolated from the serum of blood of all mice.

Step III:
When mice were injected with heat-killed virulent type III S pneumococci alone, again none of the mice died, showing that virulence is lost after heat-killing.

Step IV:
When mice were injected heat-killed type III S pneumococci (virulent when alive) plus live type II R pneumococci (non-virulent), some of the mice died due to pneumonia; pneumococci cells isolated from the dead mice were of the type III S.

Since it is known that non-capsulate type R cells can mutate back to virulent encapsulated type S cells, the resulting cell will be type II S, not type III S. Thus the transformation of non- virulent type II R cells to virulent type III S cells cannot be explained by mutation, rather, some component of the dead type III S cells (the “transforming principle”) must convert living type II R cells to type III S.

This leads to a change in the trait of cells and helps to bring some new characters in the transformed cell. Hence the transforming principle must contain some genetic material.

(b) Transforming Principle is DNA:
Avery, Macleod and McCarthy experimentally proved that the transforming principle was DNA. They showed that if DNA extract from type IIS pneumococci was mixed with type IIR pneumococci in vitro, some of the pneumococci were transformed to type III S.

But DNA extract from type III S may be contaminated with a few molecules of proteins, RNA and this contaminating protein and RNA may be responsible for transformation from type II R to type III S. So Avery, Macleod and McCarthy demonstrated the most definitive experiment using bacterial culture system and specific enzymes that degrade DNA, RNA and protein.

In separate experiments (Fig. 12.2) DNA extract from type III S cells was treated with:

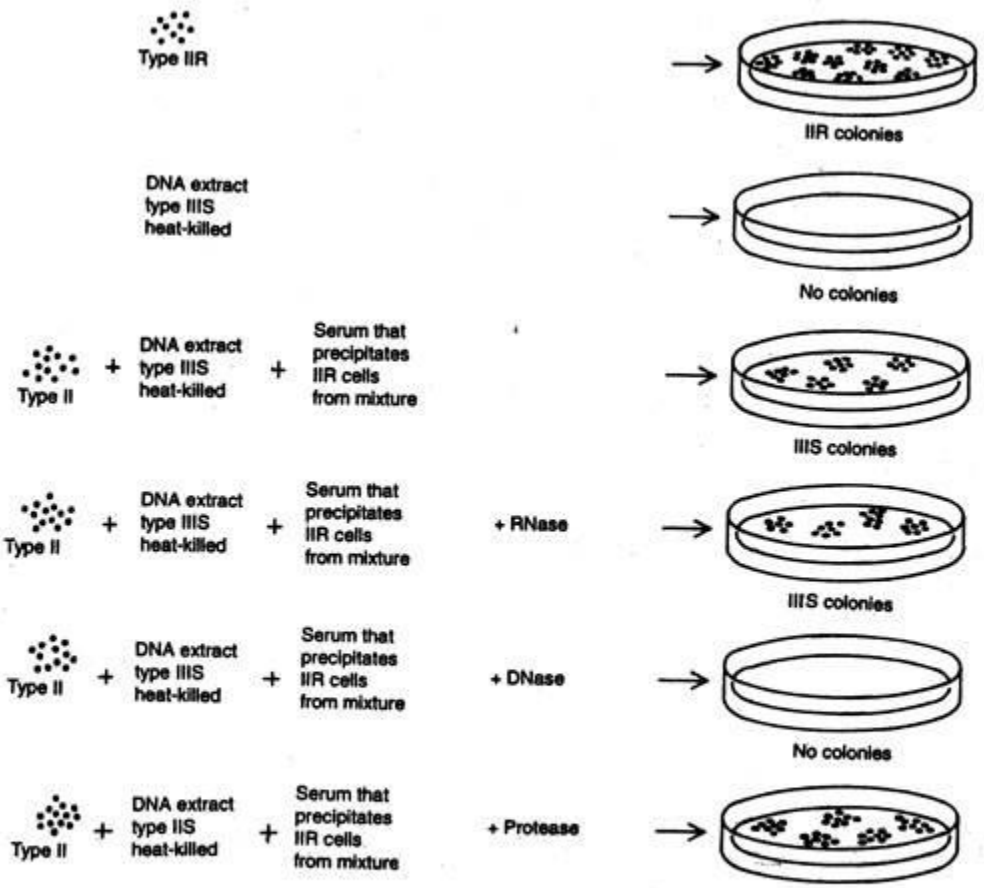


Fig. 12.2: Avery, Macleod and McCarthy's experiment.

i. DNAse which degrades DNA.

ii. RNAse which degrades RNA.

iii. trypsin, a protease which degrades protein; and then tested the treated DNA extract for its ability to transform type II R pneumococci to type III S.

iv. They observed that the treatment with RNAse or trypsin had no effect on the ability of the DNA extract to transform type II R to type III S. But DNAse treatment destroyed the transforming activity of the DNA preparation and II R cells were not transformed into III S cells. This established beyond any doubt that DNA is the transforming principle.

But these findings of Avery and co-workers was not able to explain the molecular mechanism of transformation. So some biologists were unable to appreciate the significance of these findings and they were hesitant in accepting them as an incontrovertible evidence for DNA being the genetic material.

(c) The Experiment of Hershey-Chase:

Another direct evidence indicating that DNA is the genetic material was demonstrated by A. D. Hershey and M. Chase in 1952. They first studied the life cycle of T_2 bacteriophages of Escherichia coli.
T_2 bacteriophages are composed of hexagonal box-like head coat and tail made of protein. The DNA is packed inside the proteinaceous head coat.
Bacteriophages are acellular and do not contain cytoplasm, organelles and nucleus. The DNA is present in high pure form and is not associated with RNA and protein. Bacteriophage are obligate parasite since they can reproduce only within bacterium using as host cell.

Hershey and Chase showed that, during the reproduction of bacteriophages, the DNA of the phage entered the host cell whereas most of the protein head and tail portion remained absorbed on the outside of the cell. Hence it is strongly implied that the genetic information necessary for viral reproduction was present in DNA.

DNA contains phosphorus (P) but no sulphur (S), while proteins of head and tail contains sulphur (S) but no phosphorus.

Hershey and Chase were able to specifically label the phage DNA by its growth in a medium containing the radioactive isotope of phosphorus, i.e., ^{32}P in place of normal phosphorus. Similarly, in another group of phage, the protein coats were labelled by growth in a medium containing radioactive sulphur ^{35}S in place of normal sulphur.
E. coli cells were then infected with ^{32} P labelled T_2 bacteriophage and, after being allowed 10 minutes for infection, they were agitated in a blender which sheared off the phase coats. The phase coats and the infected cells were then separated by centrifugation (Fig. 12.3).

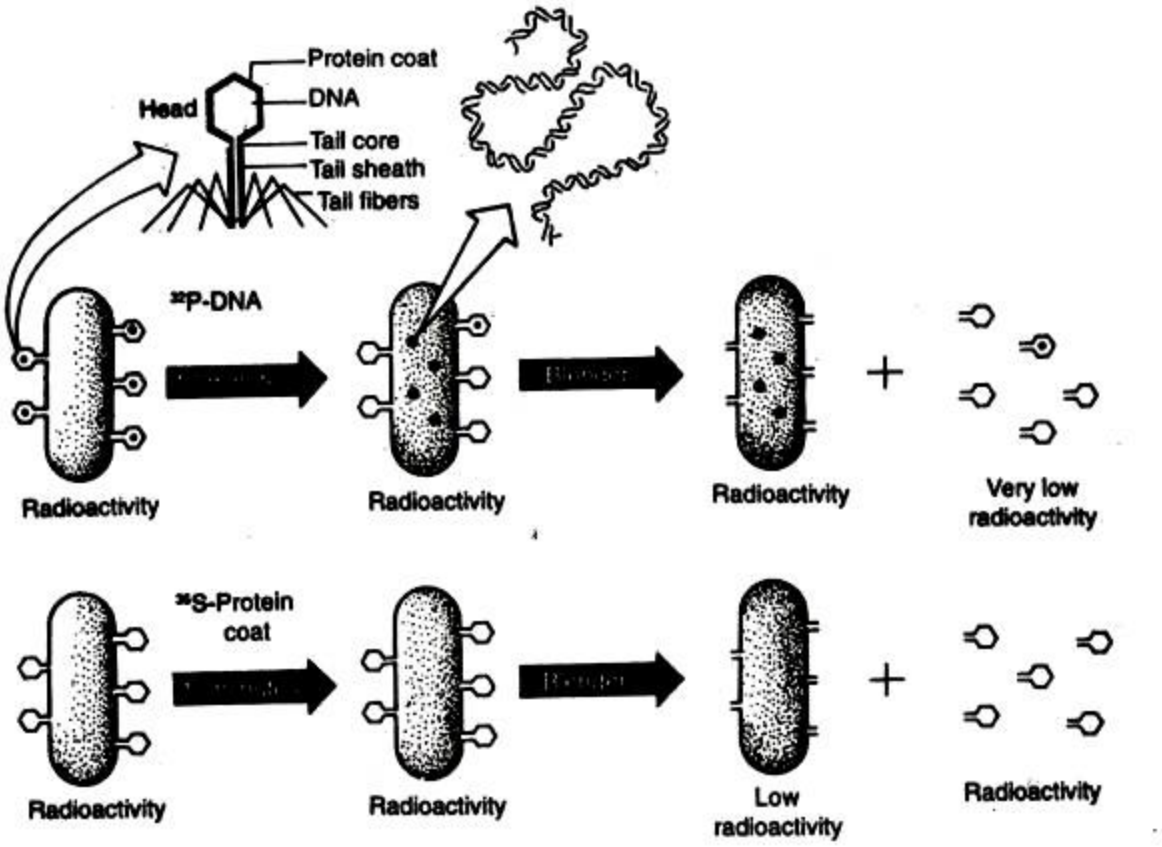


Fig. 12.3: Hershey-Chase' experiment.

Radioactivity was then measured of the sediment and in phage coat suspension. Most of the radioactivity was found in the cells. When the same experiment was done using phage with ^{35}S-labelled protein coat, most of the radioactivity was found in the suspension of phage coats; very little entered the host cells.

Since phage reproduction (both DNA synthesis) occurs inside the infected cells, and, since only the phage DNA enters the host cell, the DNA—not the protein—must carry the genetic information. As a result of the findings of Hershey and Chase led to the universal acceptance of DNA as the genetic material.

(d) Bacterial Conjugation:
Another direct evidence for DNA as the genetic material comes from the phenomenon of conjugation of bacteria. Conjugation was discovered by J. Lederberg and E. I.

Tatum in 1946. During conjugation DNA is transferred from a donor bacterial cell to a recipient bacterial cell through conjugation tube that forms between them. The donor cell—also called male—contains a F factor or fertility factor whereas recipient cells—or female cells, lack F factor, i.e., F^- cell (Fig. 12.4).

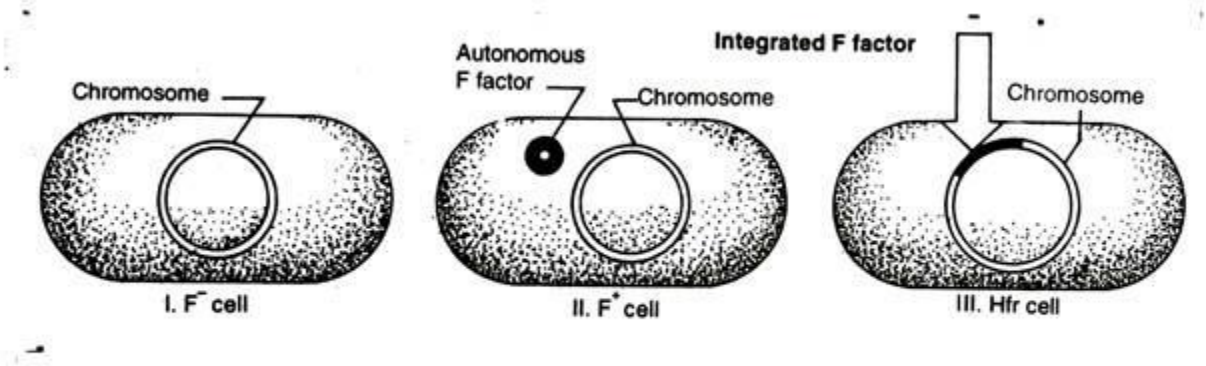


Fig. 12.4: Three forms of an E. coli with respect to the F factor, I. F^- cell, no F fator; II F^+ cell; III Hfr cells.

n male, the F factor can exist in two different states:
(1) Autonomous state and

(2) Integrated state (Fig. 12.4) where the F factor is inserted with main DNA and thus the male become Hfr male (Fig. 12.5).

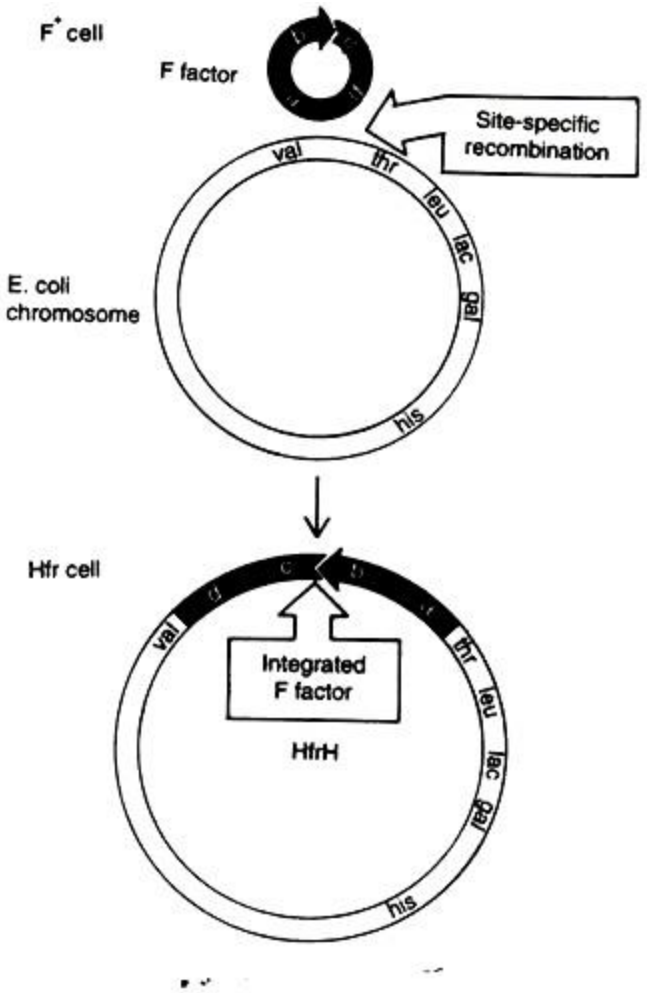


Fig. 12.5: Conversion of a F^+ cell to a Hfr cell by the integration of the F factor into the chromosome.

The F factor is a mini-circular DNA molecule. Beadle and Tatum observed that when a F^+ male E. coli cell conjugated with a F^- female E. coli cell, an unidirectional transfer of F^+ factor of male cell to F^- or female cell took place, so that the latter was covered into a F^+ or male strain.

The F factor is actually a fragment of DNA molecule that replicates during transfer. Thus mixing a population of F^+ or Hfr cells with a population of F^+ cells results in virtually all the cells in the new population becoming F^+ or Hfr (Fig. 12.6).

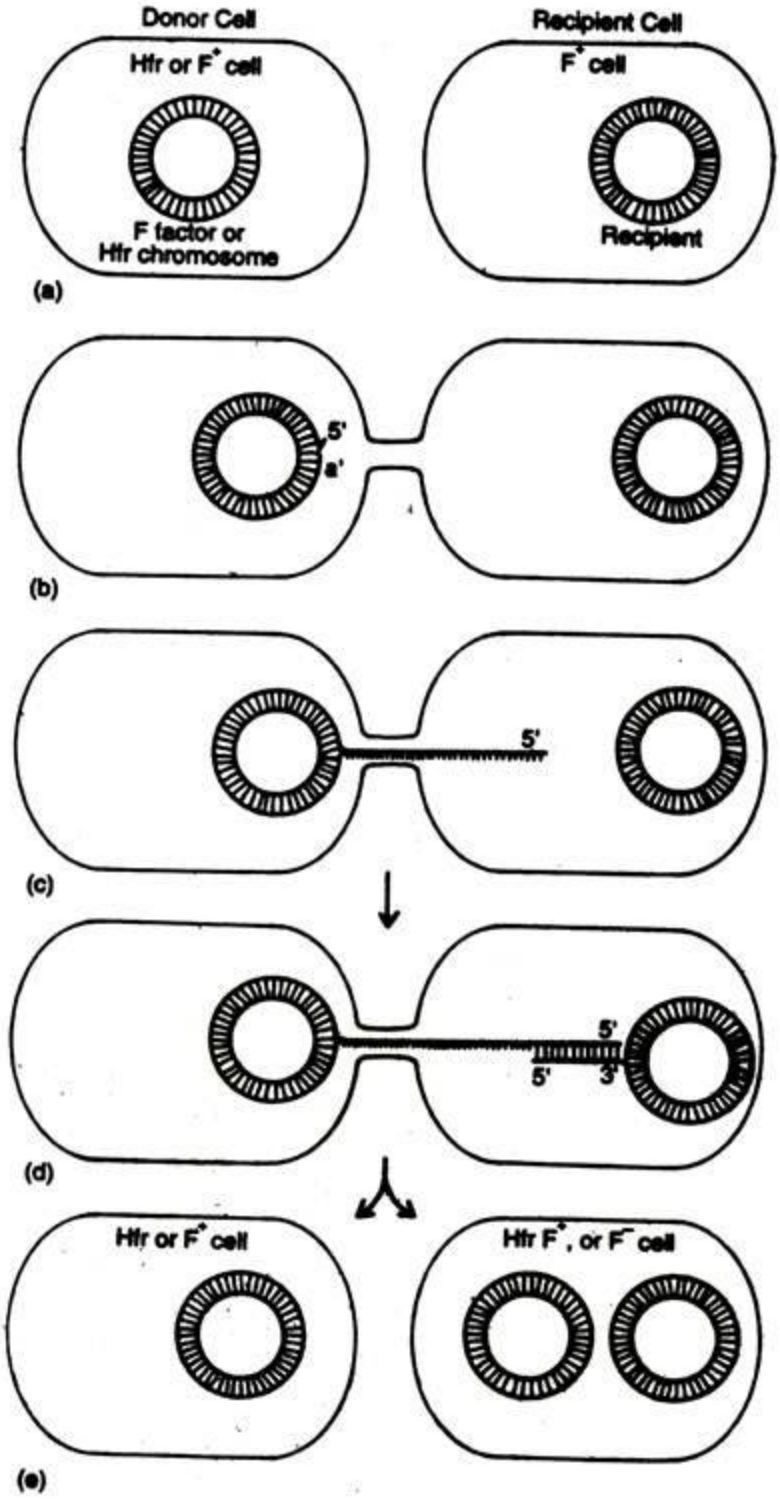


Fig. 12.6: Mechanism of DNA transfer during bacterial conjugation.

ii. Indirect Evidence:

The fact that DNA is the genetic material of higher organisms has also been supported by some indirect evidences:

(a) Localisation:

The genetic substance should have a fixed location within the cell. If it has no fixed location, then the genes are not able to function properly. It is known that the DNA, as a gene substance, is always located primarily within the chromosome in the nucleus of the eukaryotic cell.

The specific location of DNA can be studied in situ by the Feulgen reaction—which is regarded as the most specific one for DNA. Feulgen staining stains chromosome magenta colour against the clear cytoplasmic background. This technique has shown that DNA entirely remains restricted to the chromosome and it forms the major component of chromosomes.

(b) Stability:

Various macromolecules present within the cell are continuously being anabolised and catabolized. But this is not desirable for a genetic substance containing valuable hereditary information. If it happens, the genetic function will be lost. Of all the macro- molecules in the cell, DNA is the metabolically stable.

(c) Sensitivity to Mutagens:

Mutation is an important characteristic feature of the genetic material. The agents capable of inducing mutation are called mutagens. Different types of radiation (UV-ray, X-ray, *y*-ray) and a variety of chemical compounds acts as mutagens. When the cells of an organism are treated with mutagens, they cause a change in the structure of gene.

Since genes are DNA segments, the gene mutation include changes in the number and arrangement of nucleotide. Sometimes mutation causes the breaks in the DNA molecule. The changes in the DNA

structure ultimately reflect the changes of the organism's hereditary character. Therefore sensitivity of DNA to mutagens is an indirect evidence for DNA being the genetical materials.

(d) DNA Content:

One of the striking features of the genetic material is the correlation between DNA content and the number of chromosome sets. Various quantitative assay methods have shown that diploid cells contain twice as much DNA as do haploid cells of the same species (Table 12.1).

Table 12.1: DNA content and pioidy (Pollister, et. al., 1951)

Cells	Mean DNA Fegulen content (Picograms)	Presumed chromosomes set (Ploidy)
Spermatids	1.68	Haploid (n)
Liver	3.16	Diploid (2n)
Liver	6.30	Tetrapolid (4n)
Liver	12.80	Octoploid (8n)

Similarly, tetraploid and octaploid cells contains four times and eight times DNA as compared with DNA content of the haploid cells. Even the DNA content of sperm cells shows a correlation with the same or different tissues of different organisms (Table 12.2).

Table 12.2: DNA contents in picograms (gram $\times 10^{12}$) of various cells

Organism	Kidney	Liver	Erythrocytes	Sperm
Chicken	2.4	2.5	2.5	1.3
Bovine	6.4	6.4	—	3.3
Carp	—	3.0	3.3	1.6
Human	5.4	5.6	—	2.5

Thus the parallelism of behaviour in DNA and chromosome indirectly indicates that DNA is the genetic material.

(e) RNA as Genetic Material:

The genome of viruses may be DNA or RNA. Most of the plant viruses have RNA as their hereditary material. Fraenkel-Conrat (1957)

conducted experiments on tobacco mosaic virus (TMV) to demonstrate that in some viruses RNA acts as genetic material.

TMV is a small virus composed of a single molecule of spring-like RNA encapsulated in a cylindrical protein coat. Different strains of TMV can be identified on the basis of differences in the chemical composition of their protein coats. By using the appropriate chemical treatments, proteins and RNA of RNV can be separated.

Moreover, these processes are reversible by missing the protein and RNA under appropriate conditions—reconstitution will occur yielding complete infective TMV particles. Fraenkel-Conrat and Singer took two different strains of TMV and separated the RNAs from protein coats, reconstituted hybrid viruses by mixing the proteins of one strain with the RNA of the second strain, and vice versa.

When the hybrid or reconstituted viruses were rubbed into live tobacco leaves, the progeny viruses produced were always found to be phenotypically and genotypically identical to the parental type from where the RNA had been isolated (Fig. 12.7). Thus the genetic information of TMV is stored in the RNA and not in the protein.

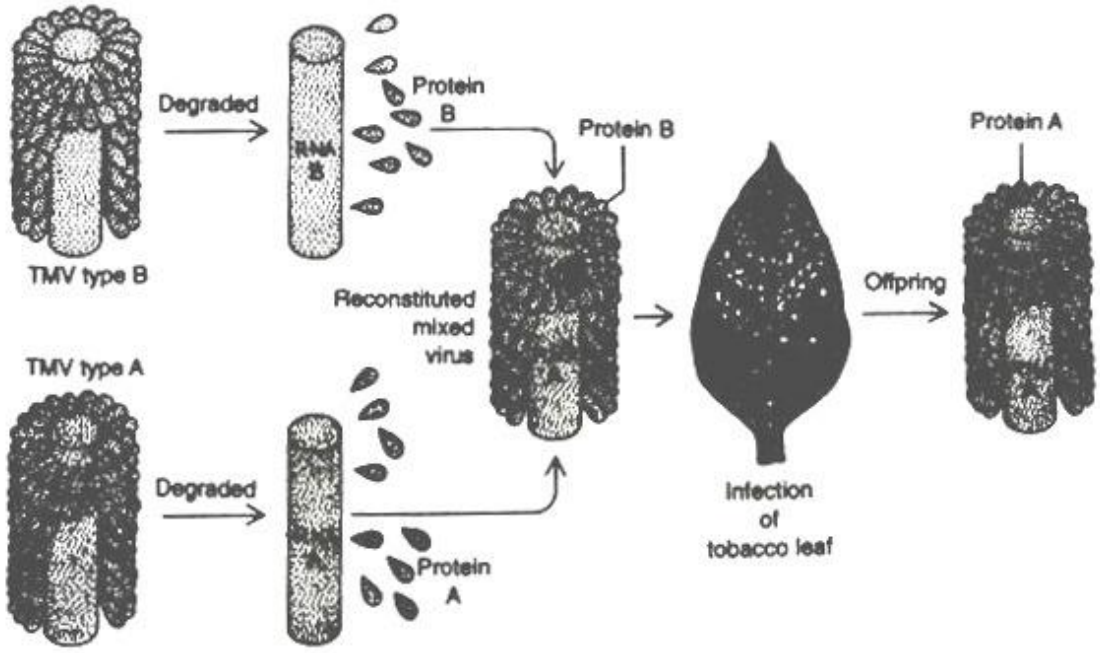


Fig. 12.7: Experimental evidence that the genetic material of tobacco mosaic virus (TMV) is RNA, not protein.

Transcription in Prokaryotes and Eukaryotes

Transcription in Prokaryotes:

In prokaryotic organisms transcription occurs in three phases known as initiation, elongation and termination.

RNA is synthesized by a single RNA polymerase enzyme which contains multiple polypeptide subunits. In E. coli, the RNA polymerase has five subunits: two α, one β, one β' and one σ subunit ($\alpha_2\beta\beta'\sigma$). This form is called the holoenzyme. The σ subunit may dissociate from the other subunits to leave a form known as the core enzyme.

These two forms of the RNA polymerase have different roles in transcription:

(i) Initiation:

Transcription cannot start randomly but must begin specifically at the start of a gene. Signals for the initiation of transcription occur in the promoter sequence which lies directly upstream of the transcribed sequence of the gene. The promoter contains specific DNA sequences that act as points of attachment for the RNA polymerase.

In E. coli, two sequence elements recognized by the RNA polymerase known as the -10 sequence and the -35 sequence arc present. The exact sequences can vary between promoters but all conform to an overall pattern known as the consensus sequence. The σ subunit of the RNA polymerase is responsible for recognizing and binding the promoter, probably at the -35 Box.

In the absence of the σ subunit the enzyme can still bind to DNA but binding is more random. When the enzyme binds to the promoter it initially forms a closed promoter complex in which the promoter DNA remains as a double helix. T he enzyme covers about 60 base pairs of the promoter including the -10 and -35 boxes. To allow transcription

to begin, the double helix partially dissociates at the – 10 box, which is rich in weak A-1 bonds to give an open promoter complex.

The σ subunit then dissociates from the open promoter complex leaving the core enzyme. At the same time the first two ribonucleotides bind to the DNA, the first phosphodiester bond is formed and transcription is initiated (Fig. 7.7).

(ii) Elongation:

During elongation the RNA polymerase moves along the DNA molecule melting and unwinding the double helix as it progresses. The enzyme adds ribonucleotides to the 3′ end of the growing RNA molecule with the order of addition determined by the order of the bases on the template strand.

In most cases, a leader sequence of variable length is transcribed before the coding sequence of the gene is reached. Similarly, at the end of the coding sequence a noncoding trailer sequence is transcribed before transcription ends.

During transcription only a small portion of the double helix is unwound at any one time. The unwound area contains the newly synthesized RNA base-paired with the template DNA strand and extends over 12-17 bases.

The unwound area needs to remain small because unwinding in one region necessitates over-winding in adjacent regions and this imposes strain on the DNA molecule. To overcome this problem, the RNA is released from the template DNA as it is synthesized allowing the DNA double helix to reform (Fig. 7.8).

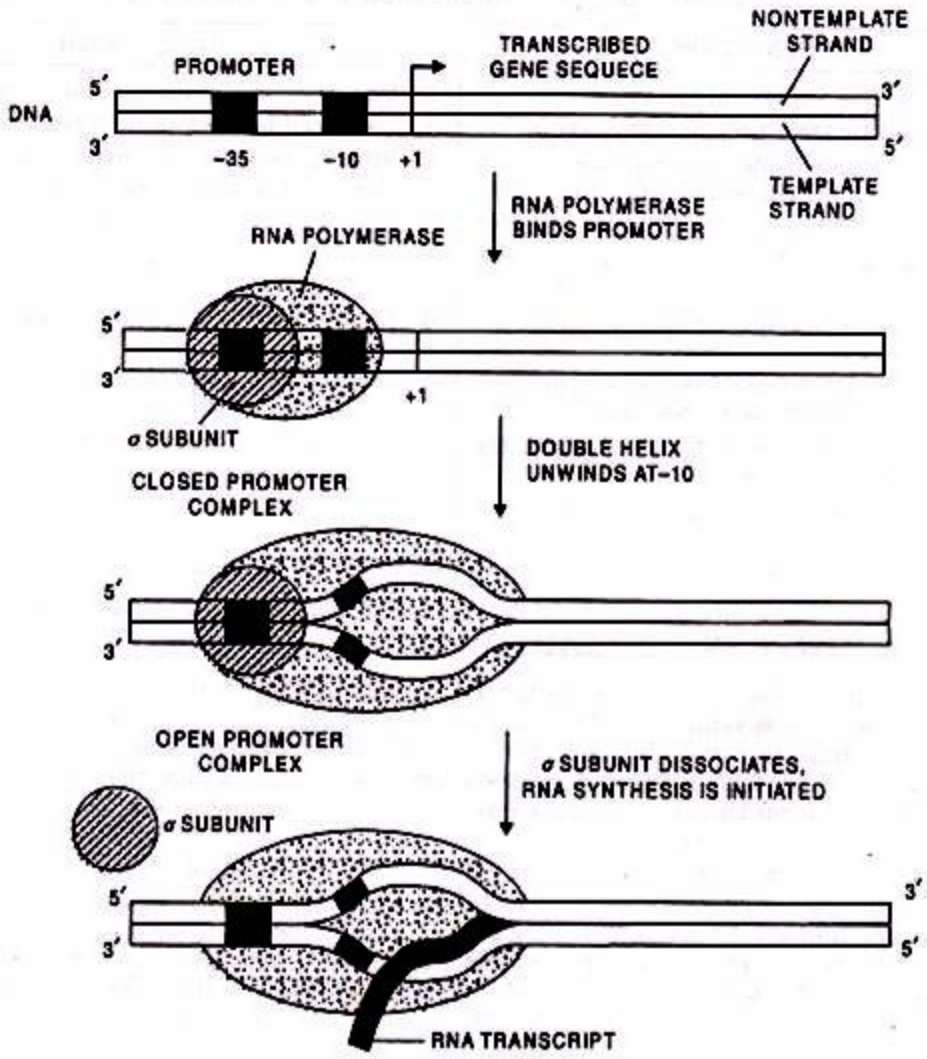


Fig. 7.7. Initiation of transcription in prokaryotes.

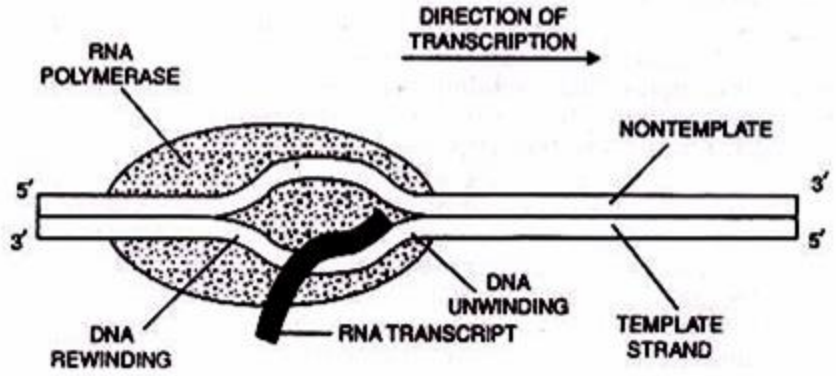


Fig. 7.8 Transcription elongation in prokaryotes.

(iii) Termination:

The termination of transcription occurs non-randomly and takes place at specific points after the end of the coding sequence. In E. coli,

termination occurs at sequences known as palindromes. These are symmetrical about their middle such that the first half of the sequence is followed by its exact complement in the second half.

In single-stranded RNA molecules this feature allows the first half of the sequence to base pair with the second half to form what is known as a stem-loop structure (Fig. 7.9). These appear to act as signals for termination. In some cases the stem-loop sequence is followed by a run of 5-10 As in the DNA which form weak A-U base pairs with the newly synthesized RNA.

It is thought that the RNA polymerase pauses just after the stem-loop and that the weak A-U base pairs break causing the transcript to detach from the template. In other cases the run of As is absent and a different mechanism occurs based on binding of a protein called Rho (ρ) which disrupts base-pairing between the template and the transcript when the polymerase pauses after the stem-loop. The termination of transcription involves the release of the transcript and the core enzyme which may then re-associate with the σ subunit and go on to another round of transcription

In many bacteria, genes of related functions are grouped together in operons. An operon acts as a single transcription unit and thus produces polycistronic mRNA. In eukaryotes, only monocistronic mRNAs are generally produced.

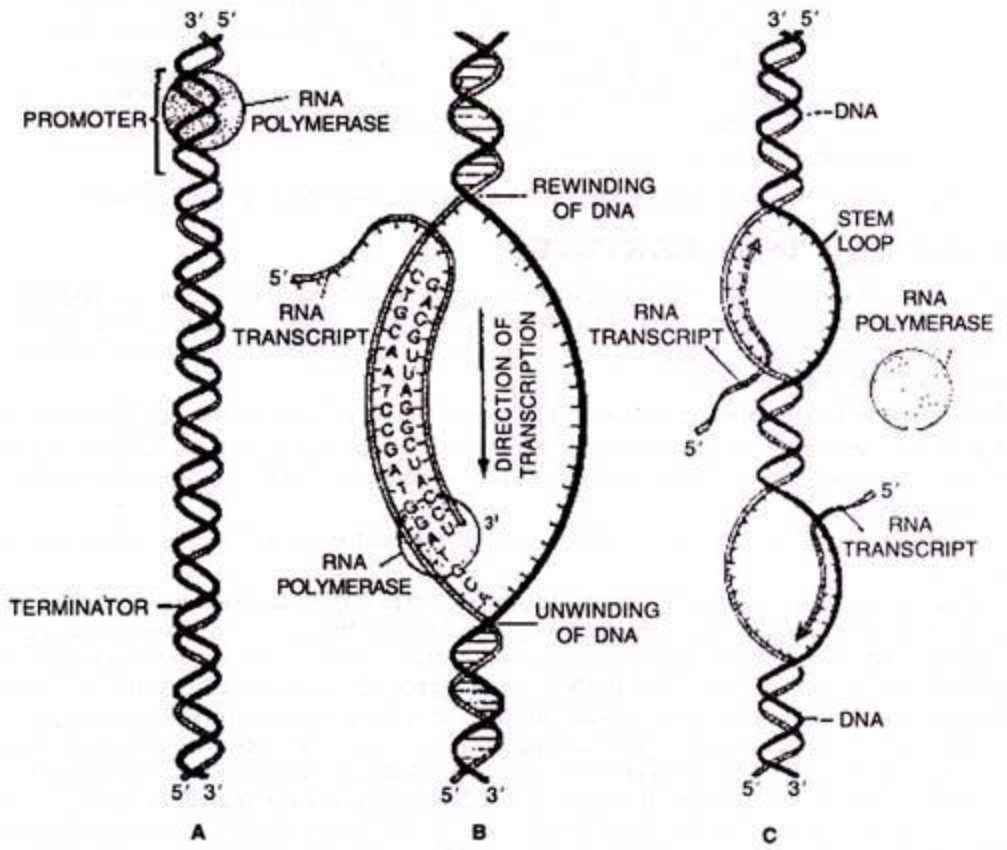


Fig. 7.9 A–C. A summarized diagram of general process of transcription in prokaryotes : **A.** Binding to promoter and RNA chain initiation : **B.** Elongation of RNA polypeptide chain, **C.** Termination of transcription.

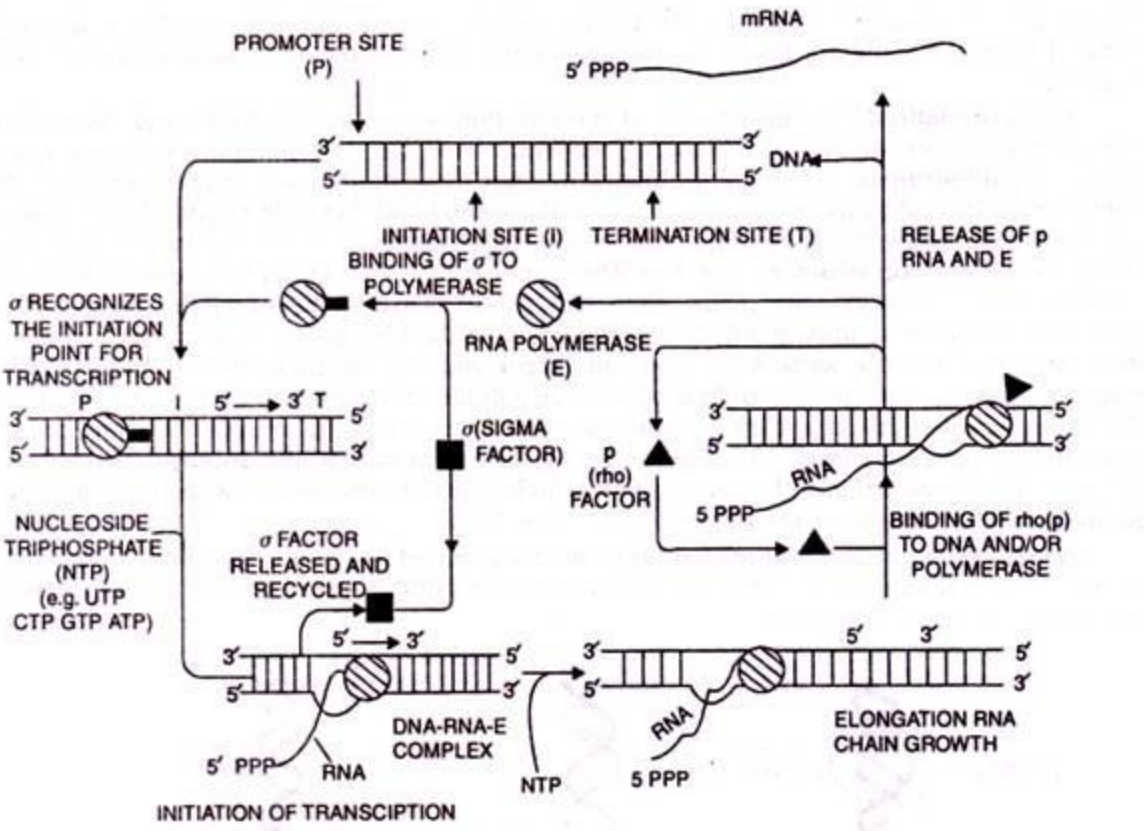


Fig 7.10. Diagrammatic presentation of the synthesis of RNA by E.coli polymerase

Transcription in Eukaryotes:

Transcription occurs in eukaryotes in a way similar to prokaryotes. However, initiation is more complex, termination does not involve stem-loop structures and transcription is carried out by three enzymes (RNA polymerases I, II and III) each of which transcribes a specific set of genes and functions in a slightly different way.

RNA polymerase I transcribes genes encoding three of the four ribosomal RNAs (18S, 28S and 5.8S). RNA polymerase II enzyme transcribes genes that encode proteins. Binding of RNA polymerase II to its promoter involves several different DNA sequence elements and a number of proteins called transcription factors. RNA polymerase III transcribes a set of short genes that encode transfer RNAs and the 5S ribosomal RNA.

Unlike the situation in prokaryotic genes, transcription in eukaryotes occurs within the nucleus and mRNA moves out of the nucleus into

the cytoplasm for translation. The initiation and regulation of transcription is more extensive than prokaryotes. Another major difference between prokaryotes and eukaryotes lies in the fact that the mRNA in eukaryotes is processed from the primary RNA transcript, a process called maturation.

Initially at the 5′ end a cap (consisting of 7-methyl guanosine or 7 mG) and a tail of poly A at the 3′ end are added (Fig. 7.11) The cap is a chemically modified molecule of guanosine triphosphate (GTP). The primary eukaryotic mRNA transcript is much longer and localised into the nucleus, when it is also called heterogenous nuclear RNA (hnRNA) or pre- mRNA.

The eukaryotic primary mRNAs are made up of two types of segments; non-coding introns and the coding exons. The introns are removed by a process called RNA splicing. Of a pair of small nuclear ribonucleoprotein (SnRNPs pronounced “snurps”), one binds to 5′ splice site and the other to 3′ splice site.

A spliceosome forms because of interaction between SnRNPs and other proteins. This spliceosome uses energy of ATP to cut the RNA, releases the introns and joins two adjacent exons to produce mature mRNA. Besides, these two post-transcriptional modifications, RNA editing may also take place before translation begins.

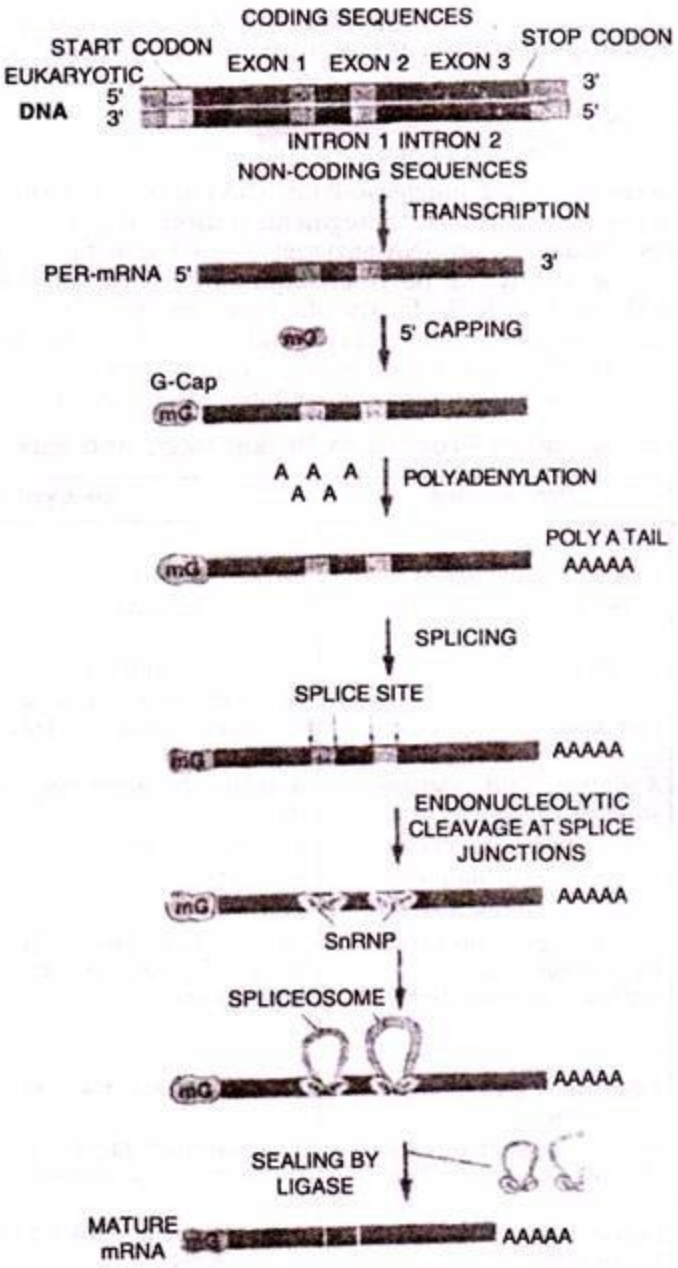


Fig. 7.11. Transcription in eukaryotes

Post-Transcription Processing:

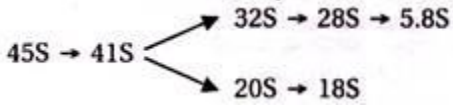
Primary transcript is often larger than the functional RNAs. It is called heterogeneous or hnRNA especially in case of mRNA.

Post-transcription processing is required to convert primary transcript into functional RNAs.

It is of four types:

(i) Cleavage:
Larger RNA precursors are cleaved to form smaller RNAs. Primary transcript of rRNA is 45S in eukaryotes.

It is cleaved to form the following:



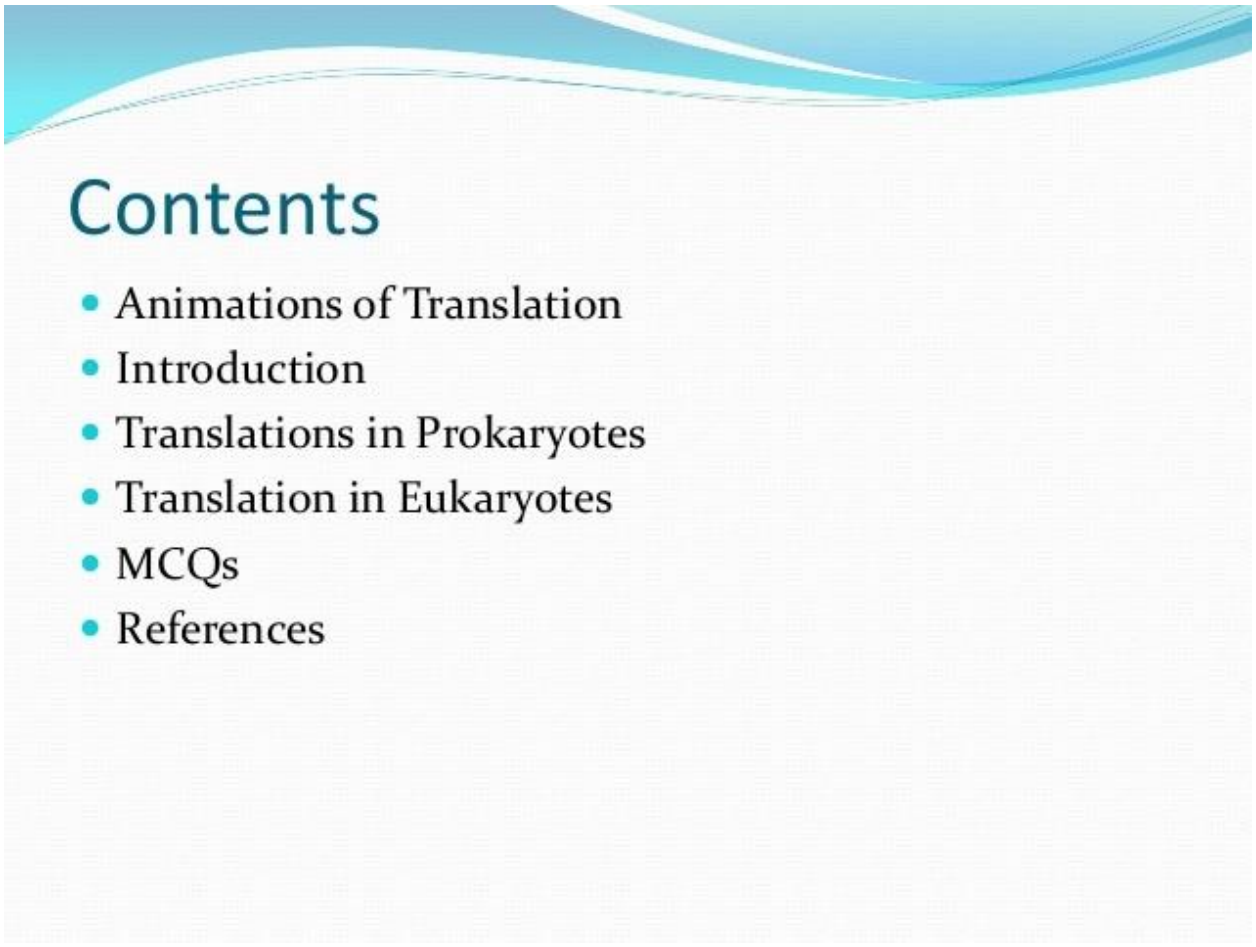
Primary transcript is cleaved by ribonuclease-P (an RNA enzyme) to form 5-7 tRNA precursors,

(ii) Splicing:
Eukaryotic transcripts possess extra segments (introns or intervening sequences). They are removed by nucleases. Ribozyme (an-RNA enzyme) is a self splicing intron involved in some of these reactions as well as catalysing polymerisation.

(iii) Terminal Additions:
Additional nucleotides are added to the ends of RNAs for specific functions, e.g., CCA segment in tRNA, cap nucleotides at 5′ end of mRNA or poly-A segments at 3′ end of mRNA.

(iv) Nucleotide Modifications:
They are most common in tRNA-methylation (e.g., methyl cytosine, methyl guanosine), deamination (e.g., inosine from adenine), dihydrouracil, pseudouracil, etc.

Translation in Prokaryotes and Eukaryotes



Introduction to Translation

- **Translation:** The biosynthesis of a protein or a polypeptide inside a living cell.

- In process of translation the language of nucleotides sequence on mRNA is translated in to the language of amino acid sequence.

- It occur in cytoplasm where ribosomes are located
- It is a universal process

Cont……

- In translation ,messenger RNA is decoded to produce a specific polypeptide
- This uses mRNA sequence as a template to guide the synthesis of a chain of amino acid that form protein
- Many types of transcribed RNA, such tRNA,rRNA,snRNA are not necessarily translated to amino acid sequence

Steps of Translation

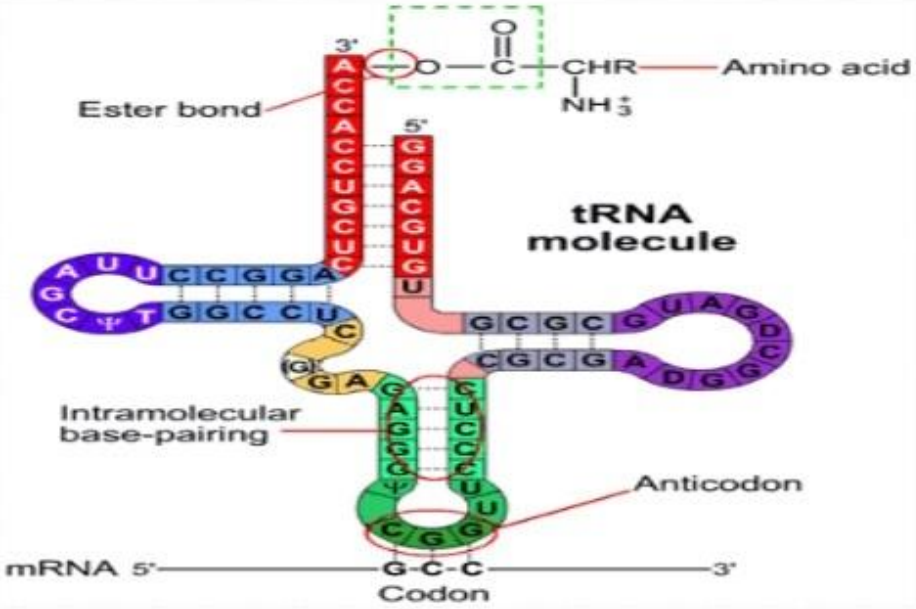
- Translation proceeds in four phases
  - Activation
  - Initiation
  - Elongation and
  - Termination

Most studies do not include activation as a step of translation

Activation

- In activation, the correct amino acid is covalently bonded to the correct tRNA
- While technically this not a step in translation, it is required for translation to proceed
- The amino acid is joined by its carboxyl group to the 3` OH of tRNA by an ester bond with help of ATP
- When tRNA has an amino acid linked to it, it is termed as "charged"

Cont.....


Ester bond
3'
5'
Amino acid
CHR
NH3+
tRNA molecule
Intramolecular base-pairing
Anticodon
mRNA 5'
3'
Codon

Initiation

- Prokaryotes initiation require the large and small subunits, the mRNA, the initiator tRNA and three initiation factors (IF-1, IF-2, IF-3) and GTP.
- IF-3 binds to the free 30s subunit, this help to prevent large subunit binding to it without mRNA and forming an inactive ribosome
- IF-2 complexed with GTP and IF-1 binds to small subunit . It will assist the charged initiator tRNA to bind

Cont.....

- The 30s subunit attached to a mRNA molecule making use of the ribosomal binding site on mRNA
- The initiator tRNA can then bind to the complex by base pairing of its anticodon with AUG codon on mRNA
- At this point,IF3 can be released, as its role in keeping the subunits apart are complete
- This complex is called *30s initiation complex*

Cont....

- The 50s subunit can now bind, which displace IF1 and IF2, and the GTP is hydrolysed in this energy consuming step

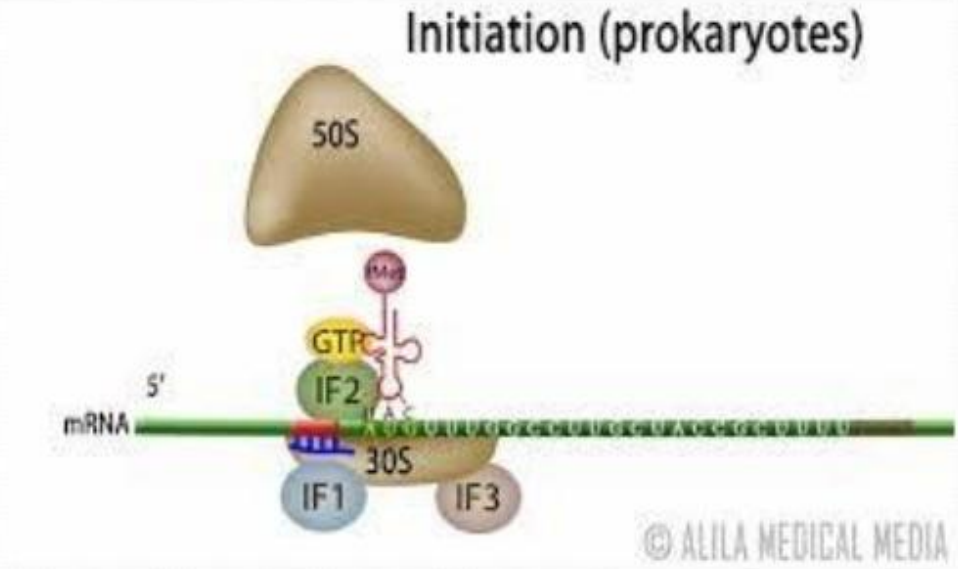
- This complex is called *70s initiation complex*

Cont.....

- The assembled ribosome has two tRNA binding sites
- These are called the A and P sites , for amino acyl and peptidyl sites and one site is E (exit site) for empty tRNA
- The A site is where incoming amino acyl tRNA molecule bind, and the p site is where the growing polypeptide chain is usually found
- The sites are in cleft of small subunit and contain adjacent codon that are being translated

Cont……

- One major of initiation is the placement of initiator tRNA in the P site
- It is the only tRNA that does this , as all other must enter the A site


Initiation (prokaryotes)
50S
GTP
IF2
5'
mRNA
30S
IF1
IF3
© ALILA MEDICAL MEDIA

Elongation

- With the formation of 70s initiation complex the elongation cycle can begin
- It involves three elongation factors EF-Tu, EF-Ts and EF-G, GTP, charged tRNA and the 70s initiation complex

Elongation is divided into 3 steps

1. ***Amino acyl tRNA delivery.***

- EF-TU is required to deliver the amino acyl tRNA to A site and energy is consumed in this step by hydrolysis of GTP
- The released EF-Tu GDP complex is regenerated with the help of EF-TS

- In the EF-Tu EF-Ts exchange cycle EF-Ts displaces the GDP and replace itself by GTP
- The resultant EF-Tu.GTP complex is now available to bind another amino acyl tRNA and deliver it to ribosome

Cont....

- All amino acyl tRNA can form this complex with EF-Tu except the initiator tRNA

Cont.....

2 .Peptide bond formation.

- After aminoacyl-tRNA delivery ,the A and P sites are both occupied and the two amino acids that are to be joined are close to each other

- The peptidyl transferase activity of the 50s subunit can now form a peptide bond between the two amino acids

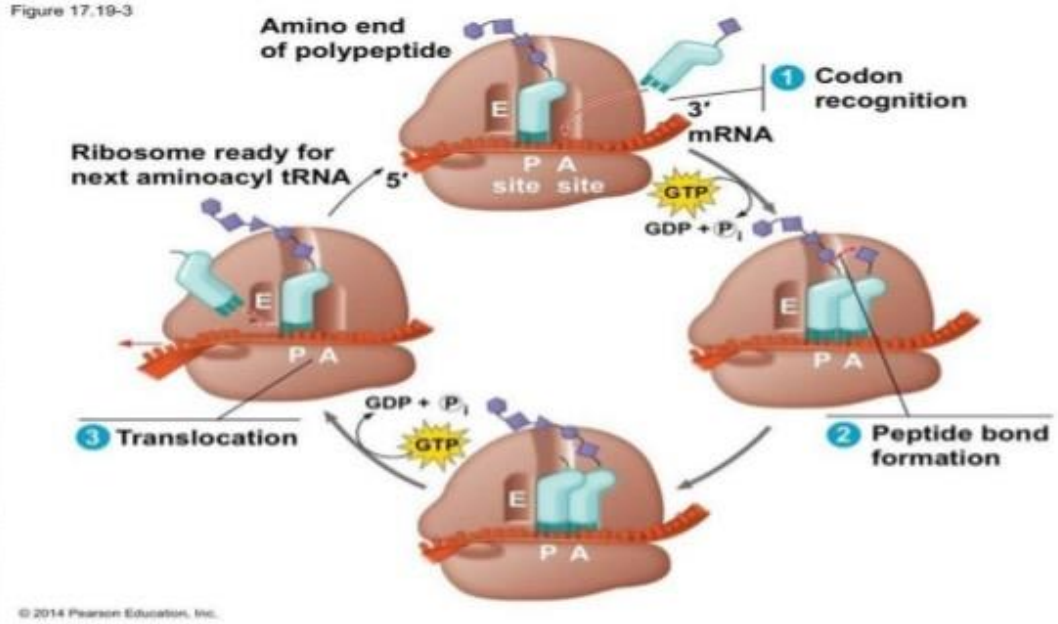
3. ***Translocation***

- A complex of EF-G(translocase) and GTP binds to the ribosome and ,is an energy consuming step, the discharged tRNA is ejected from the P site, the peptidyl-tRNA is moved from A site to P site

- The mRNA moves by one codon relative to one codon to the ribosome

- GDP and EF-G are released . A new codon is now present in the vacant site

Figure 17.19-3



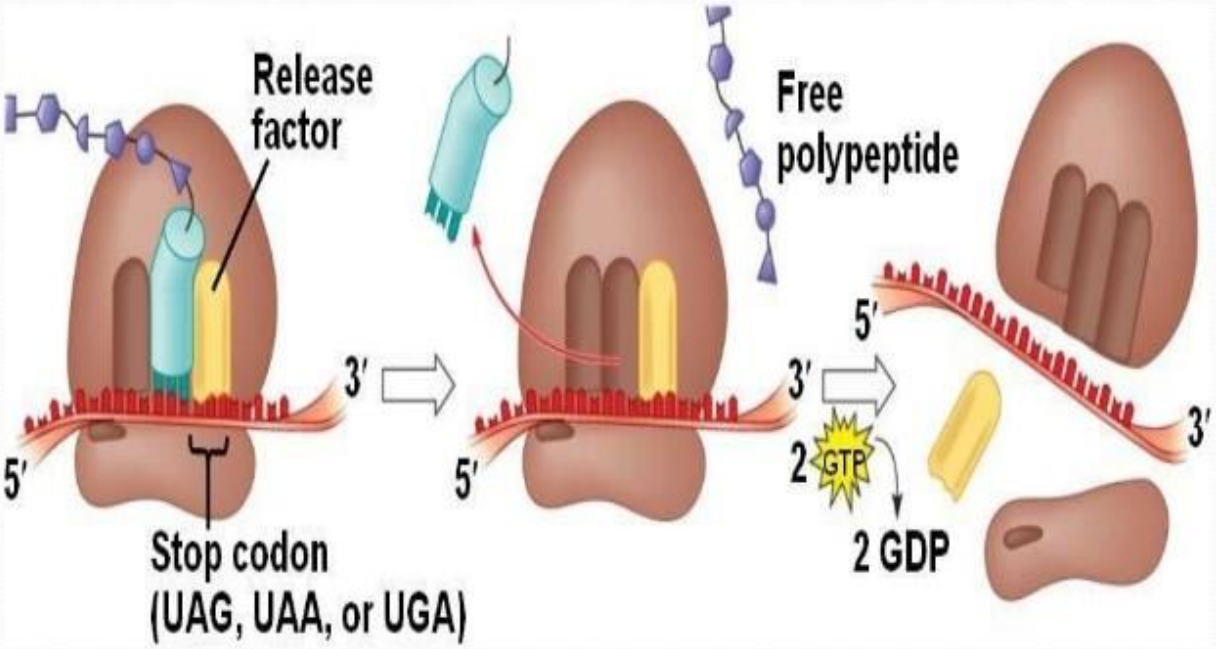
Termination

- Termination of translation happens when the A site of the ribosome faces a stop codon (UUA,UGA or UGA)
- When this happens , no tRNA can recognize it, but a releasing factor can recognize the stop codons and causes the release of polypeptide chain
- In prokaryotes once a stop codon occupies the A site, three termination or release factor (RF1,RF2,RF3) contribute to the hydrolysis of peptidyl-tRNA bond

Cont.....

- Release the free polypeptide and last uncharged tRNA from P site
- The dissociation of the 70 s into 30s and 50s subunits
- RF1 binds A site and release the polypeptide and uncharged tRNA
- RF2 releases the RF1 from A site and release itself as well from translation binding site(present on large subunit)
- RF3 function unknown
- Another factor called Ribosomal releasing factor causes the dissociation of 70s complex

Cont....


Release factor
3′
5′
Stop codon (UAG, UAA, or UGA)
Free polypeptide
3′
5′
5′
3′
2 GTP
2 GDP

Translation in Eukaryotes

Eukaryotic Translation

- In prokaryotic cell, transcription and translation are coupled, that is, translation begins while the mRNA is still being synthesized. In a eukaryotic cell, transcription occur in the nucleus , and translation occur in the cytoplasm.

- Translation process in eukaryotes involve
  - Activation (not essentially the step of translation. This occur the same way as in prokaryotes)
  - Initiation
  - Elongation and
  - termination

1. Initiation

- The initiation of translation in eukaryotes is complex, involving at least 10 initiation factors (eIFs) and is divided into three steps :

a) Formation of 43s preinitiation complex.
b) Formation of 48s initiation complex.
c) Formation of 80s initiation complex.

a. Formation of 43s preinitiation complex

- A ternary complex containing met-tRNA and eIF-2 bound to GTP attaches to 40s ribosomal subunit to form 43s preinitiation complex.
- The presence of eIF-3 and eIF-1A stabilizes this complex.

b.Formation of 48s initiation complex

- The binding of mRNA to 43s preinitiation complex results in formation of 48s initiation complex.
- eIF-4f is formed by the association of eIF-4G, eIF-4A with eIF-4E
- The eIF-4F(referred to as cap binding protein) binds to the cap of mRNA.

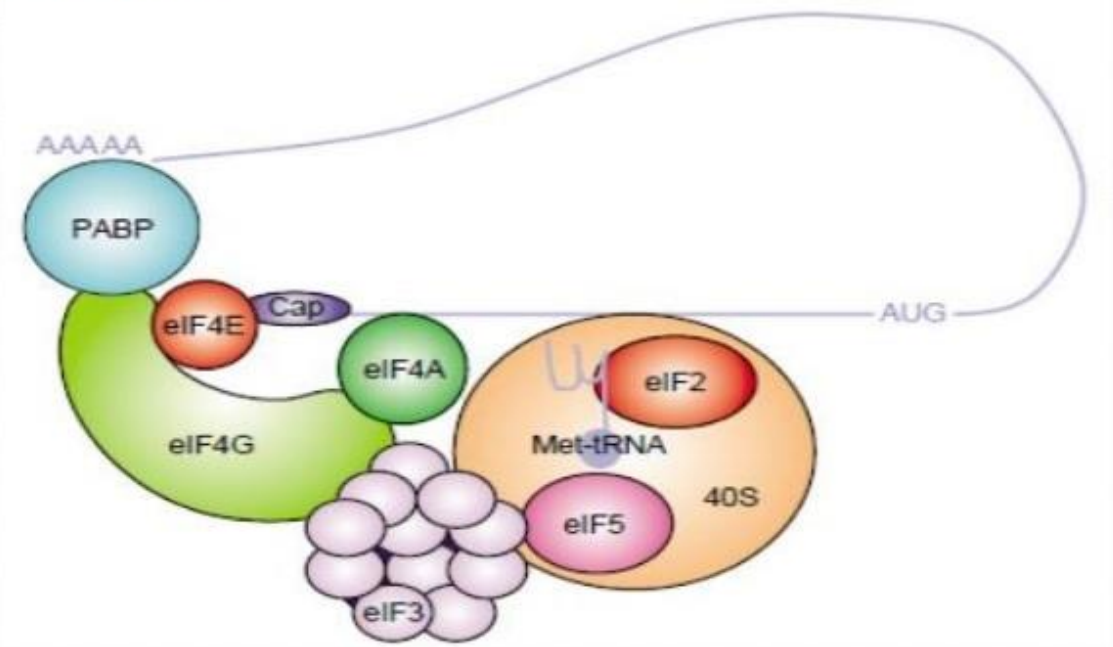
Cont.....

- Then eIF-4A and eIF-B binds to mRNA and reduces its complex structure.
- This mRNA is then transferred to 43s complex
- The ribosomal initiation complex scans the mRNA for identification of appropriate initiation codon
- 5'-AUG is the initiation codon

c.Formation of 80s initiation complex

- 48s initiation complex binds to 60s ribosomal subunit to form 80s initiation complex
- The binding involves hydrolysis of GTP(bound to eIF-2)
- This step is facilitated by the involvement of eIF-5
- As the 80s complex is formed, the initiation factors bound to 43s initiation complex are released and recycled

Cont.....


AAAAA
PABP
eIF4E
Cap
AUG
eIF4A
eIF2
eIF4G
Met-tRNA
40S
eIF5
eIF3

2. Elongation

- Ribosomes elongate the polypeptide chain by sequential addition of amino acids
- The amino acid sequence is determined by the order of the codons in the specific mRNA
- Elongation, a cyclic process involving certain elongation factors(EFs)
- Elongation may be divided into three steps

a. Binding of aminoacyl-tRNA to A-site
b. Peptide bond formation
c. translocation

a.Binding of Aminoacyl t-RNA to A-site

- The 8os initiation complex contains met tRNA in the P-site and A- site is free
- Another aminoacyl-tRNA is placed in the A site
- This require proper codon recognition on mRNA and involvement of EF-1a and supply of energy by GTP
- The aminoacyl –tRNA is placed in the A-site ,EF-1a and GDP are recycled to bring another aminoacyl-tRNA

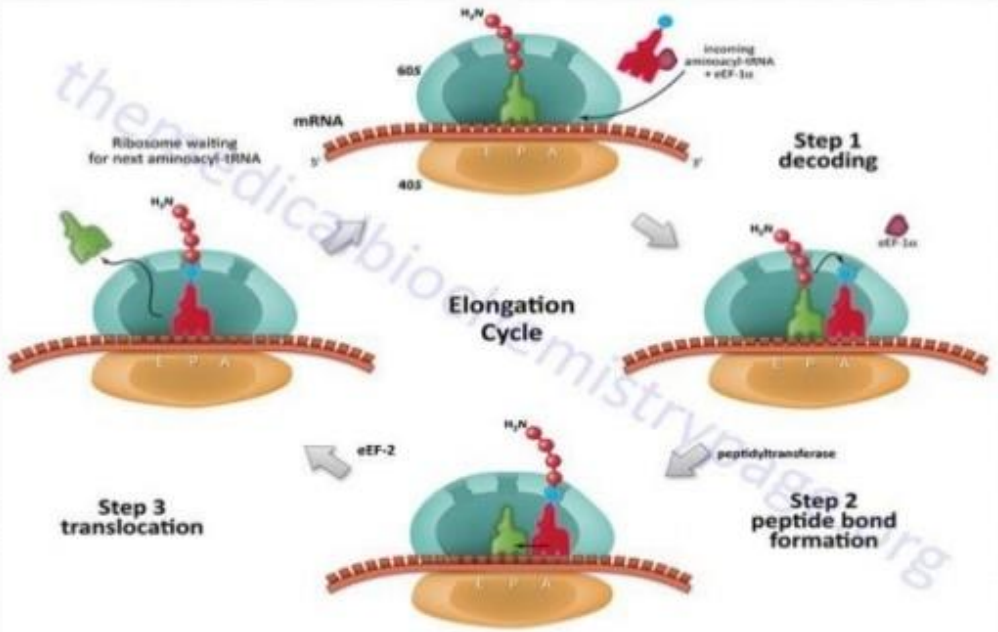
b. Peptide bond formation

- The peptidyl transferase catalyzes the formation of peptide bond
- Net result of peptide bond formation is the attachment off the growing peptide chain to the tRNA in A-site

c. Translocation

- The ribosome moves to the next codon of mRNA (towards 3' end)
- This process is called translocation, involves the movement of growing peptide chain from A-site to P-site
- Translocation require EF-2 and GTP
- GTP get hydrolyzed and supplies energy to move mRNA
- EF-2 and GTP complex recycles for translocation

Cont....


mRNA
Ribosome waiting for next aminoacyl-tRNA
Step 1 decoding
Elongation Cycle
peptidyltransferase
Step 2 peptide bond formation
eEF-2
Step 3 translocation

3. Termination

- One of the stop signals (UGA,UAA and UGA) terminates the growing polypeptide
- When the ribosome encounter s a stop codon, there is no tRNA available to bind to the A site of ribosome
- Instead a release factor binds to it
- In eukaryotes eRF1 recognizes all the three stop codons, and eRF3 stimulate the termination events

Cont…

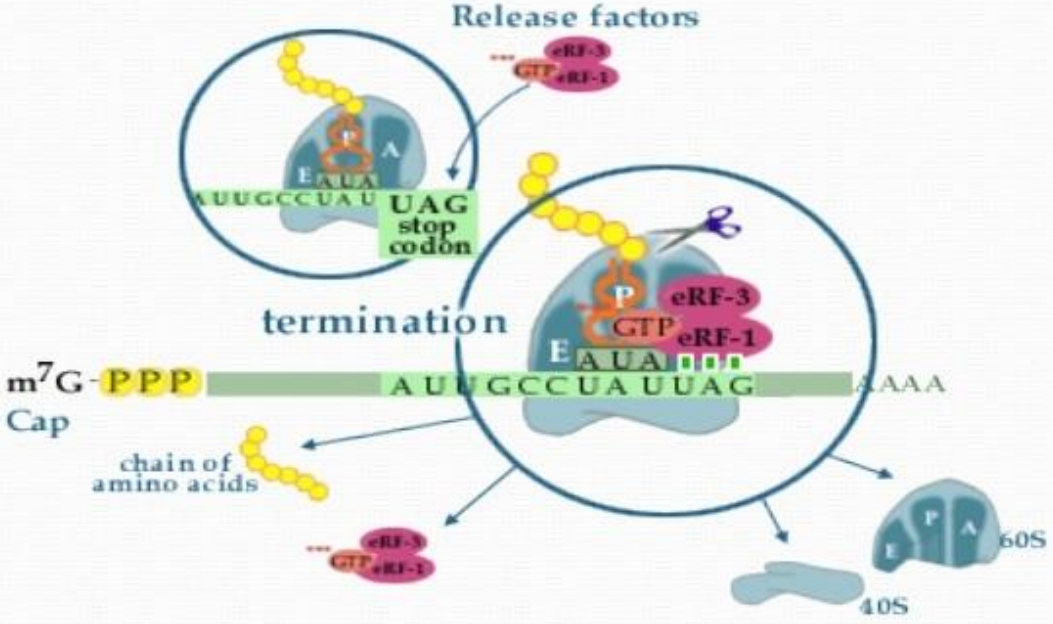
- Once the release factor binds, the ribosome unit falls apart

  - releasing the large and small subunits

  - the tRNA carrying the polypeptide is also released, freeing up the polypeptide product.

  -

Cont....


Release factors
eRF-3
GTP
eRF-1
P
A
E
A U A
A U U G C C U A U
UAG
stop
codon
termination
P
eRF-3
GTP
eRF-1
E
A U A
m7G-PPP
A U U G C C U A U U A G
AAAA
Cap
chain of
amino acids
eRF-3
GTP
eRF-1
60S
40S