(ii) **Incomplete linkage :** In majority of cases, the homologous chromosomes undergo breakage and reunion during gametogenesis. During reunion the broken pieces of the chromatids are exchanged, producing some nonparental or new combinations. Therefore, the linkage is rendered incomplete. The phenomenon of interchange of chromosome segments between two homologous chromosomes is called crossing over. Incomplete linkage is very common and has been studied in almost all the organisms.

(3) **Linkage groups :** All the genes which are linked with one another, form a linkage group. Since linked genes are present in the same chromosome, the number of linkage group in an animal or plant is equal to the haploid number of chromosomes present in its cells. This hypothesis was given by *Sutton* and was proved by experiments on Drosophila by T.H. Morgan.

Examples	Linkage groups
Drosophila	There are four linkage groups corresponding to the four pairs of chromosomes
Zea mays	The ten chromosome pairs of maize correspond with its ten linkage groups
Pisum sativum	The garden pea plant has seven pairs of chromosome and the same number of linkage groups
Man	Man has 23 linkage groups corresponding to 23 pairs of chromosomes

(4) **Strength of linkage :** The strength of linkage between any two pairs of linked genes of a chromosome depend upon the distance between them. Closely located genes show strong linkage, while genes widely located show weak linkages.

(5) **Factor affected to linkage :** Linkage is affected by the following factors.

(i) **Distance :** Closely located genes show strong linkage while genes widely located show weak linkage.

(ii) **Age :** With increasing age the strength of linkage decreases.

(iii) **Temperature :** Increasing temperature decreases the strength of linkage.

(iv) **X-rays :** X-rays treatment reduces the strength of linkage.

(6) **Significance of linkage**

(i) Due to linkage new recombinants are formed.

(ii) It helps in maintaining the valuable traits of a newly developed variety.

(iii) It helps locating genes on chromosome.

(iv) It disallows the breeders to combine all the desirable traits in a single variety.

Important Tips

- *Cinderella of genetics is Drosophila melanogaster.*
- ***H. Lamprecht (1961)*** *demonstrated that seven genes used by* ***Mendel*** *belonged to only four linkage groups (not seven as thought earlier).*
- *Blue green algae and bacteria contain one linkage group.*
- *Two dominant nonallelic genes are 50map unit apart then the linkage will be absent.*
- *Linkage decrease frequency of hybridization.*
- *In order to remain linked the distance between two genes should not increase beyond 40 map units.*
- *Linkage was first studied in Lathyrus-odoratus.*
- *Drosophilla was first animal for which a linkage map was constructed.*
- *Law of linkage is an exception to* ***Mendel's law***.

Crossing over.

Introduction : The process by which exchange of chromosomal segment take place is called crossing over. Crossing over may be defined as "*the recombination of linked genes*" brought about as a result of interchange of corresponding parts between the chromatid of a homologous pair of chromosomes, so as to produce new combination of old genes. The term was given by *Morgan* and *Cattle.* Janssen (1909) observed chiasmata during meiosis-I (Prophase). Morgan proposed that chiasmata lead to crossing over by breakage and reunion of homologous chromosomes. Crossing over results in new combination while non-cross over result in parental type, which leads to variations.

(1) **Kinds of crossing over**

(i) **Somatic crossing over :** It is found in somatic cells *e.g.*, *Curt stern* in *Drosophila* and *Potnecorvo* in *Aspergillus nidulans* shown somatic crossing over *i.e.* mitotic crossing over.

(ii) **Germinal crossing over :** It is found in germinal cells during gametogenesis. This is also known as meiotic crossing over.

(iii) **Single cross over :** It takes place at one point only on the non-sister chromatids, only 2 chromatids are involved.

(iv) **Double cross over :** It is the formation of 2 chiasmata in the same chromosome independent of each other. In a double cross over the genes lying outside the crossed regions will retain their original association.

(v) **Multiple cross over :** It is formed when more than 2 chiasmata are formed. It is very rare.

(vi) **Two-stranded crossing over :** It takes place before splitting of homologous chromosomes so all the four resultants are recombinants.

(vii) **Four stranded crossing over :** It takes place after splitting of homologous chromosomes only 2 non-sister chromatids take part in crossing over resulting in 2 parental and 2 recombinant types.

(2) **Crossing over and chiasma :** There are two views extended to explain the relationship between crossing over and chiasma formation. They are summarised here under.

(i) **Chiasma type theory :** According to Janssen, 1909 the act of crossing over is followed by chiasma formation. He suggests that the crossing over takes place at the pachytene stage and the chiasma appear at diplotene.

(ii) **Classical theory :** According to Sharp, 1934, crossing over is the result of chiasma formation. According to this view, the chiasma are organised at pachytene and crossing over takes place at diplotene stage. On the basis of evidence available from molecular biology, that is untenable and hence rejected.

(3) **Mechanism of crossing over :** There are different views put forward to explain the mechanism of crossing over.

(i) **Copy choice hypothesis :** According to *Belling,* 1928 the chromomeres represent the genes joined by interchromomeric regions. The chromomeres duplicate first and then the interchromomeric regions. The synthesis of these regions may occur in such a way that the chromomeres of the chromatid of a homologue get connected of the chromatid of the other homologue at a specific location. As a result, the adjacent chromatids of a pair of homologue are exchanged.

(ii) **Precocity hypothesis :** According to *Darlington,* the pairing of homologues occurs to avoid singleness of a chromosome. The pairing need of a chromosome could be nothing less than the replication of DNA. The crossing over takes place due to torsion on chromosome created by coiling of the two homologues around each other.

In fact, the crossing over is the event which, precisely at molecular level, results in the formation of a hybrid DNA molecule. Such models have been proposed by White house, 1963 as also by Holliday, 1964. These models mainly elaborate the mechanism of breakage and reunion of DNA helicase.

(4) **Cross over value :** The percentage of crossing over varies in different materials. The frequency of crossing over is dependent upon the distance of two genes present on a chromatid.

(5) **Coincidence :** Coincidence or coefficient of coincidence is inverse measure of interface and is expressed as the ratio between the actual number of double cross over and the expected number of such double cross. That is:

$$\text{Coincidence} = \frac{\text{Actual number of double cross over}}{\text{Expected number of double cross over}}$$

(6) **Factors controlling frequency of crossing over :** Primarily, frequency of crossing over is dependent upon the distance between the linked genes, but a number of genetic, environmental and physiological factors also affect it. These are:

(i) **Temperature :** High and low temperature increase the frequency of crossing over.

(ii) **X-ray :** Muller has discovered that exposure to X-ray and other radiations increases the frequency of crossing over.

(iii) **Age :** The frequency of crossing over decreases with increasing age in female *Drosophila*.

(iv) **Chemicals :** Certain chemicals which act as mutagens do affect the frequency of crossing over. Gene mutations may affect the frequency of crossing over. Some increase the frequency, whereas some may decrease it.

(v) **Sex :** Crossing over in *Drosophila* males is negligible. Males of mammals also exhibit little crossing over. In silk-moth, crossing over does not occur in females.

(vi) **Chiasmata formation :** Chiasmata formation at one point discourages chiasmata formation and crossing over in the vicinity. This phenomenon is known as interference.

(vii) **Inversions :** Inversions of chromosome segments suppresses crossing over.

(viii) **Distance :** Distance between the linked genes is the major factor which controls the frequency of crossing over. The chances of crossing over between distantly placed genes are much more than between the genes located in close proximity.

Figure depicts that chance of crossing over between **a** and **c** are double as compared to the chances between **a** and **b** or **b** and **c**.

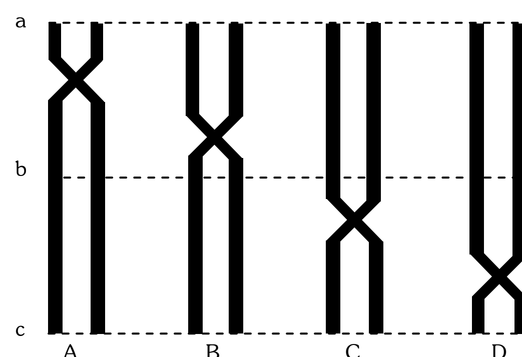


Fig : Diagram showing possibilities of crossing over between genes at different distances

(ix) **Cytoplasm :** Factor for crossing over is present in cytoplasm and is inherited to the offspring.

(x) **Nutritional effect :** Crossing over frequencies are affected by concentration of metallic ions, such as calcium and magnesium.

(xi) **Genotypic effect :** Crossing over frequencies between the same two loci in different strains of the same species show variation because of numerous gene differences.

(xii) **Chromosome structure effect :** Changes in the order of genes on a chromosome produced by chromosomal aberrations usually act as cross over suppressors.

(xiii) **Centromere effect :** Genes present close to the centromere region show reduced crossing over.

(xiv) **Interference :** If there are two doubles crossovers, then one crossover tries to influence the other by suppressing it. This phenomenon is called as interference. Due to this phenomenon, the frequency of crossing over is always lower than the expected.

(7) **Significance of crossing over :** This phenomenon is of great biological significance, which are as under:

(i) It gives evidence that the genes are linearly arranged on a chromosome. Thus, it throws light on the nature and working of the genes.

(ii) It provides an operational definition to a gene. It is deemed as the smallest heritable segment of a chromosome in the interior of which no crossing over takes place.

(iii) The crossing over is helpful in the chromosomal mapping. The percentage of crossing over is proportional to the distance between two genes.

(iv) It is the main cause of genetic variations. It's occurrence during the act of meiosis produces variations in the heritable characters of the gametes.

(v) This phenomenon has also found it's utility in breeding and evolving new varieties. The linkage of undesirable characters can be broken by temperature treatment, using X-ray or chemicals. Thus, new recombinants can be prepared.

Important Tips

- *Separation of a chromosome segment and its union to non-homologous chromosomes is called illegitimate crossing over.*
- *Two genes situated very close on the chromosome show hardly any crossing over.*
- *The most acceptable theory to explain crossing over is of Muller.*
- *Genes of Antibiotic resistance on bacteria are located on plasmid.*
- *Barr and Bertram (1949) discovered barr body in nerve cell of female cats. Later found in cells of human females*
- *Study of phenotype to DNA sequence in gene come under **forward genetics**.*
- *First chromosomal map of a plant was of maize, it was prepared by **Emerson**.*
- *Plotting of specific genes of the chromosomes is chromosomes map, linkage map, genetic map.*
- *The most important use in producing transgenic plants and animals is of **Reverse genetics**.*
- $tt \times tt \rightarrow Tt$ *, This type of inheritance is an example of **de-novo mutation**.*
- *Hugo de Vries worked on evening primrose in preparation of mutation theory.*
- ***E. coil** is an important material for genetic experiment because it is haploid in nature and also easilly cultured.*
- *The percentage of individuals with a given genotype exhibiting, the phenotype associated genotype is known as penetrance.*

Chromosomal maps.

On the basis of the following information, chromosomal maps have been prepared.

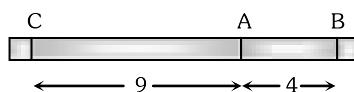
(1) The genes are linearly arranged on a chromosome and therefore, the gene order should be known.

(2) The percentage of crossing over between two genes is directly proportional to their distance. It is infact the index of their distance. The unit of crossing over has been termed as by *Haldane* as *centi Morgan* **(cM)**. One unit of map distance (cM) is therefore, equivalent to 1% crossing over. When chiasma is organised in between two gene loci, only 50% meiotic products shall be crossovers and 50% non-crossovers. Thus, the chiasma frequency is twice the frequency of cross over products *i.e.*, chiasma % = 2 (cross over %) or crossover %= ½ (chiasma %).

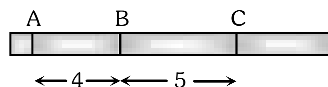
(3) Accordingly, *Sturtevant,* 1911 prepared the first chromosomal map. Infact this map is a line representation of a chromosome where the location of genes has been plotted as points at specific distances. These distances are proportional to their crossing over percentage. Suppose there are three genes on a chromosome say, **A B** and **C** which could be arranged as A, B, C; A, C, B or B, A, C. A three point test cross confirms as to which gene is located in the centre. By determining the crossing over value between A and B, B and C as also between A and C, the linkage maps can be prepared. Broadly speaking, a chromosomal map can be prepared from the following results of crossing over between the genes A, B and C:

(i) 4% crossing over taking place between A and B. (ii) 9% crossing over taking place between A and C.

Hence the genes be located as above and there should be 13% crossing over between B and C and the genes may be arranged as under:



If there is 5% crossing over between **B** and **C**, the genes are arranged in the following manner and there should be 9% crossing over between **A** and **C**.



(4) Uses of chromosomal map

(i) Finding exact location of gene on chromosomes.

(ii) Knowing recombination of various genes in a linkage group of chromosomes.

(iii) Predicting result of dihybrid and trihybrid cross.

Nucleic acids.

Two types of nucleic acids are found in the cells of all living organisms. These are DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid). The nucleic acid was first isolated by *Friedrich Miescher* in 1868 from the nuclei of pus cells and was named *nuclein.* The term nuclein was given by *Altman*.

DNA (Deoxyribonucleic Acid)

Introduction : Term was given by *Zacharis,* which is found in the cells of all living organisms except plant viruses,where RNA forms the genetic material and DNA is absent. In bacteriophages and viruses there is a single molecule of DNA, which remains coiled and is enclosed in the protein coat. In bacteria, mitochondria, plastids and other prokaryotes, DNA is circular and lies naked in the cytoplasm but in eukaryotes it is found in nucleus and known as carrier of genetic information and capable of self replication. Isolation and purification of specific DNA

segment from a living organism achieved by *Nirenberg H.Harries* is associated with DNA-RNA hybridization technique.

(1) **Chemical composition** : The chemical analysis has shown that DNA is composed of three different types of compound.

(i) **Sugar molecule** : Represented by a pentose sugar the deoxyribose or 2-deoxyribose which derived from ribose due to the deletion of oxygen from the second carbon.

(ii) **Phosphoric acid** : H_3PO_4 that makes DNA acidic in nature.

(iii) **Nitrogeneous base** : These are nitrogen containing ring compound. Which classified into two groups:

(a) **Purines** : Two ring compound namely as **Adenine** and **Guanine**.

(b) **Pyramidine** : One ring compound included Cytosine and Thymine in RNA uracil is present instead of Thymine.

Fig : Diagrammatic representation of Watson's and Crick's modal of DNA

Nucleosides : Nucleosides are formed by a purine or pyrimidine nitrogenous base and pentose sugar. DNA nucleosides are known as deoxyribosenucleosides.

Nucleotides : In a nucleotide, purine or pyrimidine nitrogenous base is joined by deoxyribose pentose sugar (D), which is further linked with phosphate (P) group to form nucleotides.

Composition of nucleoside and nucleotides of DNA and RNA

(D= Deoxyribose sugar, R = Ribose sugar, P = Phosphoric acid)

Base with its symbol	Nucleoside		Nucleotide	
	Formula	**Name**	**Formula**	**Name**
DNA Adenine = A	D – A	Deoxyandenosine	$D-A$ with P below	Deoxyandenosine monophosphate or Adenine deoxyribose nucleotide
Guanine = G	D – G	Deoxyguanine	$D-G$ with P below	Deoxygunine monophosphate or Guanine deoxyribose-nucleotide
Thyamine = T	D – T	Thymidine	$D-T$ with P below	Thymidine monophosphate or Thymidine nucleotide
Cytosine = C	D – C	Deoxycitidine	$D-C$ with P below	Deoxycytidine monophosphate or Cytosine deoxyribose nucleotide
RNA Adenine = A	R – A	Adenoside	$R-A$ with P below	Adenosine monophosphate or Adenine ribose nucleotide
Guanine = G	R – G	Guanosine	$R-G$ with P below	Guanosine monophosphate or Guanine ribose nucleotide
Uracil = U	R – U	Uridine	$R-U$ with P below	Uridine monophosphate or Uracil ribose nucleotide

Cytosine = C	R – C	Cytidine	$\begin{matrix} R-C \\ \mid \\ P \end{matrix}$	Cytidine monophosphate or Cytosine ribose nucleotide

(2) **Watson and Crick's model of DNA :** In 1953 Watson and Crick suggested that in a DNA molecule there are two such polynucleotide chains arranged antiparallal or is opposite directions i.e. one polynucleotide chain runs in $5' \rightarrow 3'$ direction, the other in $3' \rightarrow 5'$ direction. It means the $3'$ end of one chain lies beside the $5'$ end of other in right handed manner.

(i) **Important features of Watson and Crick double helical model of DNA**

There are important features of DNA double helix.

(a) The double helix comprises of two polynucleotide chains.

(b) The two strands (polynucleotide chains) of double helix are anti-parallel due to phosphodiester bond.

(c) Each polynucleotide chain has a sugar-phosphate 'backbone' with nitrogeneous bases directed inside the helix.

(d) The nitrogenous bases of two antiparallel polynucleotide strands are linked through hydrogen bonds. There are two hydrogen bonds between **A** and **T,** and three between **G** and **C.** The hydrogen bonds are the only attractive forces between the two polynucleotides of double helix. These serve to hold the structure together.

(e) The two polynucleotides in a double helix are complementary. The sequence of nitrogenous bases in one determines the sequence of the nitrogenous bases in the other. Complementary base pairing is of fundamental importance in molecular genetics.

(f) **Erwin Chargaff** (1950) made quantitative analysis of DNA and proposed "*base equivalence rule*" starting that molar concentration of A = T & G ≡ C or $\frac{A+G}{C+T}=1\&\frac{A+T}{G+C}$ which is constant for a species.

Fig : Helical structure of DNA as suggested by Watson and Crick

(g) Ten base pairs occur per turn of helix (abbreviated 10bp). The spacing between adjacent base pairs is 10Å. The helix is 20Å in diameter and DNA molecule found 360° in a clockwise.

(3) **Forms of DNA :** Five different morphological forms of DNA double helix have been described. These are A, B, C, D and Z forms. Most of these forms (except B, and Z) occur in rigidly controlled experimental conditions. Watson and crick model represents commonest form, Biotic-form (B-form or B-DNA) of DNA. Some DNA forms are inter convertible also. The differences in these DNA forms are associated with:

(i) The numbers of base pairs, present in each turn of DNA helix.

(ii) The pitch or angle between each base pair.

(iii) The helical diameter of DNA molecule.

(iv) The handedness of double helix. Which is mentioned in table.

Comparison of different types of DNA

Characters	*A*-DNA	*B*-DNA	*C*-DNA	*D*-DNA	*Z*-DNA
Base pair per turn of the helix	11	10	9.33	8	12
Tilt of pairs (γ) base	20.2^0	6.3^0	-7.8^0	-16.7^0	7 Å
Axial rise (h)	2.56 Å	3.37 Å	3.32 Å	3.03 Å	3.7 Å
Helical diameter	23 Å	20 Å	19 Å	-	18 Å
Handedness of the double helix	Right handed	Right handed	Right handed	Right handed	Left handed

(4) **Characteristics of DNA**

(i) **Denaturation or melting :** The phenomenon of separating of two strand of DNA molecule by breaking of hydrogen bond at the temp. 90^0C.

(ii) **Renaturation or annealing :** Separated strands reunite to form double helix molecule of DNA by cooling at the room temp. *i.e.* 25^0C.

These properties help to form hybrid from different DNA or with RNA.

(5) **Evidences of DNA as the genetic material :** The following experiments conducted by the molecular bioloigists provide direct evidences of **DNA** being the genetic material bacterial transformation, bacterial recombination and bacteriophage infection.

(i) **Bacterial transformation or Griffith's Experiments :** *Griffith* (1928) injected into mice with virulent and smooth (S-type, smooth colony with mucilage) form of *Diplococcus pneumoniae*. The mice died due to pneumonia. No death occurred when mice were injected with nonvirulent or rough (R-type, irregular colony without mucilage) form or heat- killed virulent form. However, in a combination of heat killed S-type and live R-type bacteria, death occurred in some mice. Autopsy of dead mice showed that they possessed S-type living bacteria, which could have been produced only by transformation of R-type bacteria. The transforming chemical was found out by *O.T.Avery, C.M. Mc. leod and M. Mc. Carty* (1944). They fractionated heat-killed S-type bacteria into DNA, carbohydrate and protein fractions. DNA was divided into two parts, one with DNAase and the other without it. Each component was added to different cultures of R-type bacteria. Transformation was found only in that culture which was provided with intact DNA of S-type. Therefore, the trait of virulence is present in DNA. Transformation involves transfer of a part of DNA from surrounding medium or dead bacteria (donor) to living bacteria (recipient) to form a recombinant.

(ii) **Evidence from genetic recombination in bacteria or bacterial conjugation :** Lederberg and Tatum (1946) discovered the genetic recombination in bacteria from two different strains through the process of conjugation. Bacterium *Escherichia coli* can grow in minimal culture medium containing minerals and sugar only. It can synthesize all the necessary vitamins from these raw materials. But its two mutant strains were found to lack the ability to synthesize some of the vitamins necessary for growth. These could not grow in the minimal medium till the particular vitamins were not supplied in the culture medium.

(a) **Mutant strain A :** It (used as male strain) had the genetic composition Met^- , Bio^-, Thr^+, Leu^+, Thi^+. It lacks the ability to manufacture vitamins methionine and biotin and can grow only in a culture medium which contains these vitamins in addition to sugar and minerals.

(b) **Mutant strain B :** It (used as female strain or recipient) has a genetic composition Me^{++}, Bio^{+}, Thr^{-}, Leu^{-}, Thi^{-}. It lacks the ability to manufacture threonine, leucine and thionine and can grow only when these vitamins are added to the growing medium.

These two strains of *E.coli* are, therefore, unable to grow in the minimal culture medium, when grow separately. But when a mixture of these two strains was allowed to grow in the same medium a number of colonies were formed. This indicates that the portion of donor DNA containing information to manufacture threonine, leucine and thionine had been transferred and incorporated in the recipient's genotype during conjugation.

This experiment of Lederberg and Tatum shown that the conjugation results in the transfer of genetic material DNA from one bacterium to other. During conjugation a cytoplasmic bridge is formed between two conjugating bacteria.

(iii) **Evidence from bacteriophage infection :** *Hershey* and *Chase* (1952) conducted their experiment on **T_2** *bacteriophage*, which attacks on *E.coli* bacterium. The phage particles were prepared by using radioisotopes of **^{35}S** and **^{32}P** in the following steps.

(a) Few bacteriophages were grown in bacteria containing ^{35}S. Which was incorporated into the cystein and methionine amino acids of proteins and thus these amino acids with ^{35}S formed the proteins of phage.

(b) Some other bacteriophages were grown in bacteria having ^{32}P. Which was restricted to DNA of phage particles. These two radioactive phage preparations (one with radioactive proteins and another with radioactive DNA) were allowed to infect the culture of *E.coli.* The protein coats were separated from the bacterial cell walls by shaking and centrifugation.

The heavier infected bacterial cells during centrifugation pelleted to bottom. The supernatant had the lighter phage particles and other components that failed to infect bacteria. It was observed that bacteriophages with radioactive DNA gave rise to radioactive pellets with ^{32}P in DNA. However in the phage particles with radioactive protein (with ^{35}S) the bacterial pellets have almost nil radioactivity indicating that proteins have failed to migrate into bacterial cell. So, it can be safely concluded that during infection by bacteriophage T_2, it was **DNA,** which entered the bacteria. It was followed by an *eclipse* period during which phage DNA replicates numerous times within the bacterial cell. Towards the end of eclipse period phage DNA directs the production of protein coats assembly of newly formed phage particles. Lysozyme (an enzyme) brings about the lysis of host cell and release, the newly formed bacteriophages. The above experiment clearly suggests that it is phage DNA and not protein, which contains the genetic information for the production of new bacteriophages. However, in some plant viruses (like TMV), RNA acts as hereditary material (being DNA absent).

(6) **DNA replication :** *Watson* and *Crick* suggested a very simple mechanism of DNA replication or DNA transcription on the basis of its double helical structure. During replication the weak *hydrogen bonds* between the nitrogeneous bases of the nucleotides separate so that the two polynucleotide chains of DNA also separate and uncoil. The chains thus separated are complementary to one another. Because of the specificity of base pairing, each nucleotide of separated chains attracts it complementary nucleotide from the cell cytoplasm. Once the nucleotides are attached by their hydrogen bonds, their sugar radicals unite through their phosphate components, completing the formation of a new polynucleotide chain.

The method of DNA replication is semi-discontinuous and described as *semi-conservative method*, because each daughter DNA molecule is a hybrid conserving one parental polynucleotide chain and the other one newly synthesized strand. DNA replication occur in *S-phage* in cell cycle.

(i) **Mechanism of DNA replication**

The entire process of DNA replication involves following steps in *E.coli.*

(a) **Recognition of the initiation point :** First, DNA helix unwind by the enzyme "Helicase" which use the energy of ATP and replication of DNA begin at a specific point, called initiation point or origin where replication fork begins.

(b) **Unwinding of DNA :** The unwinding proteins bind to the nicked strand of the duplex and separat the two strands at DNA duplex. Topoisomerase (Gyrase is a type of topoisomerase in *E.coli)* helps in unwinding of DNA.

(c) **Single stranded binding protein (SSB) :** Which remain DNA in single stranded position and also known as helix destabilising protein (HDP).

(d) **RNA Priming :** The DNA directed RNA polymerase now synthesizes the primer strands of RNA **(RNA primer).** The priming RNA strands are complementary to the two strands of DNA and are formed of 50 to 100 nucleotides.

(e) **Formation of DNA on RNA primers :** The new strands of DNA are formed in the $5' \rightarrow 3'$ direction from the $3' \rightarrow 5'$ template DNA by the addition of deoxyribonucleotides to the $3'$ end of primer RNA.

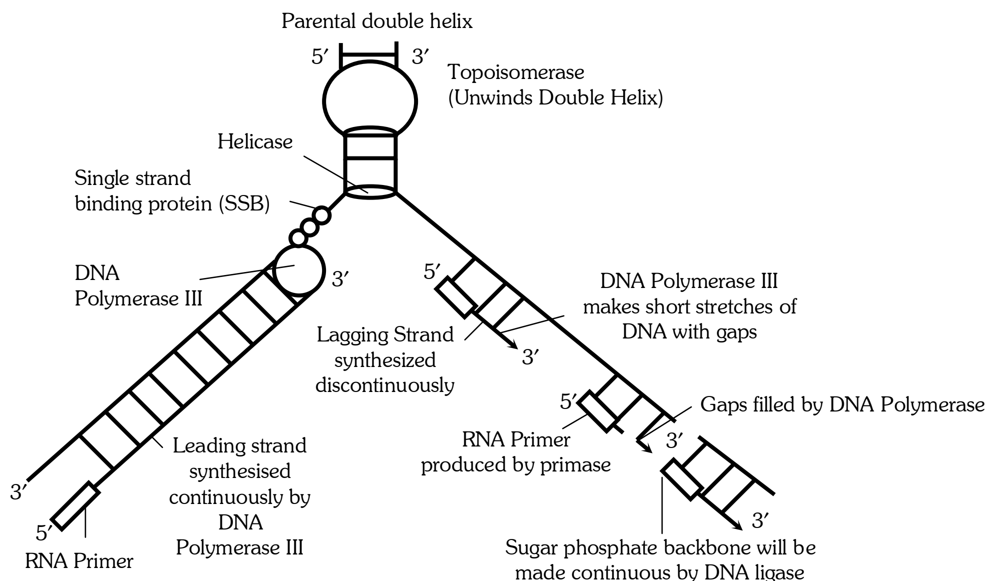


Fig : Showing continuous replication of a daughter DNA strand on leading strand and discontinuous replication of lagging strand

Addition of nucleotide is done by DNA polymerase III. The leading strand of DNA is synthesized continuously in $5' \rightarrow 3'$ direction as one piece. The lagging strand of DNA is synthesized discontinuously in its opposite direction in short segments. These segments are called Okazaki fragments.

(f) **Excision of RNA primers :** Once a small segment of an okazaki fragment has been formed. The RNA primers are removed from the $5'$ by the action of $5' \rightarrow 3'$ exonuclease activity of DNA polymerase I.

(g) **Joining of okazaki fragments :** The gaps left between Okazaki fragments are filled with complimentary deoxyribonucleotide residues by DNA polymerase-I. Finally, the adjacent $5'$ and $3'$ ends are joined by **DNA** ligase.

(ii) **DNA polymerase enzymes :** There are three DNA polymerase enzymes that participate in the process of DNA replication.

(a) **DNA polymerase-I :** This enzyme has been studied in E. coli in detail. It possesses a sulphydryl group, single interchain disulphide and one zinc molecule at the active site. DNA polymerase-I was discovered by *Kornberg* and his colleagues in 1955. It was considered to carry out DNA replication and also participates in the *repair* and *proof reading* of DNA by catalyzing the addition of mononucleotide units (the deoxyribonucleotide residues) to the free $3'$-hydroxyl end of DNA chain. A pure DNA polymerase-I can add about 1,000 nucleotide residues per minute per molecule and catalyses $5' \rightarrow 3'$ exonuclease activity and removes nucleotide residues of primer RNA at $3'$.

(b) **DNA polymerase-II :** The biological role of polymerase II is not yet known.

(c) **DNA polymerase-III :** This enzyme was discovered by *T. Kornberg* and *M.L. Gefter* (1972). It is the most active enzyme and responsible for DNA *chain elongation*.

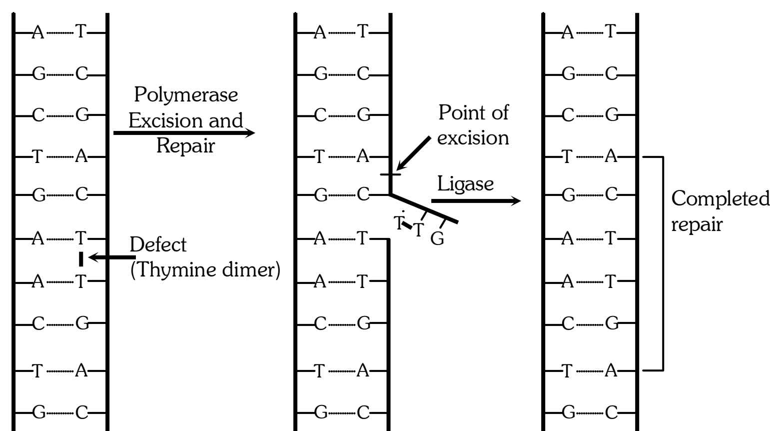


Fig : Repair of ultraviolet-induced thymine dimer which prevents replication

(iii) **DNA repair :** When DNA damaged by mutagen, a system is activate to repair damage DNA. Say for example UV light induced thymidine dimers in DNA and repair mechanism of that DNA called photoreactivation. Many enzyme involved in repair mechanism in which *endonuclease* (Chemical knives) cut the defective part of DNA then gap is filled with DNA polymerase I and finally DNA ligase seals that repaired part.

(iv) **Evidence in support of semiconservative mode of DNA replication (Meselson and Stahl's experiment) :** Meselson and Stahl (1958) cultured (Escherichia coli) bacteria in a culture medium containing $\mathbf{N^{15}}$ were isotopes of nitrogen. After these had replicated for a few generations in that medium both the strands of their DNA contained $\mathbf{N^{15}}$ as constituents of purines and pyrimidines. When these bacteria with $\mathbf{N^{15}}$ were transferred in cultural medium containing $\mathbf{N^{14}}$, it was found that DNA separated from fresh generation of bacteria possesses one strand heavier than the other. The heavier strand represents the parental strand and lighter one is the new one synthesized from the culture indicating semiconservative mode of DNA replication. circular form of replication on as characteristic of prokaryotes is *theta* replication discovered by *J. Cairns.*

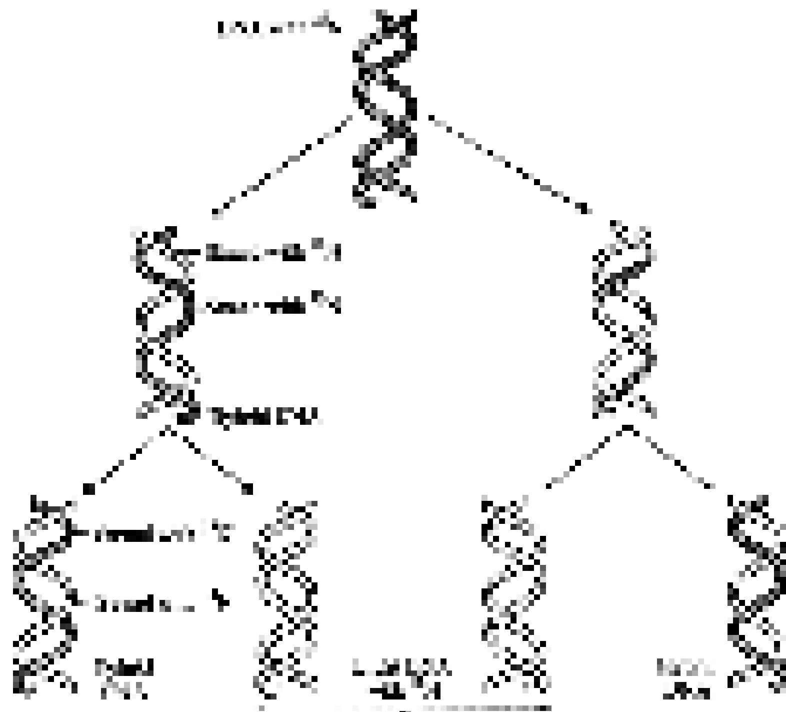


Fig : Second generation daughter molecules after DNA replication

Important Tips

- *The contribution of cytoplasmic DNA to total DNA in a cell is 1-5%.*
- ***M.H.F. Wilkins** and his associates supported DNA double helical structure using x-ray crystallography technique.*
- *Fuelgen technique used to identify the location of DNA in a cell as described by Fuelgen.*
- *F. Sanger determined base sequence in nucleic acid and synthesise of protein invitro.*
- *Fisher discovered purine and pyamidine bases in DNA*
- *Phosphoric acid found to be constituent of DNA by Levene (1910).*
- ***DNA Polymerase–III** performs the function of proof reading in which a wrong segment can be corrected by nicking with endonuclease, synthesis of a new correct segment by DNA polymerase –I and sealing by DNA ligase.*
- *Repetitive DNA or DNA finger print or satellite DNA consist 16-64 times repeated nitrogen bases in tandem and found only in eukaryotes near the centromere which have unique sequence for every organism.*
- ***Repetitive DNA or Satelite DNA** It is found in eukaryotes only.*
- ***Palindromic DNA** are inverted repetitions of bases in double stranded DNA* $5' \xrightarrow[\text{ATCGAT}]{} 3'$; $3' \xleftarrow{\text{TAGCTA}} 5'$
- *The pattern of protein binding on DNA can be studied by X-ray crystallography.*
- *Rich et-al discovered a new form of DNA in 1979 having a zig zag sugar-phosphate back bone. This is called Z-DNA. It differs from B-DNA in many characteris like helical sense.*
- *The phosphodiester bond of a polynucleotide chain can be broken by nuclease. They may remove the terminal nucleotides (Exonuclease) or break the internal bonds (Endonuclease). The restriction enzymes are those endonucleases, which breaks off specific bonds.*
- *Rodely, sasisekharan independently proposed a new model for DNA called as right – left handed helix or side by side (SBS) model.*
- *The bond between 'N' atom of nitrogen base and 'C' atom of sugar in DNA is called glycosidic bond, between sugar and phosphate is called phosphodiester bond.*
- *Nucleotide ATP is always found free in cell.*

RNA (Ribonucleic acid)

Introduction : RNA is found in the cytoplasm and nucleolus.Inside the cytoplasm it occurs freely as well as in the ribosomes. RNA can also be detected from mitochondria, chloroplasts and associated with the eukaryotic chromosomes. In some plant viruses RNA acts as hereditary material.

(1) **Structure of RNA :** More commonly RNA is a single stranded structure consisting of an unbranched polynucleotide chain, but it is often folded back on itself forming helices. DNA is a double stranded structure and its two polynucleotide chains are bounded spirally around a main axis. It is made up by:

(i) **Sugar :** Ribose

(ii) **Phosphate :** In the form of H_3PO_4.

(iii) **Nitrogenous base :** Two types: (a) Purine, (b) Pyramidine

(a) **Purine** is further divided into *Adenine* and *Guanine.*

(b) **Pyramidin** divided into *Cytosine* and *Uracil.*

(2) **Types of RNA :** RNA can be classified into two types.

(i) **Genetic RNA :** Which established by *Conrat.* In most of the plant viruses, some animal viruses and in many bacteriophages DNA is not found and RNA acts as hereditary material. This RNA may be single stranded or double stranded.

(ii) **Nongenetic RNA :** In the all other organisms where DNA is the hereditary material, different types of RNA are nongenetic. The nongenetic RNA is synthesized from DNA template. In general, three types of RNAs have been distinguished:

(a) Messenger RNA or nuclear RNA (mRNA) (b) Ribosomal RNA (rRNA) (c) Transfer RNA (tRNA)

(a) **Messenger RNA or Nuclear RNA :** mRNA is a polymer of ribo-nucleotide as a complementary strand to DNA and carries genetic information in cytoplasm for the synthesis of proteins. For this reason only, it was named messenger RNA (mRNA) by *Jacob* and *Monod* is 5% of total RNA. It acts as a template for protein synthesis and has a short life span.

(b) **Ribosomal RNA :** rRNA constitutes redundant nature upto 80% of total RNA of the cell. It occurs in ribosomes, which are nucleoprotein molecules.

Types of rRNA : Inside the ribosomes of eukaryotic cells rRNA occurs in the form of the particles of three different dimensions. These are designated 28S, 18S, and 5S. The 28S and 5S molecules occur in large subunit (60S subunit) of ribosome, whereas 18S molecules is present in the small subunit (40S subunit) of ribosome. In prokaryotic cells there are only 23S and 16S rRNA are found. Which are synthesized in Nucleolus / SAT region.

(c) **Transfer RNA (tRNA) :** The transfer RNA is a family of about 60 small sized ribonucleic acids which can recognize the codons of mRNA and exhibit high affinity for 21 activated amino acids, combine with them and carry them to the site of protein synthesis. RNA molecules have been variously termed as soluble **RNA** or *supernatant* **RNA** or adapter RNA. It is about 0-15% of RNA of the cell. tRNA molecules are smallest, containing 75 to 80 nucleotides. The **3′** end of the polynucleotide chain ends in **CCA** base sequence. This represents site for the attachment of activated amino acid. The end of the chain terminates with guanine base. The bent in the chain of

each tRNA molecule contains a definite sequence of three nitrogenous bases, which constitute the anticodon. It recognizes the codon on mRNA.

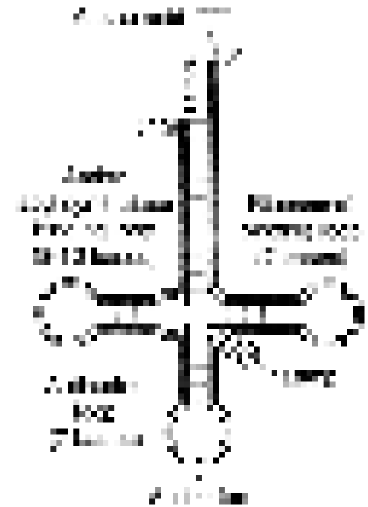


Fig : Clover leaf model of t-RNA structure

Four different region or special sites can be recognised in the molecule of tRNA.These are :

Amino acid attachement site : It occurs at the $3'$ end of tRNA chain and has OH group combines with specific amino acid in the presence of ATP forming amino acyl tRNA.

Site for activating enzymes : Dihydrouridine or DHU loop dictate activation of enzymes.

Anticodon or codon recognition site : This site has three unpaired bases (triplet of base) whose sequence is complementary with a codon in mRNA.

Ribosome recognition site ($T_{\Psi}C$) : This helps in the attachment of tRNA to the ribosome.

Important Tips

- *RNA is single stranded but it is double stranded in* ***reovirus*** *and* ***wound tumour plant*** *and* ***Rice dwarf Virus.***
- *Viroids differ from virus is naked RNA molecule.*
- *Virus particle can not be observed the stage of infection is eclipse phase.*
- *5 Bromouracil is a base analogue.*
- *Purines in RNA are* ***uracil*** *and* ***Cytosin****.*
- *CCA base sequence is present in 3′end of tRNA.*
- *Anticodons are found in tRNA.*
- *Non-genetic RNA are of three types. These are tRNA, mRNA and rRNA.*
- *Ribosomal RNA is synthesised in nucleolus of eukaryotic cells.*
- *In vitro synthesis of* ***DNA, RNA*** *and* ***Gene*** *were done by Korenberg, Ochoa and Khorana respectively..*
- *Overlapping genes reported in* $\Phi\times174$ *by* ***Linney et.al.****(1972) these genes (E.B.K.) also occur in* ***SV-40.***
- ***Ribozyme :*** *RNA acts as an enzyme having catalytic activity, discovered by* ***Altman*** *&* ***Cock.***

Genetic code.

Introduction : Defined as structure of nitrogen bases(nucleotides) in mRNA molecule which contain the information for the synthesis of protein molecule. It is discovered by frame shift mutation by Crick.

Codon is the sequence of nitrogen bases (nucleotides) in mRNA, which codes for a single amino acid. *Nirenberg* and *Mathaei* (1961) experimentally proved that a single amino acid is determined by a sequence of three nitrogen bases which is known as triplet code. Khorana has got *Nobel prize* on genetic code.

The Genetic Code Dictionary

First Letter		Second Letter								Third Letter
		U		C		A		G		
	U	UUU	Phenylalanine	UCU	Serine	UAU	Tyrosine	UGU	Cystine	U
		UUC		UCC		UAC		UGC		C
		UUA	Leucine	UCA		UAA	**Ochre** (Terminator)	UGA	**Opal** (Terminator)	A
		UUG		UCG		UAG	**Amber** (Terminator)	UGG	Tryptophan	G
	C	CUU	Leucine	CCU	Proline	CAU	Histidine	CGU	Arginine	U
		CUC		CCC		CAC		CGC		C
		CUA		CCA		CAA	Glutamine	CGA		A
		CUG		CCG		GAG		CGG		G
	A	AUU	Isoleucine	ACU	Threonine	AAU	Asparagine	AGU	Serine	U
		AUC		ACC		AAC		AGC		C
		AUA		ACA		AAA	Lysine	AGA	Arginine	A
		AUG	Methionine	ACG		AAG		AGG		G
	G	GUU	Valine	GCU	Alanine	GAU	Aspartic acid	GGU	Glycine	U
		GUC		GCC		GAC		GGC		C
		GUA		GCA		GAA	Glutamic acid	GGA		A
		GUG		GCG		GAG		GGG		G

Salient Features

(i) **Triplet :** A single amino acid is specified by a sequence of three nucleotides in mRNA *i.e.* called codon. Due to triplet nature, it consist 64 codon.

(ii) **Universal :** A codon specifies the same amino acid in all organisms from viruses to human beings.

(iii) **Commaless :** There is no pause, so it reads continously.

(iv) **Non-overlapping :** No overlapping between adjacent nucleotide.

(v) **Initiation codon :** The synthesis of polypeptide chain initiated by initiation codon, which located beginning the cistron *i.e.,* AUG or GUG, which codes to *methionine* and *valine* amino acid respectively.

(vi) **Termination codon :** Termination is done by codon. These are **UAA, UGA** or **UAG** which does not code to any amino acid. These are also called nonsense codon.

(vii) **Degeneracy :** A single amino acid may be specified by many codon *i.e.,* called degeneracy. Degeneracy is due to the last base in codon, which is known as *wobble base.* Thus first two codon are more important to determining the amino acid and third one is differ without affecting the coding *i.e.,* known *wobble hypothesis*, which establishes a economy of tRNA molecule and put forwarded by *Crick.* Degeneracy of genetic code was discovered by *Berrfield* and *Nirenberg.*

Important Tips

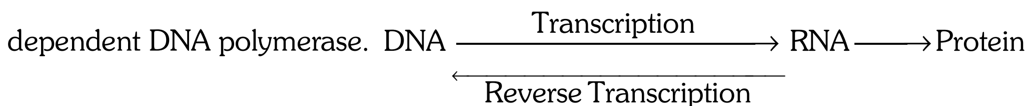
- ***George Gamow (1954)*** *first propose triplet code and given the term genetic code also proposed diamond code model.*
- *Different genetic code – in yeast mitochondria,* ***UGA*** *represent* ***tryptophan*** *while generally it is stop single. In certain ciliates* ***UAA*** *and* ***UAG*** *represent* ***Guanine****, Mitochondria DNA has genes for 22 tRNA (instead of 35 in universal code).*
- *Khorana synthesized gene of* ***tyrosine*** *suppressor tRNA gene of E.Coli in 1979, which contained 207 nucleotide pairs functional.*
- *First artificially gene synthesized* ***Alanine tRNA*** *with* ***77*** *base pairs by* ***Hargovind Khorana*** *non-functional.*
- *Protein language is composed of* ***20 alphabets.***

Central dogma.

Central dogma of molecular biology proposes a unidirectional or one way flow of information from DNA to RNA (transcription) and from RNA to protein (translation). The concept was given by *Watson* and *Crick*.

$$\text{DNA} \xrightarrow{\text{Transcription}} \text{mRNA} \xrightarrow{\text{Translation}} \text{Protein}$$

As mentioned above the first step of central dogma is transcription (synthesis of mRNA from DNA), but in case of reverse transcription DNA is synthesizes from RNA in retrovirus. That concept is given by *Temin* and *Baltimore* in Rous sarcoma virus, also known as *teminism* and enzyme catalyze this reaction is reverse trancriptase or RNA dependent DNA polymerase.



$$\text{DNA} \underset{\text{Reverse Transcription}}{\overset{\text{Transcription}}{\rightleftarrows}} \text{RNA} \longrightarrow \text{Protein}$$

Transcription.

Formation of mRNA from DNA is called as Transcription. It is heterocatalytic function of DNA. Template of DNA called sense strand (Master Strand) is involved. The segment of DNA involved in transcriptions is cistron, which have a promoter region where initiation is start and terminator region where transcription ends. Enzyme involved in transcription is *RNA polymerase-II*. Which consist five polypeptide $\alpha, \beta, \beta', \omega$ (constitute core enzyme) and σ (sigma factor). Sigma (σ) factor recognise promoter site while remaining core enzyme takes part in chain elongation. After transcription, DNA molecule reassociates to form its original structure. In eukaryotes *hn* RNA (heterogenous nuclear RNA) which consist exon (coded region) and introns (non coded region or intervening sequences) formed in nucleus and diffuse in cytoplasm is also known as split gene which goes to transcription changes for removing the introns and later formed mRNA.

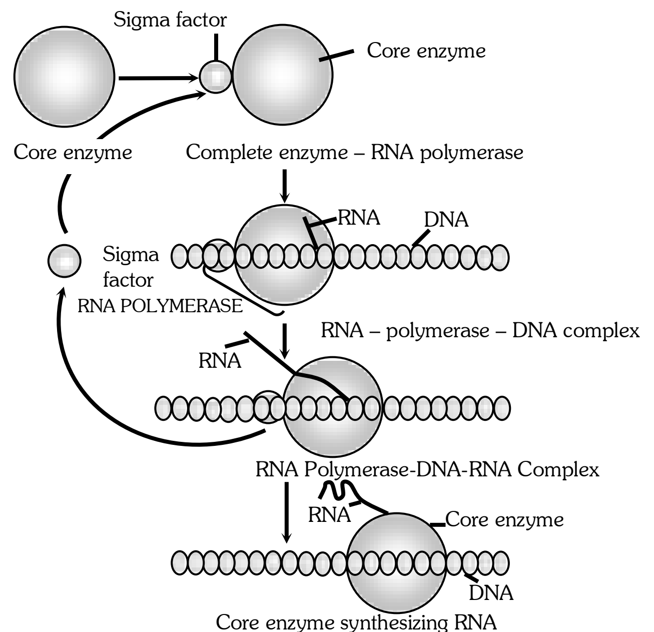


Fig : Role of sigma and core enzyme of RNA polymerase enzyme during transcription of mRNA

It consist three phenomenon: –

(1) **Initiation** : Initiation start with help of σ (*sigma*) factor of RNA polymerase enzyme. At the cap region which have 7 methyl guanosine residue at the $5'$.

(2) **Elongation :** Elongation is done by *core enzyme,* which moves along the sense strand.

(3) **Termination :** In *prokaryotes* termination is done by *rho* (ρ) factor while in *eukaryotes poly* **A** tail is responsible for termination at the $3'$.

Important Tips

- *Central dogma of modern genetics RNA →DNA→RNA→protein.*
- *Circular flow of information →DNA→ RNA→Protein→RNA→ DNA (commoner)*
- *Eukaryotic mRNA can be modified by the addition (at their 5′end) of* ***methylated argenine.***
- *Actinomycin D prevents* ***transcription.***
- *In eukaryotes three types of RNA polymerase are found which synthesizes different RNAs as RNA polymerase I, II & III formed rRNA, mRNA, & tRNA respectively in nucleolus, nucleoplasm.*
- *The terms cistron, recon and muton were proposed by* ***S. Benzer.***
- *The transcription of genes increased by* ***Glucocorticoid.***
- *When a particular gene codes for a m-RNA strand, it is said to be monocistronic or monogenic. When several genes (Cistrons) transcribe one m-RNA molecule it is called as polycistronic polygenic.*
- ***Informosomes :*** *In eukaryotes mRNA is associated with protein forming ribonucleoprotein complex. The name is given by* ***Spirin*** *and ratio of* ***protein*** *and* ***mRNA*** *is* ***4:1.***

Translation.

Formation of protein from mRNA is called translation is also known as polypeptide synthesis or protein synthesis. It is unidirectional process. The ribosomes of a polyribosome are held together by a strand of mRNA. Each eukaryotic ribosome has two parts, smaller 40S subunit (30S in prokaryotes) and larger 60S subunit (50S in prokaryotes). Larger subunit has a groove for protection and passage of polypeptide, site A (acceptor or aminoacyl site), enzyme peptidyl transferase and a binding site for tRNA. The smaller subunit has a point for *attachment* of mRNA. Along with larger subunit, it forms a P-site or peptidyl transfer (donor site). There are binding sites for initiation factors, elongation factors, translocase, GTPase, etc. The raw materials for protein synthesis are amino acids.mRNA, tRNAs and amino acyl tRNA synthetases.

Amino acids : Twenty types of amino acids and amides constitute the *building blocks* of proteins.

mRNA : It carries the coded information for synthesis of one (unicistronic) or more polypeptides (polycistronic). Its codons are recognised by tRNAs.

tRNAs : They picks up specific amino acid from amino acid pool and carrying over the mRNA strand.

Amino Acyl tRNA Synthetases : The enzymes are specific for particular amino acids and their tRNAs.

(1) **Activation of Amino Acids :** An amino acid combines with its specific *aminoacyl* ***tRNA*** *synthetase* enzyme (AA-activating enzyme) in the presence of ATP to form aminoacyl adenylate enzyme complex (AA-AMP-E). Pyrophosphate is released. Amino acid present in the complex is *activated amino acid*. It can attach to CCA or $3'$ end of its specific tRNA to form aminoacyl or AA-tRNA (*charged* tRNA / *adaptor molecule*)

Amino Acid (AA) + ATP + Aminoacyl tRNA Synthetase (E) $\rightarrow \underset{\text{amino acid adenylate enzyme complex}}{AA - AMP - E} + PPi$

AA-AMP-E + tRNA → AA—tRNA + AMP + Enzyme.

(2) **Initiation :** It is accomplished with the help of initiation factors. Prokaryotes have three initiation factors – IF_3, IF_2 and IF_1. Eukaryotes have nine initiation factors – eIF_1, eIF_2, eIF_3, eIF_{4A}, eIF_{4B}, eIF_{4C}, eIF_{4D}, eIF_5, eIF_6,mRNA attaches itself to smaller subunit of ribosome with its cap coming in contact with $3'$ end of 18 S rRNA (16S RNA in prokaryotes). It requires eIF_2 (IF_3 in prokaryotes). The initiation codon **AUG** or **GUG** comes to lie over P-site. It produces 40S – mRNA complex. P-site now attracts met tRNA (depending upon initiation codon). The anticodon of tRNA (UAC or AUG) comes to lie opposite initiation codon. Initiation factor **eIF_3 (*IF_2 in prokaryotes*)** and **GTP** are required. It gives rise to 40S-mRNA - $tRNA^{Met}$. Methionine is *nonformylated* ($tRNA_m^{Met}$) in *eukaryotic* cytoplasm and *formylated* ($tRNA_f^{Met}$) in case of *prokaryotes.* The larger subunit of ribosome now attaches to 40S-mRNA-$tRNA^{Met}$ complex to form 80S mRNA -tRNA complex. Initiation factors **eIF_1** and **eIF_4** (A, B and C) are required in *eukaryotes* and **IF_1** in *prokaryotes.* Mg^{2+} is essential for union of the two subunit of ribosomes. A-site becomes operational. Second codon of mRNA lies over it.

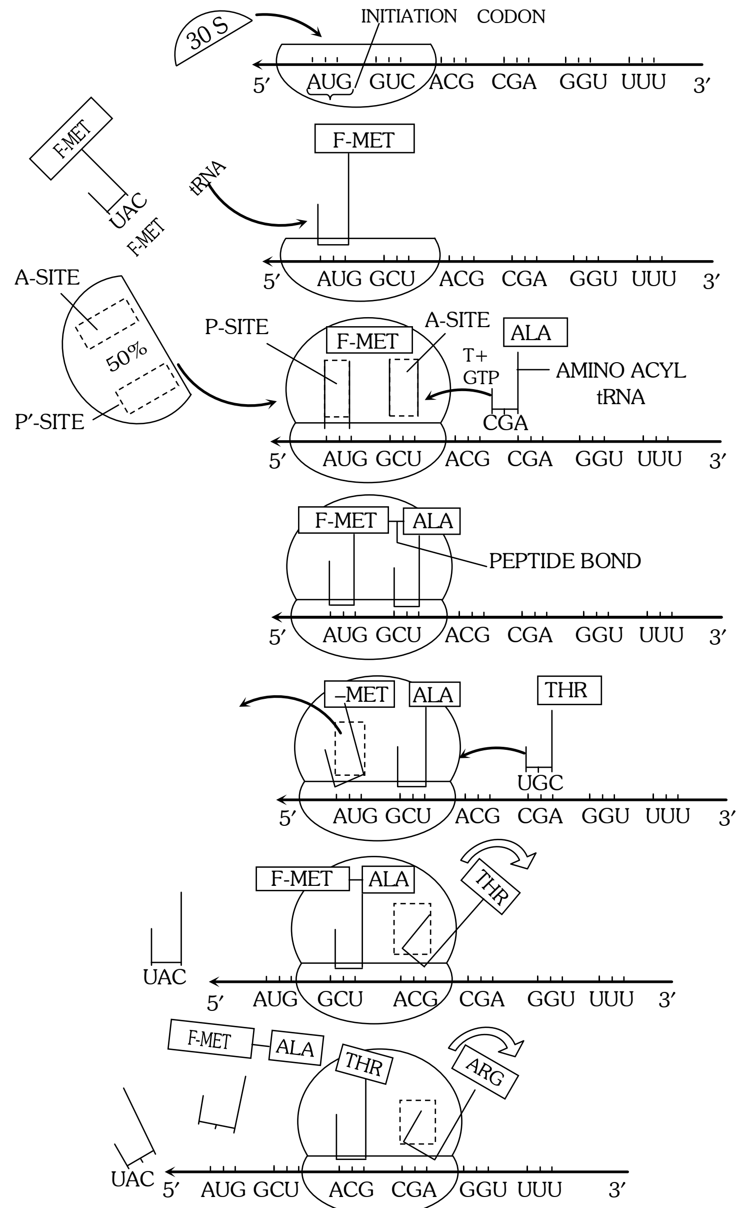


Fig : Diagramatic representation of protein synthesis in prokaryotes

(3) **Elongation/chain formation :** A new AA-tRNA comes to lie over the A site codon by means of **GTP** and elongation factor **(eEF_1** in *eukaryotes,* **EF-Tu** and **EF-Ts** in *prokaryotes*). Peptide bond (–CO.NH–) is established between carboxyl group (–COOH) of amino acid of P-site and amino group ($–NH_2$) of amino acid at A-site with the help of enzyme *peptidyl transferase/synthetase.*

Connection between tRNA and amino acid of P-site and A-site tRNA comes to bear a dipeptydl. Freed tRNA of P-site slips away. By means of *translocase* (**eEF_2** in *eukaryotes* and **EF-G** in *prokaryotes*) and **GTP**, ribosome moves in relation to mRNA so that peptidyl carrying tRNA comes to lie on P-site and a new codon is exposed at A-site.Incorporation of an amino acid in polypeptide chain thus requires one ATP and two GTP molecules. Peptide formation and translocation continue uninterrupted till the whole m-RNA code is translated into polypeptide. In a polyribosome, when a number of ribosomes are helping in translation of same mRNA code, the ribosome nearest the $5'$ end of mRNA carries the smallest polypeptide and the one towards the $3'$ end the longest. Of course, ultimately the whole polypeptide is formed by each.

(4) **Termination :** Polypeptide synthesis stops when a nonsense or *termination codon* [UAA, (ochre), UAG (Amber) or UGA (opal)] reaches A-site. It does not attract any AA-tRNA, P-site tRNA seperates from its amino acid

in the presence of release factor eRF_1 in *eukaryotes* ($RF_1$for UAG and UAA, RF_2 for UAA and UGA in prokaryotes). The completed polypeptide is released, mRNA and ribosome separate. The two subunits of ribosome also dissociate with the help of dissociation factor.

(5) **Modification :** Formylated methionine present at the beginning of polypeptide in prokaryotes and organelles is either deformylated (enzyme deformylase) or removed from chain (enzyme exopeptidase). Initially the polypeptide is elongated having only primary structure. As soon as the polypeptide comes out the groove of larger ribosome sub-unit, it forms α-helix (secondary structure) which coils further forming a number of linkages (tertiary structure). Two or more polypeptides may get associated to become β-pleated which then coil to produce tertiary and quaternary structure.

Important Tips

- ***Garrod*** *has proposed that genes control production of enzymes.*
- ***UUU*** *was first triplet codon discovered.*
- ***GUG*** *is* ***ambiguous codon,*** *which code more than one amino acid.*
- *In free ribosomes the protein is released in cytoplasm while in membrane bound polyribosomes, it is released in endoplasmic reticulum.*
- *Polysome is funtional unit of protein synthesis.*
- *Gene control both heredity and protein synthesis.*
- ***Entrance protein*** *interact with initiation complex and increase the rate of RNA synthesis.*
- *Monocistronic mRNA has codon to synthesize only one protein molecule.*
- *One gene one polypeptide theory can be explained by alkaptonuria, phenylketonuria & sickle cell anaemia.*
- *Puromycin antibiotic inhibits* ***translation.***
- *Gunter Blobel and David Sabatini proposed "Signal hypothesis" in 1971 for secretory type of proteins.*

Genes expression and its regulation.

(1) **Gene expression in prokaryotes :** Gene expression refers to the molecular mechainism by which a gene expresses a phenotype by synthesizing a protein or an enzyme. Which determines the character. The gene contains the blue print or the information for the protein or an enzyme.

The category includes mechanism involved in the rapid turn-on and turn-off gene expression in response to environmental changes. Regulatory mechanism of this type are very important in microorganisms, because of the frequent exposure of these organisms to sudden changes in environment.

Gene concept can be studied by operon model. Operon are segment of genetic material which function as regulated unit that can be switched on and switched off, which was given by French scientists. *Jacob* and *Monod* (1961) working at Pasteur institute. They were studying lactose utilisation in mutants of *E.coli*. An operon consists of one to several structural genes (three in lac operon and five in tryptophan operon of *Escherichia coli*, nine in histidine operon of *Salmonella typhimurium*), an operator gene a promoter gene a regulator gene, a repressor and inducer or corepressor. Operons are of two types, inducible and repressible.

(i) **Inducible operon system /lac operon system :** An inducible operon system is that regulated genetic material which remains switched off normally but becomes operational in the presence of an inducer. It occurs in *catabolic pathways*. The components are :–

(a) **Structural genes :** They are genes, which produce mRNAs for forming polypeptides/proteins/enzymes. Lac operon of *Escherichia coli* has three structural *genes*-**Z** (produces enzyme β-*galactosidase* for splitting

lactose/galactoside in to glucose and galactose) **Y** (produces enzyme *galactoside permease* required in entry of lactose/galactoside) and **A** (produces enzyme *galactoside acetylase/transacetylase* without any function in *E.coli*). The three structural genes of lac operon produce a single polycistronic mRNA. The three enzymes are, however, produced in different concentration.

(b) **Operator gene :** It gives passage to RNA polymerase when the structural genes are to express themselves. Normally, it is covered by a repressor. Operator gene of lac operon is small, made of 27 base pairs.

(c) **Promoter gene :** It is recognition centre / initiation point for RNA polymerase of the operon.

(d) **Regulator gene (*i* Gene) :** It produces a repressor that binds to operator gene for keeping it nonfunctional (preventing RNA polymerase to pass from promoter to structural genes).

(e) **Repressor :** It is a small protein formed by regulator gene. Which binds to operator gene and blocks passage of RNA polymerase towards structural enzymes. Repressor has two allosteric sites, one for attaching to operator gene and second for binding to inducer. Repressor of lac operon has a molecular weight of 160,000 and 4 subunit of 40,000 each.

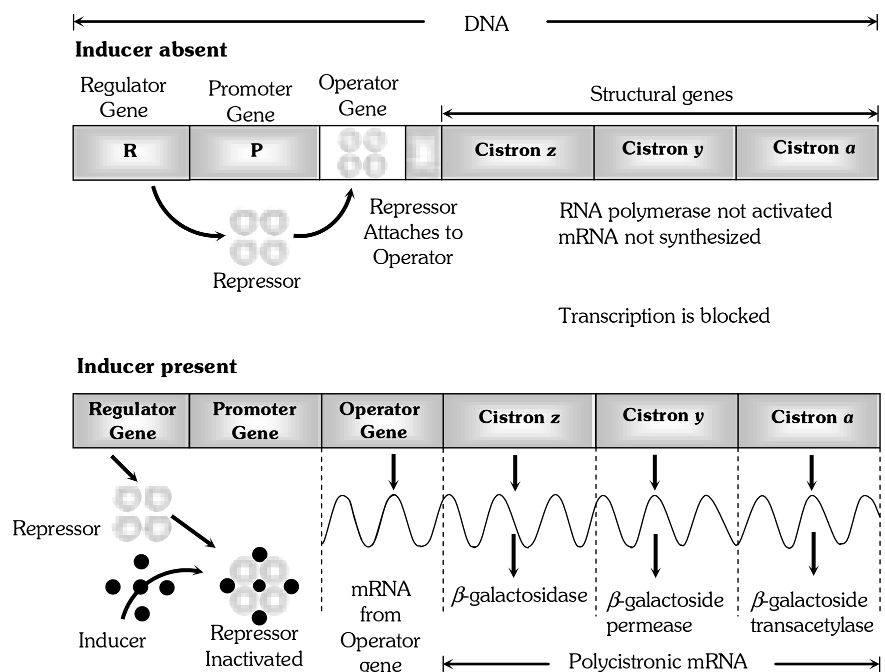


Fig : Diagram representing the function of lac operon in *Escherichia coli*

(f) **Inducer :** It is a chemical which attaches to repressor, changes the shape of operator binding site so that repressor no more remain attached to operator.

Lactose/galctoside is inducer of lac operon. As soon as the operator gene becomes free, RNA polymerase is recognised by promoter gene. cAMP is required, RNA polymerase passes over the operator gene and then reaches the area of structural genes. Here it catalyses transcription of mRNAs.

Induction	Repression
It turns the operon on.	It turns the operon off.
It starts transcription and translation.	It stops transcription and translation.
It is caused by a new metabolite which needs enzymes to get metabolised.	It is caused by an excess of existing metabolite
It operates in a catabolic pathway.	It operates in an anabolic pathway.
Repressor is prevented by the inducer from joining the operator gene.	Aporpressor is enabled by a corepressor to join the operator gene

(ii) **Repressible operon system/tryptophan operon system :** A repressible operon system is that regulated genetic material, which normally remains active/operational and enzymes formed by its structural genes

present in the cell till the operon is switched off when concentration of an end product crosses a threshold value. Repressible operon system usually occurs in *anabolic pathways, e.g.*, tryptophan operon, argnine operon. Each has the following parts.

(a) **Structural genes :** They are genes, which take part in synthesis of polypeptides/proteins/enzymes through the formation of specific mRNAs. Tryptophan operon has five structural genes – E, D, C, B and A.

(b) **Operator gene :** It provides passage to RNA polymerase moving from promoter to structural genes. Operator gene of repressible operon is normally kept switched on as aporepressor formad by regulator gene is unable to block the gene.

(c) **Promoter gene :** It is initiation/recognition point for RNA polymerase.

(d) **Regulator gene :** The gene produces an aporepressor.

(e) **Aporepressor :** It is a proteinaceous substance formed through the activity of regulator gene. It is able to block operator gene only when a corepressor is also available.

(f) **Corepressor :** The nonproteinaceous component of repressor, which can be end product (feed back inhibition/repression) of the reaction mediated through enzymes synthesized by structural genes. Corepressor of tryptophan operon is tryptophan. It combines with aporepressor, form repressor which then blocks the operator gene to switch off the operon.

(2) **Gene expression in eukaryotes :** In regulation of gene expression in eukaryotes the chromosomal proteins play important role. The chromosomal proteins are of two types. They are histones and non-histones. The regulation of gene expression involves an interaction between histones and non-histones. Histones inhibit protein synthesis and non-histones induce RNA synthesis. There are four main steps in the expression of genes. Hence regulation is brought about by the regulation and modification of one or more of these steps. They are

(i) Replication (ii) Transcription (iii) Processing (iv) Translation

(i) **Regulation of replication :** Differential gene expression is achieved by gene amplification.

(ii) **Regulation of transcription :** The regulation of the expression of gene is mainly done at transcription. Hybridization experiments clearly show that production of specialised protein is due to differential gene transcription.

(iii) **Regulation of the processing level :** Some of the RNA synthesized in the nucleus are destroyed without leaving the nucleus. 80% of the nuclear RNA has no equivalent in the cytoplasm and only 20% if the nuclear RNA is identical in the cytoplasm. All the genes in a cell are transcribed into mRNA at all times, but the mRNA produced by some genes is destroyed rapidly. But the mRNA modeled on other genes are stabilized and only these mRNAs are passed into the cytoplasm.

(iv) **Regulation of translation :** The control of mRNA-translation is a fundamental phenomenon. In sea-urchin eggs fertilisation is followed by a tremendous increase in protein synthesis; but in the unfertilised egg, there is no protein synthesis. Still the unfertilised egg has complete machinery (*i.e.,* amino acids, ribosomes, mRNA) for protein synthesis. There are two model for regulation in eukaryotes.

(a) **Frenster's model :** According to 1965, The histones act as repressor's during protein synthesis.

(b) **Britten Davidson model :** This is also called gene battery model or operon-operator model. It was proposed by Britten and Davidson in 1969. They have been proposed four type of genes namely integrator. sensor, producer and receptor.

Important Tips

- ***One gene one enzyme theory*** *was given by* ***G. W. Beadle*** *and* ***E. L. Tatum*** *they worked on Neurospora crassa (pink bread mould). Which is replaced by* ***one gene one-polypeptide theory*** *was given by* ***Yanofsky*** *et al. (1965) utilizing bacterium E. coli.*
- *Structural gene determine the primary structure of protein.*
- *cAMP- (cyclic adenosine monophosphate)- It exerts a positive control in Lac-operon because in its absnce RNA polymerase is uriable to recognise promoler gene.*
- *In inducible system when inducer is not present, no m-RNA is transcribed. But, when it is present the m-RNA synthesis occurs.*
- *In repressible system when co-repressor is present, no m-RNA is transcribed. But, when it is absent the transcription occurs.*
- *In both the system the proteins synthesis is controlled by regulator gene through operator gene. The end product may also stop it's synthesis by feed back inhibition.*
- *Two types of genes;*

  (1) ***Constitutive genes :*** *It constantly express themselves e.g. enzymes of glycolysis, which are also known as* ***house keeping gene,*** *which lacks TATA boxes.*

  (2) ***Non constitutive genes :*** *They express themselves only when needed, known as* ***luxury genes*** *Example– Inducible and Repressible genes.*