Uttar Pradesh Rajarshi Tandon
Open University

Bachelor of Science

UGZY-103

Genetics and Cell biology

COURSE INTRODUCTION

We welcome you to the study of this course on genetics and cell biology, genetics is the science of heredity that relates to the study of the structure and function of genes and the mechanisms of their transmission from one generation to the next.

Genetics and cell biology is the science of heredity.

It is studied at the level of whole organisms (classical or transmission genetics), the DNA itself (molecular genetics) or whole populations (population and evolutionary genetics).

Cell biology is the study of cell structure and function, and it revolves around the concept that the cell is the fundamental unit of life.

With our increasing knowledge of genetics and cell biology, we can better understand what controls and contributes to our development and individuality. Our improved understanding of the genetic basis for life has opened up new approaches for the investigation, diagnosis and treatment of disease.

1. Transmission genetics deals with the study of gene from one generation to the next.
2. Molecular genetics is the study of molecular structure of genes as well as nature, expression and regulation of these molecules.
3. Population genetics deals with the study of behaviour of genes in population. The population genetics define evolution as changes in gene frequencies.

The course aims at presenting the subject matter of genetics and cell biology. It examines not only the inheritance of genes which affect the characters of organisms but also the development processes where by the characters are produced.

The course divided into two blocks. The first block deals with genetics and second block deals with cell biology.

Uttar Pradesh Rajarshi Tandon
Open University

Bachelor of Science

UGZY-103

Genetics and Cell biology

BLOCK

1

GENETICS

UNIT-1

Molecular Basis of Genetic Information

UNIT-2

Blood Group, DNA and RNA

UNIT-3

DNA Polymerase and In Vitro DNA Synthesis, Transcription, Genetic Code, Gene Cloning Experiment

Course Design Committee

Prof. Ashutosh Gupta — **Chairman**
Director, School of Science
UPRTOU, Prayagraj

Dr. Shubhra Malviya — **Member**
Asst. Prof. Department of Zoology
S.S. Khanna Girls Degree College.
Prayagraj

Dr. Sippy Singh — **Member**
Asst. Prof. Department of Zoology
S.S. Khanna Girls Degree College.
Prayagraj

Dr. Deepa Chaubey — **Member/Secretary**
Acadmic Consultant School of Science
UPRTOU, Prayagraj

Course Preparation Committee

Dr. Sangeeta Srivastava Asst. Professor A.K.P.G College, Varanasi	**Author**	**Block-1** (Unit : 1, 2) **Block-2** (Unit : 7, 8)
Dr. Sudhir Kumar Srivastava Asst. Prof. Dept of Zoology Art's Commerce and Science College, Taloda, Dis. Nandubar, Maharashtra,	**Author**	**Block-1** (Unit : 3) **Block-2** (Unit : 6)
Dr. Deepu Chaubey Acadmic Consultant, School of Science UPRTOU, Prayagraj	**Author**	**Block-2** (Unit : 4, 5)
Prof. (Dr.) G.S. Shukla Prof. Director Health Science UPRTOU, Prayagraj	**Editor**	(Unit : 1 - 8)
Dr. Deepu Chaubey Acadmic Consultant, School of Science UPRTOU, Prayagraj		**Coordinator**

ISBN :

Printed and Published by Dr. Arun Kumar Gupta Registrar, Uttar Pradesh Rajarshi Tandon Open University, 2021.

Printed By : K.C.Printing & Allied Works, Panchwati , Mathura -281003.

BLOCK INTRODUCTION

Block-1. Genetics- This is the first block of the course that contains three units dealing with the basic concept of transmission of genetic material.

Unit-1 describes the genetic variation and molecular basis of genetic information. Also induced in this unit human chromosomes and human chromosomal abnormalities, sex linkage. In this unit describes the genetic basis of sex determination, dosage compensation mechanism that equalizes the x-linked gene activity in both the sexes is also discussed.

Unit-2 deals with blood group and haemoglobin, DNA and RNA structure. DNA is the genetic material (Harchey chase experiment) (separation of two DNA strands) DNA replication also introduced in this unit.

Unit-3 describe the DNA Polymerase, transcript genetic code and gene cloning.

DNA Polymerase is an enzyme that essential for DNA replication.

Process of transcription which a gene's DNA sequence is copied to make an RNA molecules. The genetic code means by which DNA and RNA molecules carry genetic information in living cells. The gene cloning means exact copies of a particular gene.

UNIT-1 MOLECULAR BASIS OF GENETIC INFORMATION

1.1 INTRODUCTION

Genetics is one of the most important branch of biological science. Genetics is the study of how living things receive common traits from previous generations. These traits are described by the genetic information carried by a molecule called DNA. The term ‗Genetics' was given by William Batson in 1906.

Each new generation resemble its ancestors, and possesses this specific and occasionally, individual traits and characteristics. It is an inherent property of living organisms to transmit these traits and characteristics by inheritance.

To inherit correctly, each cell depends on thousands of proteins to do their functions in the right places at the right times. Sometime gene mutations prevent one or more of these proteins from working properly. By changing a gene's instruction for making a protein to malfunction or to be missing entirely. Chromosomes are structure that hold our genes. If a chromosome or piece of a chromosome is missing or duplicated, there are missing or extra genes respectively that results chromosomal disorder.

Chromosomes are of two types; Autosomes and sex chromosomes. Any abnormal (recessive) trait when present on these chromosomes results autosomal abnormalities or chromosomal abnormalities respectively. These chromosomes play important role in sex determination.

Objective

After studying this unit you should be able to –

- Understand how DNA transport genetic information.
- Understand how different mechanism working together and result an amazingamount of potential variation.
- Learn about human chromosomes, chromosomal abnormalities and sexlinked inheritance.
- Learn in brief about sex determination , dosage compensation in *Drosophila* and Man.

1.2 GENETIC VARIATION

The term ―Genetics‖ was used for the first time by W.Bateson in 1905 Genetics is a branch of biology concerned with the study of genes, genetic variation, and heredity in organisms.

The first clear evidence pointing to what we now call genes came from the work of Gregor Johann Mendel, a scientist and Augustinian friar working in the 19^{th} century, was the first to study genetics scientifically.

Gregor Johann Mendel is regarded as the ―Father of Genetics‖. His experiments with garden pea, *Pisum sativum*, Constitute the foundation of modern genetics.

Genetics is often described as a science which deals with heredity and variation.

Heredity is the passing on of traits from parents to their offspring, either through the asexual reproduction or sexual reproduction. The offspring cells or organisms acquire the genetic information of their parents and ensure continuity of genetic material or germ plasm between successive generations. Therefore, heredity accounts for similarity in brothers, sisters, siblings and their parents.

Variation on other hand are differences between the individuals. Because of variation, the brothers and sisters who resemble each other in many respect, are unique in certain features. Even the identical or monozygotic twins have certain distinctive features.

Variations are of two types.

1) Hereditary variations refer to differences in inherited traits, such variations are found not only in progenies of different parents but also among progeny from the same parents (genetic variations)

2) Environmental variations are those which are merely due to environment. The genotype interacts with the environment to form the phenotype. Factors such as climate, light, temperature, moisture, minerals, nutrients in diet, vitamins, hormones and other aspects of environment play a major role in gene expression that cause variations in individuals.

Genetic variation is the variation in alleles and genes, occurs both within and among populations. Genetic variation is important because it provides the genetic material for natural selection. Genetic variation is the essence of evolution. Without genetic differences among individuals, ―survival of the fittest‖ would not be likely. Everyone would be exactly the same. How would it be determined who could or would survive? Either all survive or all die.

Meiosis and Genetic Variation – Meiosis is the process by which sex cells or gametes are created. Genetic variation occurs as alleles in gametes are separated and randomly united upon fertilization.

Sexual reproduction results in offspring that are genetically unique. They differ from both parents and also from each other. This occurs through a number of mechanisms, including crossing-over, the independent assortment of chromosomes during anaphase and random fertilization.

During prophase I of meiosis I whom homologous chromosomes form pairs, crossing-over can occur. Crossing-over is the exchange of genetic material between non-sister chromatids of homologous chromosomes. It results in new combinations of genes on each chromosomes.

When cells divide during meiosis, homologous chromosomes are randomly distributed during anaphase I, separating and segregating independently of each other. This is called independent assortment. It results in gametes that have unique combinations of chromosomes.

In sexual reproduction, two gametes unite to produce an offspring. But which two of the millions of possible gametes will it be? This is likely to be a matter of chance. It is obviously another source of genetic variation in offspring. This is known as **random fertilization**.

Random fertilization, refers to the fact that if two individuals mate, and each is capable of producing over 8 million potential gamete, the random chance of any one sperm and egg coming together is a product of these two probabilities-some70 trillion different combinations of potential off springs.

All of these mechanisms working together and as a result, an amazing amount of potential variation.

Crossing-Over- It is the exchange of genetic material between non-sister chromatids of homologous chromosomes. During prophase I, homologous chromosomes lineup in pair, gene-for-gene down their entire length, forming a configuration with four chromatids, known as a **tetrad**. The process of pairing the homologous chromosomes is called **synapsis**. During synapsis non-sister chromatids may cross-over at points called **chiasmata**(fig.1.1). Within a chiasma the genetic material from two non-sister chromatids actually intertwine around each other, and some material from non-sister chromatids switch chromosomes, that is the material breaks off and reattaches at the same position on the homologous chromosomes.

This exchange of genetic material can happen many times within the same pair of homologous chromosomes, creating unique combinations of alleles. This process is also known as homologous recombination.

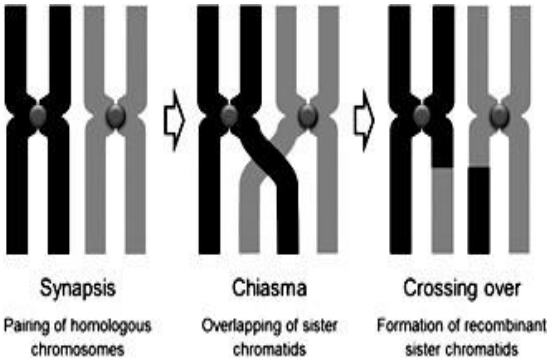


Fig.1.1 crossing over (source: www. Chegg.com)

During crossing over, segments of DNA are exchanged between non-sister chromatids of homologous chromosomes. Diagram shows that any allel (A) on one chromosomes. The four chromatids compose tetrad, with a chiasma at point of exchange.

Interdependent Assortment and Random Fertilization- In humans, there are over eight (8) million configuration in which chromosome can lineup during metaphase I of meosis. It is the specific process of meosis, resulting in four unique haploid cells, that result in these many combinations. This independent assortment in which chromosome inherited from either their father or mother can sort into any gamete produces the potential for tremendous genetic variations. This process underlines the chromosomal basis of inheritance. Gregor J. Mendels‘s findings led to the development of two laws of inheritance, the law of independent assortment.

The law of segregation state that when any individual produces gametes, the copies of a gene separate so that each gamete receives only one copy(one allele) of that gene.

The law of independent assortment state that separate genes for separate traits are passed independently of one another from parents to offspring.

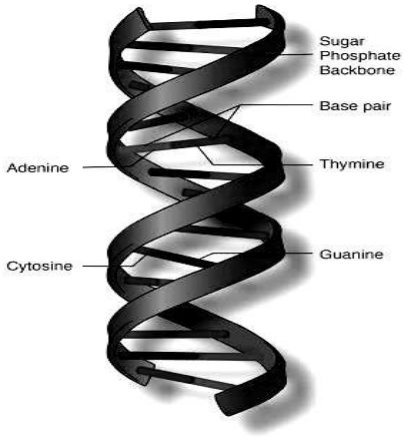
Together with random fertilization, more possibilities for genetic variation exist between any two people than the number of individuals alive today. Sexual reproduction is the random fertilization of a gamete, the female using a gamete from the male. In humans, over eight million chromosome combinations exist in the production of gametes in both the male and female. During random fertilization, a sperm cell, with over eight million possible chromosomes, fertilizes an egg cell, which also has over eight million possible chromosome combinations. Together, there are over 64 trillion unique combinations. In other words , each human couple could produce a child with over 64 trillion unique chromosome combinations.

1.3 MOLECULAR BASIS OF GENETIC INFORMATION

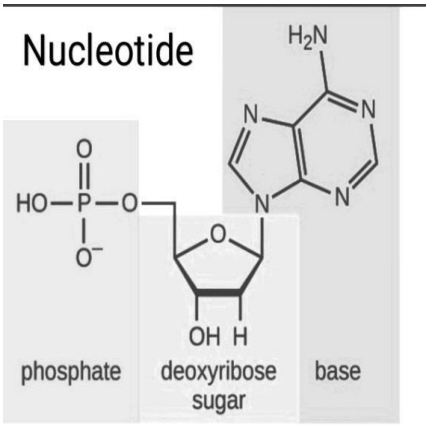
DNA and RNA are long linear polymers, called nucleic acids that carry information in a form that can be passed from one generation to the next (fig.1.2.).

These macromolecules consist of a large number of linked nucleotides, each nucleotide made up of a pentose sugar, a phosphate and a nitrogenous base.

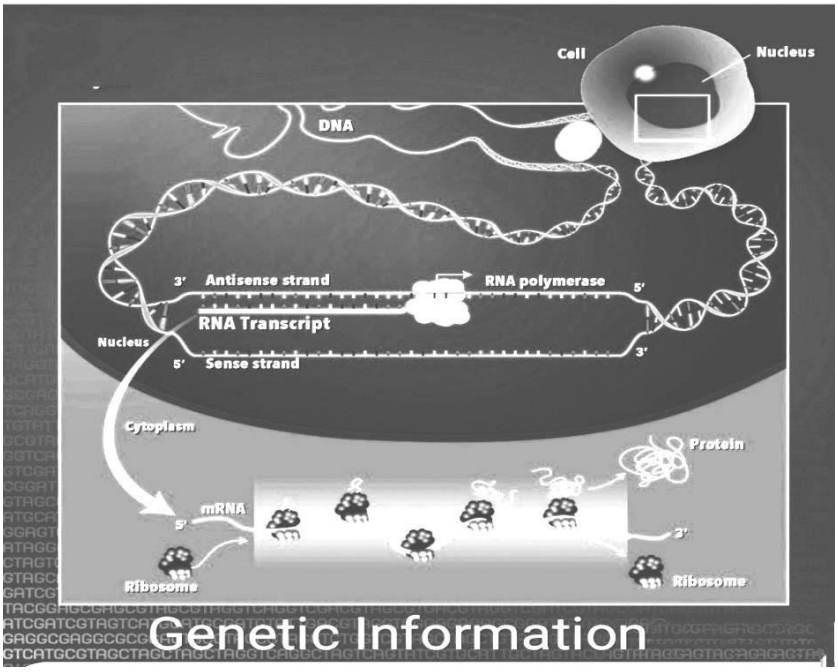
Sugars are linked by phosphates form a common backbone, whereas the bases vary among four kinds (A,G, C, T or U). *Genetic information is stored in the sequence of bases along a nucleic acid chain.*



(A)



(B)



(C)

Fig.1.2- A. DNA Double-Helix B. Nucleotide C. The flow of genetic information in a cell from DNA to mRNA to protein.(source:Genome.com)

The bases have an additional special property : they form specific pairs with one another that are stabilized by hydrogen bonds. The base pairing results in the formation of a double helix, a helical structure consisting of two strands. *These base pairs provide a mechanism for copying the genetic information in an existing nucleic acid chain to form a new chain.*

Although RNA probably functioned as the genetic material very early in evolutionary history, the genes of all modern cells and many viruses are made of DNA. DNA is replicated by the action of DNA polymerase enzymes. These exquisitely specific enzymes copy sequences from nucleic acid templates with an error rate of less than 1 in 100 million nucleotides.

Genes specify the kinds of proteins that are made by cells but DNA is not the direct template for protein synthesis. Rather the template for protein synthesis are RNA.

In particular, a class of RNA molecules called messenger RNA (mRNA) are the information carrying intermediates in protein synthesis. Other RNA molecules, such as transfer RNA (tRNA)and ribosomal RNA (rRNA), are part of the protein synthesizing machinery. All forms of cellular RNA are synthesized by RNA polymerases that take instructions from DNA templates. This process of transcription is followed by translations, the synthesis of protein according to instructions given by m RNA templates. Thus the flow of genetic information, or gene expression, in normal cell is (fig.1.3):

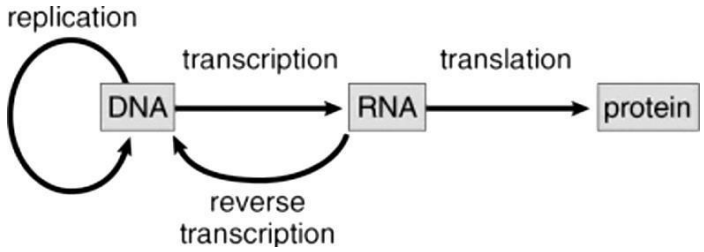


Fig 1.3 – The central dogma: Two step process, transcription and translation by which the information in genes flow into protein

This flow of information is dependent on the genetic code, which defines the relation between the sequence of amino acids in a protein. The code is nearly the same in all organisms : a sequence of three bases, called a **codon**, specifies an amino acid. Codons in mRNA are read sequentially by t RNA molecules, which serve as adaptors in protein synthesis. Protein synthesis takes place on ribosomes, which are complex assemblies of rRNAs and more than 50 kinds of proteins.

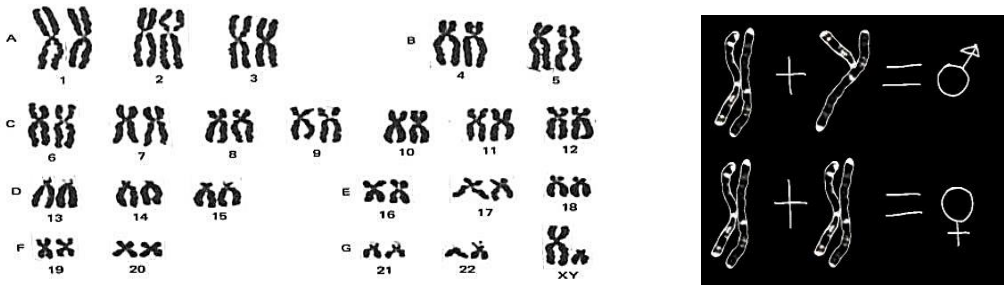
The last topic to be considered is the interrupted character of most eukaryotic genes, which are intertwined nucleic acid sequences called introns (non coding sequences) and exons (coding sequences). Both are transcripted, but introns are cut out of newly synthesized RNA molecules (nascent RNA or primary transcript RNA) leaving mature RNA molecules with continuous exons. The existence of introns and exons has crucial implications for the evolution of protein.

1.4 HUMAN CHROMOSOMES AND HUMAN CHROMOSOMAL ABNORMALITIES

Human Chromosome- The human chromosome is the basic building block of life and is one of the most important components of the cell to be transmitted from generation to generation. It is essentially an organized structure of DNA that exists within the nucleus of all human cells and comprises a single chain of DNA that is coiled and super coiled to form dense thread like pieces. Like all other eukaryotes humans contain a fixed number of chromosomes within each of the nuclei in all their cells. There are essentially two types of chromosomes as characterized by karyotyping at the metaphase of cell division. These include :

Autosomes – There are 22 pairs of autosomes in human. These code for most of the genetic traits in the body.

Gonosomes or Sex chromosomes- Humans contain two types of sex chromosomes including X and Y. Males have an X and a Y chromosome, and female possess two X chromosomes.



(A) (B)

Fig. 1.4- A. Human Karyotype and B.sex chromosomes

The 22 autosomes are numbered by size. The other two chromosomes, X and Y, are the sex chromosomes. This picture of the human chromosomes lined up in pairs is called a karyotype.

Each human cell thus contains 46 chromosomes in 23 pairs. The gametes or ovum produced by the female ovaries and the sperm produced by the male testicles, however contain only 23 chromosomes. This ensures that when the egg and the sperm get fertilized to form a baby it contains 23 pairs and restores the total chromosomal count to 46 (fig.1.4).

Human Chromosomal Abnormalities- A chromosomal abnormalities disorder, anomaly, aberration or mutation is a missing, extra or irregular portion of chromosomal DNA.

It can be from a typical number of chromosomes or a structural abnormality in one or more chromosomes.

Chromosome mutation was formerly used in a strict sense to mean a change in a chromosomal segment involving more than one gene. The term ―karyotype‖ refers to the full set of chromosomes from an individual. This can be

compared to a normal karyotype for the species via genetic testing. A chromosome anomaly may be detected or confirmed in this manner. Chromosome anomalies usually occur when there is an error in cell division following meiosis or mitosis. There are many types of chromosomal anomalies.

A. Autosomal Chromosome abnormalities

Aneuploidy- Aneuploidy is the change in chromosome number i.e extra or missing chromosome in a cell.

Aneuploidy can be caused by loss of one or more chromosomes (hypoploidy) or by addition of one or more chromosomes to the complete chromosome set (hyperploidy).

Hypoploidy may be of two types :-

i) Monosomy (2n-1) – The subtraction (or loss) of a single chromosome. A monosomic individual froms gametes of two types, (n) and (n-1). Double monosomics (2n+1+1) or triple monosomics (2n-1-1-1) may also be produced in polyploids such as wheat. Double monosomic means that the chromosome number is 2n-2 but the missing chromosomes are non-homologous same as in triple monosomics. Example – Turner Syndrome 45x is the only full monosomy in human.

ii) Nullisomy – (2n-2) - The loss of a pair of chromosome in an organism is a nullisomy. Nullisomy means that the chromosome number is 2n-2 but the missing chromosomes are homologous. Humans with this condition will not survive.

Hyperploidy – May be of three types

i) Trisomy – Diploid organisms which have an extra chromosome (2n+1) are trisomics. In human beings, the following three syndromes have been studied.

   a) Down‘s syndrome or trisomy – 21

   b) Edward‘s syndrome or trisomy – 18

   c) Patau Syndrome or Trisomy – 13

   (a) **Down’s syndrome or Mongloid idiocy-(Triplo -21)-** This autosomal aneuploidy was described by John Langdon Down (1866) under the name of mangoloid idiocy or mongolism, because the facial features of the victim studied showed resemblance to the oriental features. Down‘s syndrome is a congenital syndrome originating from the non disjunction of chromosomes of pair 21 during meiosis. The mongoloids, therefore, have 47 chromosomes instead of 46 with three copies of chromosomal 21 instead of usual two.

   Children with down‘s syndrome tend to share certain physical features such as a flat facial profile, an upward slant to the eyes,

small ears, and a protruding tongue. Low muscle tone (called hypotonia) is also characteristics of children with down's syndrome.

(b) **Edward's Syndrome - (triplo-18)-** This syndrome was first described by Edward. It is characterized by several abnormalities some of which are ; malformed low set ears, small receding lower jaw, flexed fingers, cardiac malformations, deformities of skull, face and feet. Death takes place usually around 3 to 4 months of age but sometimes it may be extended for nearly two years. The individuals showing this syndrome have an additional chromosome and thus the total number of chromosome is 47. The extra chromosome is autosome 18.

(c) **Patau's Syndrome - (Tripto – 13)-** This is also a case of trisomy described by Patau et al (1960) resulting from an extra autosomal taking the total chromosome number to 47. The individuals with Patau's syndrome exhibit many abnormal characters like mental retardation, sloping forehead, deformed face, polydactyl on hands and feet; deformed hands and feet, cardiac and other internal defects. Death occurs within hours or days but may abort spontaneously.

B. Structural Chromosomal Abnormalities- All kinds of structural chromosomal abnormalities like translocation, deletion, duplication, ring chromosome, inversion and isochromosomes have been observed in man and are associated with abortion and congenital disorder.Some of them are as follows-

(a) Traslocation

i. Traslocation between chromosome 21 and either chromosome 14 or 15 or X- The genetic material of chromosome 21 is present in triple dose and such cases are phenotypically indistinguishable from those with 21-trisomy.

The translocation mongolism runs in families. Usually the mother is a carrier of a balanced 21/14 translocation and phenotypically she is normal.

ii. Translocation between chromosome 9 and 22- In this case , the unequal end pieces of long arms of 9 and 22 chromosomes are exchanged. This results in shortened 22 chromosome, called philadelphia chromosome. The break point in chromosome 9 occurs in the cellular proto-oncogene (c-abl) and in chromosome22 occurs in a gene called breakage cluster region (bcr). The fused gene in philadelphia chromosome is transcribed and translocated into an abnormally long protein. Philadelphia chromosome is associated with chronic myelogenous leukemia(CML). If affects middle and old age people and is invariably fatal.

Peter Nowell and Hongrford (1960) had described this leukemia to arise by deletion of a part of 22 chromosome.

iii. **Translocation between chromosome 8 and 14/22/2-** In this translocation chromosome 8 breaks near proto-oncogene c-mycand other break point occur in either chromosome 14, 22, or 2 .The exchange results in Burkitt lymphoma (BL).

(b) Deletion- A deletion involving loss of large number of genes. Some of examples of deletion of a portion of chromosome in man are as follows-

i. Cri-duchat Syndrome or Cat cry Syndrome is the effect of loss of a portion of the no. 5 chromosome.

ii. Ataxia-telangiectasia is caused by chromatid breaks or formation of dicentric and acentric chromosomes.

iii. Deletion of some parts of one X-chromosome has been found to produce some sex anomalies. The deletion of short arm produces clinical symptoms similar to XO Turner‘s Syndrome.

iv. Isochromosome of the long arm of X- chromosome also produces Turner‘s syndrome because the short arms are lost during isochromosome formation in second meiotic division.

C. Sex Chromosomal Abnormalities : Non disjunction of sex chromosome- Some of the sex chromosomal abnormalities are as fololows-

i. **Turner’s Syndrome-(Gonadaldysgenesis, 45XO)-** Turner‘s syndrome is a sex chromosomal disorder in which a female (sterile) is born with only one X-chromosome but their interphase nucleus is without Barr body. Turner‘s syndrome can cause a variety of medical and developmental problems, including short height, failure of ovaries to develop and heart defects. Intelligence is usually normal but affected individuals may experience certain learning disabilities. Turner‘s syndrome happens when a female, is missing certain genes that are normally on the x-chromosome. They possess 45 chromosomes (44+xo0 instead of normal 46 chromosomes(44+xx).

ii. **Klinefelter’s Syndrome (44+XXY)-** Klinefelter syndrome is a sex chromosomal abnormalities in which a sterile male is born with extra one x-chromosome and the interphase nuclei of their cells possess one Barr body.It means they possess XXY-sex chromosomes instead of XY.

This syndrome arises by the nondisjunction of xx-chromosomes. When an abnormal egg with xx-chromosomes is fertilized with a sperm having Y chromosome, the zygote possesses XXY-chromosomes.

iii. **Triplo- X Female-** Such females with three x-chromosomes (44+xxx) are known as super females. These are usually

mentally retarded and infertile females. However, some triplo-x females are apparently normal fertile .The children born to such females are found to be normal.

1.5 SEX LINKAGE AND DETERMINATION IN DROSOPHILA AND MAN

In Mendel‘s cross the progeny of a cross between two individuals of pure lines is the same regardless of which individual is female or which individual is male or we can say that sex makes no difference in Mendel‘s crosses. But the Mendel‘s laws are not applicable on those genes which are exclusively located either in X or Y chromosome. It has been observed that genes located only on the X- chromosomes are represented twice in female (2X chromosomes) and once in male (male has only one X chromosome). Moreover, if the recessive type of genes occur in X-chromosome of males, they express themselves phenotypically, Because in such case Y chromosome contain no dominant allelomorph or gene to overcome recessive gene of X chromosome. The genes which occur only on the X-Chromosome (Mammals, Drosophila etc.) are called X-linked genes. The genes which only occur on Y-chromosomes are called holandric genes. The inheritance of X and Y linked genes is called sex-linked inheritance.

Linkage- Linkage is the tendency for alleles of different genes to be passed together from one generation to the next. Only genes situated on the same chromosome can show linkage. Genes on nonhomologous chromosomes are, unlinked and always show 50% recombination. Parental gametes carry the same set of alleles as were inherited together from one parent. Recombinant gametes carry alleles derived from both parents. The degree of linkage between two genes depends on the frequency of cross-overs that occur between them during meiosis.

Sex Linkage- Sex linkage describes the pattern of inheritance and presentation when a gene mutation (an allele) is present on a sex chromosome (an allosome) rather than an non-sex chromosome (an autosome). They are characteristically different from the allosomal forms of dominance and recessiveness.

- In mammals (Human) the homogametic sex is female (XX) and the heterogametic sex is male (XY), thus the sex liked genes are carried on the X-chromosomes.
- Sex linkage was introduced by Thomas H. Morgan in 1910, while studying inheritance of eye colour (red/white) in the fruit fly *Drosophila melanogaster*.

Types of Sex-linked inheritance

1) X-linked Inheritance– The genes which occur only on the X-chromosome (Mammals, *Drosophila*etc) are called X-linked genes and their mode of inheritance is called X-lined inheritance. Example – colourblindness, haemophilia in man.

2) Y-Linked Inheritance – The genes which only occur on y-chromosome are called y-linked genes. This is confined to males only. Hence they are

called holandric genes (Holos = whole, Andros = male). These genes are transmitted directly from father to the son. Their mode of inheritance is called Y-linked inheritance. Example – Hypertrichosis, ichthyosishystrix etc. Hypertrichosis is characterized by the presence of tufts of hairs on the ear pinna in man only. Ichthyosishystrix is the presence of scales on the body.

3) XY-Linked Inheritance – Certain sex linked genes are located on both X and Y-chromosomes. They are called XY linked genes or pseudoautosomal genes and their mode of inheritance is called X-Y linked inheritance. These genes are present on the homologous part of both X and Y chromosomes. This homologous area is called pseudo-autosomal region. The genes present in this area are : antibody gene MIC 2, zinc finger gene ZFY and sexdeterminingregion SRY. Example – Xerodermapigmentosum, retinitis pigmentosa (Macular degeneration) and epipermolysisbullosa.

4) Completely Sex-linked Inheritance: The X and Y chromosomes are not similar. The X-chromosome is larger and straight while the Y-chromosme is smaller and its one end is straight (in man) and curved (in Drosophila). The lower part of X-chromosome similar to that of Y-chromosome is smallerand its one endis straight (in man) and curved (in Drosophila). The lower part of X-chromosome is similar to that of Y- chromosome. These two parts are called homologous regions. They have the same types of genes. The remaining parts of X and Y chromosomes are not similar. Hence they are called non-homologous regions or different regions. They do not contain similar types of genes. The genes located on non-homologous region inherit together because crossing over does not occur in these regions. The genes located on non-homologous regions are called completely sex linked inheritance. Example – haemophilia, colour blindness etc.

5) Incompletely sex-linked inheritance – The genes located on homologous regions of sex chromosomes do not inherit together because crossing over may occur in these regions. So these are called incompletely sex-linked genes and their mode of inheritance is called incompletely sex linked inheritance. Example Niphritis etc.

Sex linked Genes in Man- In human, gender is controlled by the sex chromosome X and Y. However, the X and Y chromosomes are not equal. The Y chromosome is significantly smaller than the X chromosome and carries few alleles on it.This create interesting patterns of inheritance called sex-linked traits, traits that affects males and females in different proportion because they are coded for genes on the sex chromosome.Haemophilia and colour blindness are most common example of sex linked inheritance.

Haemophilia(Bleeder's disease) (X-linked recessive trait)- Haemophilia is a sex linked recessive genetic disorder.The sex linked recessive traits are more common in males than in females. In this disease the clotting of the blood do not take place in case of injury. Only males are victim of this disease and females are

considered to be the carrier. In human beings the males have XY and females have XX sex chromosomes. The X chromosome may carry a recessive gene for haemophilia. This gene does not show up if there is a dominant gene for the clotting of blood in the other chromosome. In women haemophilia will not be expressive in case the gene for haemophilia is present in one of the X chromosome. But such women are carriers of haemophilia.

In males there is a single X chromosome. If the bad gene for haemophilia is present on that chromosome they became haemophilic (Bleeder). If a haemophilic man marries a normal woman, then all the daughters born out of the marriage will carry in one of their X chromosomes the defective gene for haemophilia. This X chromosome will obviously be handed over to the daughter by the father. Such daughters are carrier of haemophilia. The carrier daughter when married to a normal man will produce 50% sons who will be haemophilic (fig.1.5). A woman will become haemophilic only if her father is haemophilic and her mother is carrier. This case is called criss-cross inheritance. A very popular haemophilic family is the royal family of Britain.

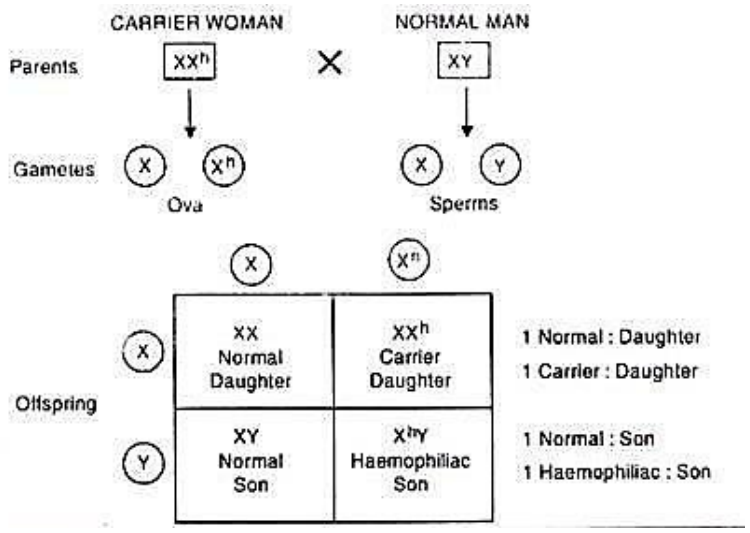


Fig. 1.5. Inheritance of haemophilia

Colour Blindness(Daltonism)(X linked recessive trait)- Colour blindness is a common sex linked human disease. In this disease one cannot differentiate between red and green colour. Colour blindness is also found common only in man than in woman.

Colour blindness is caused due to a recessive allele of a gene present on the X chromosome. The normal allele is denoted CB(Dominant allele) and the mutant allele responsible for colour blindness is cb(recessive allele).

A colour blind man must have inherited the cb allele with his X chromosome from his mother. If she had normal vision then she must have been a heterozygote CB/cb. A colour blind man cannot transmit this cb allele to his son because as per definition his son must inherit Y chromosome from his father. By the rule he must pass cb to all of his daughter. Any daughter that inherit the cb allele from her father and a normal allele from her mother will be a carrier and transmit their disease to 50% of her sons .

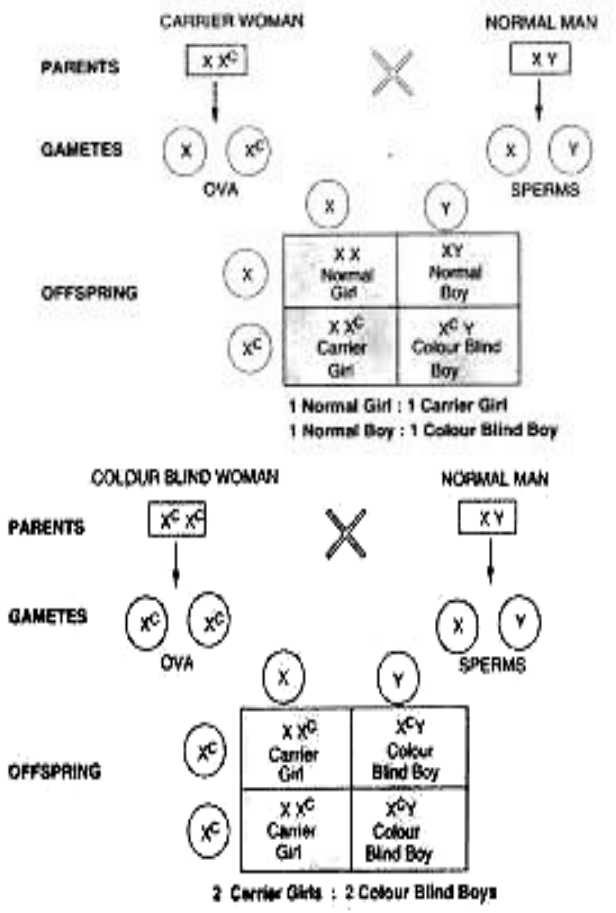


Fig.1.6.-Inheritance of colour blindness

Sex linked inheritance in *Drosophila*- The sex linked inheritance of red eye and white eye colour in *Drosophila*was discovered by T.H. Morgan in 1910.The eye colour gene located on x chromosome .The red eye colour is dominant and white eye colour is recessive.

1. X chromosome with allele for red eye colour = X+
2. 2.X chromosome with allele for white eye colour=Xw
3. Y chromosome without any allele for eye colour
4. Female has two alleles for eye colour one on each X chromosome
5. Male has only one allele for eye colour because it has only one X chromosome.

1. **A cross between Red Eyed Female and White Eyed Male *Drosophila*-** When white eyed male is crossed with red eyed female all F1 flies (both male and female) were red-eyed female. When these F1 flies mated freely, the red and white eyed flies were produced in 3:1 ratio. But all the white eyed flies were male. The red-eyed flies were both males and females, but in number females were double the red eyed males (fig 1.7).

2. **Reciprocal cross: A cross between White Eyed Female and Red Eyed Male** *a **Drosophila***- When a white eyed female *Drosophila* is crossed with a red eyed male, all the females are red eyed and all the males are white eyed. When these red Eyed F1 carrier females are crossed with white eyed males both red and white eyed males and females are formed in equal proportion (fig1.7).

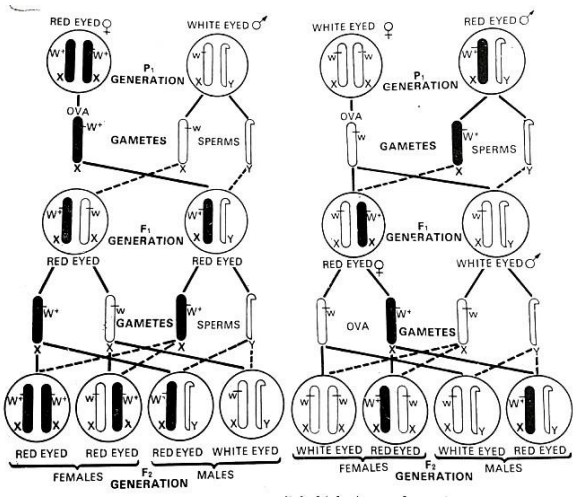


Fig.1.7- Results of reciprocal crosses showing sex linked inheritance of eye colour in *Drosophila:* A- Across between red eyed female and white eyed male ; B- A cross between white eyed female and red eyed male. (Source:-V. B. Rastogi)

Sex determination- Members of almost all species are often divided into two sections accordingly to the kind of gamete or sex cell produced by them i.e. male sex and female sex.

The word sex has been derived from the Latin word sexus meaning section or separation. Sex is an emblem of life, which can be determined at several levels. It is a fundamental quality recognized in living organisms especially in higher forms of life. Primarily, sex determination relates to whether an individual develops testes (singular=testis) or ovaries. Secondary sexual characters develops at certain periods of life, like the development of body hairs, spermatogenesis, oogenesis etc. Which are also under genetic control. All higher animals are present in two distinct forms of life i.e male and female which is an evolutionary outcome. Evidences are there which prove that sexual diamorphism has developed from a single origin.

Sex Determination in Man – In case of man total number of chromosomes is 23 pairs or 46

In male (man) 44 + XY

In Female (women) 44 + XX

Sperm produced by male are of two types : (a) 22+x and (b) 22+Y, whereas the over all have 22+X chromosomes. So, in man determination of sex of offspring depends on the kind of male gamete (either 22+X or 22+Y) which involved in fertilization process. The key to sex determination in humans is the

SRY (for sex region on the Y) gene located on the short arm of the Y-chromosome (fig.1.8) that produce TDF (Testes-determining factor).

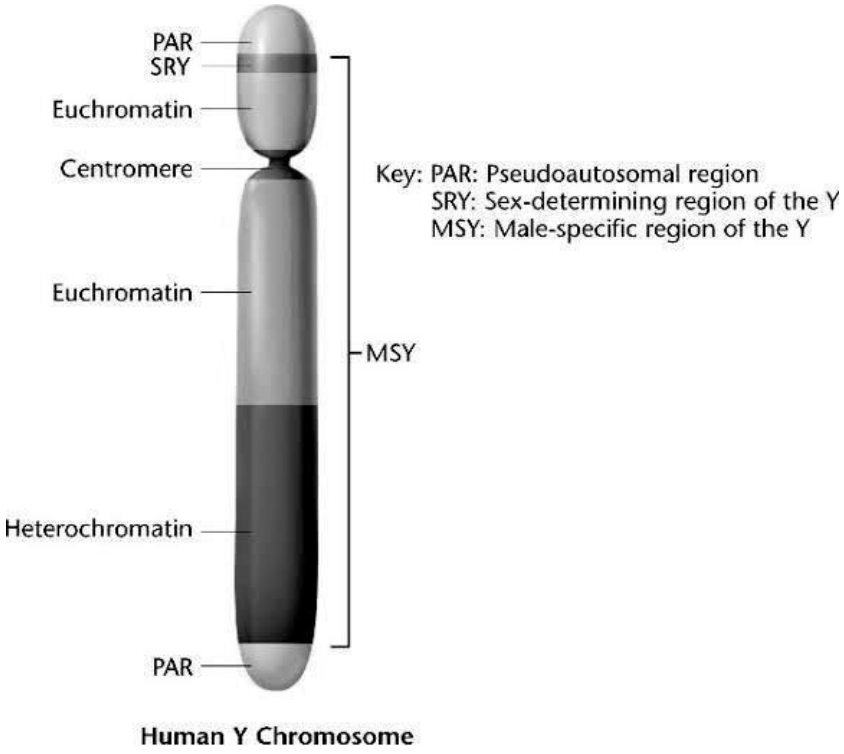


Fig.1.8. Human Y Chromosome(www.studyblue.com)

Sex determination in *Drosophila*- In *Drosophila* total numbers of chromosome is eight of which 3 pairs or six are autosome, which are common in both male and female. The fourth pair is of sex chromosome. In male this is represented by XY i.e Karyotype of male *Drosophila* 6+XY and in female XX i.e, 6 +XX. Ova produced by female are all similar possessing 3+X chromosomes, whereas the sperm produce by male are 3+X and 3+Y in equal number.

Function of X and Y chromosomes in *Drosophila*- In *Drosophila*, X has been found to process female determining genes and Y has no influence in determination of sex. The sex in Drosophila, is therefore, determined by the ratio of X-chromosomes to autosome. X/2A = 0.50 gives rise to male. The fact has been established by the occurrence of XO-males and XXY females. Which are formed as a result of nondisjunction of sex chromosome,

C.B bridges worked out genic Balance theory or ratio theory of sex determination in *Drosophila*.

Genic Balance Theory of Sex determination- Bridges crossed the experimentally produced triploid (3n individual having three whole sets of chromosomes) female Drosophila (3A:3X) to diploid males (2A: XY)

Possible gametes of triploid female are 2A+XX & A+X or @A+X &A+XX

Possible gametes of diploid male A+X and A+Y. The results obtained from such a cross are as follows-

Table. 1.0 Ratio of X-chromosome to autosome sets and their effect on sex determination in *Drosophil*a.

Sex	Number of X-chromosomes	Number of autosomal set	Sex index X/A ratio
Super Female	XXX (3)	AA (2)	3/2 = 1.5
Normal Females			
Tetraploid	XXXX(4)	AAAA(4)	4/4=1.0
Triploid	XXX (3)	AAA(3)	3/3=1.0
Diploid	XX (2)	AA(2)	2/2 = 1.0
Haploid	X(1)	A(1)	1/1 = 1.0
Intersex	XX (2)	AAA (3)	2/3 = 0.66
Normal male	X (1)	AA (2)	1/2 = 0.5
Super male	X(1)	AAA (3)	1/3 = 0.33

Gynandromorphs in *Drosophil*- In *Drosophila*, occasionally flies are obtained in which a part of the body exhibits female characters and the other parts exhibits male characters. Such flies are known as gynandromorphs. These are formed due to misdivision of chromosomes. The zygote starts as female with 2A + 2X – chromosomes. One of the X-chromosomes is lost during the division of the cell with the result that one of the daughter cells possesses 2A+2X chromosomes and the other 2A+X. If this event happens during first zygotic division, two blastomeres with unequal number of X-chromosome are formed. The blastomere with 2A+2X – chromosomes develops into female half, while the second blastomere 2A+X chromosomes produces male half and the resultant fly is a bilateral gynandromorphy.

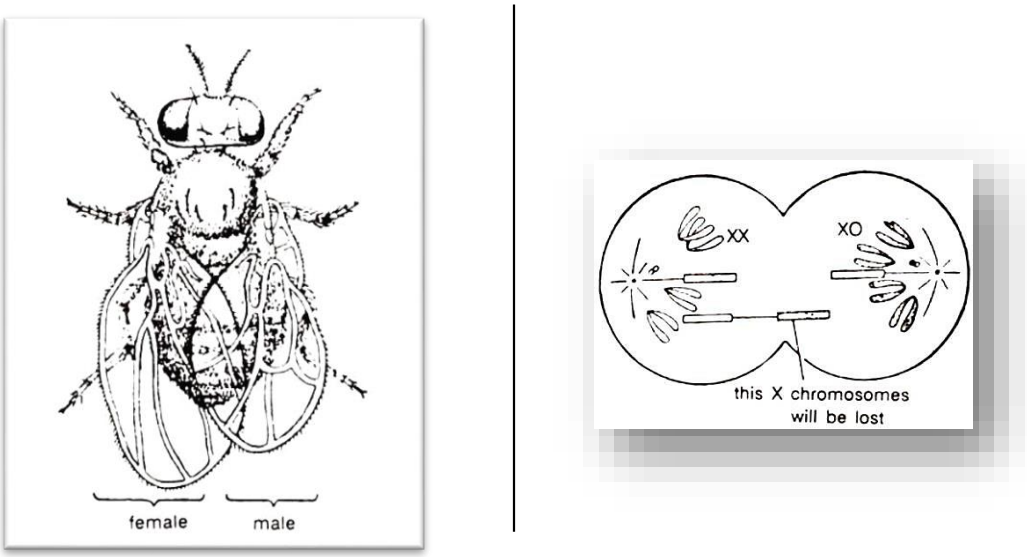


Fig 1.9 - A gynandromorphy of *Drosophila* in which right half is male and left half is female. (Source : V.B. Rastogi)

1.6 SEX CHROMATIN BODIES : BARR BODY

Sex chromatin or Barr body (after the name of its discoverer Murray Barr, 1940) is a small condensed mass of the inactivated X-chromosome in a female somatic cell, rendered inactive in a process called Lyonization, in those species in which sex is determined by the presence of the Y(including humans) or W chromosome rather than the diploidy of the X. The Lyon hypothesis states that in cells with multiple X chromosomes, all but one are inactivated during mammalian embryogenesis. This happens early in embryonic development at random in mammals, except in marsupials and in some extra- embryonic tissues of some placental mammals, in which the father‘s X chromosome is always deactivated.

Barr bodies are usually located just inside the nuclear membrane of the interphase nucleus. The number of sex chromatin bodies or Barr bodies per nucleus is one less than the number of X-chromosomes. For example, Normal female have one sex chromatin and normal male have none sex chromatin but normal females with Turner‘s syndrome (XO) have none sex chromatin. Normal males with Klinefelter syndrome (XXY) have one and XXX female have two sex chromatin (Barr body).

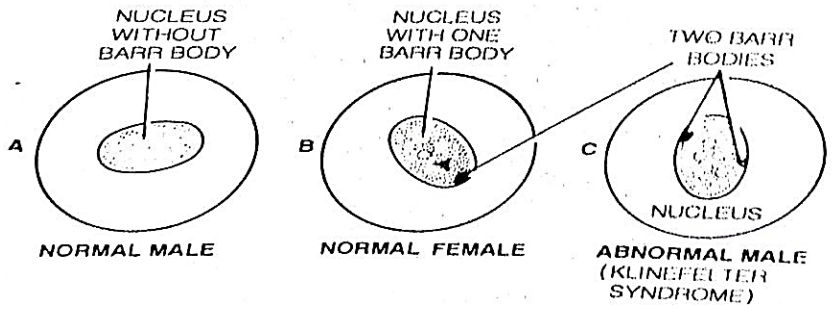


Fig 1.10– Recognition of sex in man by Barr body; (A) Nucleus of normal male with no Barr body; (B) Nucleus of normal diploid female with one Barr body; (C) Nucleus of triploid female with two Barr bodies. (Source – V.B. Rastogi)

1.7 DOSAGE COMPENSATION AND LYON’S HYPOTHESIS

Dosage Compensation- Dosage compensation is the process by which organisms equalize the expression of genes between members of different biological sexes.

In human there are two X-chromosomes in normal females and only one in males. This situation should create a genetic problem for sex linked genes between males and females. The dosage compensation is the procedure by which the effective dosage of X-linked genes in two sexes is made equal or nearly equal , so that the x-linked genes produce the same phenotypic effect in single or double dose. The dosage can be achieved in two possible ways.

i. Inactivation or facultative heterochromatisation of one of two X-chromosomes in females (Lyonization in mammals).

ii. Enhancement of gene expression by stimulated rate of gene transcription of sex-linked gene from single X-chromosome of males (In *Drosophila*).

The Lyon‘s hypothesis or dosage compensation was independently proposed by Mary Lyon (1961) and Liane Russell which states that only one of the two x-chromosomes in the homogenetic sex is functional while the other condenses and is inactivated. The X inactivation in some cells would be that from the mother.

Objectives of Lyon‘s Hypothesis

i. In normal mammalian females, one of the two X‘s is genetically inactive in the somatic cells.

ii. Inactivation is random i.e, irrespective of paternal and maternal origin (random inactivation).

iii. The particular X which has thus become inactivated, remains inactive in all the succeeding cell generation (fixed Differentiation).

1.8 SUMMARY

- Genetic variation is the variation in alleles and genes, both within and among population.
- Genetic variation can be caused by mutation, random mating, random fertilization and recombination between homologous chromosomes during meiosis.
- Genetic information is stored in the sequence of bases along a nucleic acid chain.
- The flow of genetic information depends on the genetic code.
- Sex linkage is the phenotypic expression of an allele that is dependent on the gender of the individual and is directly lied to the sex chromosome.
- In man and *Drosophila* the homogametic sex is female (XX) and the heterogametic sex is male (XY).
- The sex in *Drosophila* determined by the ratio of X-chromosome to autosome (quantitative or ratio theory of sex determination).
- Aneuploidy is the change in the chromosome number, that can caused by loss of one chromosome (hypoploidy) or by addition of one or more chromosomes to complete set (hyperploidy).
- The numberof sex chromatin bodies or Barr body per nucleus is one less than the number of X-chromosomes.

1.9 TERMINAL QUESTIONS

Q-1 What are two main sources of genetic variation ? Explain in brief.

……
……
……
……

Q-2 Describe sex chromosomal abnormalities.

……
……
……
……

Q-3 What is ―crossing over‖? At which sub-stage does it occur?

……
……
……
……

Q-4 Describe Trisomy in man.

……
……
……
……

Q-5 explain Bridge‘s genic balance theory of sex determination in *Drosophila.*

……
……
……
……

Q-6 Describe haemophilia in man.

……
……
……
……

Multiple Choice Questions:-

i. The chromosome number in human beings is-

   a. 46+XX or XY
   b. 46+XX only
   c. 46+XXY only
   d. none of these

ii. The number of autosomes in humans-

a. 44

b. 21 pairs

c. 46

d. 45

iii. Which of the following is a sex linked character in human-

a. Colour blindness

b. Haemophilia

c. a and b both

d. none of these

iv. Both *Drosophila* and mammals have XY system of sex determination. Dosage compensation Y inactivation of one X chromosome is seen-

a. mammal

b. Drosophila

c. Metaphase

d. Telophase

v. Which chromosomal abnormality is known as Down‘s syndrome ?

a. Trisomy 13

b. Trisomy 18

c. Trisomy 21

d. 47 XXX

vi. In *Drosophila* sex is not determined by-

a. X-chromosome

b. Y-chromosome

c. a and b both

d. none of these

vii. A gynendromorph is a –

a. male Drosophila

b. female Drosophila

c. Half male and half female Drosophila

d. None of these

viii. Which of the following chromosomal abnormalities is known as Turner‘s syndrome?

a. Trisomy 21

b. 47, xxy

c. Trisomy 13

d. 45 XO

ix. Which syndrome is also known as trisomy 18 ?

a. Down‘s syndrome

b. Edward syndrome

c. Patau syndrome

d. Klinefelter syndrome

x. Barr body is found in following phase of life cycle-

a. Interphase

b. Metaphase

c. G1 phase

d. Telophase

1.10 ANSWERS

i-a, ii-a, iii-c, iv-b, v-c, vi–b, vii-c, viii-d, ix-b, x-a

UNIT-2 BLOOD GROUP, DNA AND RNA

2.1 INTRODUCTION

The term ―blood group‖ refers to the entire blood group system comprising red blood cell (RBC) antigen whose specificity is controlled by a series of genes which can be allelic or linked very closely on the same chromosome. Landsteiner has been credited for the discovery of ABO blood group system in 1900. Genes in a person‘s DNA code for the specific glycosyltransferasesto allow for the addition of antigen A and/or B to the O antigen.

Mutation in genes that code for enzymes can cause inborn errors of metabolism in man. Enzyme deficiency or in activity leads to accumulation of substrate precursors or metabolites or to deficiencies of the enzyme‘s products. Fructose intolerance, Galactosemia, Maple, Syrup urine disease, phenyl ketonuria, Alkeptonuria and Albinism are examples of inborn error of metabolism.

After Avery‘s findings, many scientists were unconvinced of the conclusion that DNA was the genetic material. The question as to whether it is the DNA or one of the protein components which specifies the information for the synthesis of new phage particles was answered in 1952 by the American geneticist Harshey and Chase that the genetic material of phage T_2 was shown to be DNA.

In recent years a large and convincing body of evidence has accumulated to show that DNA is also the genetic material of eukaryotes. However, in certain viruses which infect animals, plants and bacteria, DNA is replaced by the chemically similar ribonucleic acid (RNA) as genetic material. Nucleic acids are the biopolymers, or small biomolecules, essential to all known forms of life. The term nucleic acid is the overall name for DNA and RNA. They are composed of nucleotides which are the monomers made of three components: a 5-carbon sugar, a phosphate group and a nitrogenous base. Before a cell divides, its DNA is replicated (duplicated). DNA replication is the process by which DNA makes a copy of itself during cell division. DNA replication is said to be semi-conservative, where the resulting double helix is composed of both an old strand and a new strand. In the semiconservative hypothesis, where the resulting double

helix is composed of both an old strand and a new strand. In the semiconservative hypothesis, proposed by Watson and Crick, the two strands of a DNA molecule separated during replication and each strand then acts as a template for synthesis of a new strand. The Messelson-Stahl experiment is an experiment which supported Watson and Crick‘s hypothesis that DNA replication was semiconservative.

Objective

After studying this unit you should be able to –

1) Understand the type and inheritance of blood group and haemoglobin in man.
2) Understand about inherited diseases caused by abnormal haemoglobin.
3) Understand about rare genetic disorders caused by defects in specific proteins (enzymes).
4) Know the molecular structure of nucleic acids : DNA and RNA
5) Know about some experiments that helped to confirm that DNA is genetic material and replication of DNA.

2.2 BLOOD GROUP AND HAEMOGLOBIN, GENETICS IN MAN

Blood Group- A blood group (blood type) is a classification of blood, based on the presence and absence of antibodies and inherited antigenic substances on the surface of red blood cells (RBCs). These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Several of these red blood cell surface antigens can stem from one allele (or an alternative version of a gene) and collectively form a blood group system.

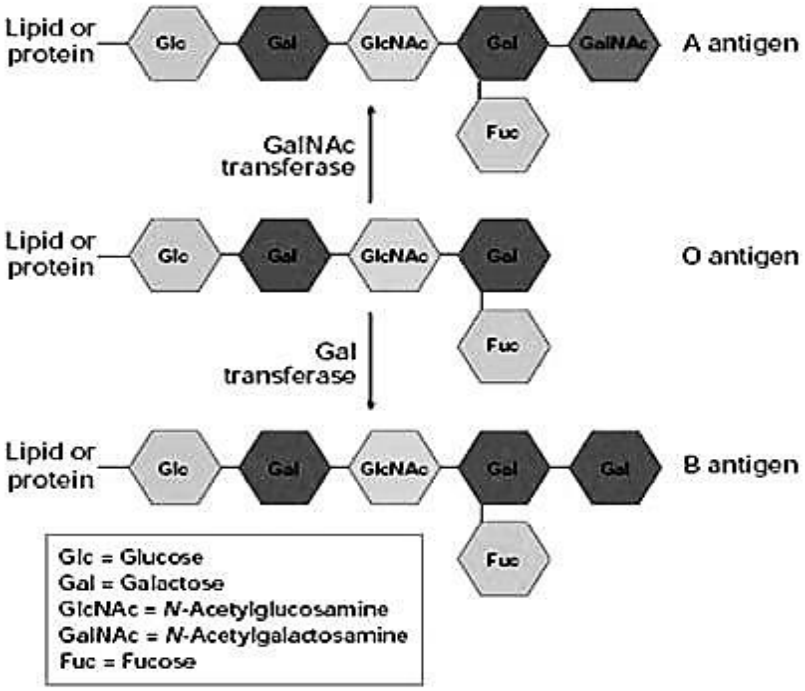


Fig.2.1. The structure of terminal sugars in the A,B and O blood antigen. (Biology Stack exchange)

There are two most important blood group systems are ABO and Rh; they determine someone‘s blood group as A, B, AB and O with +ve, -ve or null denoting RhD status.

Genetics- Blood groups are inherited from both parents. The ABO blood type is controlled by a single gene (the ABOgene) with three types of allelesinferred from classicalgenetics: *i*, I^A, and I^B. The *I*designation stands for **isoagglutinogen**, another term for antigen.The gene encodes a glycosyltransferase—that is, an enzyme that modifies the carbohydrate content of the red blood cell antigens.
The gene is located on the long arm of the ninth chromosome (9q34).
The I^A allele gives type A, I^B gives type B, and *i* gives type O. As both $I^A$and I^B are dominant over *i*, only *ii* people have type O blood. Individuals with I^AI^A or I^Ai have type A blood, and individuals with I^BI^B or I^Bi have type B. I^AI^B people have both phenotypes, because A and B express a special dominance relationship: codominance, which means that type A and B parents can have an AB child. A couple with type A and type B can also have a type O child if they are both heterozygous (I^Bi,I^Ai). The *cis-AB* phenotype has a single enzyme that creates both A and B antigens. The resulting red blood cells do not usually express A or B antigen at the same level that would be expected on common group A_1 or B red blood cells, which can help solve the problem of an apparently genetically impossible blood group.

Haemoglobin – (Hb or Hgb)- Haemoglobin is the iron-containing oxygen transport metalloprotein in the red blood cells of almost all vertebrates well as the tissue of same invertebrates. Haemoglobin in blood carries oxygen from the lungs or gills to the rest of the body. The normal range of haemoglobin in men is 13.5 to 17.5 grams per deciliter and for women, 12.0 to 15.5 grams per deciliter.
Each haemoglobin molecule is made up of four haeme groups surrounding a globin group forming a tetrahedral- structure.
Tetrahedral Structure – There are four iron atoms in each molecule of haemoglobin, which accordingly combined four atoms of oxygen. Globin consists of two linked pairs of polypeptide chains.
The HBB gene provides instructions for making a protein called beta-globin. Beta- globin is a component (sub unit) of a larger protein called haemoglobin, which is located inside red blood cells. The HBA gene provides instruction for making a protein called alpha-gloin. These HBB and HBA genes are located on chromosome 11 and 16 respectively(fig2.2).

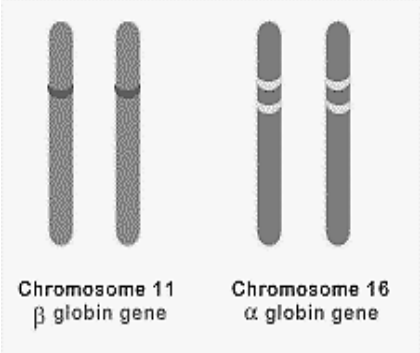


Fig.2.2. The gene of Beta chain is located on short arm of chromosome 11 and the genes of Alpha chain are located on short arm of chromosome 16. (Thalassamia.com)

Haemoglobin disorders are inherited blood diseases that affect the quality or amount of haemoglobin and the capacity to carry oxygen around the body. They fall into two main categories

i) Sickle cell Anaemia

ii) Thalassaemias

Sickle cell Anaemia- The inheritance of sickle cell anaemia in human beings is an example of incomplete dominance, lethal effect, pleiotropism and polymorphism. This abnormality occurs by an autosomal mutant allele Hbs. In homozygous condition this produces abnormal haemoglobin, called haemoglobin S (Hbs). It differs from normal haemoglobin.

Hba in its electrophoretic mobility and oxygen carrying capacity. Under low oxygen concentration RBCs with Hbs become narrowand sickle – shaped. Such sickle-shaped RBCs have lower life span, clump together and often cause vascular obstruction Vernon Ingram (1957) showed that in B-chain of HbAHaemoglobin, the amino acid on 6^{th} position of B-polypeptide chain is glutamic acid but in Hbs this amino is substituted by amino acid valine.

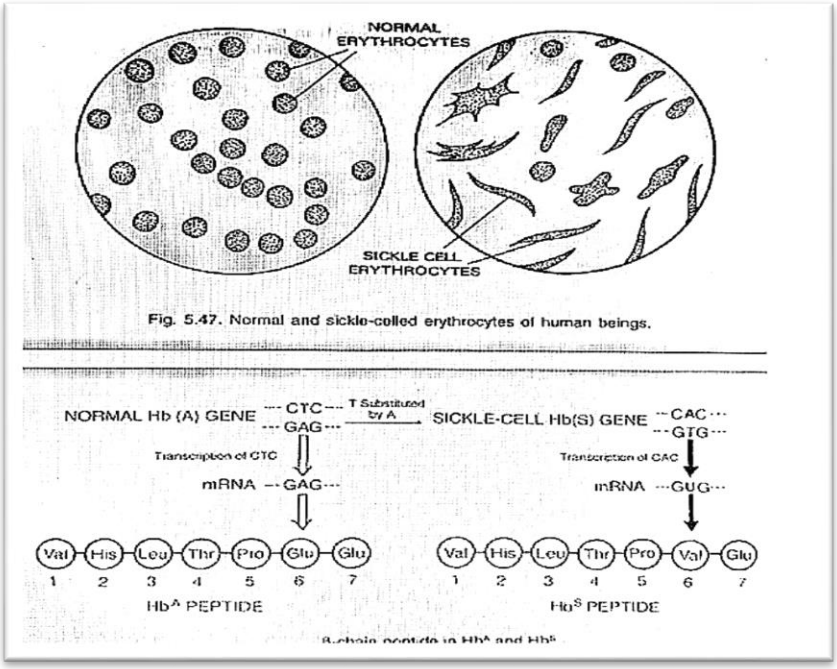


Fig2.3.Sickle Cell Anaemia:Red Blood Cells contort into a sickle shape.

(mayoclinic.org)

Thalassemia is an inherited blood disorder in which body makes an abnormal haemoglobin by mutations in the DNA of cell that make haemoglobin. There are two main types of thalassemia.

i. Alpha thalassemia occurs when a gene or genes related to the alpha globin protein are missing or changed (mutated)

ii. Beta thalassemia occurs when similar gene defects affects production of the beta globin protein.

2.3 INBORN ERRORS OF METABOLISM IN MAN

In born errors of metabolism are rare genetic disorders in which the body cannot properly turn food into energy. The disorders are usually caused by defects in specific proteins (enzymes) that help in metabolism of food. Several inborn errors of metabolism cause developmental delays or other medical problem if they are not controlled.

There are many different types of inborn errors of metabolism – Fructose intolerance. Galactosemia, Maple syrup urine disease (MSUD), Phenylketonuria (PKU), Alkeptonuria, Albinism.

Fructose Intolrance- Hereditary fructose intolerance is an inborn error of fructose metabolism caused by a deficiency of the enzyme aldolose B. It is an autosomal recessive condition caused by mutations in the ALDOB gene, located at 9q31.

Galactosemia is a condition in which the person is unable to metabolize the simple sugar galactose. It is a autosomal recessive disorder caused by mutation in GALT gene. There are three forms of the galactosemia.

a) Galactose -1-phosphate uridyl transferees deficiency

b) Galactose kinase deficiency.

c) Galactose – 6 phosphate epimerase deficiency.

People with galactosemia cannot tolerate any form of milk. They must be careful about eating food containing galactose.

Maple syrup urine disease (MSUD)

- In this condition people cannot break down the amino acids leucine isoleucine and valine. This leads to a buildup of these amino acids in the blood. The urine of people with this disorder can smell like maple syrup.

- It is an autosomal recessive metabolic disorder affecting branched-chain amino acids. It is a type of organic academia.

Phenylketonuria (PKU)- PKU is an inherited autosomal recessive disorder that increases the level of phenylalanine hydroxylase (PAH). PAH is necessary to metabolize, the amino acid phenylalanine (Phe) to the amino acid tyrosine (fig 2.4). In this condition symptoms may include mental retardation light colouring of skin, eczema (an itchy skin rash) and a musty odor.

Alkeptonuria- Black urine disease is a rare inherited autosomal recessive disorder of phenylalanine and tyrosine metabolism due to a defect in the enzyme homogentisate 1, 2- dioxygenase. Mutation in the HGD gene causealkeptonuria(fig2.4). The HGD gene gives instructions for making an enzyme called homogentisate oxidase. Following signs and symptoms are as follows –

- It is asymptomatic, but later sclera of the eyes may be pigmentated
- The skin may be darkened at/on/in sun- exposed areas
- Urine may turn brown if collected and left exposed to open air.
- The accumulation of homogentisic acid in tissues.

Albinism- Albinism is a congenital disorder in which complete or partial absence of melanin pigment in the skin, hair and eyes due to absence of an enzyme tyrosinase involved in the production of milanine (fig.2.4). It is due to autosomal recessive alleles (genes) passed from both parents of an individual.

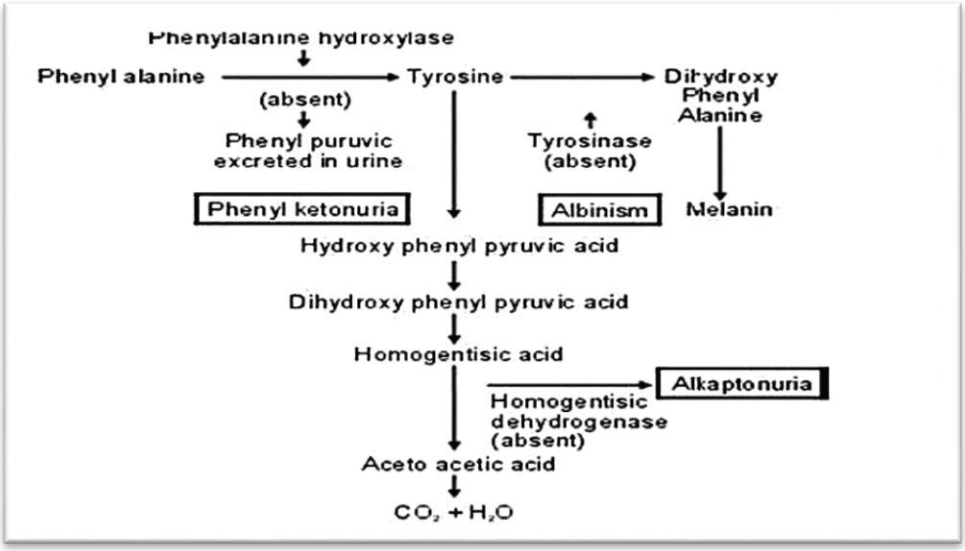


Fig.2.4. Inborn errors (Phenylketonuria, Alkeptonuria and Albinism)of metabolism in Man.

2.4 DNA AND RNA STRUCTURE

DNA Structure-

1) Primary structure of the DNA : covalent backbone and bases aside.
   i) Phosphoric acid
   ii) Pentose sugar
   iii) Nitrogenous bases (Pyrimidine‘s and Purines)
2) Secondary and tertiary structures of the molecule : Three dimensional conformation of DNA
   i) Dinuclotides
   ii) DNA molecule
      a) Hydrogen bonds : bases pairing
      b) Major groove and minor groove

iii) Non-B DNA

a) Z-DNA

b) Cruciform DNA and hairpin DNA

c) H-DNA or triplex DNA

d) G4-DNA

1) **Primary structure of the DNA-** A nucleotide is made of a phosphate + a pentose sugar + a nitrogenous base.

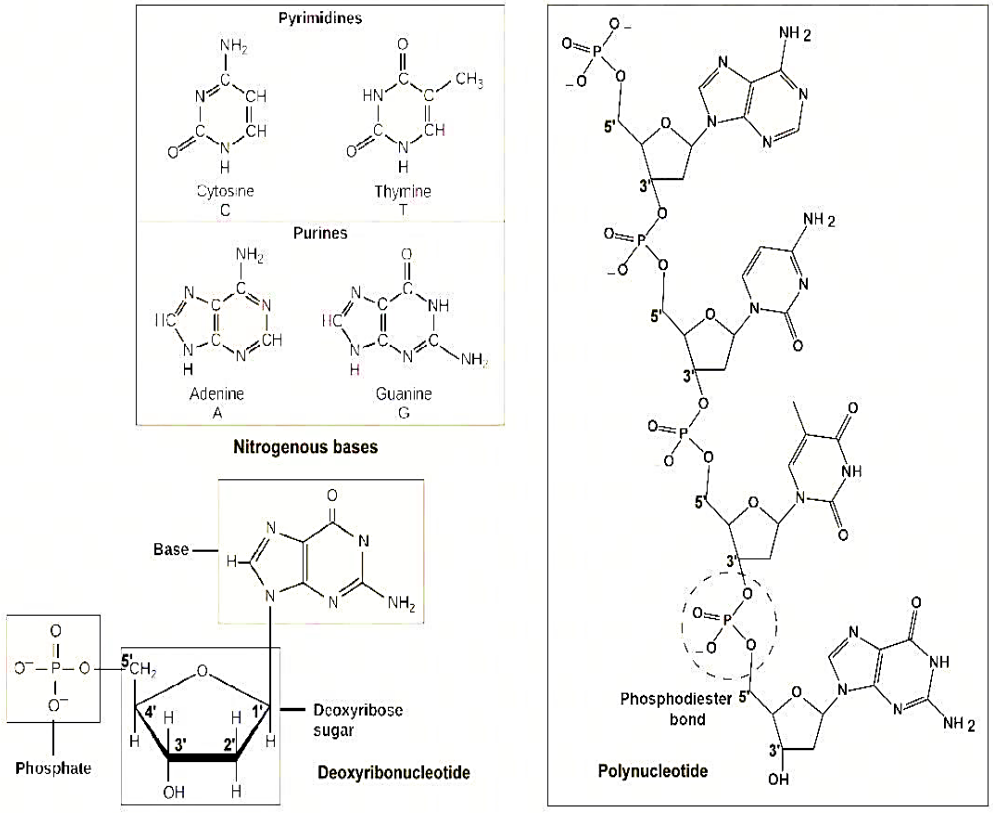
i) Phosphoric acid :- give a phosphate group

ii) Pentose sugar :- Deoxyribose, which is a cyclic pentose (5 carbon sugar, carbons in the sugar are noted from 1‘ to 5‘. A nitrogen atom from the nitrogenous base links to C1‘ (glycosidic link) and the phosphate links toCs‘ (ester link) to make the nucleotide. The nucleotide is therefore – C5‘ sugar C1 – base.

iii) Nitrogenous bases– Aromatic heterocycles; there are purines and pyrimidines.

Purines :- adenine (A) and guanine (G)

Pyrimidines : Cytosin (C) and thymine (T) (note : thymine is replaced by uracyle (U) in (RNA).



(A) (B)

Fig 2.5. Primary structure of DNA; (A) Deoxyribonucleotide ; (B) Single strand of polynucleotide

2) Secondary and tertiary structures of the molecule : Three-dimensional conformation of DNA

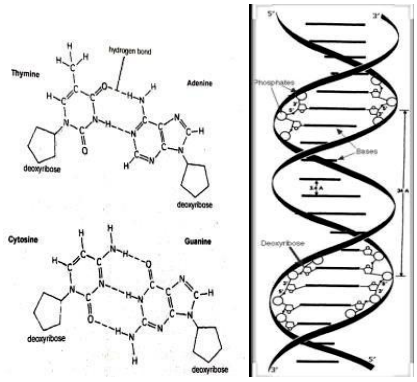
i) **Dinucleotides**– Dinucleotides form from a phosphodiester link between 2 mononucleotides. The phosphate of a mononucleotide (in C5‘ of its pentose sugar) being linked to the C3‘ of the sugar of the previous mononucleotide. Polynucleotides are made of the successive addition of monomers in a general 5‘-3‘ configuration. The backbone of the molecule is made of a succession of phosphate-sugar (nucleotide n)- phosphate-sugar (nucleotide n+1) and so on, covalently linked, the bases being aside.

ii) **DNA Molecule-** DNA is made of two dextrogyre (like a screw ; right handed) helical chains or strands (the double helix), coiled around an axis to form a double helix of $20A^0$ of diameter. The two strands are antiparallel. The general appearances of the poltmer shows a periodicity of 3.4 A^0; corresponding to the distance between 2 bases, and another one of 34 A^o, corresponding to one helix turn (and also to 10 bases pairs).

a) **Hydrogen bonds : bases pairing-** The bases (hydrophobic) are stacked on the inside, there planes are perpendicular to the axis of the double helix (fig.2.6). The outside (phosphate and sugar) are hydrophilic. Hydrogen bonds between the bases of one strandand that of the other strand hold the two strands together. A purine on one strand shall link to a pyrimidine on the other other strand. As a corallans, the number of purines residues equals the number of pyrimidine residue.

A binds T with 2 hydrogen bonds binds C with 3 hydrogen bonds (more stable links) fig.2.6

The content in ‗A‘ in the DNA is therefore equal to the content in ‗T‘ and the content in ‗G‘ equals the content in ‗C‘. This strictcorrespondence (A-T) and G-C make the 2 strands complementary. One is the template of the other one, and reciprocally this properly will allow exact replication (semi-conservative replication: one strand – the template- is conserved, another is newly synthesized, some with the second strand, conserved, allowing another one to be newly synthesized).



(A) (B)

Fig2.6.DNAstructure; A) Hydrogen bonding between Adenine-Thymine and Guanine-Cytosine base pairs; B) Diagrammatic representation of Double helical DNA molecule as proposed by Watson and Crick

b) **Major groove and minor groove**- The double helix is a quite rigid molecule. It presents a major groove and a minor groove. The major groove is deep and wide, the minor groove is narrow and shallow. DNA – protein interactions are essential processes. Protein bind at the floor of the DNA grooves, using specific binding : hydrogen bonds, and non specific binding: Van der Walls interaction. Some proteins bind DNA in its major groove, some other in the minor groove, and some need to bind to both.

The two strands are called ―plus‖ and ―minus‖ strands or ―direct‖ and ‗reverse‘ strands DNA is ionized in vivo and behave like apolyanion.The double helix as described above is the ―B‖ form of the DNA; it is the form the most commonly found in vivo, but other forms exist in vivo or in vitro. The ‗A‘ form resemble B-DNA but it is less hydrated than B-DNA, ‗A‘ form is not found in vivo.

iii) Non B-DNA

a) Z-DNA
b) Cruciform DNA & hairpin DNA
c) H – DNA or triplex DNA
d) G4 – DNA

Z – DNA is a left handed double helix with a zig-zag conformation of the backbone (less smooth than B-DNA). Only one groove is observed, resembling the minor groove. The bases are here at the outer surface phosphates are closer together than in B-DNA. Z DNA cannot form nucleosome.

Cruciform DNA and hair pin DNA; Holiday junctions (formed during recombination) are cruciform structures. Inverted (or minor) repeats (palindromes) of poly purines. Poly pyrimidine DNA stretches can also cruciform or hairpin structures through intrastrandpairing (fig 2.7).

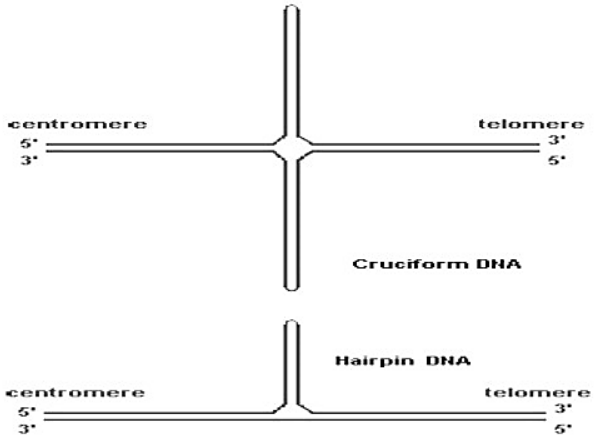


Fig. 2.7. Cruciform DNA & Hairpin DNA

H-DNA or triplex DNA- Inverted repeats (palindromes) of poly purine/poly pyrimidine DNA stretches can form triplex structures (triple helix). A triple – stranded plus a single stranded DNA are formed. H-DNA may have a role in functional regulation of gene expression as well as on RNAs (eg repression of transcription).

G4-DNA or Quadruplex DNA- folding of double stranded GC rich sequence onto itself forming Hoogsteen base pairing between 4 guanines (_Gu‘) a highly stable structure often found near promoters of genes and at telomeres.

Adenine and Guanine are purines, cytosine and uracil are pyrimidines.

RNA structure- RNA is typically single stranded and is made of ribonucleotides that are linked by phosphodiester bonds. A ribonucleotide in the RNA chain contains ribose (the pentose sugar), one of the four nitrogenous bases(A,U,G and C) and a phosphate group. RNA have much shorter chains of nucleotides.

A phosphate group is attached to the 3‘ position of one ribose and the 5‘ position of the next. The phosphate groups have a negative charge each, making RNA a charged molecule (polyanion). The bases form hydrogen bonds between cytosine and guanine between adenine and uracil and between guanine and uracil. An important structure component of RNA that distinguishes it from DNA in the presence of hydroxyl (OH) group at the 2‘ position of the ribose sugar (fig2.8). The presence of this functional group causes the helix to mostly take the A-form geometry; The A-form geometry results in a very deep and narrow major groove and shallow and another consequence of the presence 2‘ OH group is that in conformationally flexible regions of an RNA molecule (that is not involved in formation of double helix), it can chemically attack the adjacent phosphodiester bond to cleave the backbone.

RNA is transcribed with only four bases (A,C,G & U), but these bases and attached sugars can be modified in numerous ways as the RNAs mature Pseudouridine (Ψ), in which the linkage between uracil and ribose is changed from a C-N bond to a C-C bond, and ribothymidine (T) are found in various places (the most notable ones being in the TΨC loop of RNA. Another notable modified base is hypoxanthine, a deaminated adenine base whose nucleoside is called inosine (I).Inosine plays a key role in the wobble hypothesis of the genetic code.

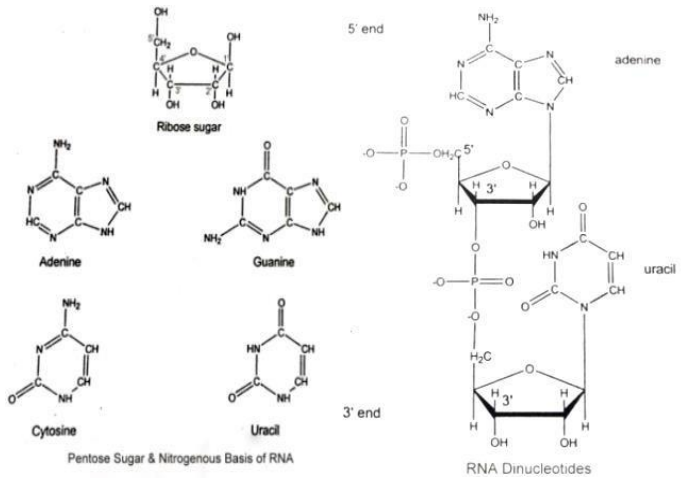


Fig 2.8 - Molecular Structure Of RNA

Different Types of RNA- The cells of all eukaryotes and prokaryotes contain three classes of RNA molecules which can be distinguished by structural and functional criteria. These are ribosomal RNA (rRNA), transfer RNA (tRNA) and messenger RNA (mRNA). Both rRNA and tRNA are metabolically stable and are concerned with deciphering the genetic code. But they do not code for proteins themselves. On the other hand, mRNA is generally unstable (i.e constantly being synthesized and degraded). It carries the nucleotide code which specifies the primary structures of protein. All of these RNAs are transcribed from DNA templates.

Ribosomal RNA (rRNA)- As the name implies, rRNA is structural component of ribosomes. Some of the commonly found structural elements are as follows.

Duplexes : Duplex RNA consists of a right handed double helix stabilized by hydrogen bonds between the bases on opposite complementary strands and by stacking between adjoining bases. The helices are of the A-form. The A-form RNA helix has 11 bp per turn, as opposed to 10 bp per turn for the usualB-form DNA helix.

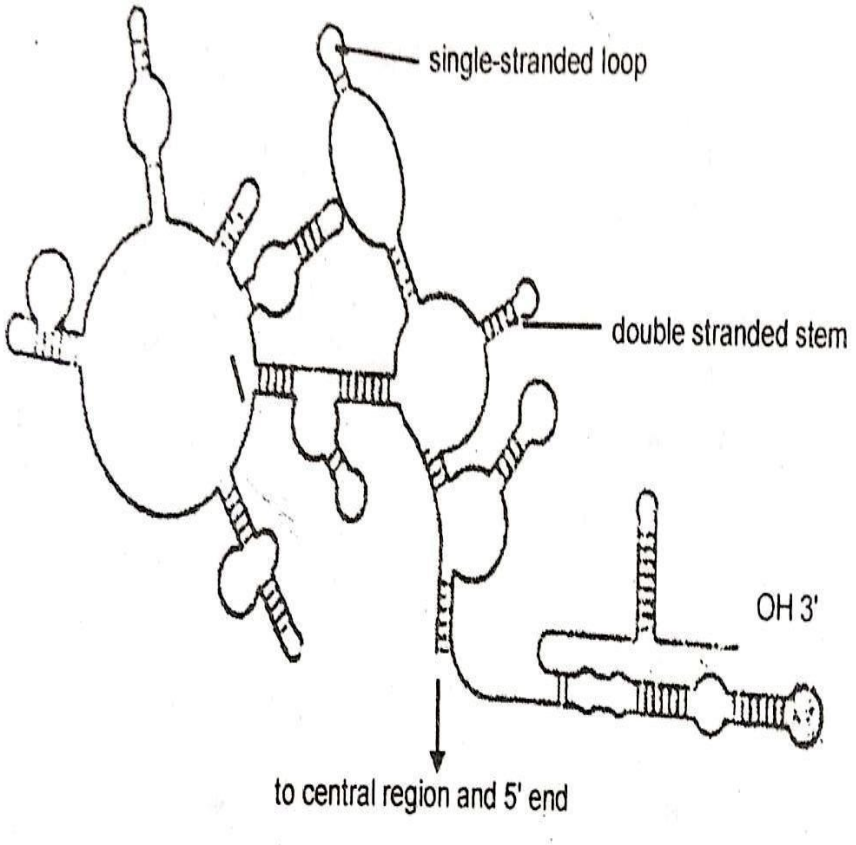


Fig.2.9 - Secondary structure of rRNA of *E.coli*.

Single Stranded regions: Stretchesof RNA which are not self-complementary exist as single stranded regions because the bases here are unpaired. These stretches can be relatively long.

Hairpins- Stretches of RNA which possess complementary nucleotides flanking a small stretch without any self-complementarity give rise to what are called variously ‗hairpins' or ‗stem-and-loop' structures. In this case the two self complimentary regions will pair and intervening non-complimentary sequence will loop out. This is a hairpin structure. Hairpin thus consist of a duplex bridged by a loop of unpaired nucleotides.

Bulge loops- Bulge loop are formed by unpaired nucleotides in one strand of a double stranded region. Bulge can also be form by a single base. Bulge loops give rise to a bend in the duplex and thus can affect the long range structure of RNA.

Internal Loops- Internal loops contain several nucleotides not capable of forming Watson – Crick base pairs. Symmetrical internal loops contain an equal no of unpaired nucleotides in each strand and Asymmetrical internal loops contain an unequal number of unpaired nucleotides.

Junction- The structure formed by three or more duplexes coming together at a point is called junction. This structure is in fact a multi branch loop. The loop forming the junction are formed by a variable no of nucleotides in each of the single strands.

Transfer RNA (tRNA)- Transfer RNA molecules varying length between 60 and 95 nucleotides, with the majority measuring about 75 nucleotides (much smaller than the normal mRNA strand). Regions of self-complementarity within tRNA create a cloverleaf-shaped structure.

The Various regions of the clover leaf model of tRNA are as follows:

1) Amino acid arm : It has seven pairs stem formed by base pairing between 5‘ and 3‘ ends of tRNA. At 3‘ end a sequence of 5‘-CCA-3‘ is added. This is called CCA arm or amino acid acceptor arm. Amino acid binds to this arm during protein synthesis.

2) D-arm : Going from 5‘ to 3‘ direction or anticlockwise direction, next arm is D-arm. It has a 3 to 4 base pair stem and a loop called D-loop or DHU-loop. It contains a modified base dihydrouracil.

3) Anticodon arm : Next is the arm which lies opposite to the acceptor arm. It has a five base pair stem and a loop in which there are three adjacent nucleotides called anticodon which are complementary to the codon of mRNA.

4) An extra arm : Next lies an extra arm which consist of 3-21 bases. Depending upon the length, extra arms are of two types, small extra arm with 3-5 bases and other a large arm having 13-21 bases.

5) T-arm or TΨC arm : It has a modified base pseudouridine Ψ. It has a five base pair stem with a loop. There are about 50 different types of modified bases in different tRNAs, but four bases are more common. One is ribothymidine which contains thymine and is not found in RNA. Other modified bases are pseudouridine Ψ, dihyrouridine and inosine.

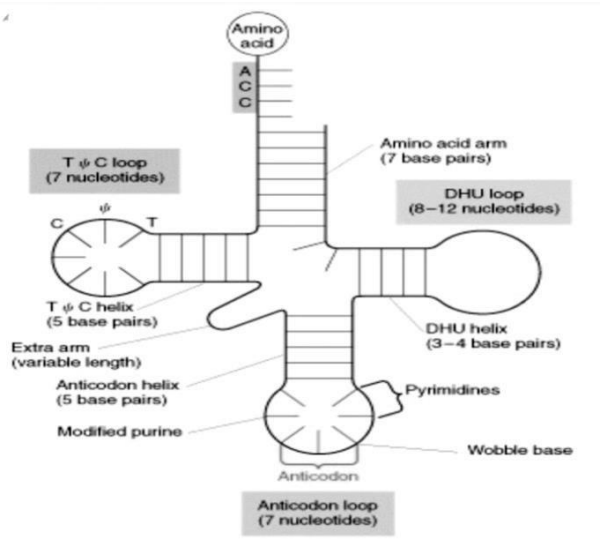


Fig 2.10 - Clover Leaf model of tRNA(Source– Wikipedia)

Messenger RNA (mRNA)- In most eukaryotic and prokaryotic mRNAs not all of the mRNA sequence is translated into proteins. The translated sequence is flanked on either side by non-translated sequences which may be several hundred nucleotides in length. Eukaryotic mRNAs also possess two other common features.

a) The base at the 5‘ end is guanine which has been methylated at the 7 position on the purine ring (m7G _cap‘)

b) The 3‘ end of the molecule contain a sequence of 100-200 adenylic acid residues (the poly(A)‘tail‘). Both of these modifications are post-transcriptional, and are thought to confer stability on the mRNA. Additionaly, the cap enhances the binding of ribosomes to the mRNA.

2.5 HERSHEY-CHASE EXPERIMENTS

The Hershey chase experiments were a series of experiments conducted in 1952 by Alfred Hershey and Martha Chase that helped to confirm that DNA is genetic material. They demonstrated that the DNA injected by phage particle into a bacterium contains the information required to synthesize progeny phage particle.

A single particle of phage T2 consists of DNA (now known to be a single molecule) encased in a protein shell. The DNA is the only phosphorous containing substance in the phage particle; the proteins of the shell, which contain the amino acids methionine and cysteine, have the only sulphur atoms. In these experiments, phage DNA was made radio-active by growing infected bacteria on a medium containing radioactive phosphate ($^{32}PO_4$). Since DNA do not contain phosphorus, only DNA would be labelled. Similarly, phage proteins were labelled with the help of $^{35}SO_4$. Since DNA does not contain sulphur, protein would be labelled with ^{35}S. Such differential labelling would enable one to distinguish between DNA and proteins of the phage without performing any chemical tests.

Hershey and Chase then allowed both kinds of labelled phage particles to infect E. coli bacteria. The infected bacteria were immediately agitated in a waring blender. After shaking, only radioactive ^{32}P was found associated with bacterial cells and 35S was found only in surrounding medium and not in bacterial cells. When phage progeny was studied for radioactivity in this experiment, it was found that the phage progeny carried labelled only with 32P. The progeny was not labelled with ^{35}S. This clearly indicates that only DNA and not protein is injected into bacterial cells. The empty protein coat (ghost) left outside. Thus, Hershey and Chase experiment proved that DNA entering the host cell carries all the genetic information or synthesis of new phage particles, hence, is the sole genetic material in DNA bacteriophages.

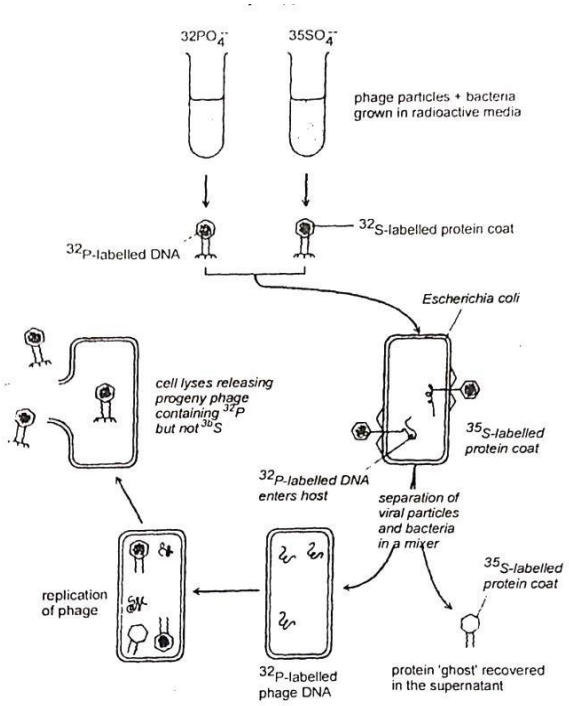


Fig 2.11 - The Hershey &Chase Experiment (source : B.A Ansari)

2.6 REPLICATION OF DNA – MESSELSON AND STAHL'S EXPERIMENT

One of the important feature of living organism is that they are capable of reproducing themselves with a high degree of accuracy. It follows that the genetic material, DNA, must also possess this quality. In their classic paper on DNA structure, Watson and Crick stated that it has not escaped our notice that the specific (base) pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

The idea was that the two complementary strands could separate from each other by disruption of the relatively weak forces joining them (hydrogen bonds) and because of the specificity of base pairing each strand could then function as a template for the synthesis of a new complementary strand.

Experimental support for this notion was provided by Matthew Messelson and Franklin Stahl (1957-58) using the newly developed technique known as **Density labelling**.

Messelson and Stahl's Experiment – In this experiment *E. coli* cells were grown for many generations in a medium containing the heavy stable isotope of nitrogen. 15N(in the form of 15NH4Cl) such that all the nitrogen atoms of the bases contained 15N, thereby making the DNA more dense than ordinary DNA. If such ‗heavy' DNA is mixed with normal ‗light' DNA and centrifuged to equilibrium is a concentrated solution of caesium chloride, the DNA molecules redistribute in the gradient of the caesium chloride concentration which forms, and band at positions in the gradient at which the density of the solution is equal to their own buoyant density. Hence two DNA bands of different buoyant density are resolved, corresponding to the ‗heavy' and ‗light'DNA's.

The 15N-labelled cells were transferred to a normal medium containing 14N and allowed to continue to grow. Samples of the bacteria were taken at different times during the experiment. DNA extracted from them and their buoyant densities determined in the ultracentrifuge. It was seen that the cells grown entirely in 15N containing medium contain all ‗heavy' DNA. After one cell generation following transfer to 14N medium the DNA has a density intermediate between that of ‗heavy' DNA and ‗light' DNA. After two generations, two bands are visible, a ‗light' band and a band of intermediate density. In successive generations the proportion of ‗light' DNA increases relative to that of intermediate density DNA. The interpretation of these results is that the replication of DNA is semi-conservative that is the two parental DNA strands serve as templates for the synthesis of complementary daughter strands as predicted by Watson and Crick.

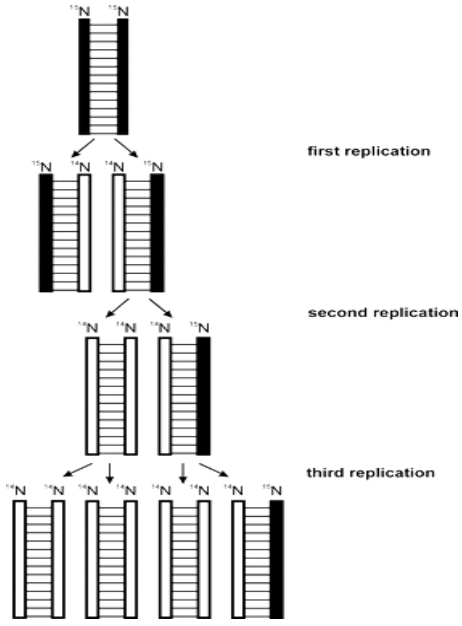


Fig 2.12 - Density distribution of DNA molecules generated during the first 3 replication cycles of the Messolson –Stahl experiment. (Source:IUBMBJournal)

Mechanism of Replication- In 1956, Arthur Kornberg discovered that cell-free extracts of *E. coli* contained an enzyme, DNA polymerase I, capable of synthesizing DNA from its precursor. The main features of the activity of this enzyme are.

a) The DNA precursors must be in the form of deoxyribonucleotide 5‘ triphosphates and all four of these (dATP, dCTP, dTTP and dGTP, known collectively as dNTPs) must be present.

b) The enzyme adds dNTPs to the 3‘-OH terminus of a pre-existing DNA (or RNA) strand, known as the primer. During this reaction a new phosphodiester linkage is formed between the 3‘-OH terminus of the primer and the innermost phosphate group of the dNTP, liberating pyrophosphate. The subsequent hydrolysis of pyrophosphate provides the energy to drive the reaction.

c) The polymerase takes its instructions from a single-stranded DNA template which is essential for activity. The dNTPs align themselves on the DNA template by complementary base pairing which follows the Watson-Crick rules. Thus the precise sequence of the newly synthesized strand is determined by the template strand.

Following the discovery of DNA polymerase I, it was assumed that this enzyme was chiefly responsible for replicating DNA. However, mutants of *E. coli* which were almost totally lacking DNA polymerase I activity (termed pol A1 mutants) were isolated, and shown to replicate their DNA at a rate comparable to the wild type. It is now known that another enzyme, DNA polymerase III, is mainly responsible for polymerization.

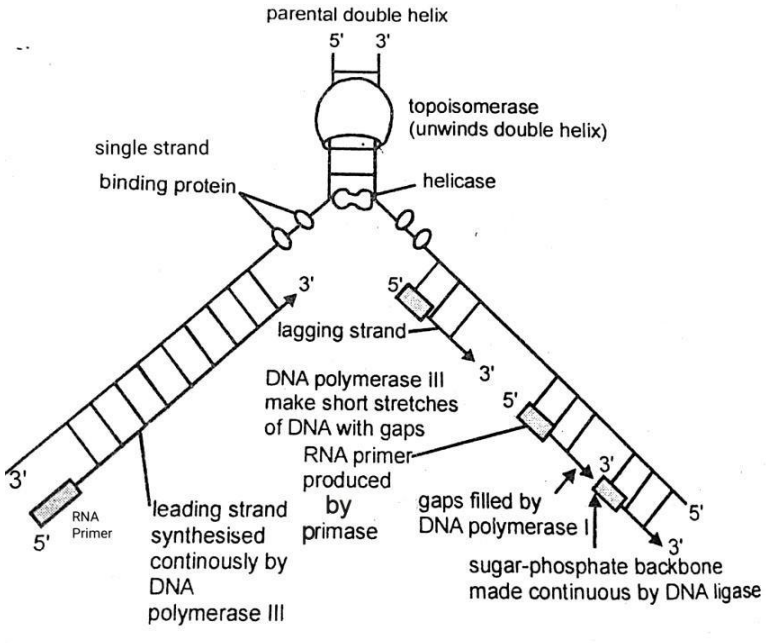


Fig.2.13. Some of the enzymatic events at the Y-shape replicating fork of *E. coli* (Source – B.A. Ansari)

The pol A1 mutants were shown to be far less capable of repairing damage to DNA sustained by ultra-violet light than the wild type. The main functions ascribed to DNA polymerase I are DNA repair and the filling in of gaps between the precursor fragments of DNA.

Autoradiography of replicating prokaryotic chromosomes labelled with 3H-thymidine, a precursor of dTTP, has shown that DNA replication is confined to regions called replicating Y-forks, so called because of their Y-shaped structure. As DNA replication progresses, the Y-forks move along the parental DNA helix. If DNA synthesis were to occur continuously on both of the single stranded template strands made available for the polymerase at the Y-fork , one daughter strand would grow in a 5‘ to 3‘ direction while the other would have to grow in a 3‘ to 5‘ direction (because the parental strands are antiparallel). However, DNA polymerase can only synthesize new strands in the 5‘ to 3‘ direction. The solution to this problem is achieved in the following way:

a) The so called leading strand is synthesized continuously in a 5‘ to 3‘ direction.

b) The so called lagging strand is synthesized in short stretches (1000-2000 nucleotides long in bacteria) again in a 5‘ to 3‘ direction, thereby creating a discontinuous molecule containing gaps. These gaps are later _filled in‘ by DNA polymerase I and the continuity of the molecule is restored by sealing the ends between adjacent stretches of DNA with an enzyme called DNA ligase. In fact, DNA polymerase III cannot initiate DNA chains de novo but requires a primer molecule. This primer is RNA synthesized by an RNA primer as a DNA strand. The RNA primer is subsequently degraded and the gap filled in by DNA polymerase I.

Table-2.0 Some of the important enzymes involved in DNA replication are summarized below :

DNA polymerase III	The major enzyme responsible for DNA replication.
DNA polymerase I	Fills in gap between the fragments of DNA synthesized by DNA polymerase III
Helicase	Uses energy released by ATP hydrolysis to disrupt bonds between base pairs and thereby creates single-stranded regions.
DNA binding protein	Stabilizes single-stranded DNA, preventing re-formation of the double helix.
Topoisomerase	Unwinds the double helix.
DNA ligase	Uses ATP to seal breaks in sugar-phosphate backbone.
RNA primase	Produces short RNA sequences to prime DNA polymerase III

ACCURACY OF DNA REPLICATION- DNA replication is an exceedingly accurate process, and it has been estimated that only one error is made in about 10^9 base pair replication. This high accuracy is due, in large part, to the finding that DNA polymerase has a proofreading activity in addition to its polymerizing activity. One such proofreading activity results from the fact that DNA polymerase can hydrolyse phosphodiester bonds in single strands if mismatched regions occur at the 3‘-OH terminus of the primer strand. Thus an incorrect base is removed before further polymerization occurs.

2.7 SUMMARY

- There are two blood group systems; ABO and Rh
- Inheritance of blood groups A,B, AB and O is governed by three genes; LA, LB and l……correction
- Sickle cell anaemia and Thalassaemia are inherited blood diseases.
- Inborn errors of metabolism in man are rare genetic disorder caused by defects in specific enzymes.
- Primary structure of the DNA is composed of along chain of a monomer called Nucleotides.
- Nucleotides contain–a phosphoric acid +a deoxiribose sugar +a nitrogenous bases (Purines and Pyrimidines) .
- Nucleoside is made of a pentose sugar and a nitrogenous base
- B-DNA is the most common double helical structure found in nature , the double helix is right handed with 10-10..5base pairs per turn and contains a major groove and minor groove.
- Harshey and Chase conducted that DNA, not protein, was the genetic material.They used radioactive isotopes of phosphorus-32 (labelled the DNA) and sulfur-35(labeled the protein of bacteriophages) as markers.
- Bacteriophages are composed of only two substances: Protein and DNA.
- Messelson and Stahl demonstrated that bacterial DNA replicated semiconservatively, means that each strand in a DNA molecule serves as a template for synthesis of a new, complimentary strand. The first replication in the 14N medium produced a band of hybrid (14N and 15 N) DNA. This result eliminated the conservative model of replication.

2.8 TERMINAL QUESTIONS

Q 1. On what basis is the blood type classified ?

……
……
……
……

Q 2. What are haemoglobin disorders ?

……
……
……
……

Q 3. What are metabolic disorders?

……
……
……
……

Q 4. How is DNA is formed?

……
……
……
……

Q 5. What did Harshey and Chase experiment prove ?

……
……
……
……

Q 6. How do we know that DNA replication is semiconservative ?

……
……
……
……

Q 7. Draw the clover leaf structure of tRNA.

……
……
……
……

Multiple Choice Questions

i) DNA replicates during

G- Phase

S-phase

G-phase

M-phase

ii) Production of RNA from DNA is called

Translation

RNA splicing

Transcription

Transposition

iii) Nucleic acids contain

Alanine

Adenine

Lysine

Arginine

iv) What is the structural units of nucleic acids?

N-bases

Nucleotides

Nucleosides

Histones

v) How many types of blood groups are present?

a) 1

b) 2

c) 3

d) 4

vi) The antigens for ABO and Rh blood groups are present on

a) Plasma

b) WBC‘s

c) RBC‘s

d) Platelets

vii) Blood disease which is caused by occurance of mutation in haemoglobin genes is

a) Leukemia

b) Bleeding disorders

c) Thalassemia

d) Hepatitis

viii) Inability to metabolize fructose due to absence of aldolase B enzyme is

a) Galactose intolerance

b) Sugar intolerance

c) Fructose intolerance

d) Carbohydrate intolerance

ix) Inborn errors of metabolism are referred to as

a) Congenital metabolic diseases

b) Inherited metabolic diseases

c) Both A and B

d) None of the above

x) Which of the following enzymes are used to join bits of DNA?

a) DNA ligase

b) DNA polymerase

c) Primase

d) Endonuclease

xi) Pick the right difference between a DNA and RNA

a) sugar and phosphate

b) sugar and purines

c) purines and phosphate

d) sugar and pyrimidines

xii) In a nucleotide the nitrogen base is jointed to the sugar molecule by

a) Phosphodiester bond

b) Glycosidic bond

c) Hydrogen bond

d) Covalent bond

xiii) Hershey and Chase experiment providing DNA as the genetic material was based on the

a) Transduction

b) Transformation

c) Transcription

d) Translation

(xiv) At which end are the DNA bases added?

a) 5‘ triphosphate end

b) 3‘ triphosphate end

c) 5‘-OH end

d) 3‘-OH end

(xv) Which is the most processive of prokaryotic DNA polymerases?

a) Pol I

b) Pol II

c) Pol III

d) Klenow fragment

2.9 Answers

i) b, ii) c, iii) b, iv) c, v) d, vi) c, vii) c, viii) c, ix) c, x) a, xi) d, xii) b, xii) a, xiv) d, xv) c

UNIT-3 DNA POLYMERASE AND *IN VITRO* DNA SYNTHESIS, TRANSCRIPTION, GENETIC CODE, GENE CLONING EXPERIMENT

Structure:

3.1 DNA POLYMERASE AND *IN VITRO* DNA SYNTHESIS

The DNA polymerase is an enzyme discovered by Author Kornberg (1957) in E. coli. This enzyme was found to be responsible for the polymerization of deoxyribonucleotide triphosphate on a DNA template to form a new complementary strand. In prokaryotic cells there are 3 types of DNA polymerase known as polymerase-I, polymerase-II and Polymerase-III. The DNA polymerase-I and II are meant for DNA repair and DNA Polymerase-III is meant for actual DNA replication. Eukaryotic cells have 5 types of DNA polymerase alpha (α), beta (β), gamma (γ), delta (δ) and epsilon (ε).

Prokaryotic DNA polymerase

(1) **DNA polymerase I or Pol I :** This enzyme was isolated around 1960 by Author Kornberg and was the first enzyme suggest to be involved in DNA replication It is also called Kornberg enzyme. The DNA polymerase-I is a single polypeptide chain with a molecular weight of 1,09,000. There is a one atom of Zn present per chain. Thus DNA polymerase are metallo-enzyme.

The electron microscopic studies shows that DNA polymerase is slightly spherical in shape with a diameter of about 65Å. It is attached at regular intervals to the DNA chain. The DNA polymerase contain 4 sites which have functional importance.

(i) Template site : Which attaches to the DNA template and holds a section of DNA in place.

(ii) Primer site : Which contains the primer, a short complementary segment of RNA on which the newly synthesized DNA strand grows.

(iii) Primer terminus site : It is present at the tip of the primer, which has a terminal 3‘OH group.

(iv) Triphosphate site : Where an incoming nucleoside triphosphate matches a complementary nucleotide on the DNA template and is bound to the 3‘OH position of the primer.

DNA polymerase-I has 3 functions.

(a) Polymerization activity : It has been observed that DNA polymerase-I is not essential for DNA replication. It can synthesize only short segments of DNA and takes part in repair synthesis.

(b) 3’→ 5’ exonuclease activity : Polymerase-I catalyses the breakdown of one of the DNA strands into its nucleotides in the 3‘→ 5‘ direction i.e. opposite to the polymerization. That is why it is called 3‘→ 5‘ exonuclease activity. It function as a ―proof reader‖ and ―edits‖ mismatched nucleotides at the primer terminus before proceeding with resynthesis of the strand error made during polymerization are corrected by polymerease-I. It thus acts as repair synthesis.

(c) 5’→ 3’ exonuclease activity : Polymerase-I can also remove nucleotides in the 5‘→ 3‘ direction. This 5‘→ 3‘ exonuclease activity has an important role in the removal of thymine primers. It is also important for removing the RNA primer and filling the gap by deoxyribonucleotides.

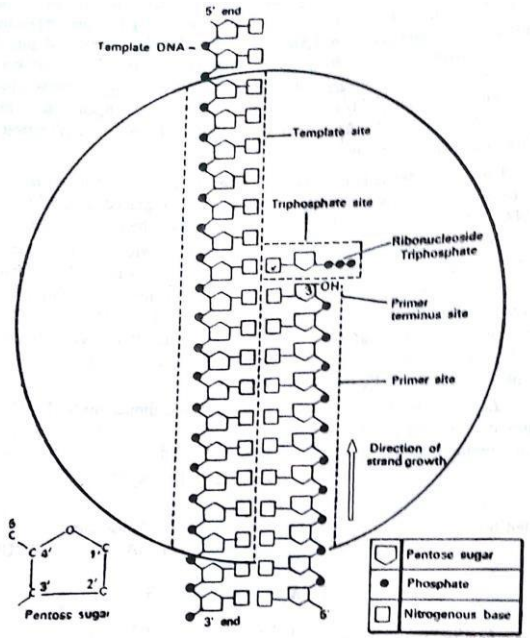


Fig: 3.1 Polymerase I

(2) DNA polymerase-II : It is a single polypeptide chain with molecular weight of 90,000. This enzyme resemble DNA polymerase-I in its activity, but it is a DNA repair enzyme. It bring about the growth in the 5‘→ 3‘ direction using free 3‘OH group. Its polymerization activity is much less than that of polymerase-I. It also shows 3‘→ 5‘ exonuclease activity.

(3) **DNA polymerase-III :** It is the main polymerizing enzyme and plays an essential role in DNA replication. It is a multimeric enzyme or holoenzyme having ten subunits such as alpha(α), beta (β), epsilon (ε), theta (θ), tau (η), gamma (γ), delta (δ), delta dash (δ‘), chi (χ) and psi (ψ). All these ten subunits are needed for replication and having different function. α-subunit has 3‘→ 5‘ exonuclease proof reading or editing activity. The core enzyme consist of three subunit α, β and θ and remaining seven subunits increase processivity.

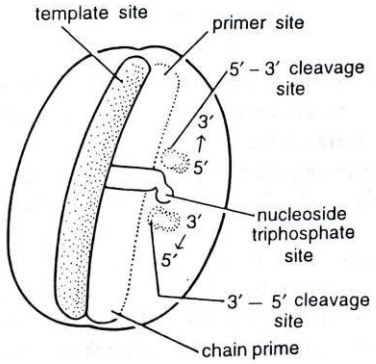


Fig: 3.2 A model of DNA polymerase–I enzyme,

showing its four different active site

Eukaryotic DNA polymerase

Eukaryotic have 5 types of DNA polymerase

(1) DNA polymerase α (alpha) : It is also called cytoplasmic polymerase or large polymerase. It is found both in nucleus and cytoplasm.

(2) DNA polymerase β (beta) : It is also called nuclear polymerase or small polymerase and is found only in vertebrates.

(3) DNA polymerase γ (gamma) : This is called mitochondrial polymerase and is encoded in the nucleus.

(4) DNA polymerase δ (delta) : This enzyme is found in mammalian cells and is PCNA dependent for DNA synthesis processivity (PCNA = proliferating cell nuclear antigen)

(5) DNA polymerase ε (epsilon) : This enzyme is PCNA independent and occurs in mammalian HeLa cell and budding yeast.

The DNA polymerase α is predominant DNA polymerase enzyme in Eukaryotic cells and was believed for long time to be only involved in DNA

replication. But now one more polymerase δ is also found to be involved in eukaryotic DNA replication.

***In vitro* DNA synthesis**

The molecular biologist understanding the mechanism of DNA synthesis inside the living cell (*in vivo*), they have tried *in vitro* synthesis of DNA. The synthesis of DNA is called replication and replication is the process of formation of new DNA strand from the parental strand. Replication of DNA is a semiconservative process in which each of the two double helices formed from the parent double strand have one old and one new strand. For the synthesis of DNA require a DNA template, deoxyribo-nucleoside triphosphate (dATP, dGTP, dCTP, dTTP), Mg^{++}, DNA unwinding protein (DNA helicases), single strand DNA binding proteins or SSBPs, DNA gyrases or topoisomerases, RNA primer and the product of dna-A, dna-B, dnaC – D, dna-E and dna-G genes and polynucleotide ligase.

DNA helicases : DNA helicases are ATP dependent unwinding enzyme which promote separation of the two parental strands and established replication forks that will progressively move away from the origin.

Single strand DNA binding proteins or SSBPs : Behind the replication fork the single DNA strand are prevented from rewinding about one another by the action of SSBPs. SSBProteins bind to exposed DNA strand without covering the bases, which therefore remain available for the templating process.

Topoisomerases : The DNA helicases introduce a positive supercoil into the duplex DNA ahead of the replication fork, the topoisomerases relax the supercoil by attaching to the transiently supercoil duplex nicking one of the strands and rotating it through the broken strand.

RNA primer : The DNA polymerase cannot initiate synthesis of DNA without the availability of a primer RNA strand so before the replication start, a short RNA segment called as RNA primer, synthesized by DNA primase or RNA polymerase enzyme. The difference between the RNA primer and typical RNA is that RNA primer after its synthesis remain hydrogen bonded to the DNA template. The primers are about 10 nucleated long in Eukaryotes. The primer are later removed and filled by DNA. In bacteria RNA polymerase are used to synthesis RNA primer on the leading strand and DNA primase are used to synthesis RNA primer on the lagging strand.

Replisome & Primosome : N.K. Sinha and A. Kornberg have suggested that the DNA polymerase, RNA primer and helicases associated together to form a multi-enzyme complex called replisome which carries out the synthesis of leading and lagging strands in a co-ordinate fashion. The primase molecule is linked to the helicases to form a unit on the lagging strand called a primosome which moves with the fork, synthesizing RNA primers as it moves.

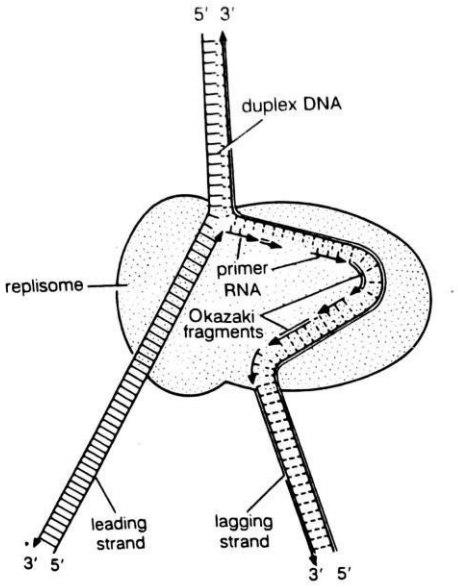


Fig: 3.3 Model of the replisome

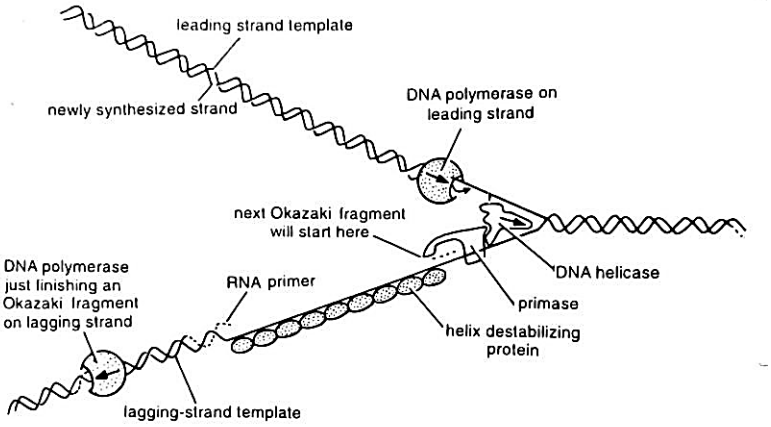


Fig: 3.4 A primosome in action

Mechanism : The initiation of the DNA replication start with the recognition of origin _O'. The dna-A (or initiator protein) - ATP complex recognize a specific sequence of 9 base pair of inverted repeat region and it bind to this site. After binding to this site it promotes the DNA duplex to open the duplex in a region of three repeats of 13 base pair sequence (13- mers). As the duplex open, the `dna-B (helicases) is transferred to exposed single stranded DNA in the presence of ATP, SSB protein and DNA gyrases. This result causes unwinding of DNA duplex and the replication proceeds. The SSB protein bind to the single stranded region. As the helicases move in the **5'→ 3'** direction, it generates a replication fork by opening the DNA duplex. The DNA strand having helicases become the lagging strand and the other strand become leading strand. The synthesis or elongation of lagging strand and leading strand takes place by different method.

(a) Discontinuous synthesis on lagging strand: - The DNA primase together with DNA helicases to synthesize a 10 – 20 base pair long RNA primer on the lagging strand. The primer having 5‘ triphosphate end 3‘-OH end. The RNA primer are recognized by DNA polymerase-III which help in the synthesis of new DNA strand by adding DNA nucleotide to theb 3‘OH group of the last ribonucleotide of the RNA primer. So in the lagging strand having a large no of lagging strand are formed called **Okazaki** fragment. The synthesis of DNA strand take place in the 5‘→ 3‘ direction. After the synthesis of DNA strand the RNA primer is hydrolyzed by the **5’→ 3’** exonuclease activity of DNA polymerase-I. The gap is filled in by DNA nucleotide by the activity of DNA polymerase-I. The newly made DNA is joined by DNA ligase which form the phospho-diester bond that links the free 3‘ end of the primer replacement to the 5‘end of okazaki fragment.

(b) Continuous synthesis on leading strand: - The RNA polymerase enzyme help in the formation of RNA primer in leading strand, then DNA polymerase-III causes elongation of DNA strand.

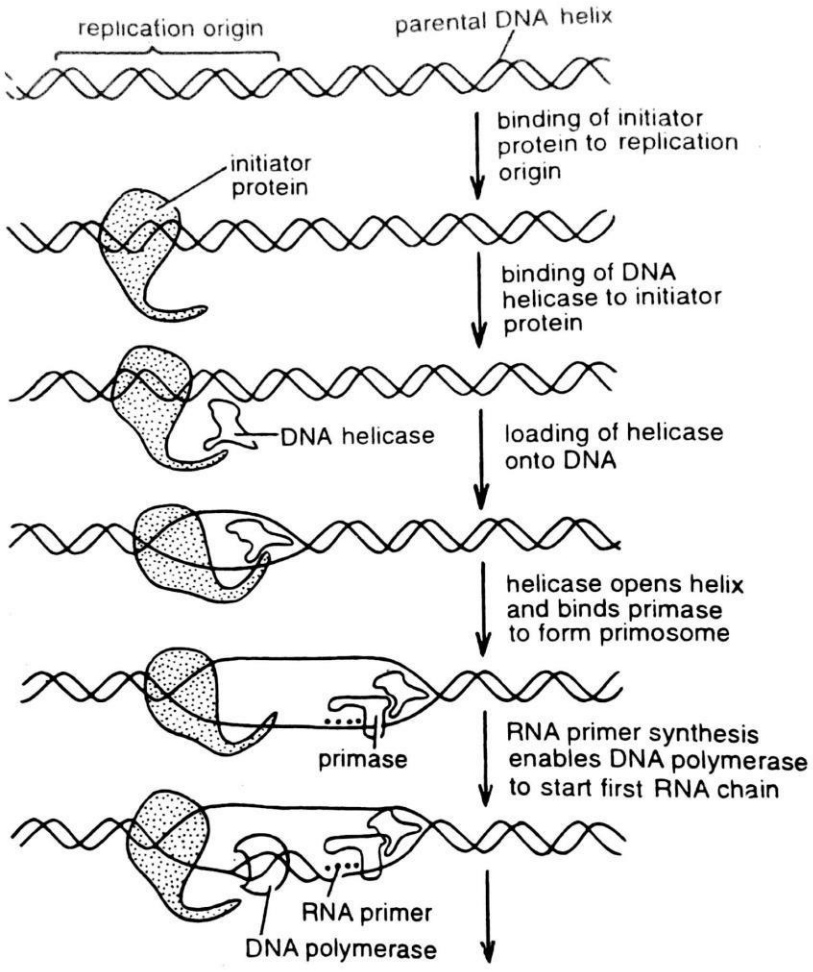


Fig: 3.5 Initial steps leading to the formation of replication forks at the *E. coli*

Replication has direction

The replication may proceed in one or both direction from the point of origin. Replication in one direction is called unidirectional replication, while in both direction is called bidirectional replication. The bidirectional replication has been found in *E.coli., Bacillus substilis, Salmonella typhimurium, Mammalian virus* SV 40. The unidirectional replication has been found in bacteriophage P 2 and 186.

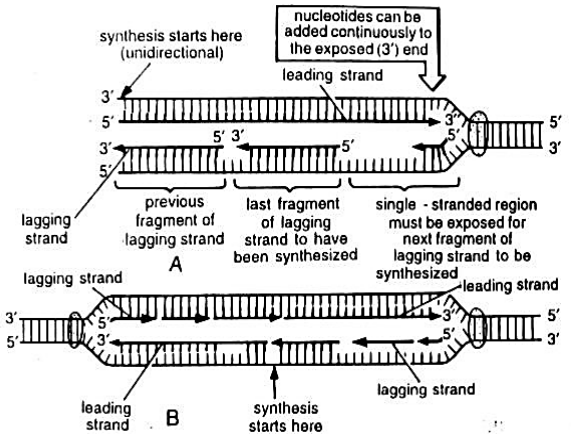


Fig : A – 3.6 Unidirectional DNA replication B - Bidirectional DNA replication

SAQs 1.

Complete the following sentences by inserting appropriate words in the blanks.

(i) The experiment of DNA utilizing ^{15}N proved that its replication is ---------

(ii) The ---------- enzymes unwind DNA strand.

(iii) The ----------enzyme break and reseal the strands.

(iv) The new strands of DNA are formed in the ---------- direction only.

3.2 TRANSCRIPTION

Transcription is the copying of a complementary messenger RNA strands on a DNA strand. The DNA strand unwinds and one of the two strands form an m-RNA strand. Transcription requires

(i) Template :- The two strand of DNA of which only one strand called the ―sense‖ strand transcribe m-RNA.

(ii) Activated precursors :- The synthesis of the m-RNA strand requires all four ribonucleoside triphosphate - ATP, GTP, UTP and CTP.

(iii) Divalent metal ions:- Mg^{++} or Mn^{++} are effective in transcription. *In vivo* synthesis requires Mg^{++}.

(iv) RNA polymerase :- The RNA polymerase (holoenzyme) consist of a core enzyme and sigma factor (ζ). The core enzyme consist of 2 identical alpha (α) subunits and one chain of each of beta (β) , beta dash (β‘), omega (ω).

The sigma factor (ζ) help in the recognition of start signals on DNA molecule and direct RNA polymerase in selecting the initiation site (promoter). Once RNA synthesis is initiated and RNA molecule become 8 – 9 bases long, the sigma(ζ) factor dissociated from the holeenzyme and then the core enzyme bring about elongation of m-RNA. The beta dash (β‘) subunit involve in binding of RNA polymerase to DNA. The beta (β)subunit of the core enzyme is required for interaction with sigma factor.

The overall polymerization reaction can be writ as follow

$$_{n}NTP + XTP \xrightarrow[Mg^{++}]{DNA,\ RNA\text{ - }P} (NMP)_n\ XTP + nPP_1$$

(*In which XTP represents the first nucleotide at the 5' terminus of the RNA chain, NMP is a mononucleotide in the RNA chain. RNA – P is the RNA polymerase, and PP_1 is the pyrophosphate released each time a nucleotide is added to the growing chain. The Mg^{++} is required for all nucleic acid polymerization reactions.*)

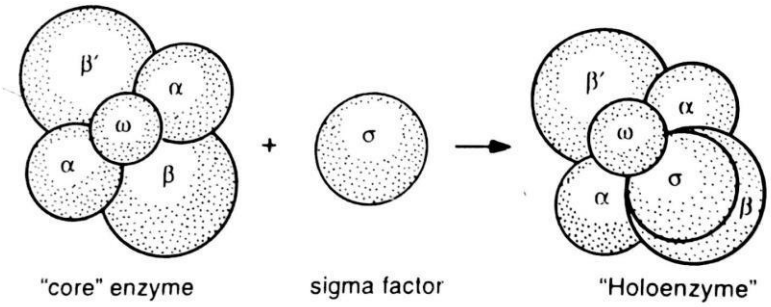


Fig: 3.7 A model of the structure of prokaryotic RNA polymerase showing association of 6 subunits or polypeptides.

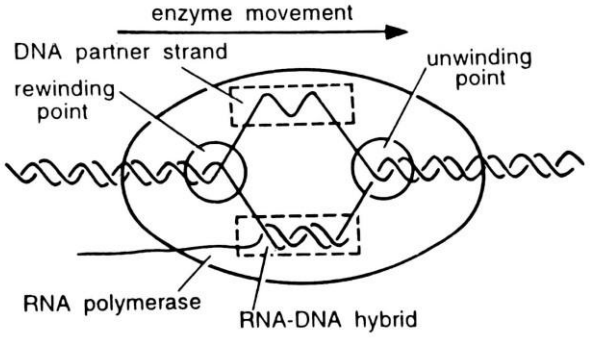


Fig: 3.8 Active centres in the core enzyme of bacterial RNA polymerase enzyme

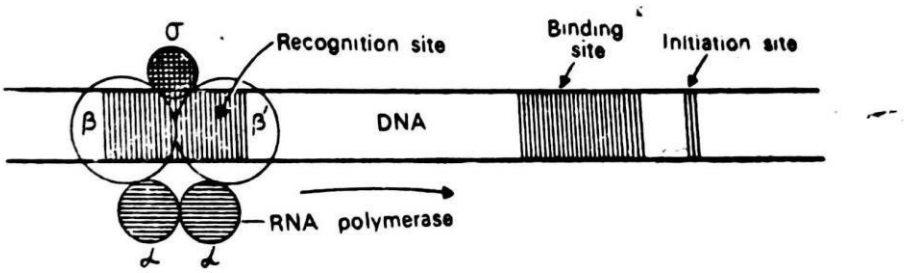


Fig: 3.9 Recognition, binding and initiation sites on DNA

The first step in the transcription is binding of RNA polymerase to a DNA molecule. The sigma (ζ) subunit of RNA polymerase binds to the -35 sequence (recognition sequence) then it move along the DNA strand then bind to the TATA box (-10 sequence) or binding site (binding sequence TATAATG), then core enzyme of RNA polymerase come and complete the whole enzyme or holoenzyme. As the RNA polymerase bind to the binding site, the initiation site, which is near the binding (6 or 7 base away), start the double helix to open to form the open promoter complex.

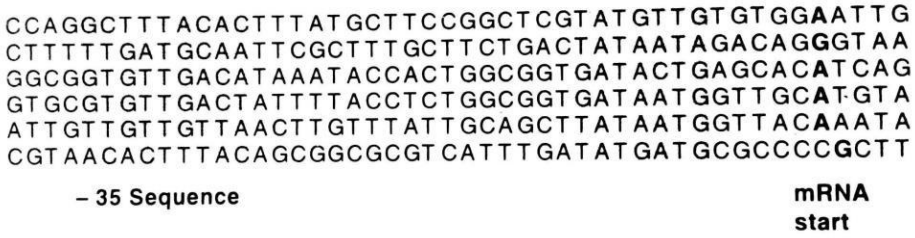


Fig: 3.10 Base sequences in the noncoding strand of six different *E. coli* promoters, showing the three important regions: mRNA start, Pribnow box (including -10 sequence) and -35 sequence

Once an open promoter complex formed, RNA polymerase is ready to initiate RNA synthesis. RNA polymerase contains two nucleotide binding site, the initiation site and the elongation site. The initiation site binds only purine triphosphate ATP or GTP and one of these is the first nucleotide in the growing RNA chain. The first DNA base that is transcribed is usually Thymine (T). The initiation nucleoside triphosphate binds to the enzyme in the open – promoter complex and form a hydrogen bond with the complementary DNA base. The elongation site is then filled with a nucleoside triphosphate that is selected strictly by its ability to form a hydrogen bond with the next base in the DNA strand. The two nucleotides are then joined together, the first base is released from the initiation site, and initiation is completed. The dinucleotide remains hydrogen bonded to the DNA. The elongation phase begins when the polymerase releases the base and then moves along the DNA chain. The RNA is synthesized in the 5‘→ 3‘ direction from the single stranded region of the DNA template.

After addition of several nucleotides to the growing chain, RNA polymerase changes its structure and loses the sigma factor. Then elongation is carried out by the core –enzyme. The core – enzyme moves along the DNA, binding a nucleoside triphosphate that can pair with the next DNA base and opening the DNA helix as it moves, thus during elongation phase addition of 40 bases per second at 37^{o} C take place. The open region extends only over a few base pair that the DNA helix recloses just behind the enzyme. The newly synthesized RNA is released from its hydrogen bonds with the DNA as the helix reforms.

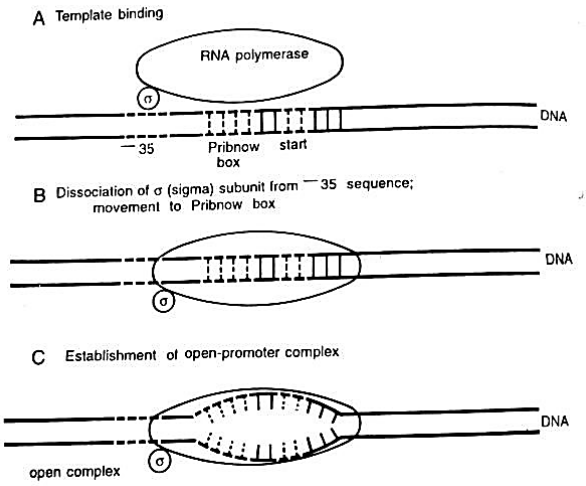


Fig: 3.11 A model for the binding of RNA polymerase to a promoter to form open- promotor complex: PB = Pribnow box

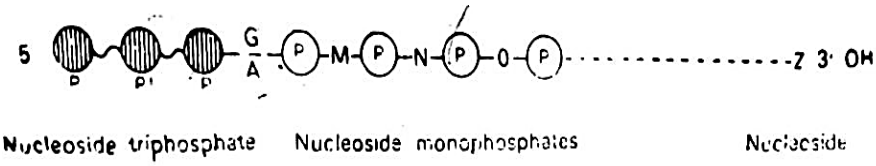


Fig: 3.12 New formed RNA chain

Termination of RNA synthesis occurs at specific base sequences in the DNA molecule. The termination region consists of the following 3 important region. (1) First there is an inverted repeat base sequence containing a central non-repeating segment, the sequence in one strand would be read like

A B C D E F ~~XYZ~~ ~~F'~~ ~~E'~~ D' C' B' A'

In which A and A', B and B' are complementary bases. This sequence is capable of intra-stand base pairing forming ―stem and loop‖ configuration in the transcript (RNA) and possible in the DNA strands. (2) The second region is near the loop end of the presumed stem and is a sequence having a G+C content. (3)A third region is a sequence of A T pairs that yields in the RNA a sequence of 6 to 8 uracil(U) often followed by adenine .

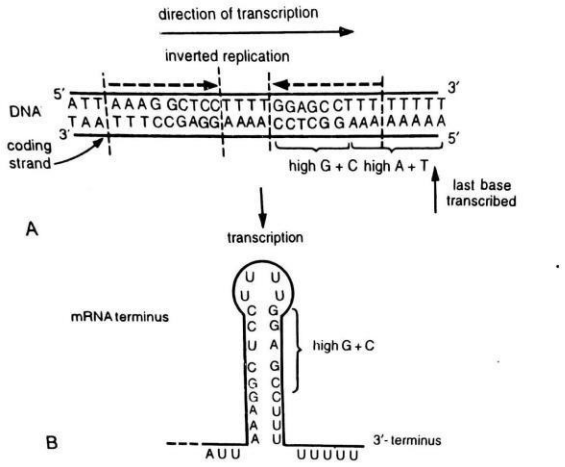


Fig : 3.13 Base sequence of (A) DNA of the *E. coli* trap operon at which transcription termination occurs

(B) 3‘ terminus of the mRNA molecule is folded to form a stem and loop structure thought to exist

There are 2 types of termination events. Those that depend only on the DNA base sequence and those that require the presence of termination protein called rho [(p) discovered by J. Roberts 1969]. According to Rho model RNA polymerase initiate the RNA chain and moves along the DNA template. The nascent RNA elongates and the rho binding site becomes exposed. Rho moves along RNA from its binding site to RNA polymerase. This movement may be brought about by hydrolysis of ATP. Rho now comes into contact with RNA polymerase. RNA & Rho dissociated from RNA polymerase. So Rho primarily act as a release factor and RNA polymerase is finally released.

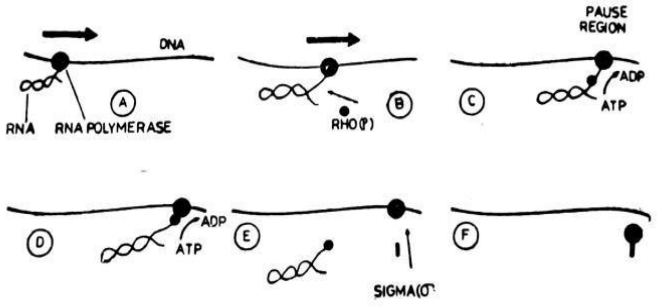


Fig: 3.14 Richardson‘s (1978) model of RNA chain termination by rho factor

Transcription in Eukaryotes- Transcription in eukaryotes differs from that in prokaryotes. In Eukaryotes 3 distinct types of RNA polymerase which transcribe three different classes of genes, the promoters for each class of the genes are also distinct. In addition, the RNA polymerase does not bind to the promoter by itself, it binds to certain transcription factor which must bind to the appropriate promoter sequences. The promoter itself can be the target of regulation, in addition the activity of several promoters is either controlled or enhanced several fold by

another sequence called enhancer. The enhancer itself may be located upto several base pair away from the target promoter. The primary mode of regulation of transcription in eukaryotes seems to be of the positive type i.e. transcription initiation exists only when the regulatory protein binds to the target sequence called response element, located in the promoter or enhancer.

SAQs 2.

Complete the following sentences by inserting appropriate words in the blanks.

(i) The process of formation of RNA from DNA is called ----------
(ii) The one strand of DNA transcribed is called ----------
(iii) TATA box found in prokaryotic DNA is concerned with ----------
(iv) Which works as stop signals during transcription ----------

3.3 GENETIC CODE

The genetic code is defined as the relationship between the sequence of bases in DNA/ RNA and the sequence of amino acids in a polypeptide chain. There are various views that how many bases can code a single amino acid. According to singlet code that each base can specify one amino acid. So for this only 4 out of 20 amino acid can code. According to doublet code two bases would specify one amino acid so 16(4x4) codon can code for 20 amino acid. The last triplet code was given by physicist Gamow in 1954. According to this 3 bases specify one amino acid, so 64(4x4x4) codon are formed which could codes for 20 amino acid. This was experimentally proved by Crick and co-worker in 1961. When they added or deleted single or double base pairs in a particular region of DNA of T_4 bacteriophage of *E.coli.* they found that bacteriophage cannot perform their normal function. But if there is addition or deletion of three bases pairs it perform normal function. From this experiment they conclude that genetic code was triplet, because the addition of one or two base has disturb the reading of codon, while the addition or deletion of third nucleotide causes proper reading of message.

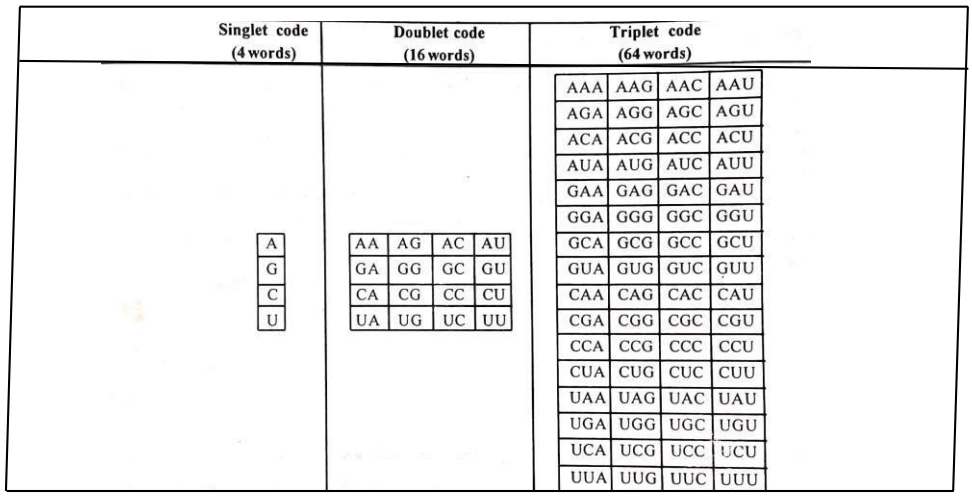


Fig: 3.15 The possible singlet, doublet and triplet codes of m-RNA

Characteristic of genetic code

(i) The genetic code is a triplet code :- The nucleotide of m-RNA are arranged in a linear sequence of codons, each codon consisting of three successive nitrogen bases i.e. the code is a triplet codon.

(ii) The code is non-overlapping :- The DNA is a long chain of nucleotides it could be read either in an overlapping or non over-lapping manner, so the genetic code could be overlapping or non-overlapping. If the reading is done by non-overlapping the 6 nucleotide would code for 2 amino acid, while in the non-overlapping it will code for 4 amino-acid.

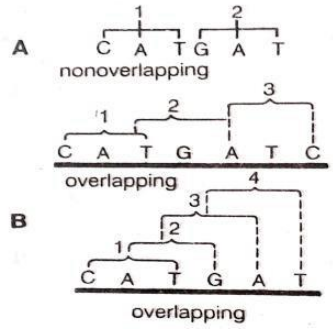


Fig: 3.16 A- Non-overlapping codon,

B- Overlapping of codon due to one base & due to two bases

In the non-overlapping code each letter is read only once while in the overlapping code it would be read three times.

By the gene mutation studies it has been shown that code is the non-overlapping type

Recently it has also been shown that in bacteriophage ϕ x 174 there is possibility of overlapping of genes and codons.

(iii) The code is commmaless :- The genetic code is commaless, which mean that no codon is reserved for punctuations. It means that after one amino-acid is coded, the second amino-acid will be automatically coded by the next three letters and that no letters are wasted as the punctuation mark.

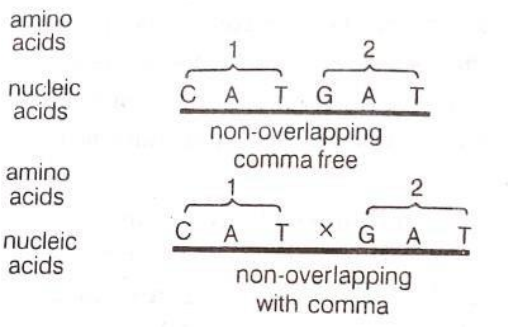


Fig: 3.17 Two form of genetic code

(a) Genetic code without comma

(b) Genetic code with comma

(iv) The code has polarity :- The code is always read in a fixed direction i.e. in the **5'→ 3'** direction i.e. the codon has polarity. It is apparent that if the code is read in opposite direction, it would specify two different proteins, since the codon would have reversed base sequence.

Codon -	UUG	AUC	GUC	UCG	CCA	ACA	AGG
Polypeptide →	Leu	Ile	Val	Ser	Pro	Thr	Arg
	Val	Leu	Leu	Ala	Thr	Thr	Gly ←

(v) Codon and anticodons :- The m-RNA is read in a polar manner 5'→ 3' direction. But anticodon is written in the 3'→ 5' direction so as to bring about an easier correlation between the bases of the codon and anticodon. The anticodon of AUG is written as 3' UAC 5' or UAC. This indicate the codon and anticodon pairing is antiparallel.

Base number		1	2	3	
Codon(m-RNA)	5'	A	U	G	3'
Anticodon (t-RNA)	3'	U	A	C	5'
Base number		3	2	1	

(vi) The code is degenerate :- When more than one codon specify the same amino acid is called degeneracy of the code. Except for methionine and tryptophan, which have single codon.

Amino acid **No of codons**

(1) Tryptophan, methionine ----------**1**

(2) Phenylalanine, tyrosine, histidine, glutamine, asparagine----------**2**

lysine, aspartic acid, glutamic acid, cysteine

(3) Isoleucine----------**3**

(4) Valine, proline, threonine, alanine, glycine ---------- **4**

(5) Leucine, arginine, serine----------**6**

The code degeneracy is basically of two types, partially and complete. The partially degeneracy occurs when first two nucleotides are identical but the third (i.e. 3' base) nucleotide of the degenerate codon differs e.g. CUU and CUC code for leucine. The complete degeneracy occur when any of the four bases can take third position and still code for the same amino acid (e.g. UCU, UCC, UCA and UCG codes for serine). Degeneracy of genetic code has certain biological advantages. Degeneracy provides a mechanism of minimizing mutational lethality.

(vii) Initiation codon :- In most organism AUG codon is the start or initiation codon i.e. the polypeptide chain start either with methionine (Eukaryotic) or N-formyl methionine (prokaryotes). The methionine or N-formyl methionine t-RNA specifically binds to the initiation site of m-RNA

containing the AUG initiation codon. In rare cases GUG also serves as the initiation codon e.g. bacterial protein synthesis. Normally GUG code for valine, but when normal AUG codon is lost by deletion, only the GUG is used as ignition codon.

(viii) Termination codon :- The 3 codons UAA, UAG, UGA are the chain termination codon. They do not code for any of the amino acids, so they are also called nonsense codon.

The UAG was the first termination codon to be discovered by Sidney Brenner (1965). It was named amber after a graduate student named Bernstein (= German word for ‗amber' and amber means brownish yellow) who helped in the discovery of a class of mutation. Apparently to give uniformity the other two termination codons were also named after color such as Ochre for UAA and Opal or Umber for UGA (Ochre means yellow red or pale yellow, Opal means milky white and umber means brown).

Termination codons do not code for any amino acids and hence causes termination and release of polypeptide chains. Apparently no t-RNA species has anticodons complementary to the termination codons. Termination codon are not read by any t-RNA molecule but read by some specific protein called release factors. In prokaryotic there are three release factor RF-1, RF-2 and RF-3. RF-1 recognizes UAA and UAG, while RF-2 recognizes UAA and UGA. RF-3 stimulates RF-1 and RF-2. In Eukaryotes a single release factor (RF) recognizes all three termination codons.

(ix) The code is universal :- The genetic code is valid for all organisms ranging from bacteria to man. Such universality of the code was demonstrated by Marshall, Caskey and Nirenberg (1967) who found that in *E. coli* (bacterium), *Xenopus laevis* (amphibian) and guinea pig (mammal) aminoacyl t-RNA use almost the same code. Nirenberg has also stated that the genetic code may have developed 3 billion years ago with the first bacteria, and it has changed very little throughout the evolution of living organism.

Wobble Hypothesis- To explain the possible cause of degeneracy of codon, Cricks (1966) proposed the wobble hypothesis. Since there are 61 codons specifying amino acids, so the cell should contain 61 different t-RNA molecule, each with a different anticodon. But the number of t-RNA molecule was found much less than 61. This explain that anticodons of some t-RNAs read more than one codon on m-RNA. According to wobble hypothesis only the first two positions of a triplet codon on m-RNA have a precise pairing with the bases of the t-RNA anticodon. The pairing of the third position bases of the codon may not clear (ambiguous) and varies according to the nucleotide present in this position. Thus a single t-RNA type is able to recognize two or more codons differing only in the third base. The anticodon UCG of serine t-RNA recognizes two codons, AGC and AGU. The bonding between UCG and AGC follows the usual Watson-Crick pairing pattern. In UCG – AGU pairing, hydrogen bonding take placed between G and U. This is a difference from the usual Watson- crick pairing mechanism where G pairs with C and A with U. Such interaction between the third bases is referred to as ―wobble pairing‖.

m-RNA codon	5‘ A G C 3‘	5‘ A G U 3‘
t-RNA anticodon	3‘ U C G 5‘	3‘ U C G 5‘

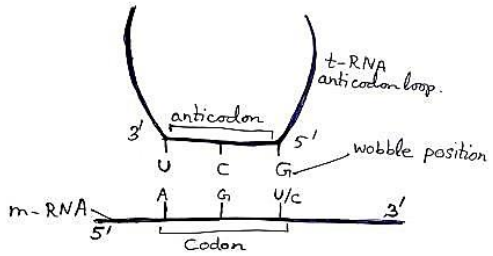


Fig: 3.18 Wobble hypothesis, In the third position (5‘end) of anticodon is in wobble position

Thus Crick‘s wobble hypothesis states that the bases at 5‘ end of the anticodon is not spatially confined as the other two bases allowing it to form hydrogen bonds with any of several bases located at the 3‘ end of a codon.

SAQs 2.

Complete the following sentences by inserting appropriate words in the blanks.

(i) The flow of information from DNA to mRNA and then to protein is called ----------

(ii) AUG is found ----------

(iii) Wobble hypothesis was proposed by ----------

(iv) Codons are composed of ----------

3.4 GENE CLONING EXPERIMENT

In genetic engineering when there is identification and selection of useful DNA segment and then its isolation and synthesis. After synthesis useful DNA segment is incorporated to a vector i.e. plasmid gives rise to the recombinant-DNA (r-DNA) molecule. This recombinant DNA molecule introduced to the bacterial protoplast where it replicates, many copies of the recombinant DNA molecule is made which are called as the clones and the process is called gene cloning.

The following steps for constructing and cloning a hybrid or recombinant DNA (r-DNA) are as :-

(1) Identification and selection of useful gene or DNA fragment.

(2) Isolation and synthesis of gene or DNA segment.

(3) Attachment of isolated synthesis gene or DNA fragment to the vector DNA or synthesizing recombinant DNA .

(4) DNA cloning (entry of recombinant-DNA into a bacterial cell)

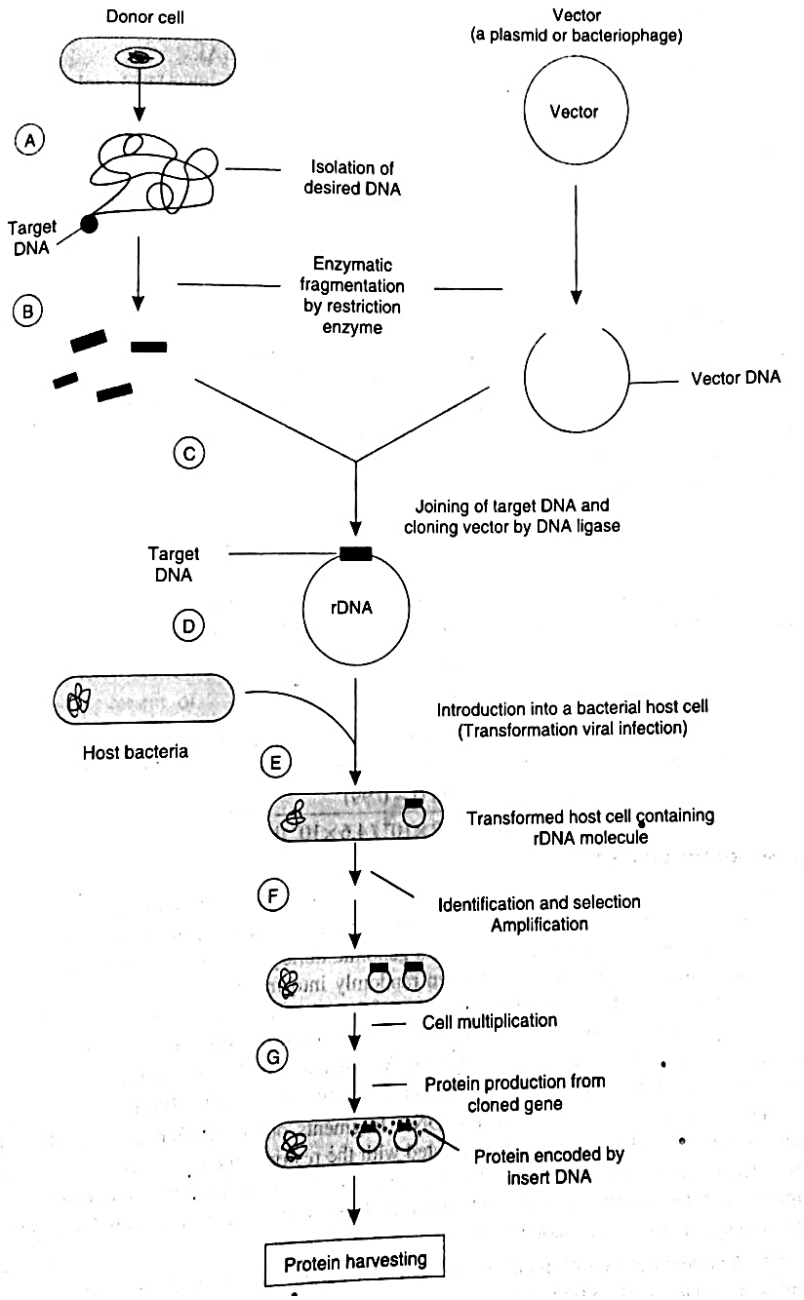


Fig: 3.19 Diagrammatic representation of gene cloning

(1) Identification and selection of useful gene or DNA fragment :- Out of billon of DNA fragment known it is very difficult to locate a particular gene or DNA fragment. However certain modern technique has made it possible to identify and select DNA fragment. These are as follows

(a) Gel electrophoresis

(b) Southern blotting technique

(c) Northern blotting technique

(d) Western blotting technique

(a) Gel electrophoresis:- The gel electrophoresis technique is used in recombinant DNA technology to separate specific DNA fragment which is cleave by restriction endonuclease enzyme. In gel electrophoresis involves movement of DNA fragment or molecules in gel plate. The gel plate is made up of either agarose or polyacrylamide. The agarose gel are used to separate large DNA fragment while polyacrylamide gel are used to separate smaller DNA fragments.

When DNA fragment are subject to a gel plate, they migrate through the gel at rate dependent upon their sizes. The smaller DNA fragment move faster than a larger DNA fragment. The rate of movement of fragments is inversely co-related with the size of fragments or molecule. So that heavier fragment will remain closer to the site of loading and lighter fragments will move away. Fragment of different size will appear as bands in the gel. Then gel is soaked in the solution of a dye like ethidium bromide which complexes with DNA by intercalating between stacked base pairs. When the gel is illuminated with UV irradiation. DNA bands can be detected on gel as visible fluorescence.

A new technique called pulsed field gel electrophoresis (PFGE) is develop to separate a large sized DNA molecules. In this technique, short pulse of electricity are used in two different directions. The DNA is embedded and used in the form of agarose plugs to avoid fragmentation of large DNA molecules.

Fig: 3.20 Gel electrophoresesd DNA in several lines

By gel electrophoresis a mixture of DNA or RNA or protein fragment can be separated. They are stained and visualized directly on the gel. Now to confirm the identity of these bands they can be hybridized with a labelled probe. So to facilitate this hybridization the bands are transferred to the nitro-cellulose membrane through a technique called blotting. When DNA is blotted, the technique is called southern blotting technique, when RNA is blotted is called northern blotting technique, when protein is blotted it is called western blotting.

(b) Southern blotting technique :- Southern blotting is a technique used to identify a particular DNA fragment or its portion with the help of a specific probe (radio actively labeled single strand DNA) which is homologous to the DNA fragment of its portion of interest. This technique was develop by E.M. Southern in 1975. The basic principal of this technique is first isolate a complete DNA molecule and then treated with restriction endonuclease, so that it is fragmented and are then separated by agarose gel electrophoresis. Now the gel plate is immerse in alkali (NaOH

solution) so that they are denatured in single strand. Now the gel plate is placed on the top of a buffer saturated filter paper. After that nitrocellulose membrane is placed on the top of gel plate and then many sheet of dry filter paper are placed above the nitro-cellulose membrane. Now leave the experiment for few hours to whole night. The buffer solution is drawn up by the dry filter paper. As the buffer solution will passes through the gel it will come in contact with the denatured DNA (i.e. single strand DNA) and it will carries the single strand DNA along with it which will bind on to the nitro-cellulose membrane. Now remove the nitrocellulose membrane having single stranded DNA band blotted on to it, is baked at $80^{o}C$ for 2 – 3 hours to fix the DNA permanently on the membrane. This membrane now has a replica (copy) of DNA bands from agarose gel. The nitrocellulose membrane is now placed in a solution of radio-actively labelled single stranded DNA (probe) which is complementary in sequence to the nitrocellulose membrane bound single stranded DNA. The probe hybridizes with the complementary DNA (c-DNA) on nitrocellulose membrane. When they hybridization is finished the nitro-cellulose membrane is washed to remove any unbound probe. The region of hybridization can be detected by autoradiography placing the nitro-cellulose membrane in contact with photographic.

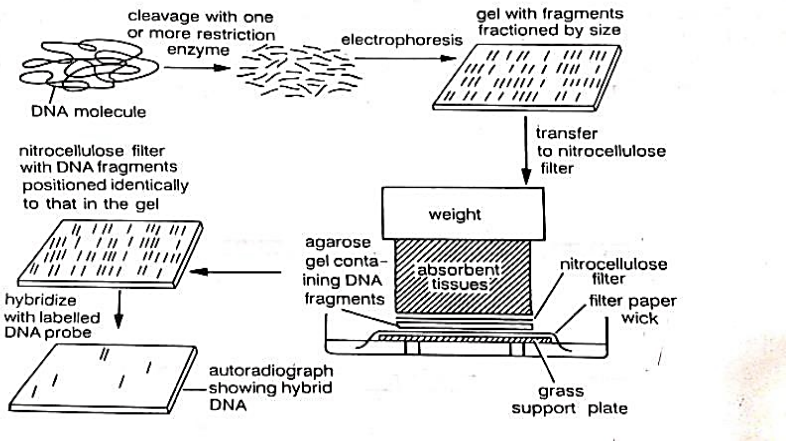


Fig: 3.21 Various steps of Southern blotting technique

(c) Northern blotting technique :- It was develop by Alwine in 1979. Northern blotting technique has been used to detect a particular RNA fragment. The technique is similar to the Southern blotting technique, the problem is that RNA fragment does not bind to the nitrocellulose membrane. So they are blotted in the reactive paper which is prepared by diazotization of aminobenzyloxymethyl paper of what man 540 paper. First take RNA strand from the transformed cell and treated with restriction enzyme to cleave it and then gel electrophoresis is done to separate RNA fragment. Now the gel plate is placed on the top of a buffer saturated filter paper and after that reactive paper is placed on the top of gel plate and then place filter paper. As the buffer solution move up it also carries the RNA fragment which is bounded to the reactive paper and then baked and it is hybridized with radio labeled DNA probe and then can be detected by autoradiography.

Now the technique have been develop in which RNA is transferred to nitrocellulose membrane and the hybridized with a single stranded nucleic acid probe of precisely known structure. The hybrid is then treated with nuclease that specially digest single stranded nuclei acid probe but do not affect the double stranded part of hybrid which has been formed as a result of the hybridization between RNA and complementary sequence of the probe. The probe may be single strand DNA or RNA and nuclease. The common nuclease used are mung bean nuclease or S_1 nuclease which digest single stranded DNA and RNAase-A and RNase-T_1 nuclease which digest single stranded RNA.

(d) Western blotting technique :- The western blotting technique has been developed by Towbin in 1979 to detect specific protein. In this technique take a mixture of protein and the gel electrophoresis in polyacrylamide gel to separate different protein. Now the gel plate is placed on buffer saturated filter paper, and Above it placed nitro-cellulose membrane. Now as buffer solution move, protein also move upward and bind to the nitro-cellulose membrane then baked and hybridized with specific labelled antibody probe. The antibody may be labelled with I and is detected by autoradiography. It is also detected by a second antibody that is tagged with an enzyme e.g. horse radish per oxidase which gives a colour reaction when its substrate is added in the presence of suitable reagent.

DNA sequencing- Sequencing a DNA mean mapping the DNA in terms of its nucleotide sequencing. It becomes possible when methods were developed for generating DNA fragments with a definite nucleotide terminus. There are two method used for determining the DNA sequences.

(a) Chemical method

(b) Chain terminator method

(c) Direct DNA sequencing by using PCR

(a) Chemical method or Maxam Gilbert method :- It include following steps

(i) First label the 5‘ ends of dsDNA with ^{32}P.

(ii) The two strands are separated by dissociated by mild alkali or heat. Both strand are labelled at 5‘end.

(iii) Now take the mixture divide it into four test tubes. Each test tube will contain different reagent having the property of destroying either only G or only A or C or T and C. So that fragments of different size having ^{32}P are produced.

(iv) Now electrophoreses is done in each of the four sample in four different lanes of the gel.

(v) Autoradiograph the gel and determine the sequence from the position of bands in four lanes.

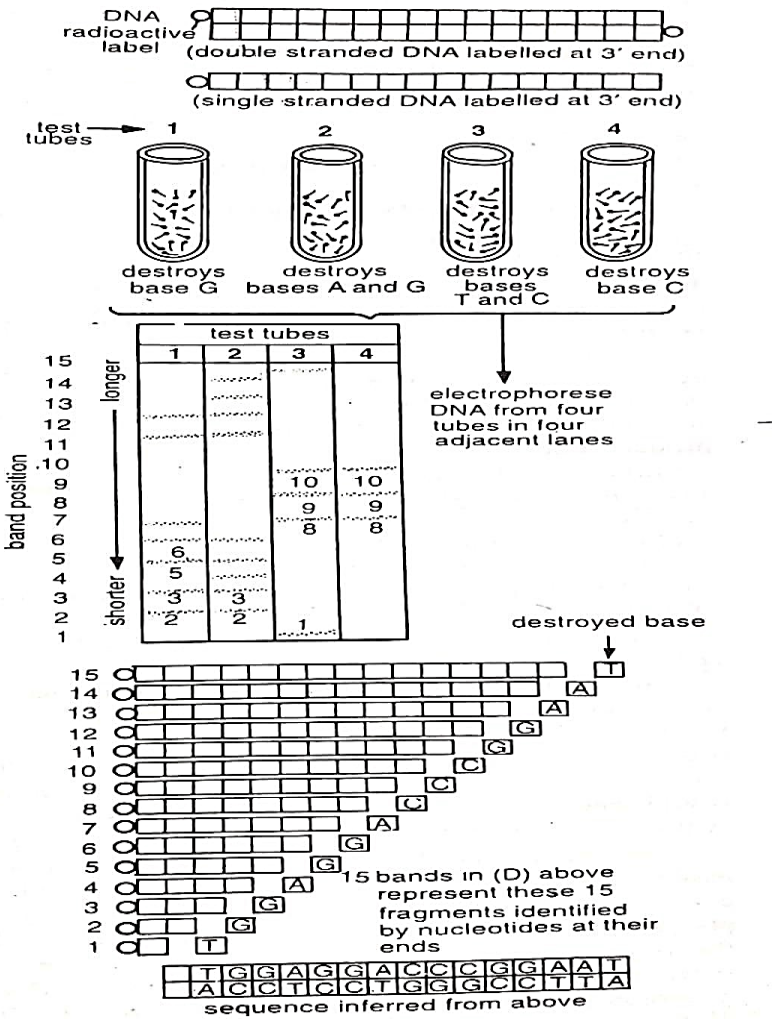


Fig: 3.22 Maxam and Gilbert‖ chemical degradation method for sequencing of DNA

(b) Chain terminator method or Sanger and coulson's enzymatic method :- It is also called plus- minus method. The method is based on synthesizing a labelled strand of DNA starting from a primer annealed to a template and terminating the synthesis at one of the four nucleotide. The terminators is 2',3' dideoxynucleotide. This dideoxynucleotide are used as triphosphate (ddNTP) which has no 3' OH, so that it cannot form a phosphodiester bond next incoming nucleotide.

The terminator is mixed with the corresponding deoxynucleotide in a proportion that allows the terminator to be corporate randomly in at least one of each of the site for the particular nucleotide. The reaction mixture thus contains at completion time, fragments, represent lengths of DNA from one identical end to each of the site for a particular base.

The gel are run, autoradiography and interpreted as in chemical method.

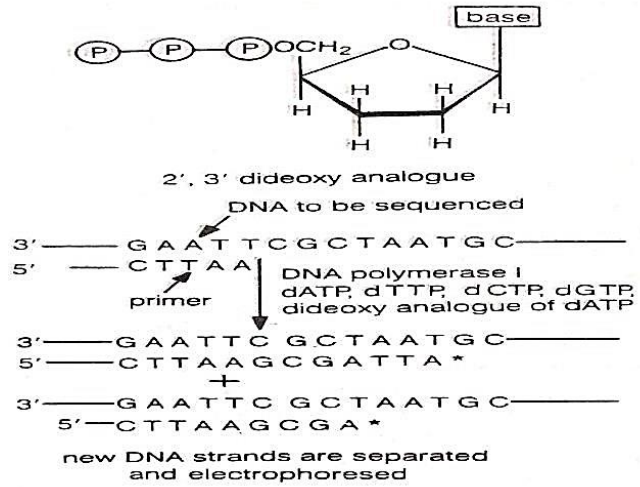


Fig: 3.23 A-Dideoxynucleotide

B- Technique involved in Sanger‘s chain termination method for sequencing of DNA

(c) Direct DNA sequencing by using PCR :- The polymerase chain reaction(PCR) was introduce by Karry Mullis in 1985. This PCR has also been used for sequencing the DNA. This method of sequencing is faster and more reliable. It can utilize either the whole genomic DNA or the cloned fragments for sequencing a particular DNA. The DNA sequencing using PCR involves two main steps

(i) Generation of sequencing template (i.e. double stranded or single stranded) using PCR.

(ii) Sequencing of PCR products either with the thermostable Taq DNA polymerase (Taq = thermos aquaticus).

The amplified DNA is obtain by repeating the reaction, which is made possible by regular denaturation of freshly synthesized double stranded DNA molecules by heating it to 90 – 98^0C. At this high temperature the two strands separate. Once the double strand DNA separate the mixture with two primers (deoxyoligonucleotides) recognizing the two strands and bordering the sequence to be amplified, is cooled to 40 – 60^0C. This permits the primers to bind to their complementary strand through renaturation. The presence of Taq DNA polymerase enzyme and all four essential nucleoside triphosphate in the ―eppendrof tube‖ allows synthesis of complementary strands in the usual manner. In an automatic thermal cycler, this process is automatically repeated 20 – 30 times, so that in a single afternoon a billion copies of the sequence flanked by the left and right primers can be produced. In order to continue the synthesis the temperature of the mixture is alternately increased (for denaturation) and decreased (for renaturation). The use of Taq DNA polymerase and some other DNA polymerase have allowed automation of the entire PCR reaction.

The DNA sequencing method using PCR eliminates the need of cloning the DNA in single stranded DNA phage vectors i.e.M13

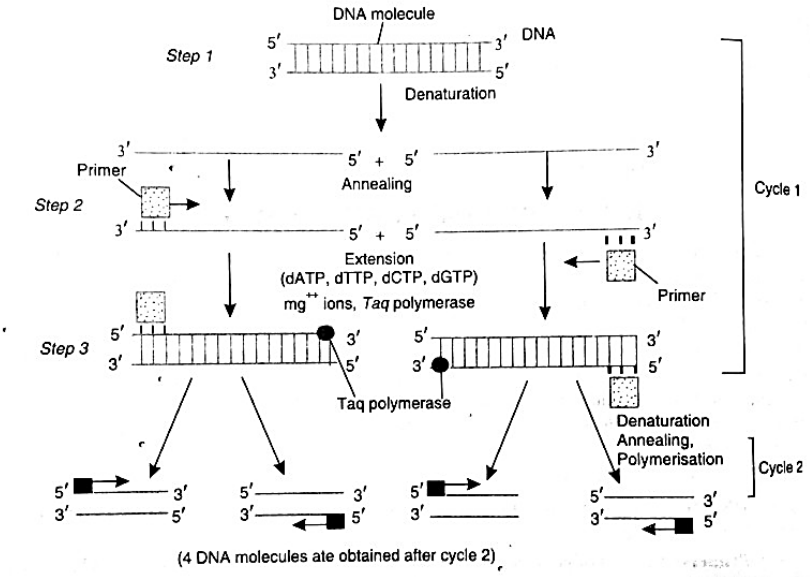


Fig: 3.24 Working of PCR

(2) Isolation and synthesis of gene or DNA segment :- Gene or DNA segment can be obtain both from the prokaryotic cell and Eukaryotic cell. There are various method by which DNA segment can either by isolated or synthesized

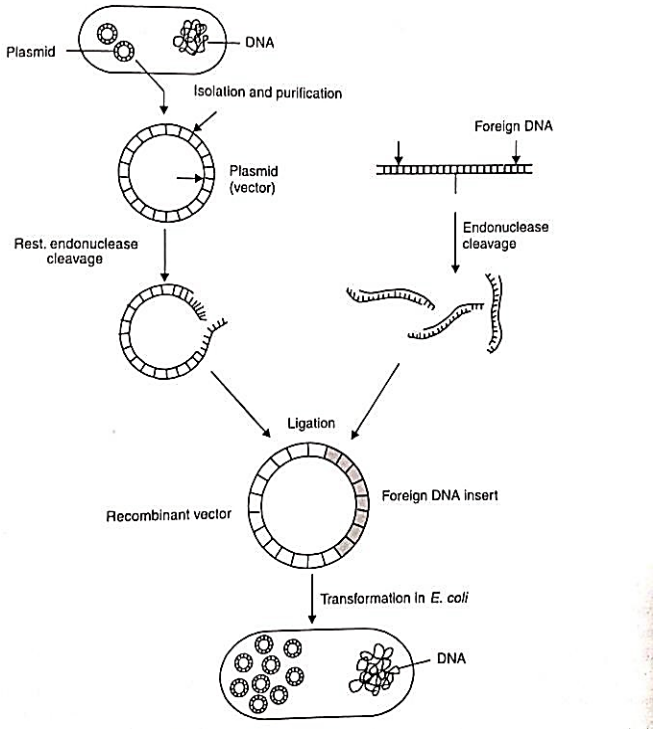


Fig: 3.25 A typical recombinant DNA experiment showing isolation and cloning of eukaryotic DNA fragments in *E.coli* cell

(1) Beckwith's methods : -This technique was develop by group of scientist of Harved university which was headed by Johanam Beckwith. In this technique transducing phages or virus which carry out transduction are usually used to isolate specific DNA segment. Shapiro has used Lambya phage ϕ 80 to isolate lactose gene from E. coli. The beckwith method can only be used in the case of prokaryotes. In Eukaryotes it cannot be use because transducing phages are not reported.

(2) Shot gun method : - In this technique the nuclear material of the organism and plasmid of the organism both are subjected to restriction endonuclease due to its action various smaller fragment of DNA are formed. These fragments of DNA are then joined with ligase. The sticky end of one end joins to the complementary sticky end of the other DNA. This process take place randomly resulting in the formation of recombinant DNA. The recombinant DNA synthesis by the shot gun method is called as random DNA or the r-DNA. This recombinant DNA is then put in the $Cacl_2$ solution make the bacterial cell wall permeable and the recombinant DNA is incorporated in the bacterial protoplast. The bacterial cells are then removed and big binary fission the bacteria divides and many identical copies of the recombinant DNA are formed. These are called as clones which can then used in genetic engineering. The shot gun method is not a very useful process because specific DNA sequence are not incorporated in the recombinant DNA.

(3) Chemical method :- If the DNA segment are synthesized by purely chemical method then it is called chemical methods technique of synthesis of DNA. This was done by Hargovind Khorana by which he synthesizes two t-RNA gene. In this technique two processes takes place.

(a) ***Chemical technique*** **: -** In this process nucleotide are synthesized and then joined together to form a single strand DNA by employing phosphate triester method and phosphite triester method.

(b) ***Enzymatic technique*** **:-** The single strand DNA is transformed into a double strand DNA by the help of enzyme polymerase, kinase etc. By this technique only short sequences of DNA can be synthesized which are of 10 – 100 nucleotide sequence.

(4) Selected method :- By this method long sequence of DNA can be synthesized. This method can be achieved by two ways.

(a) Synthesis of DNA by gene automated machine

(b) By reverse transcriptase method

(a) Synthesis of DNA by gene automated machine :- The long sequence of DNA can be synthesized by the help of gene automated machine. The machine consists of a microprocessor, specific sequence of DNA are used fed by in a key board. This key board automatically opens the valve of different reagent & solvent together

with the nucleotide resulting in the formation of specific sequence of DNA.

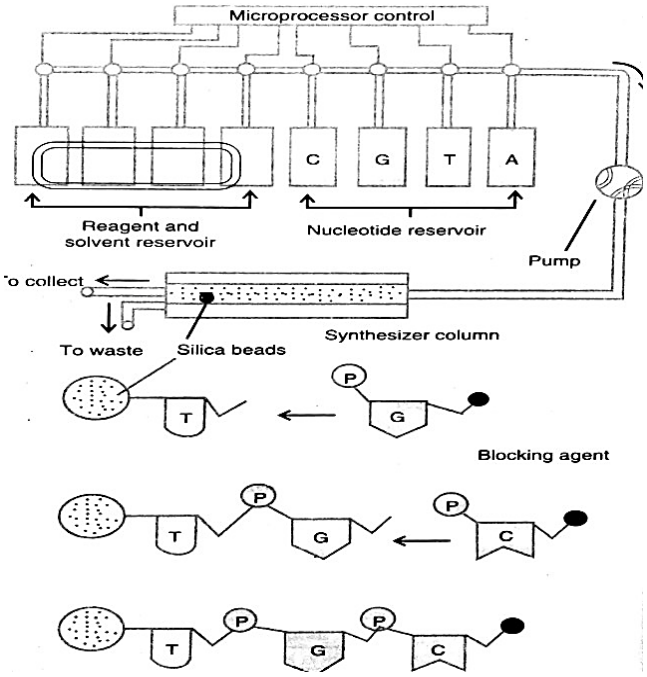


Fig: 3.26 A gene automated machine and its working mechanism

(b) By reverse transcriptase method :- When there is a synthesis of DNA by the help of m-RNA. This newly form DNA is called complementary DNA. The process of synthesis of complementary DNA is as follows –

The process of synthesis of complementary DNA is required by a very important enzyme reverse transcriptase. This enzyme has been discover by Temin & Baltimore in Rous sarcoma virus which causes tumor in chicken whose function is to synthesize DNA upon a RNA template. It is easy to use m-RNAs that carry a poly (A) tail at 3‘ end or adenine rich at 3‘ end to which a short sequence of thymine nucleotide can be annealed and its purpose is to provide a free 3‘ end that can be used for extension by the enzyme reverse transcriptase so it is clear that reverse transcriptase cannot initiate formation of a polynucleotide chain without a pre-existing primer. Now the reverse transcriptase start synthesizing polynucleotide sequence in the presences of all four deoxynucleosite triphospahte (4dNTPs). The immediate product of all the reaction is a RNA-DNA hybrid, consisting of a template m-RNA strand base paired with complementary DNA (c-DNA) strand.

A useful reaction trends to occur at the end of reverse transcriptase upon reaching the end of the m-RNA the enzyme may cause the reverse transcript to ―loop back‖ on itself by using the last few bases of the reverse transcript as a template for synthesis of a complementary strand i.e. the end of the complementary DNA is used to direct synthesis of a short sequence of bases i.e. identical with the m-RNA. This result in a short ―hair pin‖ loop at the 3‖ end of the complementary DNA.

Now the template m-RNA strand is degraded by the treatment with alkali (alkaline hydrolysis) leaving a single stranded complementary DNA having a short hair pin loop at its 3‘ end. However the alkaline hydrolysis does not affect c-DNA strand as the latter is resistant to it. In next step the single stranded c-DNA is converted into the double stranded forming of its complementary strand using the enzyme DNA polymerase and the hair pin loop at its 3‘ end provides a natural primer for this synthesis. The product of this step however is a double stranded c-DNA molecule with hair pin loop at one end. The hair pin loop is treated away by treatment with enzyme S_1 nuclease (which specifically degrade single DNA) and a traditional double stranded DNA is obtained.

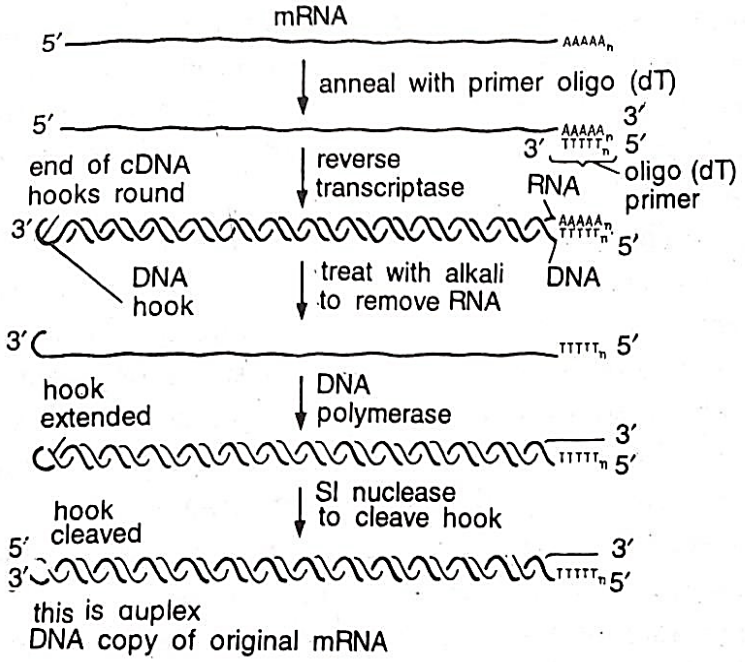


Fig: 3.27 Synthesis of c-DNA from m-RNA using reverse transcriptase enzyme

(3) Attachment of isolated synthesis gene or DNA fragment to the vector DNA or synthesizing recombinant DNA :- There are two types of DNA one is used as a carrier i.e. vehicle DNA and the other fragment which is useful is called a passenger DNA.

(a) Vehicle DNA :- This is used to carry the passenger DNA. In vehicle DNA usually the following DNA are used (i)Plasmid DNA, (ii)Bacteriophage DNA , (iii) Cosmid DNA

(b) Passenger DNA : - This is useful fragment of gene or DNA which has been isolated or synthesis. It can be of 3 types. According to the method in which it is synthesized

(i) Random DNA :- By shot gun method

(ii) Synthetic DNA :- By chemical method

(iii) Complementary DNA :- By reverse transcriptase.

The passenger DNA or vehicle DNA are usually isolated by bacterial cell. The bacterial cell is lysed by employing an enzyme. The protoplast thus obtain is ultracentrifuge at a particular sedimentation rate. The DNA is isolated. The attachment of passenger DNA to the vehicle DNA can be achieved by following technique.

(i) By restriction endonuclease method

(ii) By linker method

(iii) By extending tail method

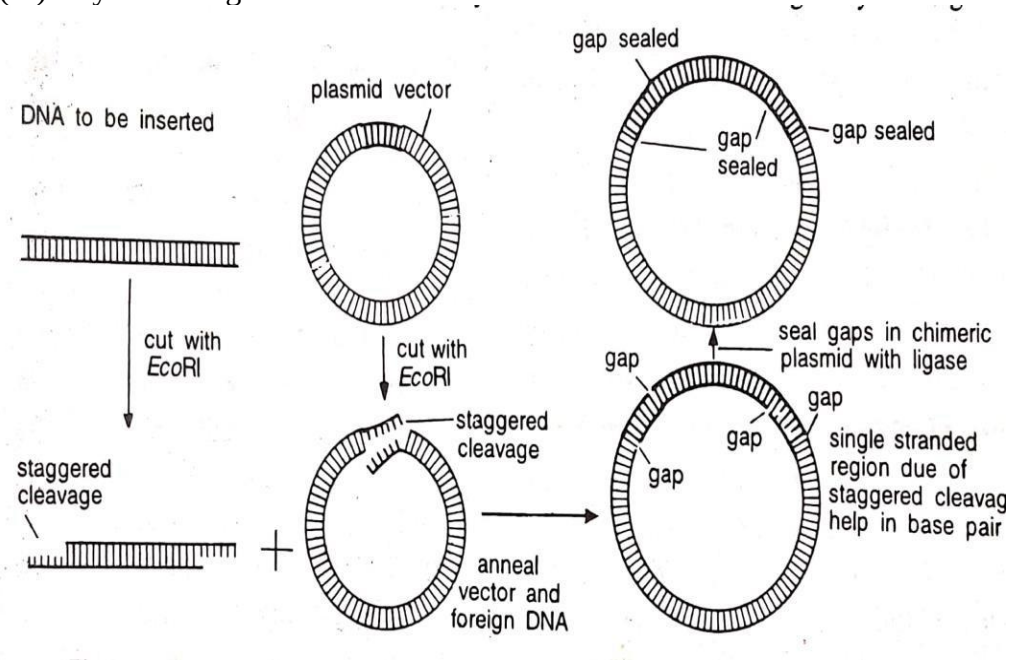


Fig: 3.28 Cloning of a DNA fragment in a plasmid vector by staggered cleavage both in vector DNA and foreign DNA to be cloned

(4) DNA cloning (entry of recombinant-DNA into a suitable bacterial cell and obtaining its identical copies): - The recombinant-DNA containing the useful gene is introduce inside the bacterial cell in order to obtain many identical copies of it. The exact identical copies are called as clone and the process of obtaining them is called cloning. Usually the bacterial protoplast is used because its multiplication rate is fast by fission (Binary fission) for introducing recombinant-DNA inside the bacterial cell. The process of transformation is usually used. The bacterial cell is treated with $Cacl_2$ in order to make its wall permeable. It is grown on a artificial medium containing together with recombinant-DNA. Together with the nutrient the recombinant-DNA is also taken up by the bacterial protoplast where it multiply by replication for induction. In Eukaryotic cell microinjection technique are used by using such technique. The DNA responsible for synthesis of rat insulin, sheep somatostatin has been obtain. The DNA cloning technique was first introduced by Cohen, Boyer, and Berg of Stainford University and California University 1971-1972.

SAQs 4.

Complete the following sentences by inserting appropriate words in the blanks.

(i) Chimeric DNA is----------

(ii) Genetic engineering means ----------

(iii) Who is the father of genetic engineering ---------

(iv) The two bacteria found to be very useful in genetic engineering experiments are

3.5 SUMMARY

(1) The DNA polymerase enzyme was responsible for the formation of complementary strand of DNA on DNA template. It is 3 types in prokaryotes and 5 types in eukaryotes. DNA replication is of semi-conservative type in which one strand will be of parent and one new strand will form.

(2) Transcription is the process of formation of m-RNA from DNA. It requires DNA template activated precursor, divalent metal ions and RNA polymerase.

(3) The genetic information is carried by 4 types of N-bases. A codon is the sequence of 3-N bases on m-RNA that codes for a particular amino acid and the set of all the codons that specific 20 amino acid are called genetic code. The concept of triplet genetic code was given by George Gamow. Since there is four types of N- bases and 3N-bases takes part in the information of codon hence total no of codon will be 4x4x4 =64.

(4) The principle of biotechnology is based on a set of molecular technique collectively called r-DNA technology (recombinant DNA technology), which is popularly known as genetic engineering (manipulation of gene). It was Paul berg, who first attempted a genetic manipulation by inserting gene of SV_{40} virus into a bacterium. In this technique there are several steps like isolation and purification of DNA, fragmentation of DNA, isolation of required gene from donar DNA, synthesis of r-DNA, culture of host cells to get desired product.

3.6 TERMINAL QUESTIONS

1. Describe DNA polymerase in Eukaryotic cell.

---

2. Explain genetic code.

---

3. Describe the DNA sequencing by chain terminator method or Sanger and coulson‘s enzymatic method.

3.7 ANSWERS

SAQs

1 (i) Semiconservative

(ii) Helicases

(iii) Topoisomerases

(iv) 5‘→ 3‘

2 (i) Transcription

(ii) Template strand

(iii) Transcription

(iv) Poly A nucleotide

3 (i) Central dogma

(ii) in RNA

(iii) F.H.C.Crick

(iv) A triplet sequences of nucleotide bases in m-RNA

4 (i) Recombinant DNA formed by combining vector DNA and passenger DNA

(ii) Manipulation of genes.

(iii) Paul Berg

(iv) *Escherichia* and *Agrobacterium*

References

1. Cell biology by C. B. Powar, Himalaya Publishing House 1996.
2. Cell and molecular biology by S. C. Rastogi, New age international Publishers 2010.
3. Cytology genetics and molecular Biology by S. K. Singh, Campus Books 2008.
4. Cell biology, genetics, molecular biology, evolution and ecology by P.S. Verma and V.K. Agrawal, S. Chand & Company Ltd. 2001
5. Biotechnology and Genomics by P. K.Gupta, Rastogi Publication 2008.
6. A text book of biotechnology by R. C. Dubey, S. Chand & Company Ltd 2006.
7. A hand book of zoology by A.K.Verma, Shri Balaji Publications 2014.

Uttar Pradesh Rajarshi Tandon
Open University

Bachelor of Science

UGZY-103

Genetics and Cell Biology

BLOCK

2

CELL BIOLOGY

UNIT-4

Cell Biology and Microscopy

UNIT-5

Plasma Membrane, Nucleus and Cell Cycle

UNIT-6

Endoplasmic Reticulum, Ribosomes

UNIT-7

Golgi Body and Lysosomes

UNIT-8

Mitochondria

Course Design Committee

Prof. Ashutosh Gupta — **Chairman**
Director, School of Science
UPRTOU, Prayagraj

Dr. Shubhra Malviya — **Member**
Asst. Prof. Department of Zoology
S.S. Khanna Girls Degree College.
Prayagraj

Dr. Sippy Singh — **Member**
Asst. Prof. Department of Zoology
S.S. Khanna Girls Degree College.
Prayagraj

Dr. Deepa Chaubey — **Member/Secretary**
Acadmic Consultant School of Science
UPRTOU, Prayagraj

Course Preparation Committee

Dr. Sangeeta Srivastava Asst. Professor A.K.P.G College, Varanasi	**Author**	**Block-1** (Unit : 1, 2) **Block-2** (Unit : 7, 8)
Dr. Sudhir Kumar Srivastava Asst. Prof. Dept of Zoology Art's Commerce and Science College, Taloda, Dis. Nandubar, Maharashtra,	**Author**	**Block-1** (Unit : 3) **Block-2** (Unit : 6)
Dr. Deepu Chaubey Acadmic Consultant, School of Science UPRTOU, Prayagraj	**Author**	**Block-2** (Unit : 4, 5)
Prof. (Dr.) G.S. Shukla Prof. Director Health Science UPRTOU, Prayagraj	**Editor**	(Unit : 1 - 8)
Dr. Deepu Chaubey Acadmic Consultant, School of Science UPRTOU, Prayagraj		**Coordinator**

ISBN :

Printed and Published by Dr. Arun Kumar Gupta Registrar, Uttar Pradesh Rajarshi Tandon Open University, 2021.

Printed By : K.C.Printing & Allied Works, Panchwati , Mathura -281003.

BLOCK INTRODUCTION

In the previous block you have studied genetics, and the following block deals with cell biology and microscopy in unit-4 of this block we discuss the history of cell biology, microscopy, fixation, staining and Autoradiography.

Microscopy is the technical field of using microscopes to view objects, and areas of objects that cannot be seen with the naked eye. Electron microscopes is a technique for obtaining high resolution images of biological and non-biological specimens. It is used in biomedical research to investigate the details structure of tissues, cells organelles and macromolecular complexes.

Autoradiography is based upon the ability of radioactive substance to expose the photographic film by ionizing it.

In unit 5 of this block we discuss the structure and function of plasma membrane and cell cycle.

Endoplasmic reticulum and Ribosomes is dealt in unit 6.

About Golgibody and Lysosomes you would learn in unit 7.

In unit 8 of this block we discuss origin structure and function of mitochondria.

UNIT-4 CELL BIOLOGY AND MICROSCOPY

Structure

4.1 INTRODUCTION

In this unit we discuss the definition and history of cell biology, microscopy, Autoradiography, Fixation and staining. The cell is the structure and functional unit of all living things. Cell contains hereditary information which is passed from cell to cell during cell division. All energy flow (metabolism and biochemistry) of life occurs within cells.

Cell biology is a branch of biology, also known as the basic unit of life. Cell biology comprises both prokaryotic and eukaryotic cells. The study of cell is called cell biology, cellular biology or cytology. Cell were discovered by Robert Hook in 1665. Cell theory, first developed in 1839 by Schleiden and Schwann.

In electron microscopy uses as electron beam to create a image of a sample. The EM operates under vacuum which means the samples are placed in a vacuum system during analysis. The optical microscope after referred to as the light microscope and magnifying lenses to examine small objects not visible to the nacked eye. TEM is a microscopy technique in which a beam of electrons is transmitted through on ultra thin specimen, interacting with the specimen as it passes through it.

The scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons.

In Autoradiography can be used to study the local distribution of the radioactive isotopes. The distribution of the isotopes in the different objects and organs is studied by stripping films of X-ray films. Similar to the photo emulsions used in black and white photography, they contain silver bromide particles in a gelatin emulsion.

Radiography is the visualisation of the pattern of distribution of radiation. In general, the radiation consists of X-ray, gamma or beta rays, and the recording medium is a photographic film.

In fixation preserves biological material (tissue or cells) as close to its natural state as possible in the process of preparing tissue for examination. Staining can be performed with basic dyes such as crystal violet or methylene blue, positively charged dyes that are attracted to the negatively charged materials of the microbial cytoplasm. Eosin is the most common dye to stain in histology.

Objectives

After studying this unit you will be able to :

- explain the history of cell biology.
- describe the light microscopy and electron microscopy.
- explain the autoradiography.
- describe the fixation and staining.

4.2 DEFINITION AND HISTORY OF CELL BIOLOGY

The cell is a fundamental structural unit of organisms. The study of these cells was made possible with the help of microscope which was invented in 1950 by Z. Janssen and H. Janseen.

The cell was first discovered and named by Robert Hooke in 1665. The cell (from Latin Cella, meaning hollow space) is the basic structural, functional, and biological unit of all known organisms.

A cell is the smallest unit of life. Cell are often called the building blocks of life "The study of cells is called cell biology" cellular biology or cytology.

Cell consist of cytoplasm enclosed within a membrane which contains many biomolecules such as proteins and nucleic acids.

Organisms can be classified as unicellular consisting of a single cell (including bacteria) or multicellular (including plants and animals).

- In 1676 the Dutch microscopist Antony Van Leeuwenhock (1632 – 1723) published his observations of single – cell organisms, or "little animalcules" as he called them. It is likely that leeuwenhock was the first person to observe a red blood cell and a sperm cell.
- In 1832 Barthelemy Dumortier (1797-1878) of France described binary fission (cell division) in plants. He observed the formation of a mid-line partition between the original cell and the new cell.
- A German Zoologist Theodore Schwann (1810 – 1882) extended cell concept to animals, i.e. all organisms are composed of cells and cell products. This cell theory resulted from numerous investigations, i.e,. of Mirbel (1802) Oken (1805), Turpin (1826) and finally by Schleiden (1838) and Schwann (1839). Although Schleiden and Schwann are universally recognized as the founders of cell theory, but its significance was earlier realized by Leeuwenhoek and others.
- "Cell is an essentially functional unit (performing all the Vital activities of life), comprising the protoplasm limited by a membrane and containing one or more nuclei at some time in its life". A. G. Loewy and P.Siekevitz (1963) stated that the cell is a "unit of biological activity delimited by a semi permeable membrane and capable of self-reproduction in a medium free of other living system". This definition does not apply to the viruses which lack a definite semi permeable membrane and nucleus.

Brown (1831) established that the nucleus is a fundamental and constant component of the cell. Dujardin, Schultze, Purkinji and von Mohl concentrated on the description of the cell components, term the protoplasm. Thus the cell is a mass of protoplasm limited in space by a cell membrane and possessing a nucleus. Next "organismal theory" has been introduced, according to which an organism is regarded as a protoplasmic unit which is incompletely divided into small centres, the cells, for the performance of various biological activities.

Previously, the structure of the cell was though to be as two-phase system consisting nucleus and cytoplasm. In electron microscopy has changes this conception, that essentially the forms of a limited number of basic components. These basic structural units of the cells are (i) membranes (ii) microtubules (iii) granules membranes mainly lipoproteinous with or without pores and polymorphic in nature.

Three historical strands weave together into modern cell biology, each with important contributions to understanding cells.

- The cytology strand focuses mainly on cellular structure, and emphasizes optical techniques.
- The biochemistry strand focuses on cellular function.

- The genetics strand focuses on information flow and heredity.
- Cells provide the necessary structural support for an organism.
- The genetic information necessary for reproduction is present within the nucleus.
- Structurally, the cell has cell organelles which are suspended in the cytoplasm.
- Mitochondria is the organelle responsible for fulfilling the cells energy requirements.
- Lysosomes digest metabolic wastes and foreign particles in suspended in the cytoplasm.
- Endoplasmic reticulum synthesizes selective molecules and processes them.

The microtubules or fibres are second basic structural units of cell. The microtubules might be supposed as a rolled-up membrane.

Fibres are of two categories –

(i) Inert structural fibres:-These include intracellular fibres as chromosomal and DNA fibres, neurofilaments and extracellular collegen and elastin.

(ii) Active fibres:- In this roup are spindle fibres, fibres of cilum and flagellum and myofilaments.

The granules are third basic units of the cell the granules may be solid or hollow.

The most common and well-studied granules are ribosomes and intra-mitochondrial granules.

Thus the two phase theory of nucleus and cytoplasm is no longer valid in the light of modern researches, based on above observation cell is referred to as three – phase system comprising three basic unit.

In 19^{th} century can be considered as the "classical era of cytology", it was known that all the organs of animals were made up of tissues such as muscle, bone, cartilage and fat.

In the 20^{th} century various modern microtechniques have been employed in cytological investigation, i.e, new histochemical and cytochemical methods have been developed to detect various molecular components of the cell; various cellular components have been separated by ultracentrifugation; different biochemical events of the cell could be known in detail by autoradiography; and methods of tissue culturing have made possible the study of living cells.

4.3 MICROSCOPY

Investigations or studies by means of the microscope is called microscopy.

In electron microscope the radiation sources is located on the top of the microscope.

In light microscope, its source is located at the bottom of microscope..

There are two fundamentally different types of microscope-

(1) Light microscope

(2) Electron microscope

(1) Light microscope- use a series of glass lenses to focus light in order to form an image whereas electron microscope use electromagnetic lenses to focus a beam of electrons. (Fig.4.1)

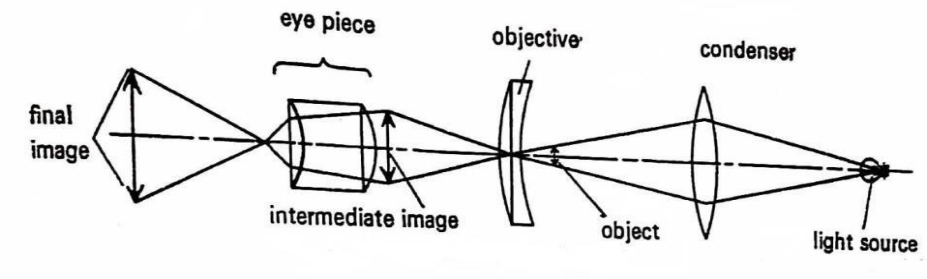


Fig.4.1 - Compound Light Microscope

(2) The electron microscope tool for studying the ultrastructure of cells because it has much greater resolving power than the light microscope (resolving power 250 times that of the light microscope). (Fig-4.3a)

The high resolution of EM images results from the use of electrons (which have very short wavelengths) as the source of illuminating radiation. (Fig-4.2)

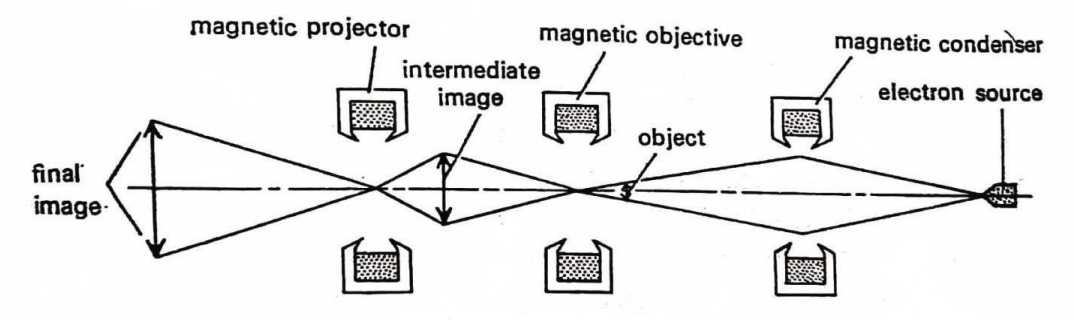


Fig.4.2 - Electron Microscope

4.4 TYPES OF ELECTRON MICROSCOPE

There are two main types of electron microscope –

(1) TEM – Transmission electron microscope

(2) SEM – Scanning electron microscope

(1) TEM – Transmission electron microscope

- The TEM (transmission electron microscope) is a very powerful tool for material science.
- The TEM operates on the same basic principles at the light microscope but uses electrons instead of light. (Fig.4.3.a)
- Because the wavelength of electrons is much smaller than that of light. TEM can be used to study the growth of layers, their composition and defects in semiconductors.
- TEM is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image.

The TEM type of electron microscope that has three essential systems.-

(1) An electron gun, which produces the electron beam, and the condenser system, which focuses the beam into the object.

(2) The image-producing system, consisting of the objective lens, movable specimen stage, and intermediate and projector lenses, which focus the electrons passing through the specimen to form a real, highly magnified images.

(3) The image– recording system, which converts the electron image into some form perceptible to the human eye. The image recording system usually consists of a fluorescent screen for viewing and focusing the image and a digital camera for permanent records. (Fig- 4.3b)

(2) SEM (Scanning Electron Microscope)

The scanning electron microscope (SEM) uses a focused beam of high – energy electrons to generate a variety of signals at the surface of solid specimens.

- The SEM is also capable of performing analyses of selected point locations on the sample, this approach is especially useful in qualitatively or semiquantitatively determining chemical compositions.
- SEMs always have at least one detector (usually a secondary electron detector), and most have additional detectors.
- The SEM is also widely used to identify phases based on qualitative chemical analysis and / or crystalline structure.
- SEM is use to examine the surface of specimen, i.e. outer cell surface and various processes, extensions and extracellular materials.
- SEM provides a three –dimensional image of a specimen.

- In SEM the electron beam does not pass through the specimen. The condenser lens focusses a fine electron beam on the surface of the specimen. The beam is moved rapidly back and forth by beam deflectors to scan the specimen surface. (Fig- 4.3c)

The main differences of TEM and SEM are-

- In the TEM, electrons that pass through the specimen are imaged. In the SEM electrons that are reflected back from the specimen (secondary electrons) are collected, and the surfaces of specimens are imaged. (Fig.4.3)

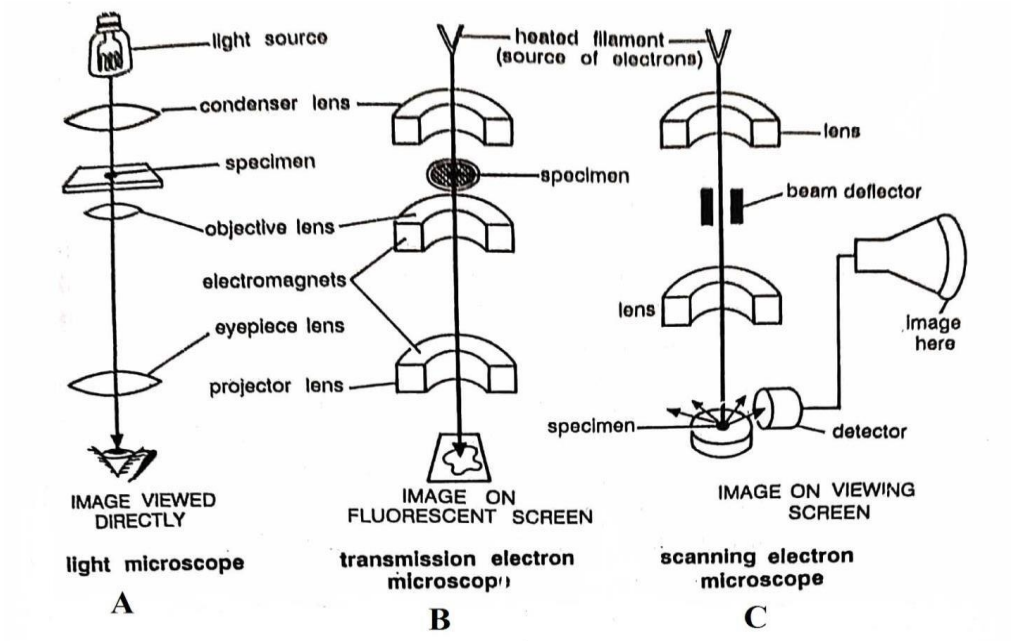


Fig.4.3 - A comparison of principal features of a light microscope, a transmission electron microscope ant a scanning electron microscope.

4.5 AUTORADIOGRAPHY

Radiography is the visualization of the pattern of distribution of radiation. In general, the radiation consists of X-rays, gamma (g) or beta (β) rays, and the recording medium is the photographic film.

Autoradiography a process in which radio active materials incorporated macromolecules, which are then placed next to a film of the photographic emulsion thus forming a pattern on the film corresponding to the location on the radioactivity. It is the study of biological events in tissue sections using radioactivity.

Autoradiography permits the localization of radioactive substances in tissues by means of emitted radiation effects on photographic emulsions.

Cairns: Autoradiography experiment

In autoradiography technique, the maternal is first supplied with a suitable radioactive material like tritiated thymidine (H^3-TDR-H^3 is heavy isotope of hydrogen and it replaces normal hydrogen in thymidine to give rise to tritiated thymidine) Tritiated thymidine is used only DNA, not RNA since thymine base is absent in RNA.

Coirns (1963) also conducted his experiments with E.coli. He used heavy isotope of hydrogen (H^3) to replace tymine of DNA with tritiated thymidine and thus labeled DNA.

The tritiated thymidine gets incorporated into DNA and replaced ordinary thymine.

The material is then sectioned the cells and prepare the slides.

These slides are then covered by photographic emulsion and store in the dark place.

The tritiated thymidine emits particles in dark due to its radioactive decay.

These particles expose the film which can be developed and interpreted.

If the exposure is light on the autoradiographs, it suggests labeling of one strand of DNA,. Which indicates, semiconservative replication.

Cairns obserbed light film exposures in E.coli which demonstrated that DNA replication is semiconservative.

The most common radioactive label is 32-phosphorus (32 P), although for certain techniques 35-sulphur (35S) and tritium (3^H) are used. These may be detected by the process of autoradiography (use of different radioisotopes in DNA).

Autoradiography emulsions are solutions of **silver halide** that can be made to set solid by the inclusion of materials such as gelatin. This can be used for example for autoradiography of microscope slides. X-ray film is the alternative and is used for gels.

4.6 Fixation

Fixation can be defined as the selective preservation of the cell or tissue structures and components for subsequent study.

Fixation should be carried out as soon as possible after removal of the tissues (in the case of surgical pathology) or soon after death (with autopsy) to prevent autolysis.

Fixation can be defined as the selective preservation of cell or tissue structures and components for subsequent study.

(1) Importance of fixation

It is impossible to underplay the importance of fixation in histopathology.

- Fixation is the foundation step behind the study of pathology and essentially exists to prevent the autolysis and degradation of the tissue and tissue.
- Fixative can be divided into two categories denaturing fixatives and cross linking (or addition) fixatives.
- The alcohol based fixatives, for example carnoy's and methacarn, are denaturing fixatives. The action of alcohol present in the solution acts to cause protein denaturation through the removal of water from the free carboxyl, hydroxyl, amino groups of proteins which results in protein coagulation and tissue shrinkage.
- Bouin's like carnoy's, was first described in the late 19^{th} century by Pol Andre Bouin, consisting of Picric acid, acetic acid and formaldehyde, it has both a coagulative as well as cross linking effect on proteins.

The mechanism of fixation is dependent on the reagent used Alcohol based fixations dehydrate cell / tissues, causing proteins to denature and precipitate in situ.

Para formaldehyde causes covalent cross links between molecules.

The process of fixation involves the following events –

(i) The proteins and other macro molecules are precipitated.

(ii) The intracellular hydrolytic enzymes are denatured, preventing autolysis.

(iii) Fixation reduced the shrinked thus macromolecules making the preparation more stable.

(iv) Fixation makes the cell suitable for staining. Fixation is generally followed by dehydration. Cell and tissue can be preserved either by chemical or by cooling method.

(2) Types of Fixation:-

There are two types of fixation –

Fixation of tissues can be achieved by chemical or physical means

(i) Physical methods- It includes heating, micro-waving and cryo-presevation (freeze drying).

(ii) Chemical Fixation- It is usually achieved by immersing the specimen in the fixative (immersion fixation) or, in the case of small animals or some whole organs such as lung, by perfusing the vascular system with fixative (perfusion fixation). Chemical fixation is a technique to fix a specimen with chemicals to prevent autolysis by the action of enzymes and deformation of morphologies during specimen preparation. Biological tissues start autolysis caused by their enzymes immediately after stopping the activities of them.

Chemical Fixation is carried out to preserve the morphologies and physical properties at the living states of the specimen as much as possible.

Fixative may be two types-

(1) Simple fixative

(2) Compound fixative

- Simple fixative is a single chemical solution such as formaline, Picric acid.
- Compound fixative is formed of two or more chemical such as Bouins fluid (aqueous solution of picric acid, formaline, Glacial acetic acid), the other hand fixation by freezing, this method is suitable for electron microscopes.

The important functions of fixatives – The fixing fluid penetrate the tissues by inward diffusion, so that the tissues can be preserved as natural shape, but they undergoes post-mortem changes.

4.7 STAINING

(1) It can define as a chemical reagent or dye that is responsible for the discolouration of the specimen. It adds contrast to the microscopic image that give a distinct view of the organism.

(2) It is a technique that is widely used for the examination of cells, tissues and cellular components. There are variety of staining method like simple, differential and special staining that are used in labs. The process of staining can be done in two ways –in-Vitro and in-Vivo

Types of stains:- The stains are classified into three groups (i) Acid stains (ii) Basic stains (iii) Neutral stains.

- Acidic stains are colouring agents which stain the cells and cellular components only at low pH or in acidic medium, therefore the cell components which are stained by acid stains are called Acidophilic. Acid stain are used to stain proteins eg cosing carbol fuschin etc.
- Where as basic stains are colouring agent which stain the cells and cell component at high pH or in alkaline medium, thus stain are called **Basophilic.** Basic stains are used to stain nucleus and nuclic acid e.g. – crystal violet, methylene blue, saframin etc.
- Neutral stains are neither acid nor basic eg. india ink, Eosinate of methylene blue and Giesma stain are example of a neutral dye stains or dyes contain salts made up of a positive ion and negative ion, depending on the type of dye, besides these stains there are some stains which are used to stain cell and tissues without killing them these stain known as Vital stains, ex. Janus Green B is a basic dye and vital stain used in histology.
- Eosin dye exclusion.

- Propidium iodide – DNA stain that can differentiate necrotic, apoptotic and normal cells.

Summary

- The cell was first discovered and named by Robert Hooke in 1665.
- A cell is the smallest unit of life. Cell are often called the building blocks of life.
- The study of cells is called cell biology.
- In the TEM, electrons that pass through the specimen are imaged.
- In the SEM electrons that are reflected back from the specimen (secondary electrons) are collected, and the surfaces of specimens are imaged.
- Acidic stains are colouring agents which stain the cells and cellular components only at low pH or in acidic medium
- basic stains are colouring agent which stain the cells and cell component at high pH or in alkaline medium

4.8 TERMINAL QUESTIONS

Q1 What is the difference between acidic and basic dyes?

……
……
……

Q2 Discuss the history of cell biology.

……
……
……

Q3 Differentiate between the light microscopy and electron microscopy.

……
……
……

Q4 what is Autoradiography? Discuss in detail.

……
……
……

Q5 Write short notes on:-

a) TEM

……
……
……

b) SEM

……
……
……

4.9 ANSWERS

SAQ 1 a) 1839**,** Schwann b) foreign particles

SAQ 2 a) Radiography b) 32-phosphorus (32 P)

SAQ 3 a) Physical methods b) Chemical fixation

UNIT-5 PLASMA MEMBRANE, NUCLEUS AND CELL CYCLE

Structure :

5.1 INTRODUCTION

In this unit we discuss the plasma membrane nucleus, cell cycle and cell division. All cells are surrounded by the plasma membrane. The membrane is composed of a phospholipid bilayer arranged back-to-back. The plasma membrane is selectively permeable. It regulates the molecules to allow, enter or exit of the cell. The plasma membrane is also dotted with proteins. some of the proteins are peripheral proteins while other are integral proteins. Carbohydrates are the third major component of plasma membranes. In general, they are found on the outer surface of cells and are bound either to proteins (forming glycoproteins) or to lipids forming (glycolipids). Nucleus is a membrane enclosed organelle found in eukaryotic cells. It contains most of cells genetic material (DNA). The nucleus is an important cell organelle, it is just like the central

nervous system of our body, so the nucleus is simply called the brain of the cell or "controlling centre of the cell" because it controls all activities of an individual cell.

Actively dividing eukaryotic cells pass through a series of stages collectively known as the cell cycle. Growth, regeneration and reproduction depend upon cell cycle and cell division. Cell division is process in which cells reproduce their own kind. During the cell cycle, M phase for the division of the nucleus and cytoplasm whereas S phase for DNA synthesis. The G_1 and G_2 phases are the preparative phases for DNA synthesis and cell division respectively. The cell cycle can be described as a repeated series of events in which cell division and replication alternate in the following order M phase, G_1 phases, S phase and G_2 phase. G_2 phase during the gap between DNA synthesis and Mitosis and G_1 phase during the gap between the M and S phase.

The combined phases of G_1, S, G_2 are called the interphase Mitosis involves the division of body cells, while meiosis involves the division of sex cells. The division of a cell occurs once in mitosis but twice in meiosis. Two daughter cells are produced after mitosis, while four daughter cell are produced after meiosis.

Objectives

After studying this unit you will be able to :-

- Describe the structure of plasma membrane.
- Explain the structure and function of nucleus.
- Explain the process of mitosis occurring in animal cell.
- Explain the process of meiosis occurring in animal cell.
- Explain the difference in mitosis and meiosis.

5.2 PLASMA MEMBRANE

Structure of Plasma Membrane

1. Plasma membranes consist mainly of phospho-lipids and proteins.
2. Membranes lipids are relatively small molecules that have both a hydrophilic and a hydrophobic. These lipid bilayer regions are barriers to the flow of polar molecules.
3. Some of the proteins are peripheral proteins that only go halfway through the membrane, while others are integral proteins that go entirely through the membrane. These proteins serve as channels to allow the molecules to enter and leave the cell.
4. Specific proteins mediate distinctive functions of membranes. Proteins serve as pumps, gates, receptor, energy transducers and enzymes. Membranes lipids create a suitable environment for the action of these proteins.

5. Membranes are noncovalent assemblies. The constituent protein and lipid molecules are held together by many noncovalent interactions, which are cooperative in character.

6. The membranes are asymmetric. The inside and outside faces of membrane are usually different.

Membrane proteins:

The major function of plasma membrane are performed by membrane proteins.

Membrane proteins are common proteins that are part of or interact with, biological membranes.

There are three ways in proteins are associated with plasma membrane.

1. **Transmembrane proteins:** Which can cross the membrane once (single pass) or multiple times (multi-pass). Transmembrane proteins extend through the lipid bilayer as a single helix (e.g., glycophorin) or multiple helices (e.g. porin).

   - When the polypeptide crosses the membrane only once, called a single pass transmembrane (e.g. glycophorin protein of human RBCs).
   - And when it crosses several times it is called a multipass transmembrane protein (e.g. band 3 protein of human RBCs)

Transmembrane proteins serve as –

1. **Channels:-** through which ions or small water, soluble substances can diffuse
2. **Carriers:-** which actively transport materials across the lipid layer.
3. **Pumps:**- which actively transport the ions across the lipid layer.

Trans-membrane proteins are the example of intrinsic membrane proteins. Intrinsic membrane proteins embeded in the hydrophobic region of the lipid layer. Hydrophobicity of some of these membrane is increased by covalent attachment \of the fatty acid chain that is inserted in the cytoplasmic leaflet.

2. **Extrinsic proteins:-** Extrinsic or peripheral membrane proteins associate loosely with the hydrophilic surfaces of the lipid bilayer or intrinsic membrane protein. Extrinsic proteins are located entirely in the cytosol and are attached to membrane by means of covalently attached fatty acid chains or oligosaccharides. Extrinsic membrane proteins may interact with the inner or outer leaflet.
3. **Covalently linked non-cytosolic extrinsic proteins -** which are found on the external surface of membrane and remain attached to the non-cytoplasmic monolayer with the help of an oligosaccharide.

   - Intrinsic proteins serve mainly as enzymes.
   - Extrinsic proteins contribute to the cytoskeleton.

4. **Non-covalently linked extrinsic proteins:-**which are present in either side of the membrane, they are attached to it by non-covalent interactions with other trans-membrane proteins. These proteins also called the peripheral membrane proteins.

Structure of plasma membrane-

Unit membrane model:- Robertson's unit membrane model was given by J.David Robertson in 1959 after the Biomembrane model was proposed by Danielli and Davson in 1935. This concept of unit membrane with three layers (two protein layers and one lipid bilayer) only supported the concept proposed by Davson and Danielli. The biomembrane model proposed by Robertson. The biomembrane are made up of four layers. Out of these four layers, two are protein layers (20-25 A thick) and two are phospholipid layers (25-35 A thick). (Fig. 5.1)

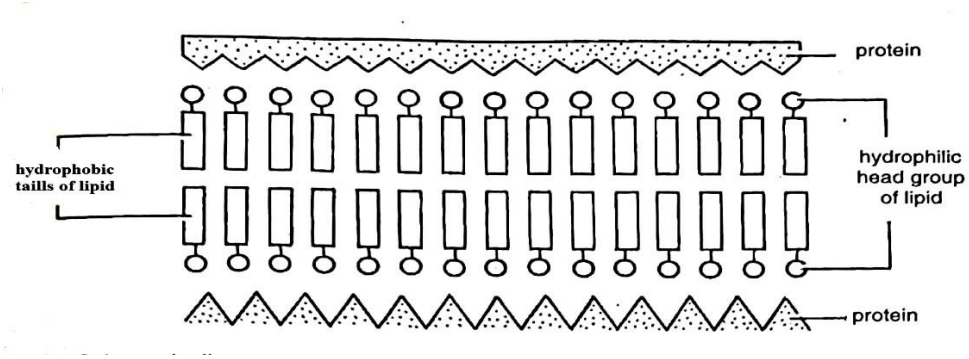


Fig.5.1 Unit membrane model (Robertson model of membrane)

On the basis of detailed electron microscopic examination, in late 1950 J.D.Robertson suggested the cell membrane as a three layered (trilaminar) structure about 7.5 to 10.0 nm in thickness,

Robertson termed this structure a "unit membrane", and he concluded that all biological membranes had unit membrane construction.

Composed of two dark electron dense lines, each 2.0 to 2.5 nm thick, separated by a light electron transparent region of 3.5 to 5.0 nm. Robertson used two distinct zones. The dark lines of trilaminar structure were proteins and polar groups of lipids and the light central region consisted of non polar groups. The globular proteins of the membrane are of two different types; extrinsic (Peripheral protein) and intrinsic (integral proteins).

Danielli-Davson model:- Proposed a model, called sandwich model, for membrane structure in which a lipid bilayer was coated on its either side with hydrated proteins (globular proteins).

The two layers of lipid molecules arranged radially with their hydrophobic hydrocarbon chains towards each other and with their respective polar groups arranged outwardly and inwardly, the entire double layer of lipid molecules.

The polar end of the lipid molecules are associated with the monomolecular layer of polar globular protein molecules. Thus the entire structure consists of a double layer of lipid molecules sandwiched between two continuous layers of proteins.

Robertson in 1960, proposed the unit membrane hypothesis. This hypothesis states that all cellular membranes have an identical trilaminar structure (or dark-light-dark or railway track pattern). The thickness of the unit membrane has been found to be greater in plasma membrane (10 nm) than in the intracellular membranes of or Golgi apparatus (i.e. 5 to 7 mm).

S.J. Singer and G.L. Nicolson (1972) postulated another model, called **fluid mosaic model**. They proposed that the care of the membrane is a lipid bilayer. Both surface of which are interrupted by protein molecules. The model also proposes that there are two types of proteins in a membrane. Proteins occur in the form of globular molecules and they are dotted about here and there in **mosaic pattern**. Some proteins are attached at the polar surface of the lipid (i.e,, the extrinsic proteins). The extrinsic proteins have essentially hydrophilic side chains. These are bound to outer hydrophilic ends of the lipid bilayers. (Fig.5.2. a & b)

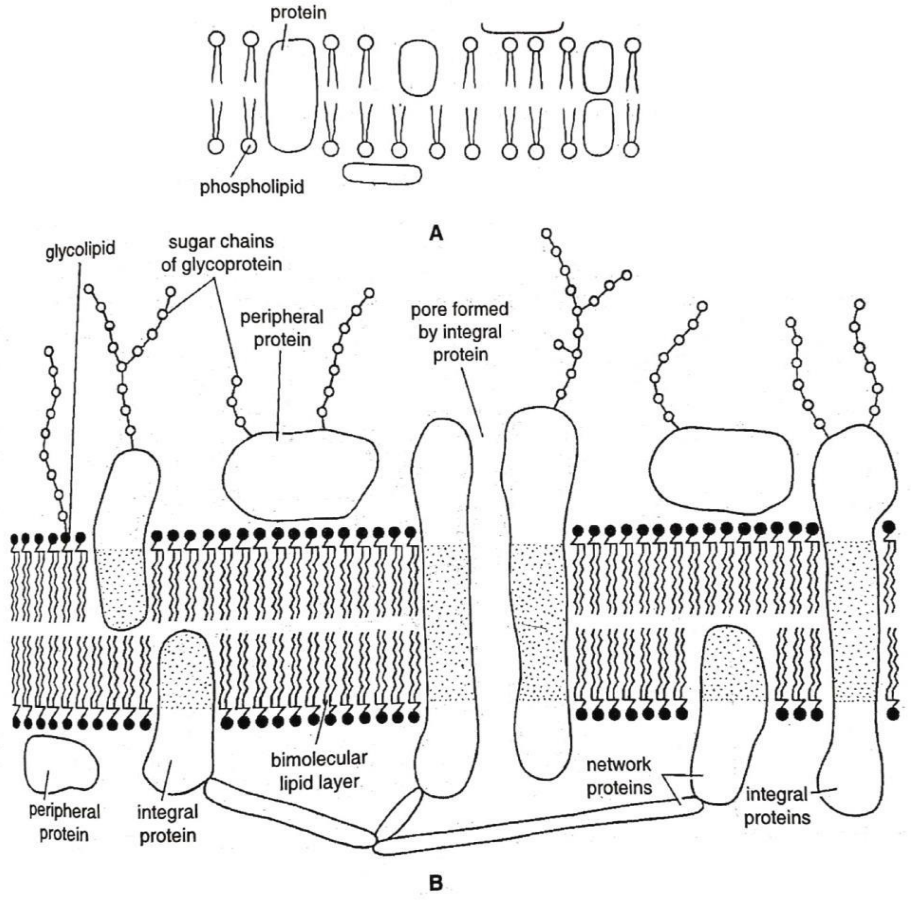


Fig.5.2 - A-B. Fluid mosaic model. **A.** Simpal view; **B.** Complex view

While other intrinsic proteins have amino-acids with hydrophobic side chain. These side chain dissolved in the hydrophobic central core of the lipid bilayer.

Plasma membrane is selectively permeable, meaning that it allows water to pass through it, but retards the passage of most solutes to varying degrees (allow only water but no solute particle to pass through).

Function of plasma membrane- The main function of plasma membrane is to regulate the flow of materials into and out of the cells. For inflow of materials, plasma membrane often functions as a semi-permeable membrane because water and other selected ions and small molecules move through it more easily than larger molecules. This inflow of materials into cytoplasm through plasma membrane occurs mainly by following types.

Transport Across Membrane:- Transport across the membrane may be passive or active.

1. **Passive transport:-** It is a type of diffusion which an ion or molecules crossing a membrane moves down its concentration gradient. No metabolic energy is consumed in passive transport.

2. **Simple diffusion:-** Solutes move from a region of higher concentration to lower concentration.

Facilitated diffusion:- Solutes move across a membrane from higher to lower concentration with the aid of transmembrane proteins. Facilitated diffusion is passive transport that uses integral membrane proteins to help larger, charged, hydrophilic and polar molecules across a concentration gradient.

There are two types of integral membrane proteins-

- The first are carrier proteins, which are proteins that bind a molecule to facilitate transport through a cell membrane.

- The second are channel proteins, which are proteins that create a passage way to transport molecules and ions through the cell membrane.

Active transport- is the movement of molecules across a cell membrane in the direction against their concentration gradient, going from a low concentration to a high concentration.

Active transport is usually associated with accumulating high concentrations of molecules that the cell needs such as ions glucose and amino-acids. In the active transport, energy is required for the movement of molecules or ions against the concentration gradient across the cell membranes. If the process uses chemical energy, such as from adenosine triphosphate (ATP), it is termed primary active transport. Secondary active transport involves the use of an electrochemical gradient.

Active transport uses cellular energy, unlike passive transport, which does not use cellular energy. Active transport is a good example of a process for which cells require energy.

Importance of active transport

Active transport includes two aspects.

1. Active transport is transport against a concentration gradient.

2. Active transport is driven by energy-yielding, spontaneous reactions of the cell; most frequently the immediate energy source for active transport is ATP.

3. Osmosis:- The plasma membrane is permeable to water molecules. The process by which the water molecules pass through a membrane from a region of higher water concentration to the region of lower water concentration is known as osmosis.

The process in which the water molecules enter into the cell is known as endosmosis, while the reverse process which involves the exit of the water molecules from the cell is known as exosmosis. Due to endosmosis or exosmosis the water molecules come in or go out of the cell.

The amount of the water inside the cell causes a pressure known as hydrostatic pressure. The hydrostatic pressure which is caused by the osmosis is known as osmotic pressure.

The plasma membrane maintains a balance between the osmotic pressure of the intra-cellular and inter-cellular fluids.

Example of Active transport:- One of the most important pumps in animal cells is the sodium – potassium pump, which moves three Na^+ out of cells, and two K^+ ions inside the cell. Because the transport process uses ATP as an energy source. It is considered an example of primary active transport.

The enzyme presence that was capable of hydrolyzing ATP to ADP and inorganic phosphate in the presence of Na^+, K^+ and Mg^{++}

This enzyme was termed the sodium-potassium ATPase Na^+ -K^+ ATPase is found associated with plasma membranes that actively transport Na^+ and K^+. Hydrolysis of ATP and transport of Na^+ and K^+ are closely linked, and ATP is not hydrolyzed unless Na^+ and K^+ are transported. Thus the hydrolysis of ATP by Na^+ - K^+ ATPase is linked to the transport of K^+ into the cell and Na^+ out of the cell.

1. The sodium – potassium pum transports sodium out of and potassium into the cell in a repeating cycle of conformational (shape) changes.

   In each cycle, three sodium ions exit the cell, while two potassium ions enter.

2. Transport that directly uses ATP for energy is considered primary active transport. Secondary active transport moves multiple molecules across the membrane.

Endocytosis:- Endocytosis method require the direct use of ATP to fuel the transport of large particles such as macromolecules.

Phagocytosis:- Parts of cells or whole cells can be engulfed by other cells process is called phagocytosis.

The ingestion of solid particles by a cell through plasma membrane is called is phagocytosis.

Pinocytosis:- In this process liquid substances are incorporated into the cell by plasma membrane and exocytosis. Both of these processes also utilize energy in the form of ATP molecules.

Overton (1902) was the first man who studied the structure of plasma membrane and stated that it is composed of a thin layer of lipid.

Gorter and Grandill (1935) suggested that plasma membrane is made up of double layer of lipid molecules. Robertson (1961) gave a "unit membrane" concept. According to which all the membranes of the cell were constructed of protein lipid protein. Singer and Nicholson (1972) proposed fluid "Mosaic Model".

Chemical composition of plasma membrane- The plasma membrane is composed of mainly proteins (60-80%) and lipids (20-40%) and a small percentage of carbohydrate (1-5%).

Proteins are the main components of plasma membrane since they provide mechanical support and act as channels for different vital or physiological activities.

Animal cell is bounded by a living extremely thin and delicate membrane called plasmalemma, or plasma membrane or cell membrane.

At molecule level, it consists of continuous bilayer of lipid molecule (phospholipid and cholesterol) with protein molecules embedded in it or adherent to its both surfaces. Some carbohydrates molecules may also be attached to the external surface of the plasma membrane, they remain attached either to protein molecules to form glycoproteins or to lipids to form glycolipids, such a fluid mosaic plasma membrane is a selectively permeable membrane, its main function is to control selectively the entrance and exit of materials. This allows the cell to maintain a constant internal environment (homeostasis). Transport of small molecules such as water, oxygen, CO_2, glucose, etc, across the plasma membrane takes place by various means such as osmosis, diffusion, and active transport.

The process of active transport is performed by special type of protein molecules of plasma membrane called transport proteins or pumps, consuming energy in the form of ATP molecules.

5.3 NUCLEUS

The cell nucleus is a large organelle in eukaryotic organisms which protects the majority of the DNA within each cells. The nucleus also produces the necessary precursors for protein synthesis.

The shape of nucleus is variable according to cell type.

It has generally spheroid but ellipsoid or flattened. According to the number of the nuclei following types of cells have been recognized. (Fig.5.3)

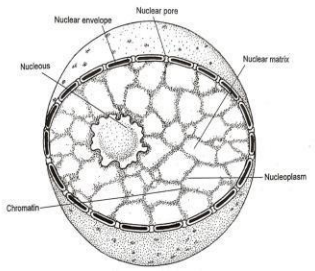


Fig.5.3. Nucleus

Mostly cell contains a single nucleus, known as mononucleate cell, cell containing two nuclei are known as binucleate cells (e.g. Paramecium). Sometimes more than two nuclei are present in a single cell, such cells are called Polynucleate cells, such cells in animals are called syncytial cells. (E.g. osteoblast). The size of nucleus is not constant and is generally correlated with DNA contents. The nuclear size is a function of chromosome number i.e. the size is variable depending upon the number of chromosomes (DNA contents).

The Nucleus consists of -

1. Nucleolemma or nuclear membrane.
2. Nucleoplasm.
3. Chromatin fibres.
4. Nucleolus.

1. **Nuclear membrane:** The nuclear membrane consists of phospholipids that form a lipid bilayer. The nucleus is separated from the cytoplasm by a limiting membrane called nuclear membrane. The membrane plays an important role for the transport of the material between the nucleus and cytoplasm. The nuclear membrane appears to be a double membrane having interruptions or pores at intervals.

   The outer nuclear membrane is generally attached with ribosome and is continuous with the membrane of rough endoplasmic reticulum. The space between the nuclear membranes is called perinuclear space and it is continuous with the endoplasmic reticulum lumen.

   The inner surface of the nuclear membrane of animal cells is smooth having no ribosomes. The outer membrane is comparatively thicker than the inner membrane.

2. **Nucleoplasm:** Nucleus contains nucleoplasm, also known as karyoplasm or nuclear sap. The nucleoplasm is a type of protoplasm, and is enveloped by the nuclear envelope (also knows as the nuclear membrane). The nucleoplasm has a complex chemical composition. It has composed of mainly the – nucleoproteins but it also contains other inorganic and

organic substances viz., nucleic acid, proteins, enzymes and minerals. The main function of the nucleoplasm is to serve as a suspension substance for the organelles, inside the nucleus. It also helps maintain the shape and structure of the nucleus, and plays an important role in the transportation of materials that are vital to cell metabolism and function.

Nucleoproteins are the most important components of nucleus. The proteins present in the nucleus are specific and are of following two types –

(i) Histone proteins
(ii) Non-histone proteins

Both histone and non-histone proteins are synthesized in a cytoplasm and enter the nucleus through the nuclear envelope.

- Histones are synthesized only when DNA is replicated, whereas non-histone proteins are synthesized continuously.
- Histones (which are of five basic types such as H_2A, H_2B, H_3, H_4 and H_1 or H_5) are highly basic proteins rich in positively charged lysine and arginine residues.
- Non-histone proteins are acidic in nature and are rich in tryptophan and tyrosin. (Fig.5.4)

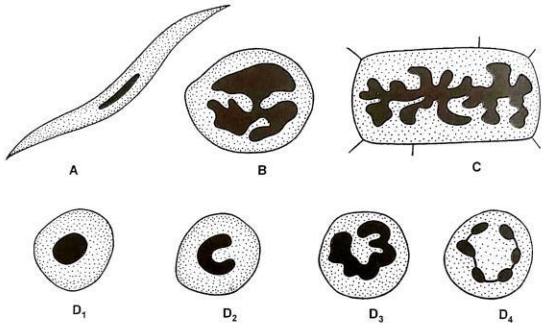


Fig.5.5. Different shapes of the nucleus in animal cell. A-Elongated in muscle cell; B- Lobed in a human neutrophil cell; C- Branched in a silk spinning cell of an insect larva; D1 to D4- Variable shapes in leucocytes.

3. **Chromatin Fibres:-** The nucleoplasm contains many thread-like, coiled and much elongated structure. These thread like structures are known as the chromatin or chromatin fibres which can be stained with basic stains.

Chromatin:- It appears as a viscous, gelatinous substances which contains DNA, RNA, basic proteins called histones, and non-histones (more acidic) proteins.

Embedded in the matrix of nucleus there is a dense rounded oval and acidophilic body called as nucleolus.

Nucleolus:- The nucleolus contained within the nucleus is a dense, membrane – less structure composed of RNA and proteins called the nucleolus.

Size:- The size of the nucleolus is related with the synthetic activity of the cell. Therefore, the cells with little or no synthetic activities (e.g.- muscle cells, spearm cells, blastomeres etc.) are found to certain smaller or no nucleoli. On the other side, the secretory cells, nurons and oocytes which synthesize proteins or other substances possess relatively large nucleoli.

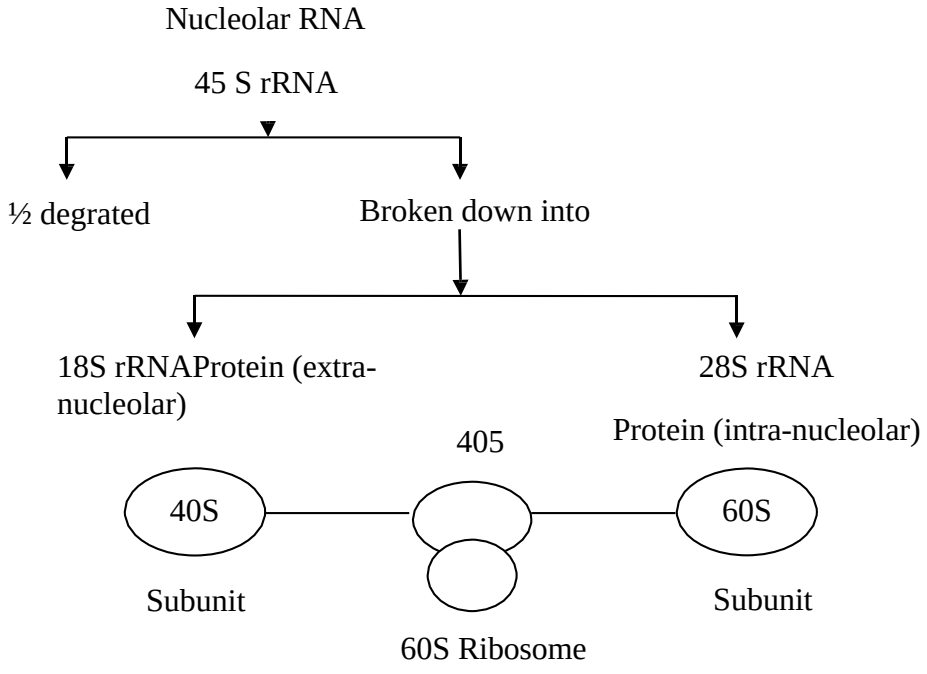
The number of nucleoli in nucleus depends upon the number of chromosomes and species. The number of the nucleoli in the cells may be one, two or four. The position of the nucleolus in the nucleus is eccentric.

Function of Nucleolus

1. **Help in Proteinsynthesis:-** The nucleolus is a factory for ribosomes. Ribosomes are made of two sub units, large and small subunits. Each subunit is manufactured separately in the nucleolus.

   The nucleoli help in the protein synthesis by the formation of ribonucleic acid. This ribonucleic acid (RNA) plays an important role in the formation of proteins. The cells with a high rate of protein, synthesis have large nucleoli with a high RNA content, whereas the cells with a low rate of protein synthesis have small undeveloped nucleoli.

2. **In enkaryotes** the gene coding for RNA contains a chain at least 100-1000 repeating copies of DNA. This DNA is given off from the chromosomal fibre in the forms of loops. The DNA loops are associated with proteins to form nucleoli. The DNA seems as a template for 45 SrRNA, half the 45 SrRNA is broken down to form 28S and 18S RNA. The other half is broken down further to nucleotide level within the nucleolus the 28S rRNA combines with proteins, to form the 60S ribosomal subunit. The 18S rRNA also associates with proteins to form the 40S subunit of the ribosome.



3. The nucleolus serves as an intermediary for carrying the genetic information from generation to generation.

   - It controls all the metabolic activities of the cell by controlling the synthesis of enzymes required.
   - It stores the cells hereditary material, or DNA, and it co-ordinates the cells activities, which include growth, intermediary metabolism, protein synthesis, and reproduction (cell division).
   - All the RNAs needed for the cell are synthesized in the nucleus.
   - So the nucleus maintains the security of the genes and controls the functions of the entire cell by regulation gene expression.

Differences between Nucleus and Nucleolus

	Nucleus		**Nucleolus**
–	Large in size	–	Very small in size.
–	Bounded by the nuclear envelope.	–	It has no limiting membrane.
–	It contains chromosomes.	–	It does not hold any Chromosomes.
–	It is rich in DNA, the genetic material.	–	It is rich in RNA.

5.4 CELL CYCLE AND CELL DIVISION

Cell cycle can be defined as the entire sequence of events happening from the end of one nuclear division to the beginning of the next.

Howard and Pelc (1953) divided cell cycle into four phases or stage-G_1, S, G_2 and M phase

(1) G_1 phase

(2) S phase

(3) G_2 phase

(4) M phase

(1) G_1 phase :- It comes after M phase and also known as *first gap or growth phase*. During this no DNA synthesis take place. In this phase synthesis of RNA, and Protein. DNA Synthesis doesn‘t occur in this period. G_1 is the period between the end of the mitosis and the start of DNA synthesis.

(2) **S phase or Synthetic phase :-** It comes after G_1 phase and occupies roughly 35-45% of cell cycle. During this phase replication of DNA and synthesis of histone protein occurs so each chromosome has two DNA molecule and duplicate set gene.

(3) **G_2 phase :-** It is called *second gap* or *growth phase*. In this phase synthesis of RNA and proteins required for cell growth take place. It occupies 10-20% time of cell cycle. G_2 phase is the interval between the end of DNA synthesis and the start of mitosis.

(4) **M phase:-** M phase involves two distinct division related process: Mitosis and Cytokinesis. M phase can be categorized into karyokinesis (the division of cell chromosome) and cytokine (the division of cell cytoplasm to form new daughter cells). In this phase the cell divids the duplicated DNA and the cytoplasm into two new daughter cells. (Fig.5.4)

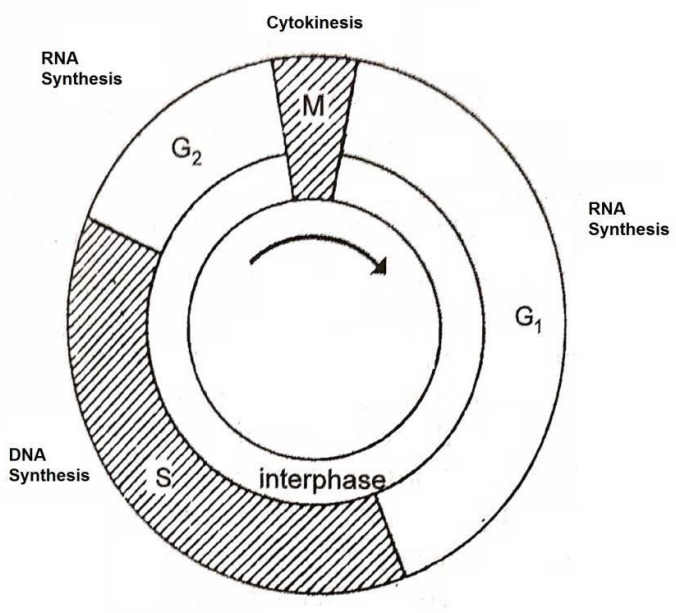


Fig.-5.5 The cell cycle of mitotic cycle

Cell division

The cell division is of two types :

(i) Mitosis

(ii) Meiosis

(1) **Mitosis-** The major purpose of mitosis is for growth and to replace wornout cells. Mitosis is part of the overall cell cycle that includes replication of DNA, division of the nucleus (Kacryokinesis) and division of the cell itself (cytokinesis). The process of mitosis has following steps:-

- Prophase
- Metaphase
- Anaphase
- Telophase
- Cytokinesis

Prophase:- This is the first stage of mitosis. In the early prophase, chromosomes become coiled, shortened and more distinct. In early prophase there are two pairs of centrioles in cytoplasm, each one surrounded by the aster, composed of microtubules that radiate in all directions. In the late prophase, each chromosome splits into two sister chromatids which remain attached only at centromere. Soon after this nuclear membrane and nucleolus completely disappears. Spindle fibres begin to appear. During prophase, two aster- covered centrosomes migrate to opposite sides of the nucleus in preparation of mitotic spindle formation. Spindle apparatus forms and attaches to the centromeres of the chromosomes.

Metaphase:- The nuclear membrane and nucleolus are absent, midline of the cell at metaphase. The spindle is formed which is made of fibres only. The spindle may be of nuclear origin or of cytoplasmic origin. It appears as two opposite polar caps outside the nuclear membrane and then extends into the nuclear area. The centromeres of the chromosomes are arranged on the equatorial plate and each is attached to the spindle fibres. At this phase, each chromosome shows two chromatids, centromere, primary constriction, etc.

Anaphase:- In Anaphase each chromatid pair separates into two identical chromosomes that are pulled to opposite ends of the cell by the spindle fibres.

Telophase: This phase is just reverse of prophase. After anaphase the chromosomes are present at both the poles of a parents cells. The chrosomes with their centromeres at poles begin to uncoil and lengthen as a result they become invisible. The chromosome increase in length, becomes thread like and form chromation network. The nuclear membrane reappears at each pole around the chromosome to form nucleus. The nucleolus also reappear in each nucleus. Thus at end two nuclei, one at each pole are present in the parent cell. (Fig.5.5.)

Cytokinesis:- This is the process of segmentation and separation of cytoplasm. At the time of cleavage of animal cells, a dense material around the aster at the equator of the spindle to become disappear during telophase.

Centrioles:- replicate during interphase, generally during the S-period at the beginning of prophase, there is a single aster surrounding the two pairs of centrioles. One of the pairs remain in position with half the original aster, while the other, along with the other half aster, migrates about 180^0 around the periphery of the nucleous two reach opposite pole.

Significance of mitosis:- Mitosis is important because it is essential for growth and repair in the body. In mitosis the constituents of the chromosomes are equally distributed to the two daughter nuclei and thus, they become qualitatively and quantitatively similar to the mother nucleus.

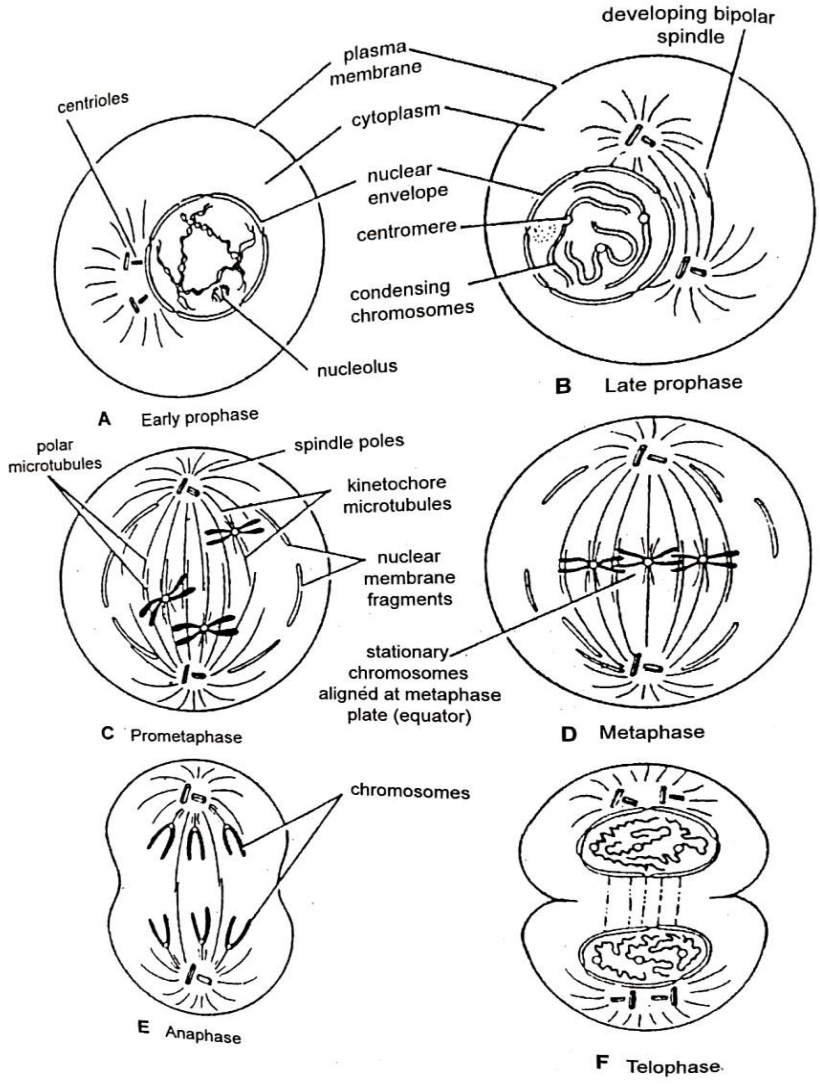


Fig.5.6 Mitosis in the animal cells.

Meiosis: - Meiosis is a cell division that is characteristic of organisms which reproduce sexually. The meiosis occurs in the germ cell in gonads and results in gamete formation, during which genetic material is duplicated once and nucleus divides twice. As a result 4 daughter cells, genetically different from the parent cells are formed. Each daughter cell has half the number of chromosome than their parents. Meiotic division involves mainly two stages. In the first stage (heterotypic), chromosome number is reduced to its half resulting in the formation of two haploid daughter cells nuclei. In heterotypic division, each chromosomes pairs with its homologue and divides longitudinally into two chromatids, so that each pair contains four chromatids (tetrads) the four chromatids of each tetrds are distributed in four nuclei formed at the close of the meiosis. Each chomatids becomes seprated or unjointed from its homologue (reduction) in one of the division (hetrotypic division) and divided equationally in the other (homeotypic division) thus for a single bivalent a equater of chromatides are formed, becoming four daughter cells or nuclei.

Stages of Meiosis

Meiosis occurs in two steps

Meiosis I		Meiosis II
Prophase I	Leptotene	Prophase II
	Zygotene	Metaphase II
	Pachytene	Anaphase II
	Diplotene	Telophase II
	Diakinesis	Cytokinesis
Metaphase I		
Anaphase I		
Telophase I		

Meiosis I: - Meiosis I is more important than meiosis II, since it is the reduction division. In this division the number of chromosome is reduced to half the parent cell. At the end of this division two daughter cells are formed. The meiosis II includes following stages:-

- Prophase I
- Metaphase I
- Anaphase I
- Telophase I

Prophase I - Prophase I is of a very long duration and complex. It is sub divided into following 5 substages :

I. Leptotene
II. Zygotene
III. Pachytene
IV. Diplotene
V. Diakinesis

I. Leptotene: - This is the first stage of prophase I of meiosis I. Nuclear membrane and nucleolus is intact. Chromosomes are long thread like structure and form chromation network.

II. Zygotene: - This is the second stage of prophase I of meiosis I. Nuclear membrane and nucleolus is still intact. Zygotene is characterised by synapsis that is pairing of homologous chromosome. Synaptonemal complex is formed as a result of synapsis. The synapsis begins at one or

more points along the length of the homologous chromosomes and at each place a pair shows two chromatids.

III. **Pachytene: -** This is the third stage of prophase I of meiosis I. Nuclear membrane and nucleolus is distinct. The chromosomes appear as thickened, coiled and thread like structure. Each chromosome shows its two chromatids. Pair of homologous chromosome is called bivalent. It is made up of four chromatids and hence known as tetrad. Pachytene stage is characterised by crossing over. It is the exchange of equal parts of chromatids of two different but homologous chromosomes.

IV. **Diplotene: -** This is the fourth stage of prophase I of meiosis I. The nuclear membrane is still intact but nucleolus is disappearing. At this stage, further thickening and shortening of chromosomes take place. The homologous chromosomes start separating from one another but still remain in contact at some points called chiasmata which indicates that crossing over has been completed at these points.

V. **Diakinesis: -** This is the fifth and last stage of prophase I of meiosis I. Nuclear membrane and nucleolus are not seen at this stage. The chromatids start separating, beginning from the centromere towards the end in zipper like manner. The chiasmata thus open. This is known as terminalization of chiasmata. (Fig.5.6)

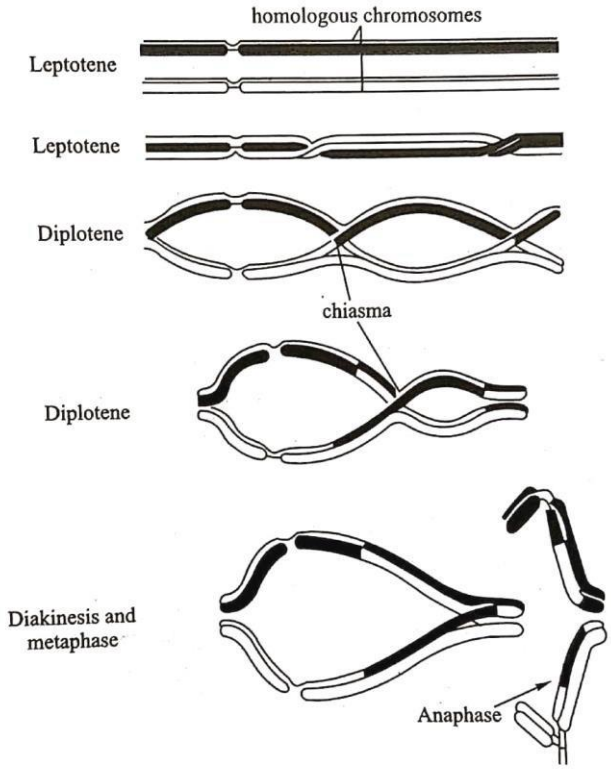


Fig.5.7. Digram of chromosomal exchange in the four-strand stage and of terminalization during first meiotic division.

Metaphase I:- At metaphase I, the spindle apparatus starts appearing and bivalents are arranged on the equatorial plate. Each chromosome of a bivalent is attached to the spindle fibres by its centromere.

Anaphase I:- At this stage, the spindle fibre contracts and pull the centromere along with chromosome to opposite pole. In the anaphase of mitosis the centromere divides logitudinally and two sister chromatids pass to two different poles but in case of anaphase I of meiosis I, the sister chromatids do not separate but go to the same pole. After anaphase I, the chromosome number is reduced and each pole has haploid number of chromosome.(Fig. 5.7)

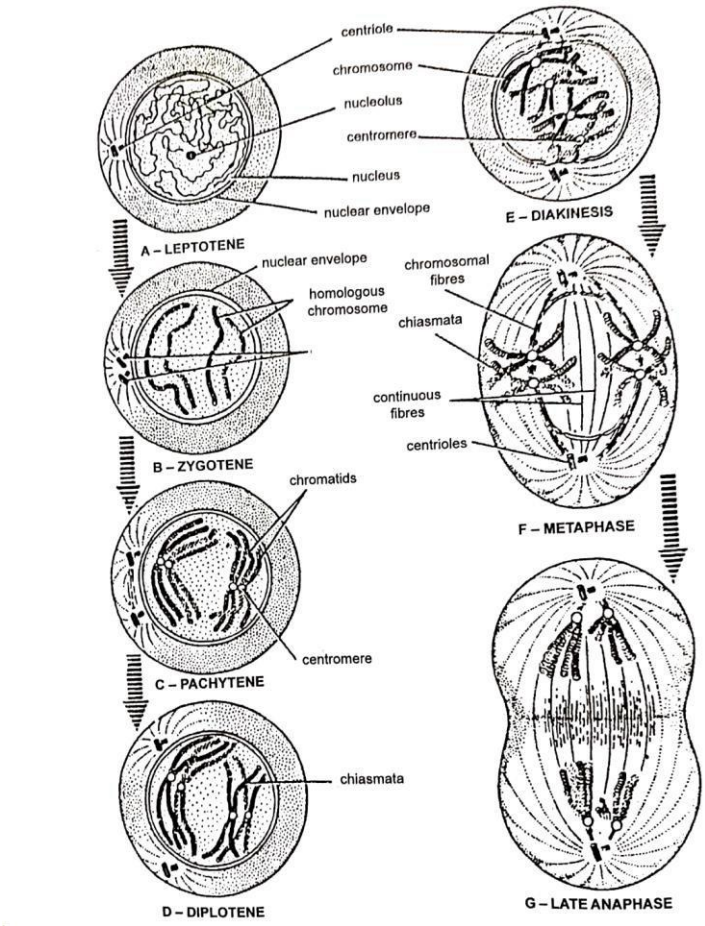


Fig.5.8. Different stages of first meiotic division.

Telophase I: - This is just reverse of prophase. The nuclear membrane and nucleolus have reappeared. The cell has two nuclei one at each pole. At telophase I, meiosis I is completed which may be followed by cytokinesis giving rise to a dyad or cytokinesis may be postponed till the end of meiosis II.

Meiosis II:- Meiosis I is followed by meiosis II which is similar to mitosis. Meiosis II results in the formation of four daughter cells, each having the same number of chromosome as was present at the end of meiosis I. The meiosis two has following stages:-

- Prophase II
- Metaphase II
- Anaphase II
- Telophase II
- Cytokinesis

Prophase II :- In the early prophase II chromosomes become short and thick. Each chromosome splits into two sister chromatids bound together by a centromere. In the late prophase II nuclear membrane and nucleolus completely disappears. Spindle fibre begins to appear.

Metaphase II :- The nuclear membrane and nucleolus are absent. Spindle fibres are formed and organised into a spindle. The centromers of the chromosome are arranged on the equatorial plate and each is attached to the spindle fibres. Each chromosome has two chromatids held together by a centromere.

Anaphase II :- At anaphase II of meiosis the centromere divides as a result the two chromatids get separated each having an individual centromere. Spindle fibres contract and each chromosome is now pulled to the opposite poles.

Telophase II :- It is just reverse of prophase. After anaphase the chromosomes are present at both the poles of a parent cell. The chromosome increase in length, becomes thread like and forms chromatine network. The nuclear membrane reappears at each pole around the chromosome to form nucleus. Nucleolus also reappears in each nucleus. Spindle fibres disappear completely.(Fig. 5.8)

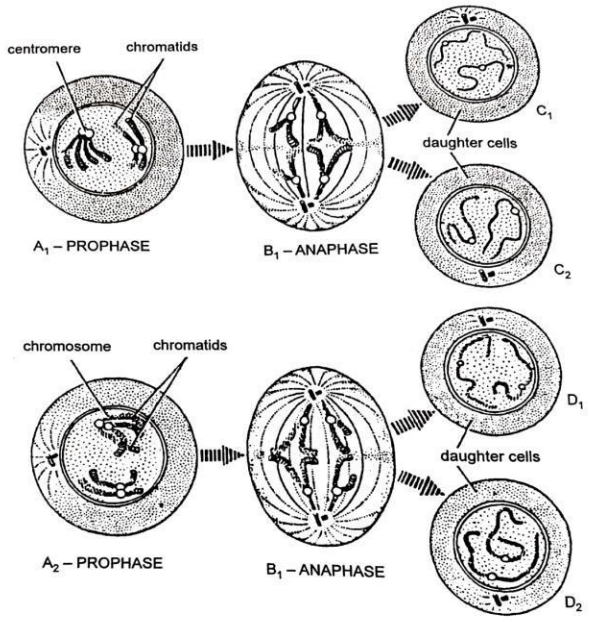


Fig.5.9. Different stages of second meiotic division.

Cytokinesis :- Cytokinesis occurs like the mitosis and results in the formation of four daughter cells each having the same number of chromosome as was present at the end of meiosis I.

Difference in Mitosis and Meiosis

	Differences	**Mitosis**	**Meiosis**
1.	Types of reproduction	Asexual	Sexual
2.	Genetically	Similar	Different
3.	Crossing over	No, crossing over and Chiasmata formation	Yes, mixing of Chromosomes can occur crossing over and chiasmata formation
4.	Number of divisions	One	Two
5.	Pairing of homologous	No	Yes, Pairing of chromosome (synapsis) occurs at zygotene
6.	Number of daughter cells produced	Adiploid cells produces two diploid cells by mitotic division	Adiploid cell produces four haploid cell by ameiotic division
8.	Chromosome number	Remains the same	Reduced by half
9.	Takes place in	Somatic cells	Germ cells
10.	Spindle fibres	Disappear completely in telophase	do not disappear completely in telophase 1
11.	Nucleoli	Reappear at telophase	do not reappear at telophse 1
12.	Step	Prophase Metaphase Anaphase Telophase	(Meiosis 1) Prophase 1, Metaphase 1, Anaphase I , Telophase I, (Meiosis II) Prophase II, Metaphase II Anaphase II Telophase II

13.	Karyokinesis	Occurs in interphase	Occurs in interphase 1
14.	Cytokinesis	Occurs in Telophase	Occurs in Telophase I and in Telophase II
15.	Function	Cellular reproduction and general growth and repair of the body	Genetic diversity through sexual reproduction

Summary

- Plasma membranes consist mainly of phospho-lipids and proteins.
- Membranes lipids are relatively small molecules that have both a hydrophilic and a hydrophobic. These lipid bilayer regions are barriers to the flow of polar molecules.
- Some of the proteins are peripheral proteins that only go halfway through the membrane, while others are integral proteins that go entirely through the membrane. These proteins serve as channels to allow the molecules to enter and leave the cell.
- Specific proteins mediate distinctive functions of membranes. Proteins serve as pumps, gates, receptor, energy transducers and enzymes. Membranes lipids create a suitable environment for the action of these proteins.
- Membranes are noncovalent assemblies. The constituent protein and lipid molecules are held together by many noncovalent interactions, which are cooperative in character.
- The membranes are asymmetric. The inside and outside faces of membrane are usually different.

5.5 TERMINAL QUESTION:

Q1. Describe the structure and function of plasma membrane.

Ans……
……
……
……....

Q2. Explain in detail the structure and function of nucleus.

Ans……
……
……
……

Q3. Process of meiosis occurring in animal cell, describe in detail.

Ans……
……
……
…….....

Q4. Process of mitosis occurring in animal cell, describe in detail.

Ans……
……
…….....
.....

Q5. Differentiate between mitosis and meiosis.

Ans……
……
……
……....

5.6 ANSWERS

SAQ.1. a) phosphor-lipids b) noncovalent

SAQ.2. a) Danielli-Davson model b) Fluid mosaic model

SAQ.3. a) Phagocytosis b) Pinocytosis

UNIT-6 ENDOPLASMIC RETICULUM, RIBOSOMES

Structure

6.1 INTRODUCTION

The eukaryotic cells are essentially two envelop systems and they are very much larger than prokaryotic cells. Secondary membranes envelop the nucleus and other internal organelles and to a great extent they pervade the cytoplasm as the endoplasmic reticulum. The eukaryotic cells are true cells which occur in the plants and animals. Though the eukaryotic cells have different shape, size and physiology, all the cells are typically composed of plasma membrane, cytoplasm and its organelles *viz*., mitochondria, endoplasmic reticulum, ribosomes, Golgi apparatus etc. and a true nucleus. Here the nuclear contents such as DNA, RNA, nucleoproteins and nucleolus remain separated from the cytoplasm by the thin perforated nuclear membranes.

Objectives :

After studying this unit you will be able to –

- Describe the structure and function of endoplasmic recticulum.
- Explain the structure and function of ribosomes.

6.2 ENDOPLASMIC RETICULUM

The term Endoplasmic reticulum (ER) was used for the first time by K. R. Porter in 1948. Porter (1961) stated, ―the endoplasmic reticulum is a complex, finely divided vacuolar system extending from the nucleus throughout the cytoplasm to the margins of the cell.‖ This cytoplasmic vacuolar system is nothing else but spaces enclosed by double membrane. Thus membranous units embedded in hyaloplasm enclosing a series of continuous and discontinuous vacuoles constitute the endoplasmic reticulum. The cavity of endoplasmic reticulum is called endoplasmic reticulum lumen or the endoplasmic reticulum cisternal space often occupies more than 10% of the total cell volume. The lumen of endoplasmic reticulum is separated from the cytoplasm by a single membrane (ER membrane). The basic structure of ER membrane is similar to that of plasma membrane. The ER membrane remains continuous with the establishes a system of channels from cells external environment to the nuclear membrane.

Occurrence :- It is found in all kinds of cells except the mature mammalian erythrocytes. It is most prominent and highly developed in cells actively

synthesizing large amounts of proteins such as the secretory cells of various glands. It is often small and relatively undeveloped in eggs and in embryonic cells but increase in size and complexity with differentiation.

Chemical composition :- The endoplasmic reticulum is composed of lipids and proteins. The amount of protein varies from 50% to 70%, while lipid content measure 30% to 50% (by weight). Out of total lipids, the phospholipids account for 70%. The 50% to 90% of phospholipids are lecithin and cephalin. Approximately 30 to 40 different enzymes are found in ER membranes such as NADH-cytochrome-c-reductase (flavoprotin), NADH diaphorase, glucose -6-phosphatase etc.

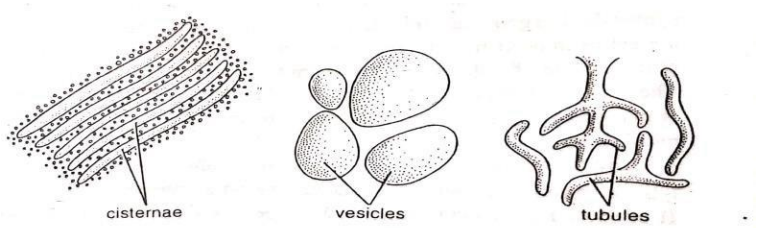


Fig : 6.1 Various types of the endoplasmic reticulum

Structure :- Endoplasmic reticulum normally occur in three main forms i.e. cisternae (lamellae), vesicles and tubules.

(1) Cisternae (lamellae) :- They are long, flattened and usually unbranched unit 40 to 53μm thick and often arranged in parallel stacks. Normally they are found in secretory cells. Ribosomes are adhered to their outer surface.

(2) Tubules :- These are of diverse shapes and irregular branched, having diameter of 50 – 100 μm. These are found in cells that are active in synthesis of steroid compounds like cholesterol and glycerides etc. These are also found in big pigmented epithelial cells of retina which are involved in the metabolism of vitamin A.

(3) Vesicles :- These are usually rounded or oval structures ranging in diameter from 25 - 500 μm. The unit membrane is of same structure as plasma membrane. Vesicles often occur isolated in the cytoplasm. They are found in abundance in pancreatic cells.

In all of these forms the spaces enclosed by the membranes comprise the vacuolar system. In some cells or in certain region of some cells, the membranes form a continuous array of connecting elements, thus enclosing a system of cavities that provides channels for the movement of materials. This membranous system of endoplasmic reticulum is composed of lipoprotein with properties of permeability. All the three forms of endoplasmic reticulum may occur either in a single cell or some may be lacking at a time.

These show different arrangements as a mammalian liver cell consists of parallel cisternae, while pancreas has different system. In striated muscle cells this endoplasmic reticulum is arranged in a network of tubules called as sarcoplasmic reticulum.

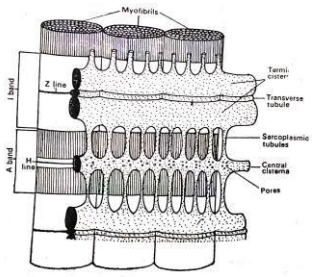


Fig : 6.2 The sarcoplasmic reticulum

Ultrastructure of Endoplasmic reticulum- The all three structures cisternae, tubules and vesicles of endoplasmic reticulum are bounded by a thin membrane of 50 to 60 Å thick. The membrane of endoplasmic reticulum is like plasma membrane and nucleus is also formed of three layers, outer and inner dense layers are composed of protein molecule, and the middle, thin and transparent layers are of phospholipids, (Robertson, 1959). Endoplasmic reticulum membrane is continuous with plasma membrane, nuclear membrane and golgi complex. Lumen of endoplasmic reticulum acts as passage for secretory products and Palade (1956) observed secretory granule in it

Types of Endoplasmic reticulum- Under electron micrographs of cells two functionally different types of endoplasmic reticulum are seen i.e. smooth endoplasmic reticulum and rough endoplasmic reticulum.

(i) ***Smooth endoplasmic reticulum*** :- The smooth endoplasmic reticulum is characterized by the absence of ribosome on the surface of endoplasmic reticulum, so its wall becomes smooth. It occurs mainly in tubular forms. These tubules measure about 500 – 1000 Å in diameter, forming irregular lattice. The smooth endoplasmic reticulum is most common in those cells which are concerned with steroid or lipid synthesis (such as adrenal and sebaceous glands, gonadial interstitial cells), carbohydrate metabolism (e.g. liver cells), electrolytic excretion (as in chloride cells of fish gills), impulse conduction (e.g. in muscle cells) and with pigment production (in retinal pigment cells). The muscle cells are also rich in smooth type of endoplasmic reticulum and here it is known as sacroplasmic reticulum.

(ii) ***Rough endoplasmic reticulum*** **:-** In rough endoplasmic reticulum, ribosome are always attached to the outer surface of the membrane i.e. the surface facing the cytoplasm. It is plate like flattened cisternae with 400 to 500 Å in width. The membrane of rough endoplasmic reticulum remains continuous with the outer membrane of the nucleus. The rough endoplasmic reticulum is found abundantly in those cells which are active in protein synthesis such as pancreatic cells, plasma cells, goblet cells and liver cells. The granular type of endoplasmic reticulum takes basophilic stain due to its RNA contents of ribosomes. In rough endoplasmic reticulum, ribosome are often present as polysomes held together by m-RNA and are arranged in typically ―rosettes‖ or spirals. Rough endoplasmic reticulum contain two transmembrane glycoprotein (ribophorins I & ribophorins II) to which are attached the ribosome by their 60S.

Annulate lamellae- The annulate lamellae were first described by McCulloch (1952). It is observed in many germ cells & somatic cells of invertebrate and vertebrates. They have been mostly observed in the cytoplasm, but have sometimes also been seen in the nucleus. They are more commonly found in embryonic and foetal cells, where high metabolic activity occurs.

Annulate lamellae consist of double membrane sheets. About 2 – 12 lamellae are arranged in stacks. They are structurally very similar to the nuclear envelope in having pores with annular masses and central granules.

It is suggested that the nuclear envelope is involved in the formation of the annulate lamellae. The outer membrane of the nuclear envelope form finger like processes which are pinched off into the cytoplasm in the form of vesicles or blebs. This process is towards the periphery of the cell and fuse to form cisternae. The matrix material becomes associated with the pores of each cisternae to form annuli resulting in the formation of an annulate lamelle. According to Hruban *et.al* (1965) that the annulate lamellae may represent an intermediate stage in the formation of the endoplasmic reticulum. The annulate lamellae are transitory cytoplasmic organelles.

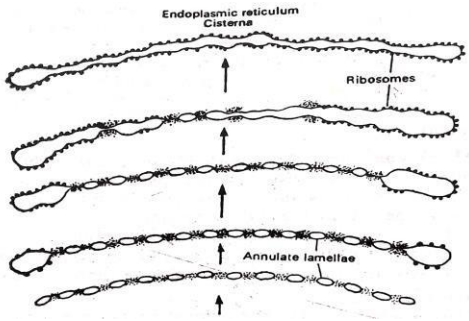


Fig : 6.3 Formation of cistern of endoplasmic reticulum from annulate lamellae

Origin of Endoplasmic reticulum- The exact process of the origin of endoplasmic reticulum is still unknown. But because membranes of endoplasmic reticulum resemble with the nuclear membrane and plasma membrane and also at the telophase stage the endoplasmic reticulum membranes are found to form the nuclear envelope. Therefore it is also normally assumed that the endoplasmic reticulum has originated by evagination of the nuclear membranes. Seikevitz and Palade (1960) have reported that the granular type of endoplasmic reticulum has originated first and later it synthesizes the agranular or smooth type of endoplasmic reticulum.

The synthesis of membranes of endoplasmic reticulum is found to proceed in the direction rough endoplasmic reticulum (RER) ⟶ Smooth endoplasmic reticulum (SER). The membrane biogenesis is a multistep process involving first, the synthesis of a basic membrane of lipid and intrinsic proteins and thereafter the addition of other constituents such as enzymes, specific sugar or lipids. The process by which a membrane is modified chemically and structurally is called membrane differentiation. The especially SER is the organelle containing the

main phospholipid synthesizing and translocating enzymes. The insertion of proteins into endoplasmic reticulum membranes occurs at the level of rough endoplasmic reticulum. Most of these proteins are formed on membrane bound ribosomes. However some of these are synthesized by free ribosomes in the cytosol and then are inserted into the membrane. For example the enzyme NAD-cytochrome-b5 reductase is synthesized in the cytosol and then becomes incorporated in various parts of the endomembrane system (i.e. RER, SER and golgi apparatus) and in the outer mitochondrial membrane.

Functions of Endoplasmic reticulum- Endoplasmic reticulum is capable of continual re-organization. Rough endoplasmic reticulum can convert to smooth endoplasmic reticulum vice versa, depending upon the metabolic requirements of the cell. The endoplasmic reticulum is a dynamic, not a static system. Functions of reticulum depend upon its location and cell type. The important functions are as follows :

(1) **Mechanical support :-** Endoplasmic reticulum, along with system of microtubules and microfilaments provide mechanical support for the colloidal areas of the cytoplasm.

(2) **Enzymatic activities :-** Membranes of reticulum provide an increased surface for various synthetic and metabolic activities within cell. Enzymes attached to its surface will act more efficiently than when dissolved in cytoplasm. A number of important enzyme are found in the endoplasmic reticulum membrane like stearases, NADH-cytochrome-c-reductase, NADH-diaphorase, glucose-6-pohosphate etc.

(3) **Transport of synthetic products :-** Endoplasmic reticulum functions as an intracellular transport system, both for nascent proteins and at least sometimes for lipids. Vacuolar system of reticulum helps in collection of synthetic products of cell. These products accumulate in reticular vacuoles and later on are transport to other parts of cell or for release from the cell in secretion. Perhaps the osmotic properties of the membranes are significant for this activity.

(4) **Storage of metabolites :-** Endoplasmic reticulum plays important role in the conversation of metabolic products such as proteins and sometimes lipids.

(5) **Formation of nuclear membrane :-** Membranes of reticulum are source of origin of some other membranes. Nuclear membrane also develops from reticulum during division.

(6) **Intracellular impulse conduction :-** Specialized modification like sarcoplasmic reticulum transmit impulses intracellularly.

(7) **Protein synthesis :-** Caro and Pallade (1964) have produced morphological evidence that granular endoplasmic reticulum is the site of protein synthesis. This suggests that endoplasmic reticulum may provide a pathway for m-RNA to move from nucleus to the cytoplasmic ribosomes. This synthesized protein later on becomes concentrated above golgi apparatus.

(8) ATP synthesis :- The endoplasmic reticulum is also a site of ATP synthesis in all the cell. This ATP is utilized as a source of energy for the intracellular transport of materials or in RNA metabolism involving ribosomes.

(9) Synthesis of glucose and glycogen :- By experiments on liver cells, it has been proved that endoplasmic reticulum is associated with the synthesis, storage and metabolism of glucose and glycogen. Membranes of smooth endoplasmic reticulum in mouse liver cells function in glycogenolysis. Smooth, tubular endoplasmic reticulum of interstitial cells shows the ability to catalyze the oxidation of progesterone to testosterone and acetic acid. This oxidation involves four enzymes found in endoplasmic reticulum.

(10) Synthesis of lipids :- Agranular endoplasmic reticulum is related with the synthesis and metabolism of lipids (Christensen,1961 and Claude 1968). Agranular endoplasmic reticulum is found in abundance in those cells in which active lipid metabolism occurs.

(11) Synthesis of non protein substances :- Agranular endoplasmic reticulum also synthesizes non protein substances such as the cholesterol, glycerides, hormones (testosterone and progesterone), etc.

(12) Sarcoplasmic reticulum :- In muscle cells, there are large amounts of smooth endoplasmic reticulum and it is known as sarcoplasmic reticulum. It is involved in the concentration of Ca^{++} by a process requiring the utilization of ATP. The Ca^{++} are stored in the sarcoplasmic reticulum and released after the muscle stimulation by nerve impulse, hormones or other means. The release of Ca^{++} leads to muscular contraction.

(13) In plant cells, during cell division, endoplasmic reticulum fragments form the new nuclear envelopes of the daughter nuclei.

SAQs 1.

Complete the following sentences by inserting appropriate words in the blanks.

(i) The endoskeleton of cell is made of ----------

(ii) The part of the cell forms the nuclear envelop during telophase ----------

(iii) Endoplasmic reticulum is mainly concerned with the ----------

(iv) Endoplasmic reticulum provides ---------- for the colloidal structure of cytoplasm.

6.3 RIBOSOMES

Palade 1955 discovered small dense granules in the cytoplasm. He called it as Palade‘s granules, which later called as ribosome. The ribosomes are small dense rounded and granular particles. They contain ribonucleoprotein. They occur either freely in the matrix of the mitochondria, chloroplast and cytoplasm or remain attached with the membrane of the endoplasmic reticulum and nucleus.

They occur in most prokaryotic and eukaryotic cells and are known to provide scaffold for the ordered interaction of all the molecules involved in protein synthesis.

Occurrence & distribution- The ribosomes occur in cells, both prokaryotic and eukaryotic cells. In prokaryotic cells the ribosomes often freely in the cytoplasm. In eukaryotic cells the ribosomes either occur freely in the cytoplasm or remain attached to the outer surface of the membrane of endoplasmic reticulum. The yeast cells, reticulocytes or lymphocytes, meristamatic plant tissue, embryonic nerve cells and cancerous cells contain large number of ribosomes which often occur freely in the cytoplasmic matrix. The cells in which active protein synthesis takes place, the ribosomes remain attached with the membranes of the endoplasmic reticulum. Such cells are the pancreatic cells, plasma cells, hepatic parenchymal cells, Nissls bodies, osteoblasts, serous cells or the submaxillary gland, chief cells of the glandular stomach, thyroid cells and mammary gland cells. The cells which synthesize specific proteins for the intracellular utilization and storage often contain large number of free ribosomes. Such cells are the erythroblasts, developing muscle cells, skin and hair.

Shape- The shape of ribosome is spherical. The ribosome of prokaryote is smaller in size than eukaryotes. In prokaryotes they are 150 Å and in eukaryotes they are 250 Å in diameter.

Size- There are mainly two size of ribosomes (i) Smaller prokaryotic found in bacteria and blue green algae, having a sedimentation coefficient 70S equivalent to the molecular weight of 2.7 x 10^6 daltons. (ii) Eukaryotic cells of plants and animals of 80S coefficient with a molecular weight of about 4 x 10^6 daltons.

Number- The number of ribosomes is directly related to the RNA content of the cell. In rabbit‘s reticulocytes, they are 1 x10^5 per cell. In one mm of liver contain 2 x 10^{13} ribosomes. In *E.coli* they are about 20,000 to 30,000 ribosome per cell.

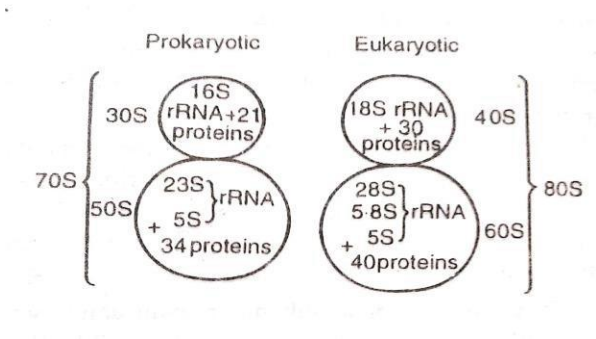


Fig : 6.4 Various components of prokaryotic (70S) and Eukaryotic (80S) ribosomal subunits

Structure of ribosomes- The ribosomes are oblate spheroid structure of 150 to 250 Å in diameter. Each ribosome is porous, hydrated and composed of two subunits. One ribosomal subunit is large in size and has a dome-like shape, while the other ribosomal subunit is smaller in size and occurring above the large subunit and forming a cap-like structure.

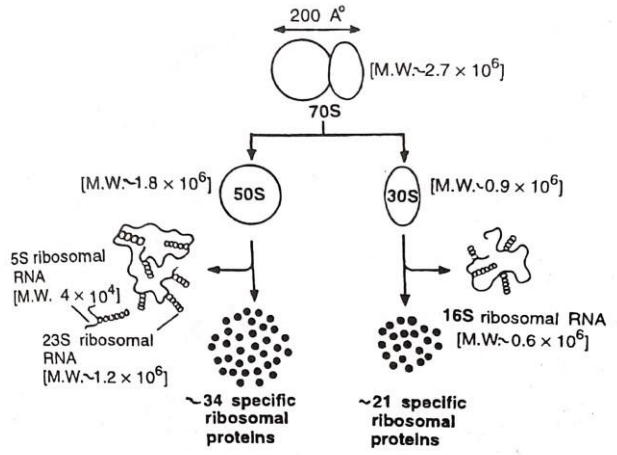


Fig : 6.5 Structure of 70S (prokaryotic) ribosome

The 70S ribosome consists of two subunits *viz.*, 50S and 30S. The 50S ribosomal subunit is larger in size and has the size of 160 Å to 180 Å. The 30S ribosomal subunit is smaller in size and occurs above the 50S subunit like a cap.

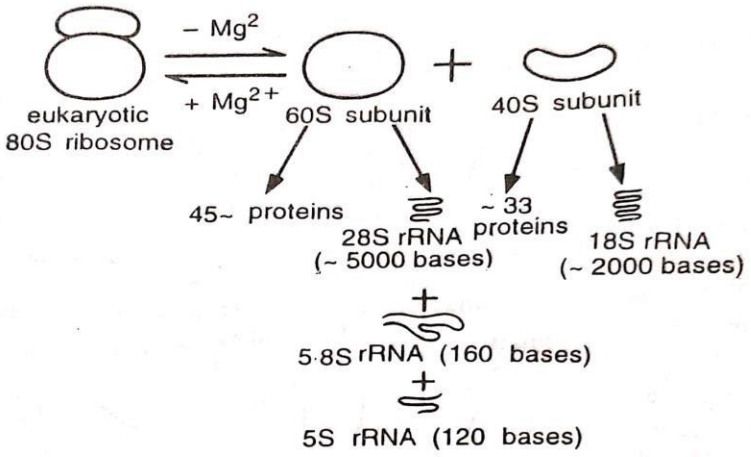


Fig : 6.6 Different RNA and protein components of Eukaryotic (80S) ribosomes

The 80S ribosome also consists of two subunits *viz.*, 60S and 40S. The 60S ribosomal subunit is dome-shaped and larger in size. In the ribosomes which remain attached with the membranes of endoplasmic reticulum and nucleus, etc., the 60S subunit remains attached with the membranes. The 40S ribosomal subunit is smaller in size and occurs above the 60S subunit forming a cap like structure. Both the subunits remain separated by a narrow cleft.

The two ribosomal subunits remain united with each other due to high concentration of the Mg^{++} (.001M) ions. When the concentration of Mg^{++} ions reduces in the matrix, both ribosomal subunits get separated. Actually in bacterial cells the two subunits are found to occur freely in the cytoplasm and they unite only during the process of protein synthesis. At high concentration of Mg^{++} ions in

the matrix, the two ribosomes (called monosomes) becomes associated with each other and known as the dimer. Further during protein synthesis many ribosomes are aggregated due to common messenger RNA and form the polyribosomes or polysomes.

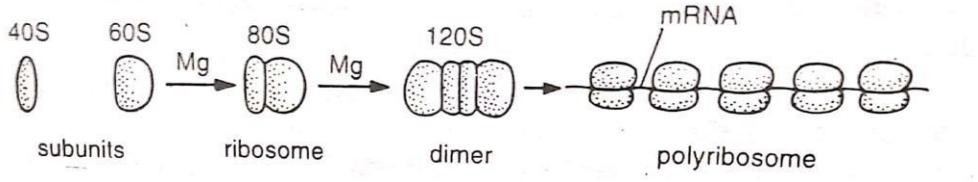


Fig : 6.7 Diagram of the subunit structure of the ribosome and the influence of Mg^{++} ions.

Ultrastructure of ribosomes- Electron microscopy indicates that ribosomes are composed of two rounded subunits fitted together to give a complete unit of about 200Å in diameter. One subunit is approximately twice the size of the other. In 70S ribosomes Nanninga (1967) observed the 50S subunit as pentagonal compact particle of 160 to 180 Å and in its centre found a round concave area of 40 to 60 Å for accommodating the small subunit. Further, Florendo (1968) revealed a pore like transparent area in the 50S subunit. It does not permit entrance of enzyme ribonuclease. These pores have also been seen in the 60S subunit of 80S ribosomes. Smaller subunits, i.e. 30S of 70S ribosomes, and 40S of 80S ribosomes, have no regular form and often divided into two portions. Both these portions are interconnected by a strand of 30 to 60 Å thickness.

The m-RNA is positioned in the gap between both ribosomal subunits. As a result ribosome protects a stretch of some 25 nucleotides of m-RNA from degradation by ribonuclease. Large ribosomal subunit may have a groove or tunnel through which nascent protein chain grows.

Types of ribosomes- The ribosomes are of two types – 70 S and 80 S. (S – Svedberg unit)

(1) 70S Ribosome : 70S ribosomes are relatively smaller than 80S. They are found in prokaryotes for e.g. bacteria, blue green algae and *E.coli*. 70S ribosome consists of two subunits – a small cap 30S subunit and large dome 50S subunit. It should be noted that the sedimentation coefficient is not achieved by a simple addition of the two subunits and in this sense it is not additive. Thus 30S and 50S subunits together make 70S and not 80S.

The length of the 70 S ribosome is 290 Å and the width is 210 Å. The cleft between the two subunits is deeper as compared to the 80 S ribosome. The 30S subunit contains 21 different proteins and 16S r-RNA. The 50S subunit contains 34 proteins and 5S and 23S r-RNA. Some proteins are identical in both subunits. The RNA : Protein ratio in the 70 S ribosome is 2 : 1.

(2) 80S Ribosome : 80S ribosomes are larger than 70S ribosomes. The 80S ribosomes are found in the cytoplasm of eukaryotes. The 80S ribosomes has 40S smaller subunit and 60S larger subunit.

The length of the 80S ribosome is 300 to 400 Å and its width is 200 to 240 Å. The cleft between the two subunits is superficial. The 40S small subunit contains 33 proteins and 16S and 18S r-RNA. The 60S large subunit contains 49 protein and 25S, 29S, 5.8S and 5S r-RNA. The RNA : Protein ratio in the 80S ribosome is 1 : 1.

Dissociation and reconstitution of the ribosomes- Nomura and Traub (1968) performed classical experiment of dissociation and reconstitution (self assembly) of the ribosome. This experiment was done to understand the three dimensional organization of ribosomal proteins in the ribosomes and also for the investigation of interactions between the molecules of r-RNA and proteins. This experiment involves to take purified 30S ribosomal subunits, dissociate them by chemical means to their component RNAs and proteins and then allow them to reassociate under appropriate ionic conditions. Dissociation of 30S subunit may be done by treatment with four molar urea and two molar LiCl, which separate the proteins. If the 16S r-RNAs previously extracted with phenol is placed in the presence of 20 different protein molecules of 30S ribosomal subunit, the reconstitution of self assembly of 30S ribosomal subunit takes place into two steps

$$\text{16S RNA} + \xrightarrow{R_1} \text{RI particles} \xrightarrow{\text{heat}} \text{RI* particles} \xrightarrow{+\text{ S protein}} \text{30S ribosomal subunit Protein}$$

In the first step, performed at a low temperature, the 16S RNA binds some of the 30S ribosomal proteins, forming an RI particles (i.e. a reconstitution intermediate) that is inactive.

In the second step, the RI particles are heated at 40^0C in the presence of the other proteins that have remained in the supernatant (i.e. S proteins) there by forming an excited intermediate, RI* within 20 minutes fully active 30S ribosomal subunits are formed. The reconstitution of 30S subunits is highly specific. It can be achieved with 16S RNA of other bacteria, but not with 16S RNA from yeast or the 23S RNA from *E.coli*. In similar manner, reconstitution of 50S ribosomal subunit is also done. Finally a complete functional ribosome is reconstituted spontaneously. In some of the these experiments when ribosomal protein is modified at a time, they show that certain ribosomal proteins require prior to the attachment of other proteins in order to become incorporated in a step wise manner. For example, some ribosomal proteins called initial nor primary binding proteins (i.e. L4 protein) bind at specific sites on the naked rRNA and without them the other proteins called secondary binding proteins cannot bind. Initial binding proteins have also been found essential in the control of synthesis of ribosomal proteins.

All the ribosomal proteins of the 70S ribosomes have been isolated and specific antibodies against them have been produced. The various immunological and chemical cross linking procedures have made possible the construction of maps of the topographical distribution of ribosomal proteins within the ribosomal subunit. Some important sites or centres of specific functions have also been indicated in some of these maps.

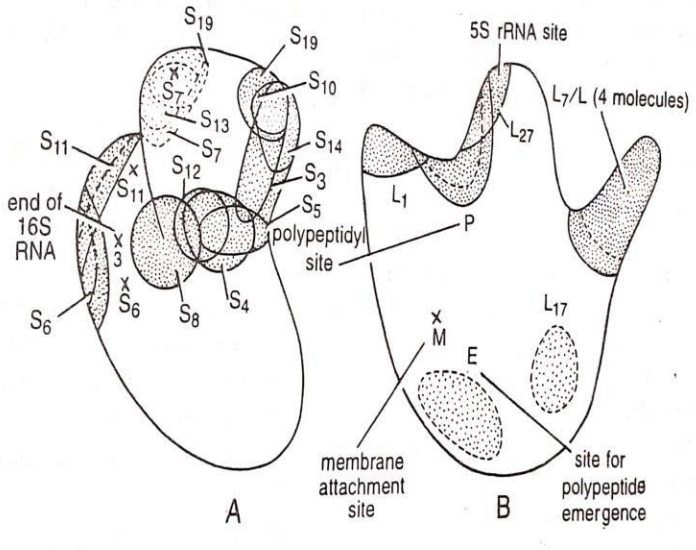


Fig : 6.8 Map of proteins in the ribosome showing their position in small subunit(A) and Large subunit (B)

6.4 SUMMARY

The endoplasmic reticulum is an important cytoplasmic organelle of cell. In the cytoplasm of most animal cells it appears as an extensive network (reticulum) of membrane limited channel. Some portion of endoplasmic reticulum remain continuous with the plasma membrane and nuclear envelop. The outer surface of rough endoplasmic reticulum has attached ribosome, where as smooth endoplasmic reticulum do not have attached ribosome. The function of smooth endoplasmic reticulum include lipid metabolism (both catabolism & anabolism, they synthesize a variety of phospholipids, cholesterol & steroids), glycogenolysis (degradation of glycogen, glycogen being polymerized in the cytol) and drug detoxification (by the help of cytochrome P-450). The rough endoplasmic reticulum synthesize membrane proteins and glycoproteins which are cotranslationally inserted into the rough endoplasmic reticulum. Thus endoplasmic reticulum is the site of biogenesis of cellular membrane.

Ribosomes are tiny spheroidal dense particles of (150 to 200 Å diameter) that contain approximately equal amounts of RNA and proteins. They are primarily found in all cells and serve a scaffold for the ordered interaction of the numerous molecules involved in protein synthesis. Ribosome granules may exist in the free state in the cytol or attached to rough endoplasmic reticulum. Ribosomes have a sedimentation coefficient of about 80S and are composed of two subunits namely 40S and 60S. The smaller 40S ribosomal subunit is prolate ellipsoid in shape and consists of one molecule of 18S ribosomal RNA and 30 proteins (named as S_1, S_2, S_3 and so on). The larger 60S ribosomal subunit is round in shape and contains a channel through which growing polypeptide chain makes its exit. It consists of three types of rRNA molecules i.e. 28S rRNA, 5.8 rRNA and 5S rRNA and 40 protein (named as L_1,L_2,L_3 and so on). Ribosome play important role in the protein synthesis.

SAQs 2.

Complete the following sentences by inserting appropriate words in the blanks.

(i) Polyribosomes are aggregation of ----------

(ii) Ribosome consist of ----------

(iii) Ribosomes help in ----------

(iv) Ribosomes on hydrolysis produce ----------.

6.5 TERMINAL QUESTIONS

1. Explain the structure of endoplasmic reticulum.

---

2. Describe the function of endoplasmic reticulum.

---

3. Describe the ultra structure of ribosomes.

---

4. Describe the types of ribosomes.

6.6 ANSWERS

SAQs 1

(i) Endoplasmic reticulum

(ii) Endoplasmic reticulum

(iii) Peptide bound synthesis

(iv) Mechanical support

SAQs 2

(i) Several ribosomes held together by string of m-RNA.

(ii) RNA + protein

(iii) Protein synthesis

(iv) Split proteins and r-RNA

Answer 6.5

1. Endoplasmic reticulum normally occur in three main forms i.e. cisternae (lamellae), vesicles and tubules.

(1) Cisternae (lamellae) :- They are long, flattened and usually unbranched unit 40 to 53μm thick and often arranged in parallel stacks. Normally they are found in secretory cells. Ribosomes are adhered to their outer surface.

(2) Tubules :- These are of diverse shapes and irregular branched, having diameter of 50 – 100 μm. These are found in cells that are active in synthesis of steroid compounds like cholesterol and glycerides etc. These are also found in big pigmented epithelial cells of retina which are involved in the metabolism of vitamin A.

(3) Vesicles :- These are usually rounded or oval structures ranging in diameter from 25 - 500 μm. The unit membrane is of same structure as plasma membrane. Vesicles often occur isolated in the cytoplasm. They are found in abundance in pancreatic cells.

2. Endoplasmic reticulum is capable of continual re-organization. Rough endoplasmic reticulum can convert to smooth endoplasmic reticulum vice versa, depending upon the metabolic requirements of the cell. The endoplasmic reticulum is a dynamic, not a static system. Functions of reticulum depend upon its location and cell type. The important functions are as follows

(i) Mechanical support.

(ii) Enzymatic activities

(iii) Transport of synthetic products

(iv) Storage of metabolites

(v) Formation of nuclear membrane

(vi) Intracellular impulse conduction

(vii) Protein synthesis

(viii) ATP synthesis

(ix) Synthesis of glucose and glycogen

(x) Synthesis of lipids

(xi) Synthesis of non protein substances

(xii) Sarcoplasmic reticulum

3. Electron microscopy indicates that ribosomes are composed of two rounded subunits fitted together to give a complete unit of about 200Å in diameter. One subunit is approximately twice the size of the other. In 70S ribosomes Nanninga (1967) observed the 50S subunit as pentagonal compact particle of 160 to 180 Å and in its centre found a round concave area of 40 to 60 Å for accommodating the small subunit. Further, Florendo (1968) revealed a pore like transparent area in the 50S subunit. It does not permit entrance of enzyme ribonuclease. These pores have also been seen in the 60S subunit of 80S ribosomes. Smaller subunits, i.e. 30S of 70S ribosomes, and 40S of 80S ribosomes, have no regular form and often divided into two portions. Both these portions are interconnected by a strand of 30 to 60 Å thickness.

The m-RNA is positioned in the gap between both ribosomal subunits. As a result ribosome protects a stretch of some 25 nucleotides of m-RNA from degradation by ribonuclease. Large ribosomal subunit may have a groove or tunnel through which nascent protein chain grows.

4. Electron microscopy indicates that ribosomes are composed of two rounded subunits fitted together to give a complete unit of about 200Å in diameter. One subunit is approximately twice the size of the other. In 70S ribosomes Nanninga (1967) observed the 50S subunit as pentagonal compact particle of 160 to 180 Å and in its centre found a round concave area of 40 to 60 Å for accommodating the small subunit. Further, Florendo (1968) revealed a pore like transparent area in the 50S subunit. It does not permit entrance of enzyme ribonuclease. These pores have also been seen in the 60S subunit of 80S ribosomes. Smaller subunits, i.e. 30S of 70S ribosomes, and 40S of 80S ribosomes, have no regular form and often divided into two portions. Both these portions are interconnected by a strand of 30 to 60 Å thickness.

The m-RNA is positioned in the gap between both ribosomal subunits. As a result ribosome protects a stretch of some 25 nucleotides of m-RNA from degradation by ribonuclease. Large ribosomal subunit may have a groove or tunnel through which nascent protein chain grows.

References

1. Cell biology by C. B. Powar, Himalaya Publishing House 1996.
2. Cell and molecular biology by S. C. Rastogi, New age international Publishers 2010.
3. Cell biology by Singh & Tomar, Rastogi Publication, 2008
4. Cell biology, genetics, molecular biology, evolution and ecology by P.S. Verma and V.K. Agrawal, S. Chand & Company Ltd. 2001
5. Cell biology by Ahirrao & others , Prasant Publication

UNIT-7 GOLGI BODY & LYSOSOMES

7.1 INTRODUCTION

Cells have extensive sets of intracellular membranes, which together compose the endomembrane system. The endomembrane system was first discovered in the late 1800s when scientist **Camillo Golgi** noticed that a certain stain selectively marked only some internal cellular membranes. Golgi thought that these intracellular membranes were interconnected, but advances in microscopy and biochemical studies of the various membrane-encased organelles later made it clear the organelles in the endomembrane system are separate compartments with specific functions. These structures do exchange membrane material, however, via a special type of transport.

Today, scientists know that the endomembrane system includes the endoplasmic reticulum (ER), Golgi apparatus, and lysosomes. Vesicles also allow the exchange of membrane components with a cell's plasma membrane.

The lysosome membrane helps to keep its internal compartment acidic and separates the digestive enzymes from the rest of the cell. Lysosome enzymes are made by proteins from the endoplasmic reticulum and enclosed within vesicles by the Golgi apparatus. Lysosomes are formed by budding from the Golgi complex.

Lysosomes are cytoplasmic organelles in which a variety of macromolecules are degraded by different acid hydrolase enzymes..If a lysosomal enzyme is absent or has reduced activity or if enzymes are not correctly targeted to lysosomes, the macromolecules normally degraded by lysosomes will accumulate, causing abnormal storage of various complex compounds and result LSDs.

Objective

After studying this unit you should be able to

1) Know about the occurance, distribution, chemical composition of Golgi body along with its structural and functional morphology.
2) Understand the relationship between the Golgi bodies and lysosomes.
3) Know that what do lysosomes and Golgi bodies have in common.
4) Know about that lysosomal enzyme and lysosomal membrane.
5) Know about the polymorphism of lysosomes.
6) Understand the origin and function‘s of lysosomes.
7) Know about the lysosomal storage diseases/disorders result from an accumulation of specific substrates, due to the inability to break them down .

7.2 GOLGI BODY

The Golgi body was one of the first organelles to be discovered and observed in detail. It was discovered in 1898 by Italian neurologist (i,e.,physician) **Camillo Golgi** during an investigation of the nervous system.

Occurance- The Golgi body, membrane-bound organelle occurs in all cells except the prokaryotic cells (e.g. mycoplasmas, bacteria and blue green algae) and eukaryotic cells (cells with clearly defined nuclei) of certain fungi, sperm cells of bryophytes and pteridophytes, cells of mature sieve tubes of plants and mature sperm and red blood cells of animals. The number of Golgi body per plant can vary from several hundred as tissues of corn root and algalrhizoids to a single organelle in some algae. Certain algal cells such as*Pinulariaand Microsterias,*contain largest and most complicated Golgi bodies. In higher plants, Golgi bodies are particularly common in secretory cells and in young rapidly growing cells.

In animal cells, usually occurs a single Golgi body , however, number may vary from animal to animal and from cell to cell. Thus, *Paramoeba* species has two Golgi body and nerve cells, liver cells and chordate oocytes have multiple Golgi bodies.

Distribution- In the cells of Higher plants, the Golgi bodies or dictyosomes are usually found scattered throughout the cytoplasm and their distribution does not seem to be localized in any particular manner. However, in animal cells Golgi body is a localized organelle. For example, in the cells of ectodermal or endodermal origin, the Golgi body remains polar and occurs in between the nucleus and the periphery (e.g. thyroid cells, exocrine pancreatic cells and

mucus- producing goblet cells of intestinal epithelium) and in the nerve cells it occupies circum-nuclear position.

Morphology- Golgi bodi is made up of a series of flattened, stacked pouches called **cisternae.** However, they can be highly **pleomorphic**, which means that they can change their shape for their function.

The detailed structure of basic components of the Golgi body can be studies as follows:

1. **Cisternae-** Depending on the type of cell, the number may vary from just a few to thousands. In general, the Golgi apparatus is made up of approximately four to eight cisternae, although in some single-celled organisms it may consist of as many as 60 cisternae. The cisternae are very small with a diameter ranging from 0.5 to 1.0 nm. Each of these is bound by a membrane and is held together by a matrix of proteins. The cisternae are held together by matrix proteins, and the whole of the Golgi apparatus is supported by cytoplasmic microtubules. The apparatus has three primary compartments, generally known as **"cis"** (cisternae neareast the endoplasmic reticulum), **"medial"** (central layers of cisternae), and **"trans"** (cisternae farthest from the endoplasmic reticulum). Two networks, the cis Golgi network and the trans Golgi network, which are made up of the outermost cisternae at the cis and trans faces, are responsible for the essential task of sorting proteins and lipids that are **received (**at the **cis face**) or **released** (at the **trans face**) by the organelle.

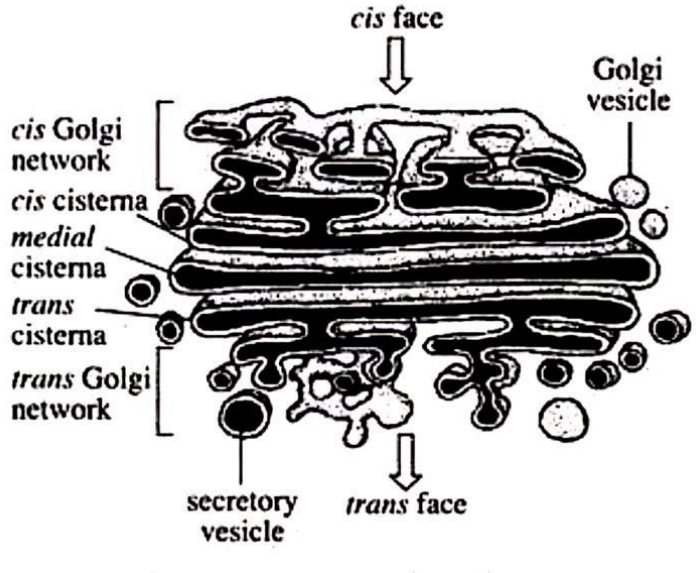


Fig: 7.1 A three dimensional view of Golgi complex.(source: zoology notes)

2. **Tubules-** From the peripheral area of cisternae arise a complex, anastomosing flat network of tubules of300 to 500 A° diameter. Clowes and Juniper (1969) have compared this tubular network to disc of lace.

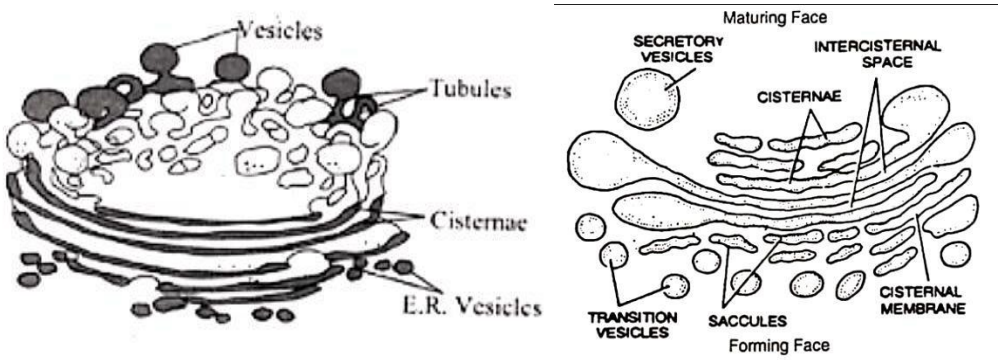


Fig: 7.2 Ultra structure of Golgi complex (source: google.com)

3. **Vesicles-** The vesicles are small droplet-like sacs which remain attached to tubules at the periphery of the cisternae.

   They are of following two types:

   (a) Smooth vesicles- The smooth vesicles are of 20 to 80µ diameter. They contain secretory material (so often are called secretory vesicles) and are budded off from the ends of cisternal tubules within the net. Often more than one tubule connection, and presumably fill, a single forming vesicle.

   (b) Coated vesicles- The coated vesicles are spherical protuberances, about 50 µm in diameter and with a rough surface. They are found at the periphery of the organelle, usually at the ends of single tubules and are morphologically quite distinct from the secretory vesicles. Their function is unknown.

4. **Golgian vacuoles-** These are large rounded sacs present on the maturing face of Golgi. These are formed either by the expanded cisternae or by the fusion of secretory vesicles. The vacuoles are filled with some amorphous or granular substance.

Chemical Composition- Regarding the chemical composition of Golgi complex, it has been demonstrated that the following substances are present:

1. **Phospholipids-** Phospholipids composition of Golgi membranes is intermediate between those of endoplasmic membranes and plasma membranes.

2. **Proteins and Enzymes-** Golgi complex from different plant and animal cells show great variations in the protein and enzyme contents. Some of the enzymes are ADPase, ATPase, NADPH cytochrome-C-reductase, glycosy1 transferases, galactosy1 transferase, thiamine pyrophosphate etc.

3. **Carbohydrates-** Both plant and animal cells have some common carbohydrate components, like glucosarine, galactose, glucose, mannose and fructose. Plant Golgi lack sialic acid, but it occurs in high concentration in rat liver. Some carbohydrates like xylulose and arabinose are present in plant cells only.

4. **Vitamin C**- The fraction of vitamin C stored in the Golgi complex has been shown by Tomitte. According to him Golgi complex stores vitamin C and liberates it slowly into the cytoplasm in sufficient amount to prevent-oxidation of the cell products.

Origin of Golgi Complex- Three different sources have been proposed from which new Golgi complex may arise:

1. **From endoplasmic reticulum**- Essner and Novikoff (1962) and Beams and Kessel(1968) have proposed that the Golgi cisternae arise from the ER. The rough endoplasmic reticulum after synthesizing specific proteins loses ribosomes and changes into smooth ER. Small transitory vesicles pinch off from smooth ER. These migrate to dictyosome.

   On reaching the forming face of dictyosome these fuse to form new cisternae and thus contribute to its growth. By the fusion of these vesicles new cisternae are formed continuously on the forming face and on the maturing face the old cisternae break down into secretory vesicles. Thus Golgi exhibits a phenomenon of membranous flow.‘

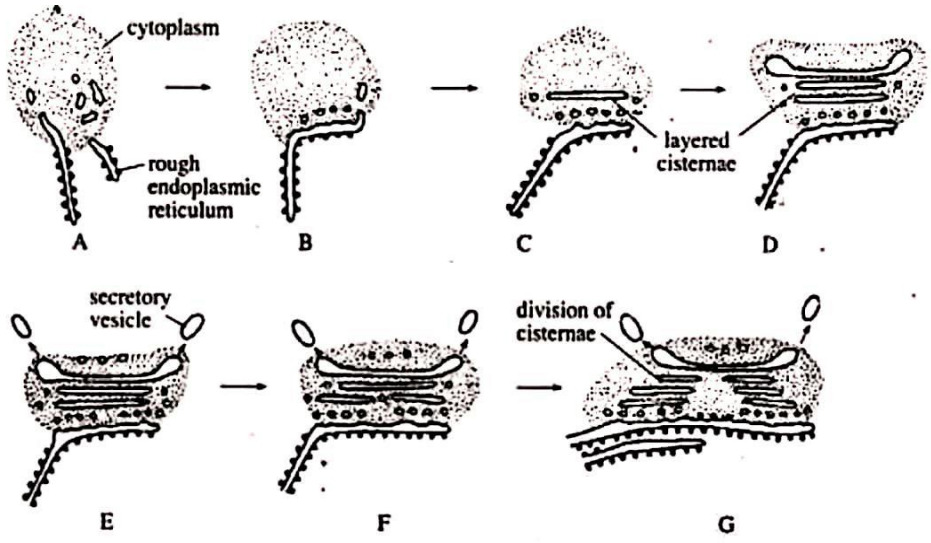


Fig: 7.3 Formation of Golgi apparatus from endoplasmic reticulum(A-C) and subsequent developmental stages: formation of stack of cisternae (D and E)formation of secretory vesicle (E and F) division of cisternae (G) (source: zoology notes)

2. **From nuclear membrane**- Bouch (1965) described the origin of Golgi from outer membrane of nuclear envelope in brown algae. Vesicles are pinched off from outer nuclear membrane which fuses to form cisternae on the forming face of dictyosome. Presence of zones of exclusion in relation with smooth ER or nuclear membrane, the occurrence of zones of exclusion in dormant seeds of higher plants and the formation of dictyosome from these zones in germinating seeds provide evidence in support of the above two theories about the origin of dictyosome.

3. **By the division of pre-existing dictyosome-** It has been observed that during cell division in both plants and animals, the number of dictyosomes increases and the number of dictyosomes in each daughter cell just after division is almost equal to the number in the parent cell prior to division, from tins and other direct observations on the dividing cells it has been presumed that dictyosomes also divide during cell division.

Q i) Discuss polarity of Golgi complex.

Q ii) Discuss secretory Function of Golgi complex.

Functions of Golgi complex-

1. **Formation of Acrosome during Spermiogenesis-** During the maturation of sperm the Golgi complex plays a role in the formation of acrosome (Burgos and Fawcett, 1955).

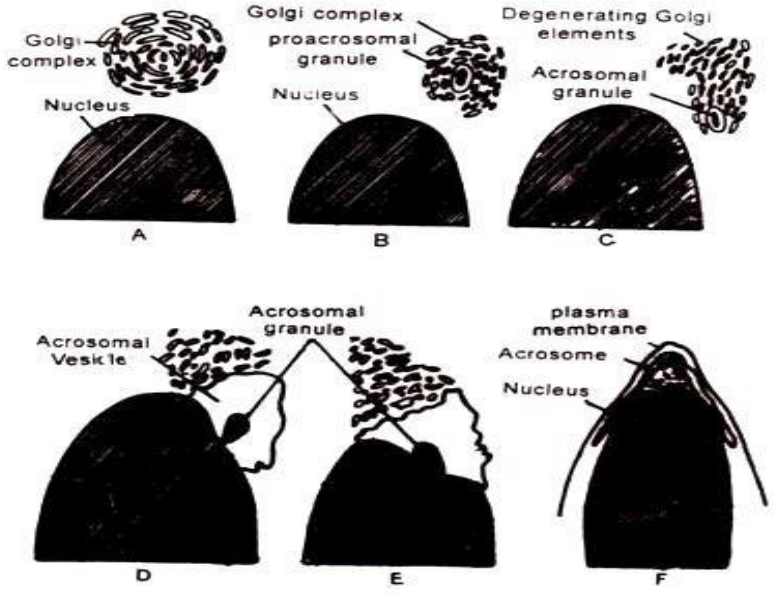


Fig: 7.4 Diagram illustrating gradual modification in Golgi complex to form acrosome during spermiogenesis. (source:V.B.Rastogi)

In early stages, the Golgi appears as a spherical body, comprising cisternae arranged in parallel stacks and numerous small vesicles. The later always pinched off from the cisternae. As development proceeds, the Golgi complex becomes irregular in shape and large vaculoles are formed by dilations of cisternal sacs.

In the centre of these large vacuole or vacuoles is present a dense granule, the proacrosomal granule. This granule which is derived from Golgi complex continues to grow within the vacuole by a process known as accretion. This vacuole and granule approaches the anterior pole of the nuclear membrane, constituting acrosomal granule.

With the elongation of the spermatid, the acrosomal vesicle spreads over the nuclear surface and finally collapses with the nuclear membrane, forming the cap material. The acrosomal granule becomes the acrosome which lies at the apex of the nucleus and apparently comprises certain enzymes involved in the process of fertilization.

2. **Synthesis and secretion of polysaccharides-** Studies on goblet cells by autoradiography and electron microscopy have established the inter-relationship between protein synthesis, carbohydrate addition and sulphation. The goblet cells of the colon produce mucigen. This secretory material contains a large proportion of carbohydrate.

   The Golgi complex is found just above the nucleus. Towards the free surface of the cell are gradually enlarging mucigen granules. The proximal cisternae of the Golgi complex do not show any swelling, but at some distance across the stack the distal cisternae are quite suddenly converted into mucigen granules. The distal cisternae continually convert into mucigen granules every 2-4 minutes. New proximal cisternae are formed in compensation.

3. **Role in secretion-** Golgi complex is considered to play some role in the secretory function of a cell. But the question is this that they are secreting or synthesizing some substances themselves or they are simply a store house in which the secretory products which are secreted somewhere else in the cell, is simply stored and concentrated.

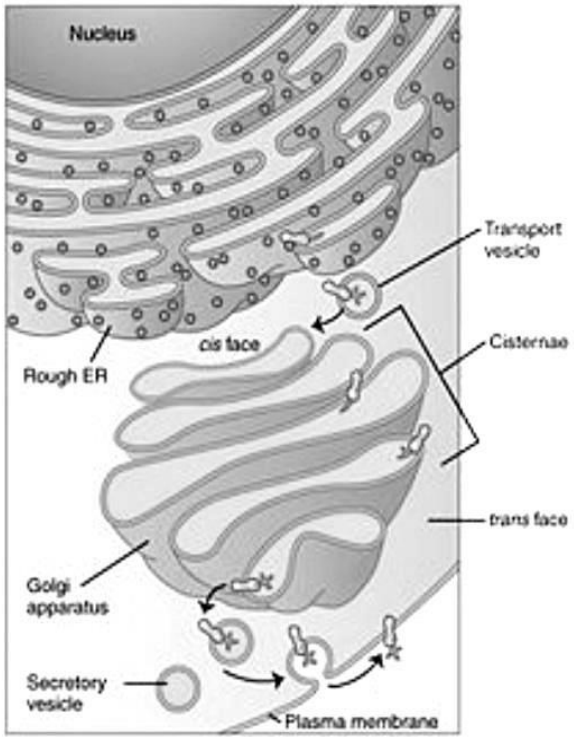


Fig:7.5 The Golgi Apparatus in context of the secretory pathway

From the studies of Palade et al. 1962 this secretory cycle is now well-defined and includes four steps in case of pancreatic acinar cells and they are:

(i) The incorporation of amino acids into protein at the surface of rough endoplasmic reticulum.

(ii) Transfer of these nascent secretory proteins into the cisternae of rough endoplasmic reticulum.

(iii) The intracellular transport of these proteins to the Golgi complex.

(iv) The migration of zymogen granules towards the apex of the cell where they discharged into lumens.

4. Role of Golgi body in oogenesis- Srivastava (1965) has given a brief review on the Golgi complex during oogenesis. According to Afzelius (1956), the Golgi complex of a sea-urchin egg, as seen under electron microscope, consists of stacks of lamellae forming walls of flat pouches, which may occasionally be swollen.

There are some indications of transverse divisions of these bodies. Sotelo (1959) and Sotelo and Porter (1959) have described the Golgi complex in rat-ovum as seen under the electron microscope and found juxtra nuclear localization of this organelle in early oocytes.

In the next stage, these resolve into fragments and in the third stage, these moves towards the cortex. In all these cases, their structure remains to be of closely packed arrays of slender, double profiles (flattened sacs) and spherical vesicles.

In the early oocytes the complex is compactly organized. In later stages, discrete bundles of profiles, surrounded by small vesicles are found scattered in the cortical zone. In the early oocytes, the Golgi complex and centrosome are closely associated.

5. Absorption of compounds- Hirsch et al., have discovered that when iron sugar is fed to an animal, iron becomes absorbed on Golgi complex (Kedrowsky). Van Teel has shown that Golgi systems also absorb compounds of copper and gold. Kedrowsky has shown that Golgi complex of Opalina can absorb bismutose (compound of albumin and bismuth) and protargol (compound of albumin and silver). Thus, Kirkman and Severinghaus state that Golgi complex acts as a condensation membrane for the concentration of products into droplets or granules.

6. Plant cell wall formation- The cell wall of plants is made up of fibrils which predominantly contain polysaccharides, along with some lipids and proteins. During cytokinesis a cell plate is formed between the two daughter nuclei, and has around it a membrane which later becomes the plasma membrane of the daughter cells. There is clear evidence that the polysaccharides are formed in the Golgi complex and transferred to the new cell wall which is laid down while the cells are still growing.

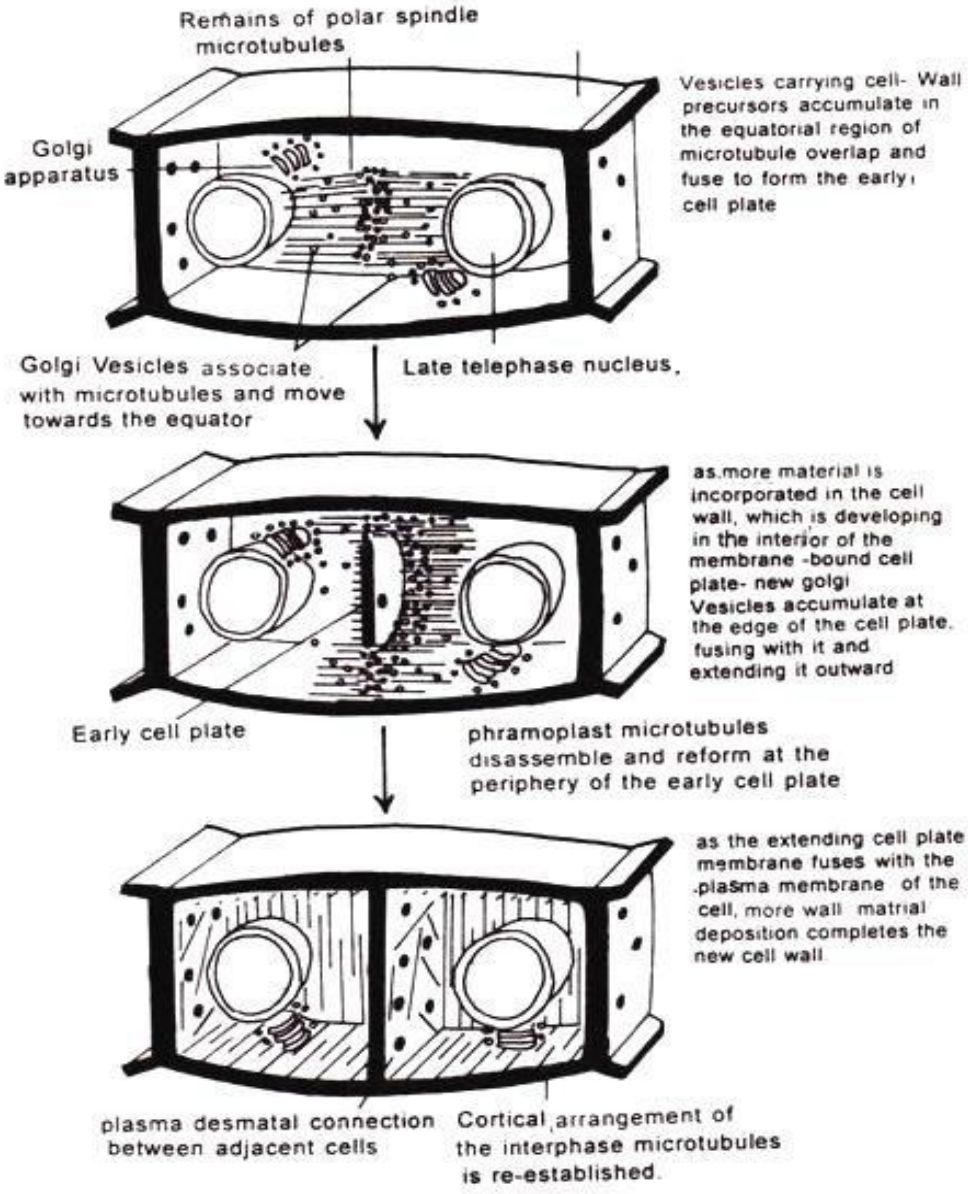


Fig: 7.6 Diagram showing the association of Golgi vesicles with microtubules result the formation of new cell wall .

Substances like pectins and hemicelluloses, which form the matrix of the cell plate separating the plasma membranes, are also contributed by the Golgi complex.

7. **Formation of intracellular crystals-** In the marine isopod, Limnorialingmorum, which is a burrowing form there are present midglands whose cells consist of crystals. These range up to 30 A° in length and 15 A° thick. It has been proved that these crystals are formed by Golgi complex and are known to contain protein and iron. They are without enclosing membrane and usually spheroidal in shape. They are concerned with the secretory activity.

8. **Milk Protein droplet formation-** In the lactating mammary gland of mice are produced protein droplets which are related with Golgi complex. These droplets usually open on to the cell surface by the fusion of their enclosing membrane with the plasma membrane.

9. **Formation of lysosomes and vacuoles-** Primary lysosomes are formed from the Golgi membranes the same way as the secretory vesicles. There is good evidence that dictyosomes accumulate hydrolytic enzymes in their more mature regions. Some vacuoles in plant cells have been found to contain small amounts of hydrolytic enzymes and these are presumed to have been derived from Golgi complex.

10. **Pigment formation-** In many mammalian tumour and cancer cells the Golgi complex has been described as the site of origin of pigment granules (melanin).

11. **Regulation of fluid balance-** A homology has been suggested between the Golgi complex and the contractile vacuole of lower Metazoa and Protozoa. The contractile vacuole expels surplus water from the cell. In certain Protozoa the Golgi complex is also concerned with regulation of fluid balance.

7.3 LYSOSOME (SUICIDE BAG)

Lysosome, subcellular organelle that is found in nearly all types of eukaryotic cells (cells with a clearly defined nucleus) and that is responsible for the digestion of macromolecules, old cell parts, and microorganisms. Lysosomes contain a wide variety of hydrolytic enzymes.

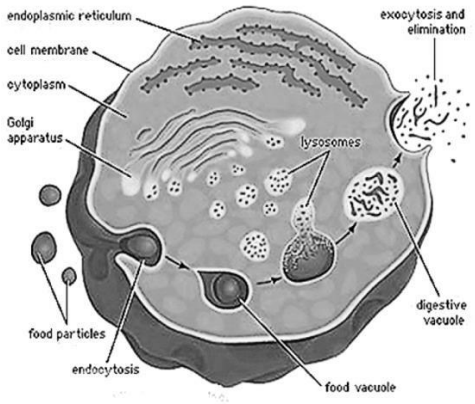


Fig:7.7 Lysosome

These are first observed in liver cells. They are 1.5 to 2 milii micron in size These are single membrane bounded structures. They were first called pericanalicular-dense bodies. "Christian De Duve" called them lysosomes in 1955. They were named as lysosomes because they contain hydrolytic enzymes.

They are present in all animal cells ,except mammalian R.B.C. They are more in liver cells spleen cells, kidney cells etc.. In prokaryotes they are absent.

They are round or spherical bodies. They are .4 to .8 milli microns in size.

Each lysosome is covered by a unit membrane. It encloses a dense matrix. It shows 2 regions, outer dense part and central less dense part.

Lysosome:

Chemical Composition- The composition of the lysosomal membrane is the characteristic of a unit phospholipid-protein membrane. The sialic acid content of lysosomes is not much greater than that found in other subcellular fractions, suggesting that sialopolymers are not involved in a special role in lysosomes. The released enzyme fraction, which contained about myc of the total protein, also contained free flavins and free amino acids in amounts very much larger than those observed for mitochondria. Fe, Zn, Cu, Mn, and MO were the principal metals concentrated in lysosomes.

7.4 KINDS OF LYSOSOMES OR POLYMORPHISM IN LYSOSOMES

Lysosomes are dynamic organelles and exhibiting polymorphism in their morphology. In the same cell at different times or in different cells four kinds of lysosome are reported.

1) **Primary lysosome or storage granule or protolysosomes or virigin lysosomes**- It is a newly formed lysosome that is single membrane bound organelle.It forms from G.E.R.L, which means 'Golgi associated with Endoplasmic Reticulum will give Lysosome''. This was stated by Dyson 1978.

   This is called original lysosome which is formed from Golgi sacs and contains one type of enzyme or another. Under radiography technique, the transfer of protein was observed to have the following sequence: ER → Golgi complex → Lysosomes.

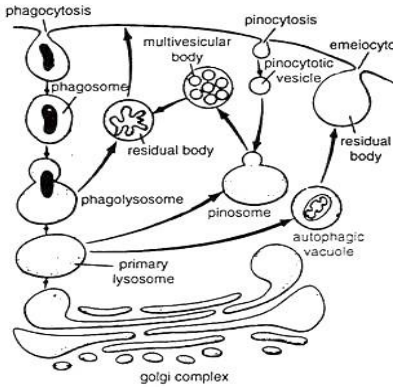


Fig:7.8 Diagram showing formation of different types of lysosomes and there functioning. (source: V. B. Rastogi)

2) **Secondary Lysosome or digestive vacuole or heterophagosome-** Secondary lysosomes are formed when primary lysosomes fuse with phagosomes or pinosomes(primary lysosome + food= secondary lysosome). They are larger in size and capable of releasing their enzymes outside the cells where they degrade foreign material.

3) **Residual body-** After the process of digestion in phagosome or autophagic vacuole some materials are not digested. Such lysosomes with undigested food is called residual body. This residual body will send the undigested matter through plasma membrane.

In nerve and muscle cells residual bodies are more in number. They are called, "Lipofucine granules". (By the estimation of these granules the age can be decided)

The polymorphic tendency of lysosome is not real, it is connected with the digestive activity of the lysosome.

4) **Autophagic vacuole or autolysosome or cytolysosome-** When the organism is in a state of starvation the lysosome will start digesting the intracellular structures. Such lysosome is called autophagic vacuole. The intracellular parts involved in digestion may include mitochondria or endoplasmic reticulum .

Q i) What are primary and secondary lysosomes?

Q ii) Differentiate between heterophagy and autophagy.

Q iii) Write short notes on LSDs.

Lysosomal Enzymes- Enzymes known to be present in the lysosomes include **hydrolases** that degrade proteins, nucleic acids, lipids, glycolipids, and glycoproteins. Hydrolases are most active at the acidic medium (pH= 5.0),maintained within the lysosome and analogous to the activity of stomach. After the material is broken down, lipids and amino acids are transported across the lysosomal membrane by permeases for use in biosynthesis. The remaining debris generally stays within the lysosome and is called a residual body.

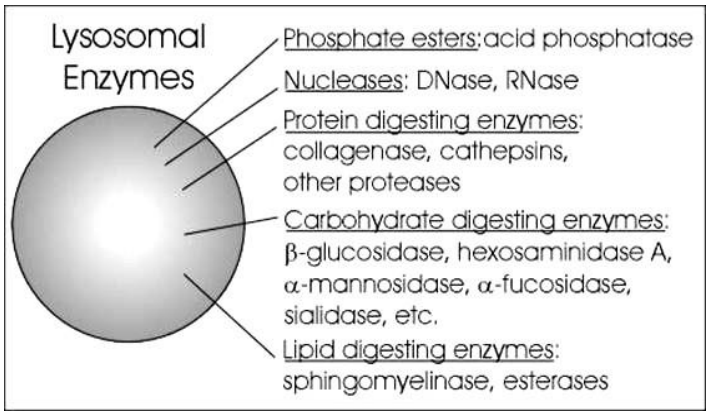


Fig:7.9 Diagram showing different hydrolytic enzymes in a lysosome

Table: Different enzymes present in Lysosomes

Sr. No	Enzymes	Substrate
1	Phosphates	Different
	A- Acid phosphatase	Most phosphomonoesters
	B- Acid phosphodiesterase	Oligonucleotides and phosphodiesterase
2	Nucleases	
	A- Acodribonuclease	RNA
	B- Acid deoxyribonuclease	DNA
3	Polysaccharides/ mucopolysaccharides hydrolyzing enzymes	
	A- beta Galactosidase	Galactosides
	B- alpha Glucosidase	Glycogen
	C- alfaMannosidase	Mannosides, glycoproteins
	D- beta Glucoronidase	Polysaccharides and mucopolyssacharides
	E- Lysozymes	Bacterial cell walls and mucopolyssacharides
	F- Hyaluronidase	Hyaluronic acids, chondroitin sulphates

	H- Arylsulphatase	Organic sulfates
4	Proteases	
	A- Cathepsin(s)	Proteins
	B- Collagenase	Collagen
	C- Peptidase	Peptides
5	Lipid degrading enzymes	
	A- Esterase	Fatty acyl esters
	B- Phospolipase	Phospholipids

LysosomalMembrane : A Simple Barrier Between the Acidic Lysosomal Environment and the Cytoplasm

Lysosomes are organelles of eukaryotic cells involved in the turnover of various macromolecules. One of their major tasks is the degradation of extracellular material as well as intracellular components that are delivered to lysosomes by endocytosis or autophagy, respectively

1. Because the products of lysosomal catabolism and certain cytosolic compounds which are destined for degradation have to be transported across the **7–10 nm thick lysosomal membrane, possess a number of highly specialized proteins**. Furthermore, it has to withstand the luminal milieu with an acidic pH of <5 and 50 potent hydrolases, maintaining a tight barrier toward the surrounding cytosolic environment. Therefore, **lysosomal membrane proteins (LMPs) are usually highly glycosylated probably forming a continuous glycoprotein layer at the luminal side** of the lysosomal membrane.
2. The most abundant **type-1 transmembrane proteins of the lysosomal membrane are the lysosomal associated membrane proteins LAMP-1 and LAMP-2** with more than 10 used glycosylation sites. The recent high resolution crystal structure of a human dendritic LAMP protein and the

possible modeling of the glycosylated luminal domains of LAMP-proteins revealed hitherto unknown β-prism folds resulting in an unexpected compact conformation in close proximity to the lysosomal membrane.

3 . Based on computational analysis it is estimated that the **thickness of the lysosomal glycoprotein coat is around 8 nm.** This is considerably lower than glycocalyces at the cell surface.

4. **The specialized glycoprotein layer may be important to regulate the stability and integrity of the lysosome**. It may indirectly modulate the fusion of lysosomes with phagosomes, autophagosomes or with the plasma membrane during exocytosis. Whereas the transmembrane segments of lysosomal membrane proteins are putatively involved in direct transport events across the membrane, the usually rather short cytosolic parts of these proteins mediate contact to ytosolic proteins and

proteins on other organelles.

Origin of Lysosomes- The origin of lysosome is not clearly known. According to 'Dyson' 1978 the lysosomes arise from Golgi complex and endoplasmic reticulum. The protein granules produced by ribosome is stored in endoplasmic reticulum. They move in to smooth endoplasmic reticulum. From there they move into Golgi. There they are concentrated and modified as primary lysosomes.

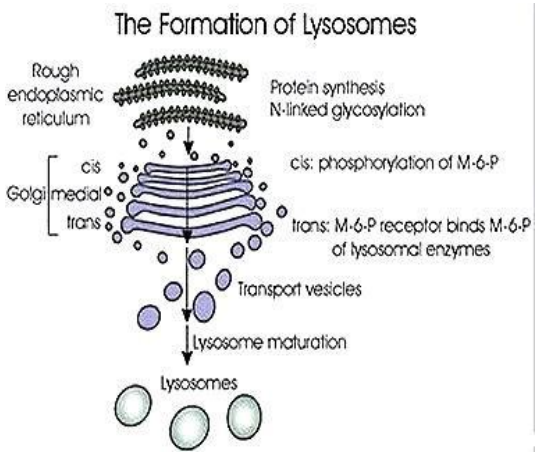


Fig:7.10 Origin of Lysosomes:Targeting of lysosomal proteins by phosphorylation of mannose residues. Proteins destined for incorporation into lysosomes are specifically recognized and modified by the addition of phosphate groups to the 6 position of mannose residues

Functions of Lysosomes- Some of the main functions of lysosomes are as follows:

1. **Intracellular digestion-** The word lysosome is derived from (lyso lytic or digestive; and soma body) thus helping in digestion. Pinocytic vacuoles formed as a result of absorption of fluid substance into cell or phagocytic vacuoles formed by absorption of solid particles into cell, carry protein material to lysosomal region.

These foreign proteins may undergo digestion within cell as a result of endocytosis. Endocytosis includes the processes of phagocytosis (Gr,phagein, to eat), pinocytosis (Gr, pinein, to drink) and micropinocytosis.

Phagocytosis and pinocytosis are active mechanisms in which cell requires energy for their operation.

Digestion of intracellular substances or autophagy- Many cellular components, such as mitochondria, are constantly being removed from the cell by lysosomal system. Cytoplasmic organelles become surrounded by membranes of smooth endoplasmic reticulum, forming vacuoles, then lysosomal enzymes are discharged into autophagic vacuoles and the organelles are digested. Autophagy is a general property of eukaryotic cells.

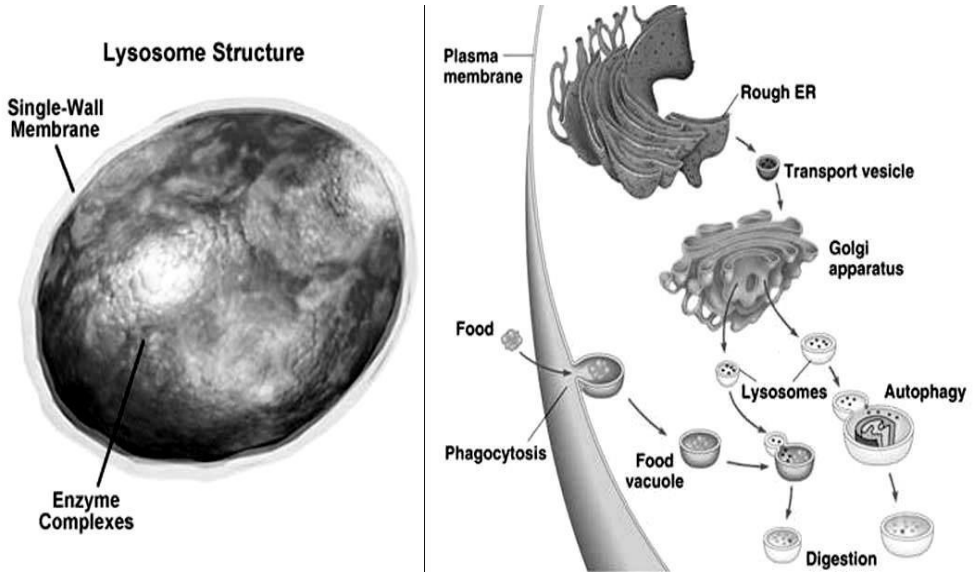


Fig:7.11 Lysosome : The stomach of Cells

2. **Removal of dead cells-** Hirsch and Cohn (1964) told that lysosomes help in the removal of dead cells in tissues such as white blood cells with engulfed bacterium in blood, cells in the outer layer of skin and mucous membrane linings of the body.

   Lysosomal membrane ruptures in these cells, releasing enzymes into body of cell, so that whole cell may be digested. Lysosomes contain a sufficient complement of enzymes to digest most types of biological or organic materials and the digestive process (autolysis) occurs quite rapidly in dead cells. This process of tissue degeneration (necrosis) is due to this lysosomal activity.

3. **Role in metamorphosis:** Recently lysosome‘s role has been discovered in the metamorphosis of frog. Disappearance of tail from tadpole larva of frog is due to lysosomal activity (action of cathepsins present in lysosomes).

4. **Help in protein synthesis-** Novikoff and Essner (1960) have suggested the possible role of lysosomes in protein synthesis. Recently, the author (Dr. Singh 1972), has correlated lysosomal activity with the protein synthesis.

5. **Help in fertilization-** During fertilization, sperms head secrete some lysosomal enzymes which help in the penetration of sperm into vitelline layer of ovum. Acrosome contains protease and hyaluronidase and abundant acid phosphatase.

6. **Role in osteogenesis-** It has been studied that formation of bone cells and also their destruction depends upon lysosomal activity. Likewise, ageing of cells is related with the lysosome activity.The osteoclasts (multinucleated cells) which remove bone, do so by the release of lysosomal enzymes which degrade the organic matrix. This process is activated by the parathyroid hormone.

7. **Malfunctioning of lysosomes-** Lysosomal malfunction may lead to diseases, for example, when glycogen taken up by lysosomes is not digested (Pompe‘s disease). Ruptures of lysosomes in skin cells exposed to direct sun light leads to pathological changes following sunburn. The enzymes liberated by these lysosomes kill cells in the epidermis, causing blistering and later to ‗peeling‘ of a layer of epidermis.

8. **Autolysis in cartilage and bone tissue-** The excess of vitamin A causes cell poisoning. It disrupts the lysosomal membrane, causing release of enzymes into the cell and producing autolysis in cartilage and bone tissue.

7.5 LYSOSOMES AND DISEASES

Synthesis of lysosomal enzymes is controlled by nuclear genes. Mutation in the genes for these enzymes are responsible for more than 30 different human genetic disorders, and that number keeps growing, which are collectively known as **lysosomal storage diseases (LSDs)**. They are a type of inborn errors of metabolism caused by malfunction of one of the enzymes.These diseases result from an accumulation of specific substrates, due to the inability to break them down. Although the different types of LSDs are rare individually, taken together they affect roughly 1 in 7,700 births, making them a relatively common health problem.

Lysosomes are cytoplasmic organelles in which a variety of macromolecules are degraded by different acid hydrolase enzymes. If a lysosomal enzyme is absent or has reduced activity or if enzymes are not correctly targeted to lysosomes, the macromolecules normally degraded by lysosomes will accumulate, causing abnormal storage of various complex compounds including **glycolipids, glycosaminoglycans, oligosaccharides,** and **glycoproteins**.

Lysosomal storage disorders are **autosomal recessive**, except for **Fabry disease and Hunter syndrome,** which are **X-linked**. Abnormal macromolecule storage leads to a variety of signs and symptoms, depending on where the storage occurs.

Some of the most common lysosomal storage disorders include:

- **Gaucher disease:** Gaucher disease often causes spleen and liver enlargement, blood problems and bone issues.
- **Fabry disease:** This disorder often causes severe burning pains in hands and feet and, in some cases, a distinctive skin rash on the legs. Untreated, this disease can cause kidney failure, heart failure, strokes and death before age 50. Although men are more likely to have severe disease, women may also be seriously affected.
- **Niemann-Pick disease:** Similar to Gaucher disease, Niemann-Pick disease involves organ enlargement, lung dysfunction and central nervous system damage for certain subtypes.
- **Hunter syndrome:** This disease is part of a group of disorders that cause bone and joint deformity as well as interference with normal growth.
- **Glycogen storage disease II (Pompe disease):** Depending on the specific subtype, Pompe disease may cause heart enlargement and heart failure in infants. It may also cause respiratory problems and severe muscle weakness in adults.
- **Tay-Sachs disease:** This disorder causes severe and fatal mental and physical deterioration, with both an early-onset and a late-onset form.

7.6 SUMMARY

- Morphologically the Golgi is composed of flattened membrane-enclosed sacs (cisternae) and associated vesicles
- A striking feature of the Golgi apparatus is its distinct polarity in both structure and function. Proteins from the ER enter at its *cis* face (entry face), which is convex and usually oriented toward the nucleus. They are then transported through the Golgi and exit from its concave *trans* face (exit face). As they pass through the Golgi, proteins are modified and sorted for transport to their eventual destinations within the cell.
- Products from the Golgi apparatus go to three main destinations: (1) inside the cell to lysosomes (2) the plasma membrane (3) outside the cell
- A lysosome is a cell organelle. They are like spheres. They have hydrolytic enzymes which can break down almost all kinds of biomolecules, including proteins, nucleic acids, carbohydrates, lipids, and cellular debris. They contain more than 50 different enzymes.
- Gaucher disease is one of the most common lysosomal storage disorders (LSDs). These fats or sugars accumulate in cell lysosomes where enzymes are active, disrupting normal function and causing lysosomal storage disorders. All LSDs except Hunter syndrome (MPS II) and Fabry disease are autosomal recessive disorders.

7.7 TERMINAL QUESTIONS

Q 1. Describe ultra structure and chemical composition of Golgi complex .

……
……
……

Q 2. Describe the occurance and distribution of Golgi body in cells and mention its functions in detail.

……
……
……

Q 3. What are lysosomes ? Describe their functional significance .

……
……
……

Q 4. Lysosomes are suicide bags in the cell. Explain.

……
……
……

Q 5. Write a note about histochemical marking of lysosomal components: membrane and enzymes.

……
……
……

Q 6. Write short notes on lysosomes and diseases.

……
……
……

Multiple choice Questions:

i) Cellular organelles containing hydrolytic or digestive enzymes that have digestive actions (hydrolases enzymes) are called or (Hydrolytic enzymes are located in the following cell organelles).

a) Lysosome

b) Microsomes

c) Ribosomes

d) Mesosomes

ii) The golgi complex originates form

a) Cell membrane

b) Mitochondria

c) Cytoplasm

d) Endoplasmic reticulum

iii) Dictyosomes are

a) Class of ribosomes

b) Place of Flagellar origin

c) Respiratory particle

d) Golgi bodies

iv) Golgi bodies help in

a) Controlling the activities of the cell

b) Controlling the activities of the nucleus

c) Synthesis of secretory substances

d) Synthesis of protein

v) Most of the hydrolytic enzymes of Lysosomes function at

a) Acidic Ph (Ph 5)

b) Base Ph

c) Neutral

d) Any Ph

vi) Cellulose and hemicellulose are the constituents of cell wall are synthesized by

a) Lysosomes

b) Microbodies

c) Smooth endoplasmic reticulum

d) Golgi apparatus

vii) The membranous bag of hydrolytic enzymes used for controlled intra cellular digestion of macromolecules is a

a) Phagosome

b) Lysosome

c) Nucleosome

d) Centrosome

viii) Golgi apparatus is absent in

a) Higher plants

b) Blue green algae

c) R.B.C

d) Liver cells

ix) Which one of the following organelles is located near the nucleus and contains a collection of flattened membrane bound cisternae

a) Golgi apparatus

b) Centrioles

c) Nucleolus

d) Mitochondrion

x) ―Phagocytosis‖ was discovered by

a) Metchnikoff

b) Altmann

c) Louis Pasteur

d) Preistley

xi) Lysosome have a high contents of

a) Hydrolytic enzymes

b) Polyribosomes

c) Lipoproteins

d) DNA ligase

xii) Acrososmes are produced by

a) Agranular endoplasmic reticalum

b) Golgi complex

c) Lysosomes

d) Centriles

xiii) Golgi complex is involved in

a) Protein synthesis

b) Cell secretary process

c) Particles movement from one part to another

d) Synapsis

xiv) The internal membrane is the extension of the infolded plasma membrane in

a) Golgibodies

b) Mitochondria

c) Endoplasmic reticulum

d) Plastid

xv) Which cellular organelle is polymorphic

a) Golgi complex

b) Peroxisome

c) Glyoxysome

d) Lysosomes

xvi) Which of the following cellular organelles breaks down complex macro molecules, such as polysaccharides and protein

a) Golgi complex

b) Lysosomes

c) Rough endoplasmic reticulum

d) Mitochondria

xvii) The process by which a cell secretes macro-molecules by fusing a transport vesicle to the plasma membrane is called.

a) Pinocytosis

b) Endocytosis

c) Phagocytosis

d) Exocytosis

xviii) Storage of enzymes for the digestion of cellular components, proteins and carbohydrates is carried out by

a) Mitochondria

b) Lysosomes

c) Centrioles

d) Ribosomes

7.8 ANSWERS

i) a, ii) d, iii) d, iv) c, v) a, vi) d, vii) b, viii) b, ix) a, x) a, xi) a, xii) b, xiii) b, xiv) a, xv) d, xvi) b, xvii) d, xviii) b.

UNIT-8 MITOCHONDRIA

8.1 INTRODUCTION

The **mitochondrion** (plural **mitochondria** : *Gr*. Mito: thread; *chondria*: granule) is granular or filamentous, double- membrane bound organelle, found in cytoplasm of all eukaryotic cells but absent in prokaryotic cells where respiratory enzymes are located on the plasma membrane.

Kolliker (1880) was the first who observed the mitochondria under light microscope as granules in muscle cell of insects**. Flemming** (1882) describe them **as fila**. **Altmann** in 1894 observed them and they were called Altmann's granules**bioplasts**(Gr, bios: life+ plast, germ).The term mitochondria were applied by **Benda** (1897-98) to these granules.

Mitochondria are typically round to oval in shape and range in size from 1.5 to 10 μm. Mitochondria are regarded as the **biochemical machines** that are associated with cell respiration and convert the potential energy of foodstuffs into kinetic energy. These are commonly known as **—Power house of the cells**‖.

The **outer membrane** covers the surface of the **mitochondrion**, while the **inner membrane** is located within and has many folds called **cristae.The folds increase surface area of the membrane, which is important because the inner membrane holds the proteins involved in the electron transport chain.**

The primary function of mitochondria is to generate large quantities of energy in the form of adenosine triphosphate (ATP). In addition to producing energy, mitochondria store calcium for cell signaling activities, generate heat, and mediate cell growth and death. The number of mitochondria per cell varies widely; for example, in human mature erythrocytes (red blood cells) do not contain any mitochondria, whereas liver cells and muscle cells may contain hundreds or even thousands.

Mitochondria itself has its own DNA and can replicate independently so it is autonomous but still depends on the nuclear DNA that's why the term is used for mitochondria as **semi-autonomous** body.

Objective

At the completion of this unit students will be able to:

- Define mitochondria.
- Describe origin of mitochondria.
- Understand the structure and functions of mitochondria.
- Describe characteristics of the mitochondria.
- Understand that mitochondria are semi-autonomous bodies.

8.2 ORIGIN OF MITOCHONDRIA

1. Mitochondria show external resemblance to plastids but neither they are originated from, nor do they give rise to plastids. Although morphology of both, at certain times during the cell cycle, shows some similarity. Both arise independently of each other.
2. Mitochondria may arise from invaginations of plasma membrane (fig:8.1) or develop from the endoplasmic reticulum. The membrane invaginates and extends into the cytoplasm as a tubular structure. It gradually becomes curved and folded, a double-walled structure, the mitochondrion.

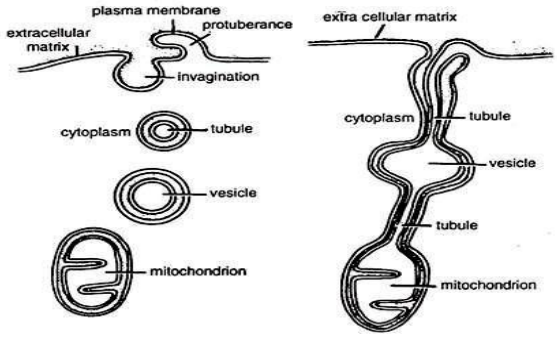


Fig:8.1 A possible mechanism for the formation of mitochondria from plasma membrane(source:V.B.Rastogi)

3. They are developed by accumulation of micro bodies in cytoplasm. A micro body consists of a single outer membrane and a dense matrix with a few cristae. Gradually micro bodies develop into fully formed mitochondria. These micro bodies are also visible in the embryo of Triturus in which cristae are few and less developed.
4. **De novo origin-** There is no evidence that mitochondria arise de novo (afresh), hence this hypothesis has been discarded.

5. **Prokaryotic origin-** The term bioplasts was employed by Altmann for mitochondria which means self-perpetuating units. This view has been supported by many recent workers showing homology between mitochondria and bacteria:

   (i) First is the localization of enzymes of respiratory chain, which in case of bacteria, are localized in plasma membrane. Plasma membrane may be compared to the inner membrane of mitochondrion.

   (ii) In some bacteria; plasma membrane forms membranous projections like cristae of mitochondria. These projections have been called mesosomes, which possess respiratory chain enzymes (Salton, 1962).

   (iii) The mitochondrial DNA is circular as found in the chromosomes of most prokaryotes (bacterial cells). Replication, process of mitochondrial DNA is also similar to bacterial DNA.

   (iv) Mitochondria contain ribosomes which are smaller and similar in size to that of bacterial ribosomes,

   (v) Protein synthesis in mitochondria as well as in bacteria is inhibited by chloramphenicol, whereas extra mitochondrial protein synthesis is unaffected by this chemical. Furthermore, in the process of protein synthesis, mitochondria depend partially on mitochondrial matrix, DNA, partially on nucleus and cytoplasm of the eukaryotic cells. It exhibits the symbiotic nature of mitochondria. These evidences support the prokaryotic origin of mitochondria.

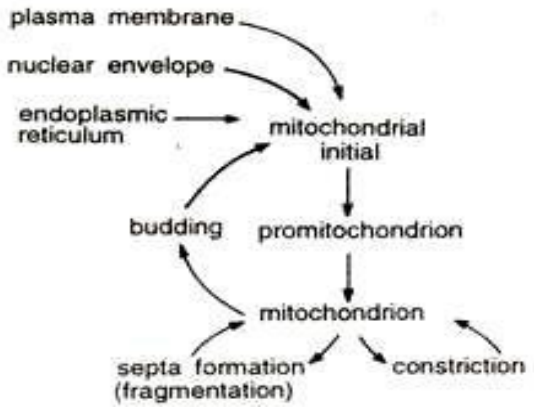


Fig:8.2 Outline of possible origin of mitochondria

6. **New mitochondria originate by the division of pre-existing mitochondria-** It has been observed that sometimes mitochondria become elongated and broken into small pieces (fig:8.3). Each piece forms a new mitochondrion in later stages. During cell division, mitochondrion divides transversely into two and each develops into a mature mitochondrion later on.

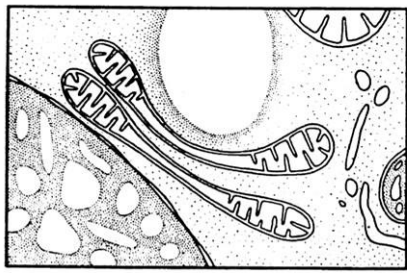


Fig:8.3 Division of mitochondria elongation

7. **Budding** of mitochondria from the preexisting mitochondria may occur in differentiated or undifferentiated cells, forming mitochondrial initials. These increase in size and develop cristae to form a mature and functional mitochondria. Initials may also be derivations from nuclear envelope or plasma membrane.

8.3 MORPHOLOGY

Mitochondria are membrane bound cytoplasmic organalles of varying shape size and number. Each Mitochondria is surrounded by 2 membranes, an outer and inner membrane around a central matrix.The two membranes have different properties. Because of this double-membraned organization, there are five distinct parts to a mitochondrion. They are:

1) The outer mitochondrial membrane,
2) The inter membrane space (the space between the outer and inner membranes),
3) The inner mitochondrial membrane,
4) The cristae space (formed by infoldings of the inner membrane).
5) The matrix (space within the inner membrane). Mitochondria stripped of their outer membrane are called **mitoplasts**.

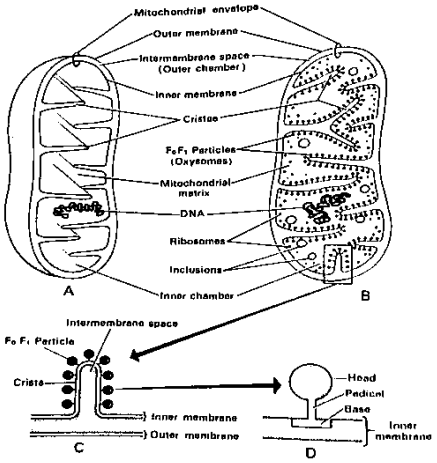


Fig: 8.4 A. Mitochondria cut open to show its membranes and cristae; B. Internal structure of mitochondria; C. An enlarged crista; D.An enlarged FoF1 particle.

1) **Outer membrane-** The outer mitochondrial membrane, which encloses the entire **organelle, is 60 to 75 angstroms (Å) thick. It has a protein-to-phospholipid ratio** similar to that of the eukaryotic plasma membrane (about 1:1 by weight). It contains large numbers of integral membrane proteins called porins (Transport Proteins). These porins form channels that permits freely the passage of cytoplasmic molecules of 5000 daltons or less in molecular weight from one side of the membrane to the other.

2) **Intermembrane space-** The intermembrane space is the space between the outer membrane and the inner membrane. It is also known as perimitochondrial space. Because the outer membrane is freely permeable to small molecules, the concentrations of small molecules, such as ions and sugars, in the intermembrane space is the same as in the cytosol. However, large proteins must have a specific signaling sequence to be transported across the outer membrane, so the protein composition of this space is different from the protein composition of the cytosol. One protein that is localized to the intermembrane space in this way is cytochrome c.

3) **Inner membrane-** The inner membrane is highly permeable even to small ions due to its high phospholipid (cardiolipin) contents. It is therefore provided with specific carrier proteins (Translocators) for the transport of substance from outer space to inner one. The inner membrane is pushed into the matrix at regular intervals in order to increase its surface area. These projections called cristae possess repiratory chain enzymes, as well as ATP synthetase which are needed for energy generation (i.e ATP production).

4) **Cristae**- The inner mitochondrial membrane is compartmentalized into numerous cristae, which expand the surface area of the inner mitochondrial membrane, enhancing its ability to produce ATP. For typical liver mitochondria, the area of the inner membrane is about five times as large as the outer membrane. This ratio is variable and mitochondria from cells that have a greater demand for ATP, such as muscle cells, contain even more cristae. These folds are studded with small round bodies known as F_1 particles or oxysomes. These are not simple random folds but rather invaginations of the inner membrane, which can affect overall chemiosmotic function.

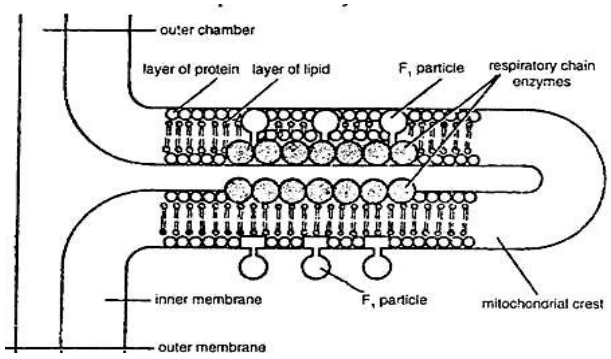


Fig: 8.5 Ultra structure of a mitochondrial crest.

6) Matrix- The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total protein in a mitochondrion.The matrix is important in the production of ATP with the aid of the ATP synthase contained in the inner membrane. The matrix contains a highly concentrated mixture of hundreds of enzymes, special mitochondrial ribosomes, tRNA, and several copies of the mitochondrial DNA genome. Of the enzymes, the major functions include oxidation of pyruvate and fatty acids, and the citric acid cycle.

Mitochondria have their own genetic material, and the machinery to manufacture their own RNAs and proteins**.**

Q.i) What is located on the cristae of the mitochondria?

Q.ii) Where would cristae be found in a cell ?

8.4 CHEMICAL COMPOSITION

Composition of mitochondrial membrane is just like the plasma membrane, i.e., phospholipids and proteins. Protein is present on two surfaces and a bimolecular layer of lipid in between the two. Outer But the cardiolipin is lower in comparison to inner membrane. Bensley gives the following composition of dried liver mitochondria :

(1) Proteins and unknowns 65%

(2) Glycerides 29%

(3) Lecithin and cephalin (phospholipids) 4%

(4) Cholesterol 2%

Bensley believes that the surface of mitochondria is composed of a mosaic of protein and lipid substances. He suggests that cortex of mitochondria should contain protein, glycerides and cholesterol molecules.

Cohn gives the following composition of dry mitochondria (by weight) :Proteins 70%, Lipid 25-30%

Of the lipids 90% is phospholipids and 10% cholesterol, carotenoids, vitamin E and other inorganic elements like S, Fe and Cu. The sulphur content is also relatively high because considerable sulphydryl (SH) is present on active groups of some enzymes.

Besides these, there are 0.5 per cent RNA and small amount of DNA, and respiratory enzymes which help in respiration like cytochrome oxidase, reductase, transaminase, coenzyme 1, octanoxidase, fatty acid oxidase, etc.

Of the proteins, the largest portion probably consists of enzymes, but structural proteins constitute about 30% of the total protein. An actomyosin-like protein that splits adenosine triphosphate (ATP) and contracts on addition of ATP has been reported from liver mitochondria. Phospholipids are also important for enzyme function, since their addition to purified enzymes also increases activity.

Mitochondrial lipids are largely phospholipids, lecithin being prominent, but triglycerides, phosphatidic acid and cholesterol are also present.

Mitochondrial enzymes- All the three major components of food (carbohydrates, proteins and lipids) degraded in cytoplasm, enter mitochondrial Krebs cycle and undergo oxidation. The electrons emitted during Krebs cycle are transported to electron transport system (ETS). Several enzymes and coenzymes are involved in the oxidative phosphorylation and electron transfer mechanism. Lehninger (1969) classifies them as follows:

1. **Enzymes of outer membrane of mitochondira**

   (i) Monoamine oxidase
   (ii) Fatty acid coenzyme A ligase
   (iii) Kynurenine hydroxylase
   (iv) NADH-cytochrome C-reductase

2. **Enzymes of outer mitochondrial chamber:**

   (i) Adenylate kinase
   (ii) Nucleoside diphosphokinase
   (iii) DNAse 1 and 5′ – endonuclease

3. **Enzymes of inner mitochondrial membrane:**

   These are of electron transfer, coupling as follows :

   (i) Respiratory chain enzymes namely NADH-dehydrogenase, Succinate- dehydrogenase, Coenzyme Q or ubiquinone, four cytochromes, cytochrome C- reductase.

   (ii) ATP synthetase oxidase

   (iii) P-hydroxy butyrate dehydrogenase

   (iv) Carnitine fatty acid acyl transferase

It has also glycerol-phosphate dehydrogenase, choline-dehydrogenase, and several carriers or translocators for permeation of phosphate, glutamate, asparate, ADP and ATP.Inner membrane contains a high concentration of cardiolipin (polyglycerophosphatides), which is important in all systems involving electron transport.

4. **Enzymes of mitochondrial matrix:**These are soluble and are involved in Kreb‘s cycle and fatty acid cycle. They include the following enzymes:

   (1) Fumarase and aconitase
   (2) Citrate synthetase
   (3) α-keto acid dehydrogenases
   (4) β-oxidation enzymes
   (5) L-malate dehydrogenase
   (6) L-glutamate dehydrogenase
   (7) Isocitrate dehydrogenase
   (8) Phosphatidic acid cytidyltransferase (CTP)
   (9) Nucleotides, K+, Mg+ +. Cl–, S04–, HPO4– etc.

The matrix also contains DNA, ribosomes and other RNA species and enzymes involved in the synthesis of protein.

Table: 8.0 Localization of enzymes, obtained in fractionation studies.(source: biologydiscussion.com)

Mitochondrial fraction	*Enzymes located*
1. Outer membrane.	Monoamine oxidase, "Rotenone-insensitive" NADH- cytochrome C reductase, Kynurenine hydroxylase, Fatty acid CoA ligase, Glycerolphosphate acyl transferase, Nucleoside diphosphokinase,
2. Inter-membrane space.	Adenylate kinase, Nucleoside diphosphokinase, Nucleoside monophosphokinase,
3. Inner membrane.	Respiratory chain enzymes. P.Hydroxybutyrate, dehydrogenase, Ferrochelatase, Camitine palm ityl-transferase, Fatty acid oxidation system, Xylitol dehydrogenase,
4. Matrix	Malate, isocitrate and glutamate dehydrogenase, Fumarase, Aconitase, Citrate synthetase, Omithine-Carbomyl transferase, Fatty acid oxidation systems, Pyruvate carboxylase,

Mitochondrial DNA (M-DNA):

Mitochondria contain one or more molecules of DNA which are circular in shape, highly twisted and measure about 5µ in length. It resembles bacterial DNA which is also circular in shape. DNA base ratio in mitochondrial DNA resemble those of prokaryotic cells, and is more than those of nuclear DNA. Mitochondrial DNA forms a loop from which replication begins and passes in one direction.

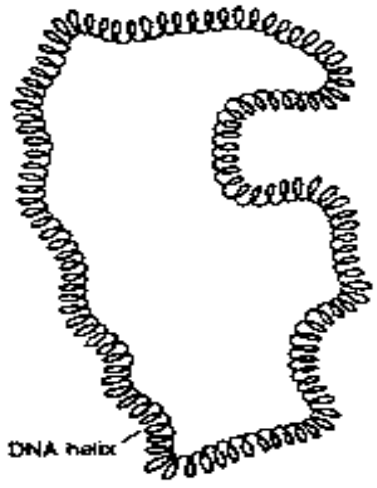


Fig:8.6 A mitochondrial DNA molecule extracted from rat liver mitochondrion . Note the circular shape which is similar to bacterial DNA.(source:V.B.Rastogi)

A large proportion of DNA may occur in looped form. However, only a small proportion of DNA replicates at any one time. Thus, replication is not synchronized and continues throughout the cell cycle and it is not in coordination with cyclic nuclear DNA replication. DNA synthesis is slower in mitochondrial than in nuclear DNA.

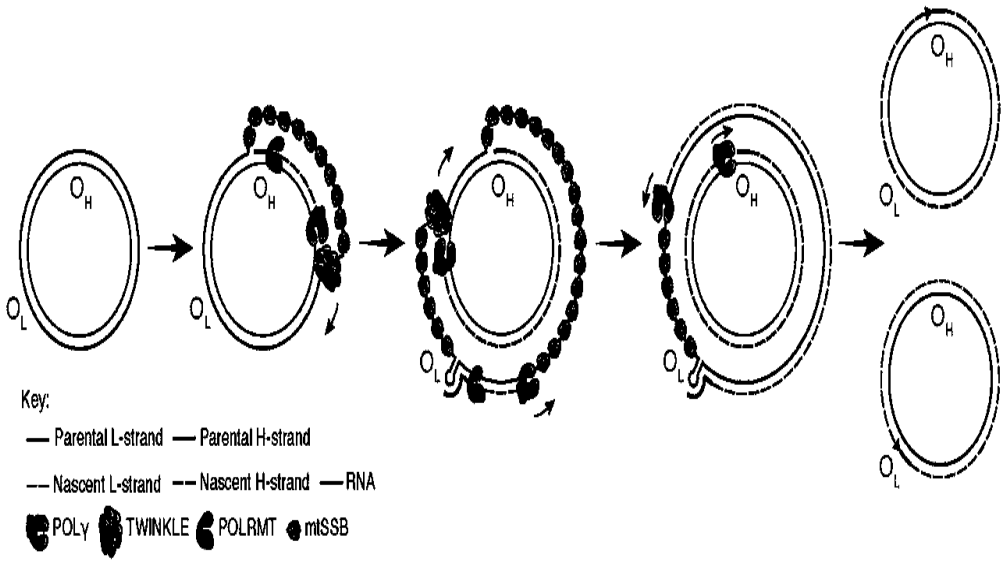


Fig:8.7 Diagram showing replication of mitochondrial DNA.(source:google.com)

Mitochondrial DNA codes for intrinsic ribosomal RNA, transfer RNA, ribosomal proteins and few other proteins, regulating enzymes and structure. Many of the mitochondrial proteins (especially enzymatic proteins) are coded by nuclear DNA.

Mitochondrion, inspite of having its own DNA is not independent of nucleus. Mitochondrial DNA is capable of mutation. M-DNA behaves like a chromosome and duplicates in usual manner into several circles. Due to DNA, mitochondria are capable to undergo self-reproduction and thus may exhibit cytoplasmic inheritance. M-DNA differs from nuclear DNA in several aspects such as :

(1) According to **Rabinowitch** (1968), mitochondrial DNA contains more guanine-cytosine (GC) contents than nuclear DNA and is of higher density.

(2) Denaturation or melting temperature of M-DNA is higher than the nuclear DNA.

(3) M-DNA is circular in shape as in bacteria and not coiled.

(4) Rate of renaturation of M-DNA is more.

(5) Molecular weight of M-DNA varies from 9 to 11 million.

Mitochondrial RNA- Similarly, there occurs mitochondrial RNA (M-RNA). It is different from RNA of nuclear origin as it is resistant to ribonuclease enzyme. It

is synthesized inside mitochondria on DNA template. Three species of M-RNA namely 23S, 16S and 4S have been isolated so far.

8.5 FUNCTION OF MITOCHONDRIA

The most prominent roles of mitochondria are to produce the energy currency of the cell, ATP (i.e., phosphorylation of ADP), through respiration, and to regulate cellular metabolism. The central set of reactions involved in ATP production are collectively known as the citric acid cycle, or the Krebs cycle. However, the mitochondrion has many other functions in addition to the production of ATP.

Energy Production- An important role for the mitochondria is the production of ATP, as reflected by the large number of proteins in the inner membrane . This is done by oxidizing the major products of glucose: pyruvate, and NADH, which are produced in the cytosol by glycolysis. This type of cellular respiration known as aerobic respiration, is dependent on the presence of oxygen. When oxygen is limited, the glycolytic products will be metabolized by anaerobic fermentation, a process that is independent of the mitochondria. The production of ATP from glucose has an approximately 13-times higher yield during aerobic respiration compared to fermentation.

Pyruvate and the citric acid cycle- Pyruvate molecules produced by glycolysis are actively transported across the inner mitochondrial membrane, and into the matrix where they can either be oxidized and combined with coenzyme A to form CO_2, acetyl-CoA, and NADH,or they can be carboxylated (by pyruvate carboxylase) to form oxaloacetate. This latter reaction ‖fills up‖ the amount of oxaloacetate in the **citric acid cycle**, and is therefore an anaplerotic reaction, increasing the cycle‘s capacity to metabolize acetyl-CoA when the tissue's energy needs (e.g. in muscle) are suddenly increased by activity.

In the citric acid cycle, all the intermediates (e.g. citrate, iso-citrate, alpha-ketoglutarate, succinate, fumarate, malate and oxaloacetate) are regenerated during each turn of the cycle. Adding more of any of these intermediates to the mitochondrion therefore means that the additional amount is retained within the cycle, increasing all the other intermediates as one is converted into the other. Hence, the addition of any one of them to the cycle has an anaplerotic effect, and its removal has a cataplerotic effect. These anaplerotic and cataplerotic reactions will, during the course of the cycle, increase or decrease the amount of oxaloacetate available to combine with acetyl-CoA to form citric acid. This in turn increases or decreases the rate of ATP production by the mitochondrion, and thus the availability of ATP to the cell.

Acetyl-CoA, on the other hand, derived from pyruvate oxidation, or from the beta-oxidation of fatty acids, is the only fuel to enter the citric acid cycle. With each turn of the cycle one molecule of acetyl-CoA is consumed for every molecule of oxaloacetate present in the mitochondrial matrix, and is never regenerated. It is the oxidation of the acetate portion of acetyl-CoA that produces CO_2 and water, with the energy thus released captured in the form of ATP.

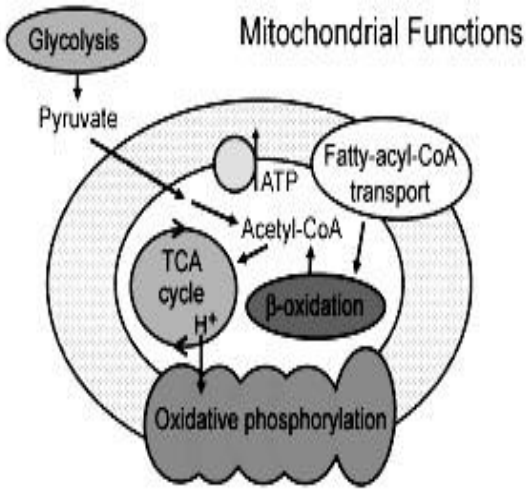


Fig:8.8 Diagram showing the energy production in mitochondria.

Q.i) How ATP produced in mitochondria ?

Q.ii) What does mitochondrial DNA do ?

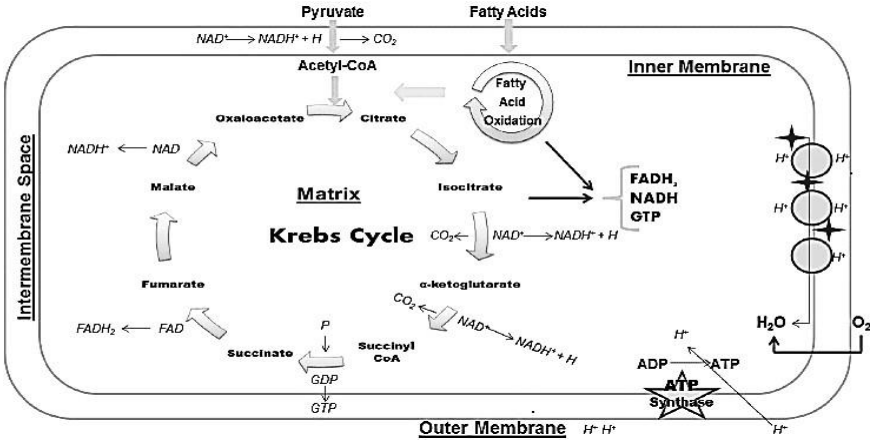


Fig:8.9 Diagrammatic representation of scheme of the structure and function of mitochondria.(source:google.com)

In the liver, the carboxylation of cytosolic pyruvate into intra-mitochondrial oxaloacetate is an early step in the gluconeogenic pathway, which converts lactate and de-aminated alanine into glucose, under the influence of high levels of glucagon and/or epinephrine in the blood. Here, the addition of oxaloacetate to the mitochondrion does not have a net anaplerotic effect, as another citric acid cycle intermediate (malate) is immediately removed from the mitochondrion to be converted into cytosolic oxaloacetate, which is ultimately converted into glucose, in a process that is almost the reverse of glycolysis. The enzymes of the citric acid cycle are located in the mitochondrial matrix, with the exception of succinate dehydrogenase, which is bound to the inner mitochondrial membrane as part of Complex II. The citric acid cycle oxidizes the acetyl-CoA to carbon dioxide, and, in the process, produces reduced cofactors

(three molecules of NADH and one molecule of $FADH_2$) that are a source of electrons for the electron transport chain, and a molecule of GTP (that is readily converted to an ATP).

NADH and $FADH_2$: the electron transport chain- Electron transport chain in the mitochondrial intermembrane space

The redox energy from NADH and $FADH_2$ is transferred to oxygen (O_2) in several steps via the electron transport chain. These energy-rich molecules are produced within the matrix via the citric acid cycle but are also produced in the cytoplasm by glycolysis. Reducing equivalents from the cytoplasm can be imported via the malate-aspartate shuttle system of antiporter proteins or feed into the electron transport chain using a glycerol phosphate shuttle. Protein complexes in the inner membrane (NADH dehydrogenase (ubiquinone), cytochrome c reductase, and cytochrome c oxidase) perform the transfer and the incremental release of energy is used to pump protons (H+) into the intermembrane space. This process is efficient, but a small percentage of electrons may prematurely reduce oxygen, forming reactive oxygen species such as superoxide. This can cause oxidative stress in the mitochondria and may contribute to the decline in mitochondrial function associated with the aging process.
As the proton concentration increases in the intermembrane space, a strong electrochemical gradient is established across the inner membrane. The protons can return to the matrix through the ATP synthase complex, and their potential energy is used to synthesize ATP from ADP and inorganic phosphate (Pi).This process is called chemiosmosis, and was first described by **Peter Mitchell** who was awarded the 1978 Nobel Prize in Chemistry for his work. Later, part of the 1997 Nobel Prize in Chemistry was awarded to Paul D. Boyer and John E. Walker for their clarification of the working mechanism of ATP synthase.

Heat production- Under certain conditions, protons can re-enter the mitochondrial matrix without contributing to ATP synthesis. This process is known as proton leak or mitochondrial uncoupling and is due to the facilitated diffusion of protons into the matrix. The process results in the unharnessed potential energy of the proton electrochemical gradient being released as heat. The process is mediated by a proton channel called thermogenin, or UCP1Thermogenin is a 33 kDa protein first discovered in 1973.Thermogenin is primarily found in brown adipose tissue, or brown fat, and is responsible for non-shivering thermogenesis. Brown adipose tissue is found in mammals, and is at its highest levels in early life and in hibernating animals. In humans, brown adipose tissue is present at birth and decreases with age.

Storage of calcium ions- TEM of a chondrocyte, stained for calcium, showing its nucleus and mitochondria.The concentrations of free calcium in the cell can regulate an array of reactions and is important for signal transduction in the cell. Mitochondria can transiently store calcium, a contributing process for the cell's homeostasis of calcium.In fact, their ability to rapidly take in calcium for later release makes them very good "cytosolic buffers" for calcium.The endoplasmic reticulum (ER) is the most significant storage site of calcium,and there is a significant interplay between the mitochondrion and ER with regard to calcium. The calcium is taken up into the matrix by the mitochondrial calcium uniporter on

the inner mitochondrial membrane.It is primarily driven by the mitochondrial membrane potential.Release of this calcium back into the cell's interior can occur via a sodium-calcium exchange protein or via "calcium-induced-calcium-release" pathways. This can initiate calcium spikes or calcium waves with large changes in the membrane potential. These can activate a series of second messenger system proteins that can coordinate processes such as neurotransmitter release in nerve cells and release of hormones in endocrine cells.

Ca_2+ influx to the mitochondrial matrix has recently been implicated as a mechanism to regulate respiratory bioenergetics by allowing the electrochemical potential across the membrane to transiently "pulse" from $\Delta\Psi$-dominated to pH-dominated, facilitating a reduction of oxidative stress. In neurons, concomitant increases in cytosolic and mitochondrial calcium act to synchronize neuronal activity with mitochondrial energy metabolism. Mitochondrial matrix calcium levels can reach the tens of micromolar levels, which is necessary for the activation of isocitrate dehydrogenase, one of the key regulatory enzymes of the Krebs cycle.

Additional functions- Mitochondria play a central role in many other metabolic tasks, such as:

Signaling through mitochondrial reactive oxygen species

Regulation of the membrane potential

Apoptosis-programmed cell death

Calcium signaling (including calcium-evoked apoptosis)

Regulation of cellular metabolism

Certain heme synthesis reactions

Steroid synthesis.

Hormonal signaling- Mitochondria are sensitive and responsive to hormones, in part by the action of mitochondrial estrogen receptors (mtERs). These receptors have been found in various tissues and cell types, including brain and heart . Some mitochondrial functions are performed only in specific types of cells. For example, mitochondria in liver cells contain enzymes that allow them to detoxify ammonia, a waste product of protein metabolism. A mutation in the genes regulating any of these functions can result in mitochondrial diseases.

Cellular proliferation regulation- The relationship between cellular proliferation and mitochondria has been investigated using cervical cancer HeLa cells. Tumor cells require an ample amount of ATP (Adenosine triphosphate) in order to synthesize bioactive compounds such as lipids, proteins, and nucleotides for rapid cell proliferation. The majority of ATP in tumor cells is generated via the oxidative phosphorylation pathway (OxPhos).Interference with OxPhos have shown to cause cell cycle arrest suggesting that mitochondria play a role in cell proliferation. Mitochondrial ATP production is also vital for cell division in addition to other basic functions in the cell including the regulation of cell volume, solute concentration, and cellular architecture. ATP levels differ at various stages of the cell cycle suggesting that there is a relationship between the

abundance of ATP and the cell's ability to enter a new cell cycle. ATP's role in the basic functions of the cell make the cell cycle sensitive to changes in the availability of mitochondrial derived ATP. The variation in ATP levels at different stages of the cell cycle support the hypothesis that mitochondria play an important role in cell cycle regulation. Although the specific mechanisms between mitochondria and the cell cycle regulation is not well understood, studies have shown that low energy cell cycle checkpoints monitor the energy capability before committing to another round of cell division.

8.6 MITOCHONDRIA AS SEMI-AUTONOMOUS CELL ORGANELLES

Autonomous means anyone who has full authority for doing anything **but** semi-autonomous means restricted authority is there. As we know that:

1. Mitochondria have their own DNA which can replicate independently. The mitochondrial DNA produces its own mRNA, tRNA and rRNA**.**
2. The organelles possess their own **ribosomes,** called **mitoribosomes.**
3. Mitochondria synthesize some of their own structural proteins. However, most of the mitochondrial proteins are synthesized under instructions from cell nucleus.
4. The organelles synthesize some of the enzymes required for their functioning. e.g. succinate dehydrogenase.
5. They show **hypertrophy** .i.e. internal growth.

However, both their structure and functioning are **controlled by the nucleus of the cell and availability of materials from the cytoplasm**. Hence, they are termed as the **'semi-autonomous'** cell organelles

Mitochondria itself has its own DNA so it can produce some proteins required to do some functions but still it depends on the nuclear DNA for other important proteins to carry out its functions. As it can replicate independently so it is autonomous but still depends on the nuclear DNA that's why the term is used semi-autonomous body.

8.7 SUMMARY

- Mitochondria are found in all eukaryotic cells. They may be lost in the later stages of the development (red blood cells and phloem sieve tubes).
- The mitochondrion is a double-membraned, rod-shaped structure found in both plant and animal cell. Its size ranges from 0.5 to 1.0 micrometre in diameter. The structure comprises an outer membrane, an inner membrane, and a gel-like material called the matrix. The outer membrane and the inner membrane are made of proteins and phospholipid layers separated by the intermembranespace.The outer membrane covers the surface of the mitochondrion and has a large number of special proteins

known as porins. It is freely permeable to ions, nutrient molecules, energy molecules like the ADP and ATP molecules.

- Mitochondria are the sites of aerobic respiration. They produce cellular energy in the form of ATP. Hence, they are called power houses of the cell.
- The most important function of mitochondria is to produce energy through the process of **oxidative phosphorylation.** It is also involved in the following process:

  1. Regulates the metabolic activity of the cell
  2. Promotes the growth of new cells and cell multiplication
  3. Helps in detoxifying ammonia in the liver cells
  4. Plays an important role in apoptosis or programmed cell death
  5. Responsible for building certain parts of the blood and various hormones like testosterone and oestrogen
  6. Helps in maintaining an adequate concentration of calcium ions within the compartments of the cell
  7. It is also involved in various cellular activities like cellular differentiation, cell signalling, cell senescence, controlling the cell cycle and also in cell growth.

- Mitochondria are partly independent or semi-autonomous as they can manufacture some of the proteins required for their functioning with the help of their DNA, RNAs, enzymes and ribosomes and obtained the others from the cytoplasm formed under the control of nuclear DNA.

8.8 TERMINAL QUESTIONS

Q1) Describe ultrastructure of mitochondria.

……
……
……

Q 2) Which of the mitochondrial enzymes are present in the matrix.

……
……
……

Q 3) Why mitochondria termed as the ―power houses‖ of the eukaryotic cells?

……
……
……

Q 4) What is the major function of mitochondria?

……
……
……

Q 5) What are the chemical composition of mitochondria ?

……
……
……

Q 6) Why are mitochondria considered to be semi-autonomous bodies ?

……
……
……

Multiple choice Questions

i) Which is a function of mitochondria?

a. Regulating metabolism

b. Producing ATP

c. Storing calcium

d. All of the above

ii) Which is NOT a reason why mitochondria are thought to have evolved from free-living bacteria?

a. Mitochondria have their own DNA.

b. reproduce through binary fission.

c. Mitochondrial DNA is inherited from the mother.

d. The genome is similar to that of bacterial DNA.

iii) Where is the mitochondrial matrix located?

a. Within the inner membrane

b. Between the inner and outer membrane

c. Inside the mtDNA

d. In the intermembrane space

iv) The site of aerobic respiration in eukaryotic cells is

a. Peroxisome

b. Plastid

c. Mitochondria

d. Cilia

v) How do the small molecules pass through the outer membrane of mitochondria?

a. ATPpump

b. Carrierprotein

c. Channel

d. Porins

vi) Mitochondria arises

a. By growth and division of pre-existing mitochondria

b. From non mitochondrial membrane

c. From precursors of the cytoplasm

d. None of the above

vii) The site for cellular respiration is

a. Nucleus

b. Ribosome

c. Mitochondria

d. Endoplasmic Reticulum

viii) Following in which mitochondria is absent ?

a. Muscle fiber

b. RBC

c. Renal tubular cells

d. Liver cells

ix) Who introduced the name Mitochondria first ?

a. James Long

b. Benda

c. Pasture

d. Harvard

x) On which factor number of mitochondria depends ?

a. Energy requirement of the cell

b. Size of the plant and animal

c. Combination of plasma membrane

d. Requirement of secretion

xi) Life spane of mitochondria is

a. 1 to 2 years

b. 2 to 4 years

c. 4 to 10 years

d. 10 to 14 years

xii) Percentage of RNA in a mitochondria

a. 1%

b. 5%

c. 1.5%

d. 0.5%

xiii) What is the purpose of the cristae ?

a. To make oxygen

b. To protect mitochondria

c. To increase surface area of inner membrane

d. To make protein

xiv) MitochondrialDNA is a

a. Simple, single stranded linear DNA molecule

b. Simple, single stranded circular DNA molecule

c. Simple, double stranded linear DNA molecule

d. Simple, double stranded circular DNA molecule

xv) Oxysomes or F0 – F1 particles occur on

a. Inner mitochondrial membrane

b. Chloroplast surface

c. Mitochondrial surface

d. Thylakoids

8.9 ANSWERS

i) d, ii) c, iii) a, iv) c, v) d, vi) a, vii) c, viii) b, ix) b, x) a, xi) c, xii) d, xiii) c, xiv) d, xv) a.

ROUGH WORK