CELL BIOLOGY

M.Sc. BOTANY

SEMESTER-II, PAPER-III

LESSON WRITERS

Prof. A. Amruthavalli
Professor
Department of Botany & Microbiology,
Acharya Nagarjuna University

Prof. K. Mallikarjuna
Professor
Department of Botany & Microbiology,
Acharya Nagarjuna University

Dr. Y.R.K.V. Tirupati Rao
Assistant Professor (C)
Department of Botany & Microbiology,
Acharya Nagarjuna University

Dr. K. Babu
Faculty
Department of Botany & Microbiology,
Acharya Nagarjuna University

LESSON WRITER & EDITOR

Prof. V. Umamaheswara Rao
Professor
Department of Botany & Microbiology,
Acharya Nagarjuna University

ACADEMIC ADVISOR

Prof. A. Amruthavalli
Professor
Department of Botany & Microbiology,
Acharya Nagarjuna University

DIRECTOR, I/c.

Prof. V. Venkateswarlu
M.A., M.P.S., M.S.W., M.Phil., Ph.D.
Professor

CENTRE FOR DISTANCE EDUCATION
ACHARYA NAGARJUNA UNIVERSITY
Nagarjuna Nagar 522 510
Ph: 0863-2346222, 2346208
0863-2346259 (Study Material)
Website www.anucde.info
E-mail: anucdedirector@gmail.com

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Prof. V. VENKATESWARLU
Director, I/c
Centre for Distance Education,
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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
M.Tech., Ph.D.,
Vice-Chancellor I/c
Acharya Nagarjuna University.

M.Sc. BOTANY
SEMESTER-II, PAPER-III
203BO24-CELL BIOLOGY
SYLLABUS

UNIT-I

Eukaryotic Cell: Organelles Chloroplast, Mitochondria, Ribosomes, Endoplasmic reticulum, Peroxisomes, Golgi apparatus, Lysosomes and plant vacuoles and Cytoskeleton.

UNIT-II

Nucleus; Ultrastructure of Prokaryotic and Eukaryotic Chromosome; chromosome banding; Karyotype; Euchromatin and heterochromatin. Special types of Chromosomes: Polytene, Lamp-brush, B-chromosomes, and Sex- chromosomes.

UNIT-III

Phases of Cell Cycle: G1, S, G2 and M phases, Check points in cell cycle - Role of cyclins; Cyclin dependent kinases; Cell division; significance of meiosis.

UNIT-IV

Apoptosis - Mechanism and Significance, oncogene and tumour suppressor genes. Genomes of mitochondria and chloroplasts. Endosymbiotic Theory.

UNIT-V

Structural Alteration in Chromosomes -Origin, Duplications, Deletions, Inversions and Translocations.

Numerical Alteration in Chromosomes: Origin, Occurrence of Haploids, Polyploids and ANEUPLOIDs.

TEXT BOOKS:

1) Arun Kumar Sharma and Archana Sharma, 1980. Chromosome Techniques. Theory and Practice. Third Edition, Butterworth - Heinemann, London.
2) Brown, W.V. and Berke, E.M. 1975.Text Book of Cytology, Saint Louis: CV Mosby Company, USA.
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(203BO24)

MODEL QUESTION PAPER

M.Sc. DEGREE EXAMINATION,
BOTANY - SECOND SEMESTER
CELL BIOLOGY

Time: Three hours **Maximum: 70 marks**

Answer All Questions **5 × 14 = 70M**

UNIT-I

1) a) Give a detailed note on the structure and functions of the Chloroplast.

OR

b) Give a detailed note on the structure and functions of the Mitochondria.

UNIT-II

2) a) Give a detailed note on the structure and functions of the Nucleus.

OR

b) Give a detailed note on a special type of chromosome.

UNIT-III

3) a) Give a detailed note on the different phases of the cell cycle.

OR

b) Give a detailed note on the different checkpoints in the cell cycle.

UNIT-IV

4) a) Give a detailed note on Apoptosis.

OR

b) Give a detailed note on the Endosymbiotic theory.

UNIT-V

5) a) Give a detailed note on structural alterations in chromosomes

OR

b) Give a detailed note on numerical alterations in chromosomes.

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LESSON-1

ULTRASTRUCTURE OF EUKARYOTIC CELL, CELL WALL AND CELL MEMBRANE

1.0 OBJECTIVE:

- Students will understand the internal structure of cell in brief and the role of cell wall and plasma membrane in protecting the cell and its internal components.

STRUCTURE:

1.1 Introduction

1.2 Ultrastructure of Eukaryotic Cell

1.2.1 Cellular Components

1.2.2 Functions of a Cell

1.3 Cell wall

1.3.1 Ultrastructure

1.3.2 Functions of Cell Wall

1.4 Plasma Membrane

1.4.1 Ultrastructure

1.4.2 Functions of Plasma Membrane

1.5 Summary

1.6 Technical Terms

1.7 Self-Assessment Questions

1.8 Suggested Readings

1.1. INTRODUCTION:

The biological science which deals with the study of structure, function, growth and reproduction along with molecular organization is called cytology (Gr., *kytos* = hollow vessel or cell; *logous* = to discourse) or cell biology. The body of all living organisms except viruses has cellular organization and may contain one or many cells. The organisms with single cell are called unicellular organisms (e.g., bacteria, blue green algae, some algae, Protozoa, etc.). The organisms with many cells in their body are called multicellular organisms (*e.g.*, most plants and animals). Cells are of two types based on the presence or absence of nuclear membranes: A. Prokaryotic cells B. Eukaryotic cells. The prokaryotic (Gr., *pro* = primitive or before; *karyon* = nucleus) are small, simple and most primitive. The eukaryotic cells (Gr., *eu*=good, *karyotic*=nucleated) are having nuclear membrane around the chromatin material. The eukaryotic cells are the true cells which occur in the plants (algae to angiosperms) and the animals (Protozoa to mammals).

The plant cell is always surrounded by a cell wall and this feature distinguishes them from animal cells. The cell wall is a non-living structure which is formed by the living protoplast. In most of the plant cells, the cell wall is made up of cellulose, hemicellulose, pectin and protein. In many fungi, the cell wall is formed of chitin and in bacteria; the cell wall contains protein-lipid-polysaccharide complexes. Plasma membrane encloses every type of cell, both prokaryotic and eukaryotic cells. Plasma membrane is an ultrathin, elastic, living, dynamic and selective transport-barrier. It is a fluid-mosaic assembly of molecules of lipids (phospholipids and cholesterol), proteins and carbohydrates. Plasma membrane controls the entry of nutrients and exit of waste products, and generates differences in ion concentration between the interior and exterior of the cell. It also acts as a sensor of external signals and allows the cell to react or change in response to environmental signals.

1.2. ULTRA STRUCTURE OF EUKARYOTIC CELL

1.2.1. Cellular Components

A eukaryotic cell is a complex structure having true nucleus and cell organelles along with large number of proteins, enzymes and minerals. The eukaryotic cell contains the following components.

Cell Wall and Plasma Membrane:

The outermost structure of most plant cells is a dead and rigid layer called cell wall. It is mainly composed of carbohydrates such as cellulose, pectin, hemicellulose and lignin and certain fatty substances like waxes. There is a pectin-rich cementing substance between the walls of adjacent cells which is called middle lamella. The cell wall which is formed immediately after the division of cell, constitutes the primary cell wall. Primary cell wall is composed of pectin, hemicellulose and loose network of cellulose microfibrils. In certain types of cells such as phloem and xylem, an additional layer is added to the inner surface of the primary cell wall at a later stage. This layer is called secondary cell wall and it consists mainly of cellulose, hemicellulose and lignin. In many plant cells, there are tunnels running through the cell wall called plasmodesmata which allow communication with the other cells in a tissue. Every kind of animal cell is bounded by a living, extremely thin and delicate membrane called plasmalemma, cell membrane or plasma membrane. In plant cells, plasma membrane occurs just inner to cell wall, bounding the cytoplasm. The plasma membrane is a three-layered structure. The plasma membrane is a selectively permeable membrane and it selectively permits the entry or exit of materials.

Cytoplasm:

The plasma membrane is followed by the colloidal organic fluid called matrix or cytosol. The cytosol is the aqueous portion of the cytoplasm also known as the extra nuclear protoplasm) and of the nucleoplasm. The cytosol serves to dissolve the great variety of molecules concerned with cellular metabolism, e.g., glucose, amino acids, nucleotides, vitamins, minerals, oxygen and ions. The cytosol of cells also contains fibres that help to maintain cell shape and mobility; these fibres are termed as the cytoskeleton.

Endoplasmic reticulum (ER):

The cytoplasm contains an extensive network of membrane limited channels called endoplasmic reticulum (or ER). Some portion of ER membranes remains continuous with the

plasma membrane and the nuclear envelope. The outer surface of rough ER has attached ribosomes, whereas smooth ER does not have attached ribosomes. Functions of smooth ER include lipid metabolism, glycogenolysis and drug detoxification. On their membranes, rough ER (RER) contain certain ribosome specific, transmembrane glycoproteins, called ribophorins I and II, to which are attached the ribosomes while engaged in polypeptide synthesis.

Golgi Apparatus:

It is a cup-shaped organelle which is located near the nucleus. Golgi apparatus consists of a set of smooth cisternae present in stacks in parallel rows. It is surrounded by spherical membrane bound vesicles which appear to transport proteins. Plant cells contain many freely distributed sub-units of Golgi apparatus, called dictyosomes, secreting cellulose and pectin for cell wall formation during the cell division.

Lysosomes:

The cytoplasm of animal cells contains many tiny, spheroid or irregular-shaped, membrane-bounded vesicles known as lysosomes. The lysosomes are originated from Golgi apparatus and contain numerous hydrolytic enzymes for intracellular and extracellular digestion. Lysosomes have a high acidic medium (pH 5) and this acidification depends on ATP- dependent proton pumps. The lysosomes of plant cells are membrane-bounded storage granules containing hydrolytic digestive enzymes, E.g. vacuoles of parenchymatous cells.

Cytoplasmic Vacuoles:

The cytoplasm of many plant and some animal cells (*i.e.,* ciliate protozoans) contains numerous small or large-sized, hollow, liquid-filled structures, the vacuoles. These vacuoles are supposed to be greatly expanded endoplasmic reticulum or Golgi apparatus. The vacuoles of animal cells are bounded by a lipoproteinous membrane and their function is the storage, transmission of the materials and the maintenance of internal pressure of the cell. The vacuoles of the plant cells are bounded by a single, semipermeable membrane known as tonoplast. These vacuoles contain water, phenol, flavonols, anthocyanins, alkaloids and storage products, such as sugars and proteins.

Peroxisomes:

These are tiny circular membrane bound organelles containing a crystal-core of enzymes i.e., urate oxidase, peroxidase, D-amino oxidase and catalase. These enzymes are required by peroxisomes in detoxification activity. Peroxisomes are also related with β-oxidation of fatty acids and also in degradation of the amino acids. In green leaves of plants, peroxisomes carry out the process of photorespiration.

Glyoxysomes:

These organelles develop in a germinating plant seed to utilize stored fat of the seed. Glyoxysomes consist of an amorphous protein matrix surrounded by a limiting membrane. The membrane of glyoxysomes originates from the ER and their enzymes are synthesized in

the free ribosomes in the cytosol. Enzymes of glyoxysomes are used to transform the fat stores of the seed into carbohydrates by way of glyoxylate cycle.

Mitochondria:

Mitochondria are oxygen-consuming ribbon-shaped cellular organelles of immense importance. Each mitochondrion is bounded by two unit membranes. The outer mitochondrial membrane resembles more with the plasma membrane in structure and chemical composition. Inner mitochondrial membrane is rich in many enzymes, coenzymes and other components of electron transport chain. Inner mitochondrial membrane gives out finger-like outgrowths (cristae) towards the lumen of mitochondrion and contains tennis-racket shaped F1 particles which contain ATPase enzyme for ATP synthesis. Mitochondria also contain in their matrix single or double circular and double stranded DNA molecules, called mt DNA and also the 55S ribosomes, called mitoribosomes. Since mitochondria can synthesize 10 per cent of their proteins in their own protein-synthetic machinery, they are considered as semi-autonomous organelles.

Plastids:

Plastids occur only in the plant cells. They contain pigments and may synthesize and accumulate various substances. Plastids are of different types: 1. Leucoplasts - are colourless plastids lacking thylakoids and ribosomes. 2. Amyloplasts – produce starch. 3. Proteinoplasts - accumulate protein. 4. Oleosomes or elaioplasts - store fats and essential oils. 5. Chromoplasts - contain pigment molecules and are coloured organelles. Chromoplasts impart a variety of colours to plant cells, fruits and petals. Chloroplasts are a type of plastids contains chlorophyll pigment.

Chloroplasts:

These involved in the process of photosynthesis. Chloroplasts have diverse shapes in green algae but are round, oval or discoid in shape in higher plants. Each chloroplast is bounded by two membranous envelopes. Chloroplast contains third system of membranes within the boundary of inner membrane, called grana. The grana form the main functional units of chloroplast and are present in the stroma matrix. Stroma contains a variety of photosynthetic enzymes and starch grains. Grana are stacks of membrane-bounded, flattened discoid sacs, arranged like neat piles of coins. They contain DNA, ribosomes and complete protein synthetic machinery.

Ribosomes:

Ribosomes are tiny spheroidal dense particles. Ribosome granules may exist either in the free state in the cytosol e.g. basal epidermal cells or attached to RER E.g.: pancreatic acinar cells, plasma cells, lymphocytes and osteoblasts. 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 viz., S1, S2, S3, 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, viz., 28S rRNA, 5.8S rRNA and 5S rRNA, and 40 proteins.

Microtubules:

Microtubules are found in the cytoplasm of all types of eukaryotic cells. They are long fibres about 24 nm in diameter. In cross section, the wall of a microtubule is made up of 13 globular subunits, called protofilaments, about 4 to 5 nm in diameter. Chemically, microtubules are composed of two kinds of protein subunits α-tubulin (tubulin A) and β-tubulin (tubulin B). The wall of a microtubule is made up of a helical array of repeating α and β tubulin subunits. Various cell organelles are derived from special assemblies of microtubules, which include Cilia and flagella, Basal bodies and centrioles.

Nucleus:

The nucleus is centrally located and spherical cellular component which controls all the vital activities of the cytoplasm and carries the hereditary material the DNA in it. The nucleus consists of 1. Chromatin-chromatin carries genes and is of two types, a) Euchromatin, which is well-dispersed form of chromatin which takes lighter DNA-stain and is genetically active b) Heterochromatin, which is the highly condensed form of chromatin which takes dark DNA-stain and is genetically inert. 2. Nuclear envelope and nucleoplasm - Nuclear envelope comprises two nuclear membranes i.e. an inner nuclear membrane which is lined by nuclear lamina and an outer nuclear membrane which is continuous with rough ER. At certain points the nuclear envelope is interrupted by structures called pores or nucleopores. Nuclear pores contain octagonal pore complexes which regulate exchange between the nucleus and cytoplasm. The nuclear envelope binds the nucleoplasm which is rich in those molecules which are needed for DNA replication, transcription, regulation of gene actions and processing of various types of newly transcribed RNA molecules (i.e., tRNA, mRNA and other types of RNA). 3. Nucleolus - Nucleus contains in its nucleoplasm a conspicuous, darkly stained, circular sub-organelle, called nucleolus. Nucleolus lacks any limiting membrane and is formed during interphase by the ribosomal DNA (rDNA) of nucleolar organizer (NO). Nucleolus is the site where ribosomes are manufactured. The ultrastructure of plant and animal cells is depicted in Figures 1.1a and 1.1b.

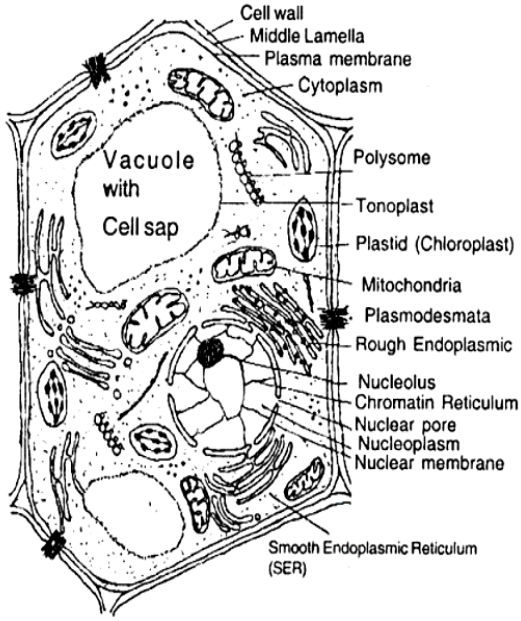

Figure-1.1a: Ultrastructure of Plant Cell

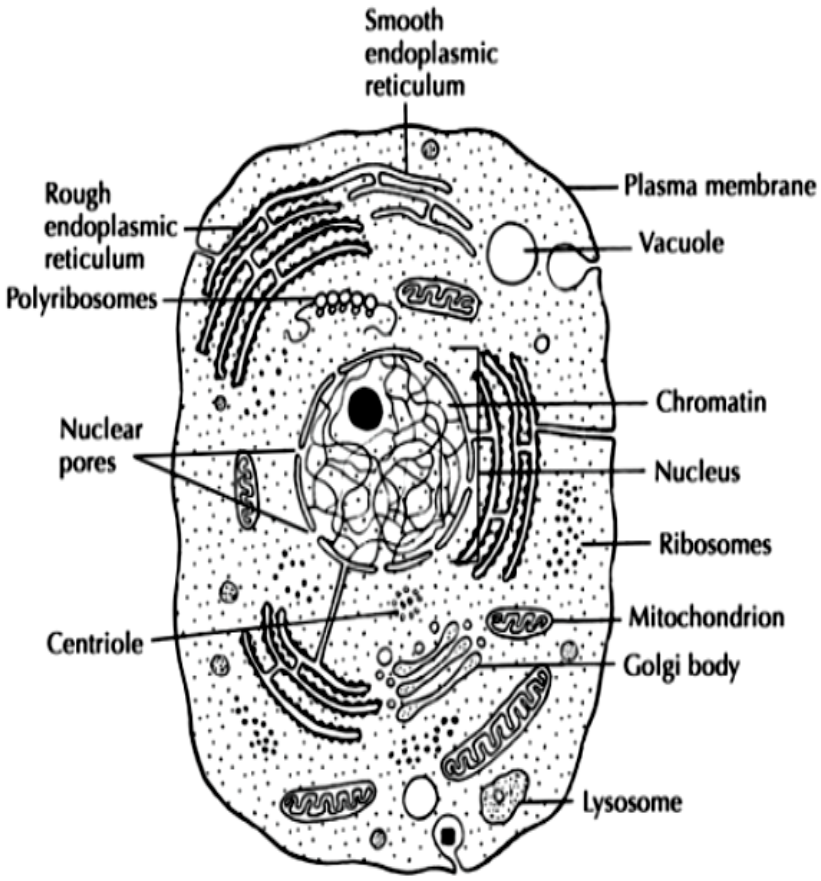

Figure-1.1b: Ultrastructure of Animal Cell

1.2.2. Functions of a Cell

Selective Permeability: Regulates the entry and exit of substances, allowing essential nutrients in and waste out.

Protection and Support: Acts as a barrier, protecting the cell from its surroundings and maintaining its structural integrity.

Cell Communication: Contains receptors that detect chemical signals (hormones, neurotransmitters) and initiate cellular responses.

Transport of Molecules: Facilitates movement through 1. Passive transport (diffusion, osmosis, facilitated diffusion – no energy required). 2. Active transport (requires energy, e.g., sodium-potassium pump). 3. Endocytosis (engulfing substances) and exocytosis (expelling substances).

Cell Recognition: Glycoproteins and glycolipids on the membrane help in immune recognition and interaction with other cells.

Cell Adhesion: Helps cells stick together to form tissues and communicate through junctions like tight junctions, gap junctions, and desmosomes.

Signal Transduction: Converts external signals into internal responses, influencing processes like growth, metabolism, and cell differentiation.

1.3. CELL WALL

1.3.1. Ultrastructure:

The cell wall is a rigid and protective layer around the plasma membrane which provides the mechanical support to the cell. The cell wall also determines the shape of plant cells. Due to the shape of cell walls many types of plant cell as the parenchymatous, collenchymatous, etc., have been recognized.

Chemical Composition:

Chemically, the plant cell wall is composed of a variety of polysaccharides (carbohydrates), lipids, proteins and mineral deposits; all exhibiting distinct staining reactions. The polysaccharides of cell wall include cellulose, hemicelluloses, pectin compounds and lignins.

1) **Cellulose:** It is a linear, unbranched polymer, consisting of straight polysaccharide chains made of glucose units linked by 1-4 β- bonds. These are the glucan chains which by intra-and intermolecular hydrogen bonding produce the structural units known as microfibrils, observable under electron microscopy and having toughness like the rubber. Each microfibril is ribbon-like flat fiber being 10 nm wide and 3 nm thick (or 25 to 30 nm in diameter) and is composed of about 2000 glucan chains in it. According to a classical estimate, each cellulose microfibril comprises three micelles or elementary fibrils: each elementary fibril contains about 100 cellulose molecules and each cellulose molecule is made up of 40 to 70 glucan chains (i.e., One microfibril = 3 × 100 × 70 = 21000 glucan chains). Often numerous microfibrils get associated to form the macrofibrils having up to 0.5 μm diameter and observable under the light microscopy. Cellulose is synthesized by a wide variety of cells that include bacteria, algae, fungi, cryptogams and seed plants.
2) **Hemicelluloses:** These are short but branched heteropolymers of various kinds of monosaccharides such as arabinose, xylose, mannose, galactose, glucose and uronic acid. Some of the common hemicelluloses go under the names xylans, arabinoxylans, glucomannans, galactomannans and xyloglucans.
3) **Pectins:** Pectins are water soluble, heterogeneous branched polysaccharides that contain many negatively charged D-galacturonic acid residues along with D-glucuronic acid residues. Because of their negative charge, pectins are highly hydrated and intensely bind cations. When Ca^{2+} is added to a solution of pectin molecules, it cross-links them to produce a semi rigid gel. Such Ca^{2+} cross-links are thought to help hold cell-wall component together.
4) **Mannan:** It is a homopolysaccharide of mannose and is found in the cell wall of yeast, fungi and bacteria.
5) **Agar:** It is a polysaccharide, found in the cell wall of sea weeds and containing D-and L-galactose residues.
6) **Lignin:** This is a biological plastic and non-fibrous material. It occurs only in mature cell walls and is made of an insoluble hydrophobic aromatic polymer of phenolic alcohols (*e.g.*, hydroxyphenyl propane).

7) **Chitin:** It is a polymer of glucosamine. Glycoproteins (present up to 10 per cent in primary cell wall) are hydroxyproline- rich proteins (like the collagen). In them, many short oligosaccharide side chains are attached to hydroxyproline and serine side chains. Thus, more than half the weight of glycoprotein is carbohydrate. These glycoproteins are known to act like the glue to increase the strength of the wall.
8) **Cutin:** It is also a biological plastic and is made of fatty acids (waxes).
9) **Suberin:** This is a water-resistant substance, comprising of fatty acids and found in the cork and cell wall of many plants.
10) **Sporopollenin:** It is a lipoidal polymer forming tough wall (with species-specific patterns) of pollen grains.
11) **Minerals:** These deposits occur in cuticle in the form of calcium and magnesium carbonates and silicates. Deposits of calcium compounds are found in the cell wall of cruciferous and cucurbitaceous plants. Silicate deposits are common in the cell wall of graminae family.

The cell wall is complex in nature and is differentiated in the following layers: (i) Primary cell wall; (ii) Secondary cell wall; (iii) Tertiary cell wall (Figure 1.2).

i) **Primary cell wall.** The first formed cell wall is known as primary cell wall. It is the outermost layer of the cell and in the immature meristematic and parenchymatous cells it forms the only cell wall. The primary cell is comparatively thin and permeable. Certain epidermal cells of the leaf and the stem also possess the cutin and cutin waxes which make the primary cell wall impermeable. The primary cell wall of the yeast and the fungi is composed of the chitin.
ii) **Secondary cell wall.** The primary cell wall is followed by secondary cell wall. The secondary cell wall is thick, permeable and lies near the plasma membrane of the tertiary cell wall, if the latter occurs. It is composed of three concentric layers (S1, S2 and S3) which occur one after another. Chemically the secondary cell wall is composed of compactly arranged macrofibrils of the cellulose, in between which sometimes occurs lignin as an interfibrillar material.
iii) **Tertiary cell wall.** In certain plant cells, there occurs another cell wall beneath the secondary cell wall which is known as tertiary cell wall. The tertiary cell wall differs from the primary and secondary cell wall in its morphology, chemistry and staining properties. Besides the cellulose, the tertiary cell wall consists of another chemical substance known as the xylan.

Middle Lamella:

The cells of plant tissues generally remain cemented together by an intercellular matrix known as the middle lamella. The middle lamella is mainly composed of the pectin, lignin and some proteins.

Plasmodesmata:

Every living cell in a higher plant is connected to its living neighbours by fine cytoplasmic channels, each of which is called a plasmodesma (Gr., *desmos* = ribbon, ligament; plural, plasmodesmata) which pass through the intervening cell walls. The plasma membrane of one cell is continuous with that of its neighbour at each plasmodesma. A

plasmodesma is a roughly cylindrical, membrane-lined channel with a diameter of 20 to 40 nm. Running from cell to cell through the centre of most plasmodesmata is a narrower cylindrical structure, the desmotubule, which remains, continuous with elements of the SER membranes of each of the connected cells. Between the outside of the desmotubule and the inner face of the cylindrical plasma membrane is an annulus of cytosol, which often appears to be constricted at each end of the plasmodemata. These constrictions may regulate the flux of molecules through the annulus that joins the two cytosols. Plasmodesmata are formed around the elements of smooth endoplasmic reticulum that become trapped during cytokinesis (of mitotic cell division) within the new cell wall that will bisect the parental cell. Plasmodesmata function in intercellular communication, *i.e*., they allow molecules to pass directly from cell to cell. For example, plasmodesmata are especially common and abundant in the walls of columns of cells that lead toward sites of intense secretion, such as in nectar-secreting glands (trichomes of *Abutilon* nectaries). In such cells there may be 15 or more plasmodesmata per square micrometer of wall surface, whereas there is often less than 1 per square micrometer in other cell wall.

Lignification:

The structure of cell wall is stabilized by the deposition of lignin in the cell wall matrix. Such a process of lignification was required in connection with the transition from aquatic to the terrestrial plant life during organic evolution of plants. A lignified cell wall is composed of microfibrils of cellulose embedded in the matrix containing large amount of lignin. Usually the primary cell wall becomes more lignified than secondary cell wall.

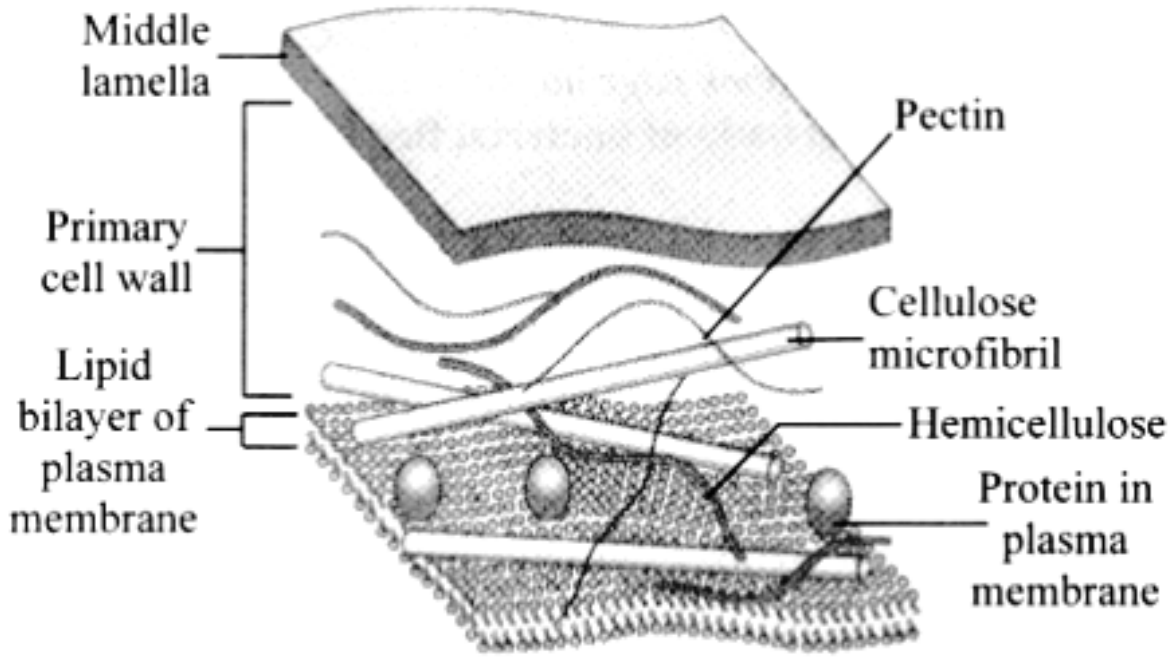

Figure-1.2: Structure of Plant Cell Wall

1.3.2. Functions of Cell Wall:

The plant cell wall is a rigid, protective, and supportive structure surrounding plant cells. It is primarily composed of cellulose, hemicellulose, pectin, and proteins, and it plays essential roles in plant growth, defense, and communication. Here are its detailed functions:

1) **Structural Support and Mechanical Strength:** Provides rigidity and shape to the plant cell, preventing it from collapsing. Supports the overall mechanical stability of the plant, allowing it to grow upright. Strengthens tissues such as wood (which contains lignin) to support large plants and trees.
2) **Regulation of Cell Expansion and Growth:** Controls the rate and direction of cell expansion, allowing controlled growth. The primary cell wall is flexible, accommodating growth, while the secondary cell wall (in mature cells) reinforces strength. Loosening and remodelling of cell wall components allow the cell to expand in specific directions.
3) **Protection against pathogens and Environmental stress:** Acts as a physical barrier against bacteria, fungi, viruses, and herbivores. Contains antimicrobial compounds (e.g., phytoalexins) that prevent infections. Protects against mechanical damage, UV radiation, and dehydration.
4) **Prevention of excessive water uptake (Osmotic Regulation):** Prevents cell bursting due to excessive water intake by exerting turgor pressure. Maintains osmotic balance by resisting excessive expansion. Works with the vacuole to regulate water pressure inside the cell.
5) **Facilitation of Cell-to-Cell communication:** Contains plasmodesmata, microscopic channels that connect adjacent plant cells. Allows for exchange of nutrients, hormones, and signalling molecules between cells. Supports coordinated plant responses to environmental signals.
6) **Molecular filtering and Selective permeability:** Acts as a selective barrier, permitting or restricting movement of molecules. The cell wall matrix filters out harmful substances while allowing beneficial compounds to pass.
7) **Wound healing and Regeneration:** When plant cells are damaged, the cell wall repairs itself by producing additional wall material. Wounded areas may become reinforced with callose and lignin to seal off infections.
8) **Storage of biochemicals and metabolites:** Some components of the cell wall, like pectins and hemicellulose, store nutrients and energy. Acts as a reservoir of signaling molecules that regulate plant responses.
9) **Facilitating Plant Cell Differentiation:** Determines the shape and function of plant cells by varying its composition and thickness. Specialized cells (e.g., xylem) develop lignified secondary walls for water conduction.
10) **Role in Photosynthesis and Gas Exchange:** In leaf epidermal cells, the cell wall allows gas exchange while preventing excessive water loss. Some plant cell walls contain specialized structures like stomata, which regulate CO_2 and O_2 exchange.

1.4. PLASMA MEMBRANE

1.4.1 Ultrastructure:

The plasma membrane is also called cytoplasmic membrane, cell membrane, or plasmalemma. The term cell membrane was coined by C. Nageli and C. Cramer in 1855 and the term plasmalemma has been given by J. Q. Plowe in 1931. Chemically, plasma membrane found to contain proteins, lipids and carbohydrates. The details are given here under.

1) **Lipids:** Four major classes of lipids are commonly present in the plasma membrane i.e. phospholipids, sphingolipids, glycolipids and sterols. The relative proportions of these lipids vary in different membranes. Phospholipids may be acidic phospholipids E.g.: sphingomyelin or neutral phospholipids (phosphatidyl choline, phosphatidylserine, etc).
2) **Proteins:** Plasma membrane contains about 50 per cent protein. According to their position in the plasma membrane, the proteins are of two main types integral or intrinsic proteins and peripheral or extrinsic proteins, both of which may be either ectoproteins or endoproteins. The intrinsic proteins tend to associate firmly with the membrane, while the extrinsic proteins have a weaker association and are bound to lipids of membrane by electrostatic interaction. On the basis of their functions, proteins of plasma membrane can also be classified into three main types- structural proteins, enzymes and transport proteins (permeases or carriers). Some of them may act as antigens, receptor molecules (e.g., insulin binding sites of liver plasma membrane), and regulatory molecules and so on. Structural proteins are extremely lipophilic and form the main bulk (i.e., backbone) of the plasma membrane.
3) **Carbohydrates:** Carbohydrates are present only in the plasma membrane. They are present as short, unbranched or branched chains of sugars (oligosaccharides) attached either to exterior ectoproteins or to the polar ends of phospholipids at the external surface of the plasma membrane. No carbohydrate is located at the cytoplasmic or inner surface of the plasma membrane. All types of oligosaccharides of the plasma membrane are formed by various combinations of six principal sugars i.e. D-galactose, D-mannose, L-fucose, N-acetylneuraminic acid, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine.

The Fluid Mosaic Model is the most widely accepted model describing the structure of the plasma membrane. It was proposed by Singer and Nicolson in 1972 and explains how the membrane is flexible and composed of various molecules that move dynamically (Figure 1.3).

Key Features of the Fluid Mosaic Model

1) **Fluid Nature:** The phospholipid bilayer behaves like a fluid, allowing lipids and proteins to move laterally. This flexibility helps the cell membrane adapt to different conditions.
2) **Mosaic Arrangement:** The membrane is a mosaic of proteins, lipids, and carbohydrates arranged asymmetrically. These molecules are not fixed but move freely within the bilayer.
3) **Phospholipid Bilayer:** Composed of phospholipids, which have a hydrophilic head (attracted to water) and hydrophobic tails (repelled by water). Forms a bilayer where the tails face inward and the heads face outward.

4) **Proteins in the Membrane: Integral proteins**: Embedded within the bilayer, some spanning across it (transmembrane proteins). Peripheral proteins: Loosely attached to the inner or outer surface. Functions: Transport, signaling, enzymatic activity, and cell communication.
5) **Cholesterol (in animal cells):** Helps maintain membrane fluidity and stability, preventing it from becoming too rigid or too fluid.
6) **Carbohydrates (Glycoproteins and Glycolipids):** Involved in cell recognition, signaling, and immune response.

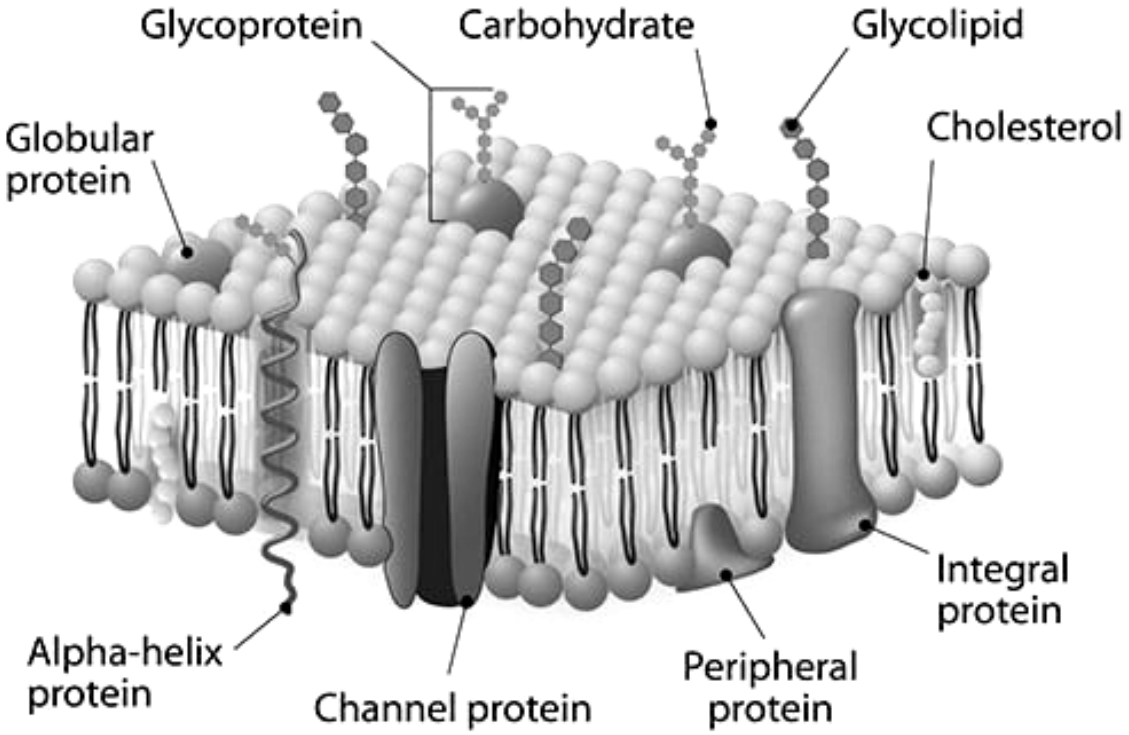

Figure-1.3: Structure of Plasma Membrane

1.4.2 Functions of Plasma Membrane

The plasma membrane acts as a thin barrier which separates the intra-cellular fluid or the cytoplasm from the extra-cellular fluid in which the cell lives. Though the plasma membrane is a limiting barrier around the cell but it performs various important physiological functions.

Functions of the Plasma Membrane

1) **Selective permeability (Regulation of transport):** Controls what enters and exits the cell. Allows essential nutrients (e.g., glucose, amino acids) to enter and waste products to exit. Uses passive transport (diffusion, osmosis) and active transport (using ATP and transport proteins).
2) **Cell communication and Signal transduction:** Receptor proteins detect and transmit signals from the environment. Allows cells to respond to hormones, neurotransmitters, and other signaling molecules. Helps in coordinating cellular activities.

3) **Structural support and Cell shape**: Maintains the cell's shape and provides mechanical support. Connects to the cytoskeleton inside the cell. Helps cells attach to each other and form tissues.
4) **Cell recognition and Immune response:** Glycoproteins and glycolipids on the membrane act as cell markers. Helps the immune system distinguish self from non-self cells (prevents autoimmune attacks). Important in organ transplant compatibility and blood type recognition.
5) **Endocytosis and Exocytosis (Bulk Transport):** Endocytosis: Engulfs large molecules or particles into the cell (e.g., phagocytosis, pinocytosis). Exocytosis: Removes waste or secretes substances like hormones and enzymes.
6) **Maintaining homeostasis:** Regulates the internal environment by balancing ion concentration, pH, and water levels. Prevents excessive loss or uptake of water (especially in osmosis).
7) **Intercellular connection and adhesion:** Helps cells adhere to each other in tissues. Forms tight junctions, gap junctions, and desmosomes for communication and support.

1.5. SUMMARY:

Cells are the basic units of life, with structures that support their function. The cell membrane surrounds and protects the cell, controlling the movement of substances in and out. Inside, the cytoplasm contains organelles such as the nucleus, which houses genetic material (DNA) and controls activities. The mitochondria generate energy, while the endoplasmic reticulum and Golgi apparatus help with protein and lipid processing. Ribosomes synthesize proteins, and lysosomes aid in digestion and waste removal. Plant cells also have a rigid cell wall, chloroplasts for photosynthesis, and a large central vacuole for storage. Together, these structures enable cells to grow, reproduce, and function efficiently. The plant cell wall is a rigid, protective layer surrounding the cell membrane, providing structural support, shape, and defense against pathogens. It is primarily composed of cellulose, hemicellulose, and pectin, forming a complex matrix that allows flexibility while maintaining strength. The wall is divided into the primary wall, which is thin and flexible in growing cells, and the secondary wall, which is thicker and more rigid in mature cells. Plasmodesmata, small channels in the wall, facilitate communication and transport between adjacent cells. The cell wall also plays a crucial role in water regulation, mechanical support, and plant growth.

The plasma membrane, also known as the cell membrane, is a selectively permeable barrier that surrounds the cell, regulating the movement of substances in and out. It is primarily composed of a phospholipid bilayer with embedded proteins, cholesterol, and carbohydrates, which contribute to its fluidity and functionality. The membrane plays a crucial role in cell communication, transport, and maintaining homeostasis. It utilizes passive and active transport mechanisms, including diffusion, osmosis, and protein-mediated transport, to control the exchange of nutrients, ions, and waste products. Additionally, it facilitates cell signaling and interaction with the external environment, making it essential for cellular function and survival.

1.6. TECHNICAL TERMS:

Cell organelles, Glycoproteins, Phopsholipids, Cell wall, Cellulose.

1.7 SELF ASSESSMENT QUESTIONS:

1) Describe the ultra-structure of eukaryotic cell.
2) Describe the 'Fluid mosaic model' of the plasma membrane. On the basis of this model explain different functions of the plasma membrane.
3) What is cell wall? Describe the chemical composition, structure, origin and function of the plant cell wall.
4) Explain the chemical compositions of cell wall and plasma membrane.
5) Brief the functions of cell wall and plasma membrane.

1.8. SUGGESTED READINGS:

1) C.B. Powar. 2010. Cell Biology. Himalaya Publishing House, Mumbai – 400004.
2) De Roberties E.D.P & De Roberties (Jr.) 2017. Cell and Molecular Biology (8^{th} Edition).
3) Wolters Kluwer (India0 Pvt Ltd., New Delhi.
4) P.S. Verma & V.K. Agarwal, 2021. Cell Biology, Genetics, Molecular Biology, Evolution
5) and Ecology. S.Chand And Company Limited, New Delhi – 110044.

Dr. YRKV Tirupati Rao

LESSON-2

EUKARYOTIC MITOCHONDRIA, CHLOROPLAST AND RIBOSOMES

2.0 OBJECTIVE:

- Students will understand the structure and functions of mitochondria, chloroplast and ribosomes of eukaryotic cells.

STRUCTURE:

2.1 Introduction

2.2 Mitochondria

2.3 Chloroplast

2.4 Ribosomes

2.5 Summary

2.6 Technical Terms

2.7 Self-Assessment Questions

2.8 Suggested Readings

2.1. INTRODUCTION:

Mitochondria, the cell's energy producers, possess a highly specialized internal structure optimized for ATP synthesis through cellular respiration. It houses mitochondrial DNA (mtDNA), which is circular and bacterial-like, supporting the endosymbiotic theory, contains 70S ribosomes for synthesizing a subset of mitochondrial proteins. Chloroplasts, the photosynthetic organelles in plants, possess a complex internal structure optimized for light-driven ATP and NADPH production. The ribosomes of both 70S (prokaryotic ribosomes) and 80S (eukaryotic ribosomes) types referred to as work benches for making the proteins. A ribosome is a small dense and granular ribonucleoprotein particle which serves as the site for protein synthesis in the cell. The ribosome reads the sequence of the mRNA and, using the genetic code, translates the sequence of RNA bases into a sequence of amino acids.

2.2. MITOCHONDRIA

The mitochondria have uniform distribution in the cytoplasm, but in many cells their distribution is very restricted. The distribution and number of mitochondria are often correlated with cell function. Typically, mitochondria with many cristae are associated with mechanical and osmotic work situations e.g., muscle fibres, kidney tubule cells, and rod and cone cells of retina. Myocardial muscle cells have numerous large mitochondria called **sarcosomes**.

Number:

The number of mitochondria in a cell depends on the type and functional state of the cell. It varies from cell to cell and from species to species. e.g., the *Amoeba*, *Chaos chaos* (50,000), eggs of sea urchin (1,40,000), oocytes of amphibians (3,00,000). The cells of green plants contain less number of mitochondria in comparison to animal cells.

Shape and size:

The mitochondria may be filamentous or granular in shape and may change from one form to another depending upon the physiological conditions of the cells. Thus, they may be of club, racket, vesicular, ring or round-shape. E.g. Granular shaped (primary spermatocyte or rat), club-shaped (liver cells) Mitochondria are remarkably mobile and constantly changing their shape. Normally mitochondria vary in size from 0.5 μm to 2.0 μm and, sometimes their length may reach up to 7 μm. Each mitochondrion is bound by two membranes that play a crucial part in its activities. Each of the mitochondrial membrane is 6 nm in thickness and fluid mosaic in ultra structure (Figure 2.1). The outer membrane is quite smooth and has many copies of a transport protein called porin which forms large aqueous channels through the lipid bilayer. Inside and separated from the outer membrane by a 6–8 nm wide space is present the inner membrane. The inner membrane is not smooth but is impermeable and highly convoluted, forming a series of infoldings, known as cristae, in the matrix space.

The inner membrane divides the mitochondrial space into two distinct chambers, 1. Peri-mitochondrial space lies between outer membrane and inner membrane. This space is continuous into the core of the crests or cristae. 2. Matrix space, which is filled with a dense, homogeneous, gel-like proteinaceous material, called mitochondrial matrix. The mitochondrial matrix contains lipids, proteins, circular DNA molecules, 55S ribosomes and certain granules. Granules are prominent in the mitochondria of cells concerned with the transport of ions and water, including kidney tubule cells, epithelial cells of the small intestine, and the osteoblasts of bone-forming cells. Further, the inner membrane has an outer cytosol or C face toward the perimitochondrial space and an inner matrix or M face toward matrix. Attached to M face of inner mitochondrial membrane repeated units of stalked particles called elementary particles, inner membrane subunits or oxysomes are present. They are also identified as F1 particles or F0-F1 particles and are meant for ATP synthesis (phosphorylation) and also for ATP oxidation.

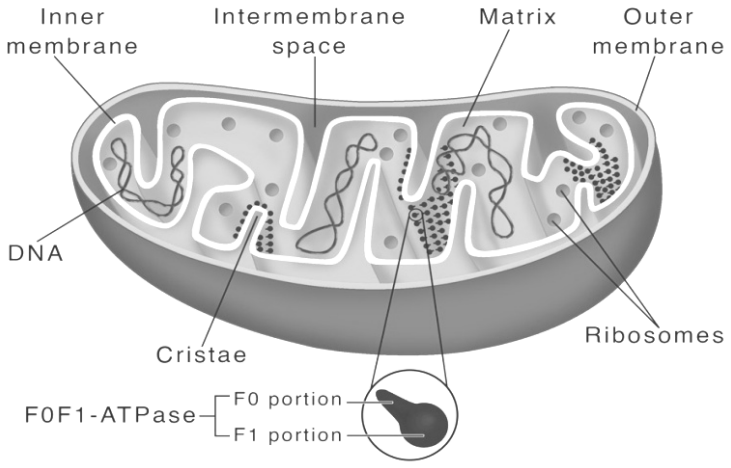

Figure-2.1: Ultrastructure of Mitochondria

F1 Particles:

Inner membrane is studded with pin head particles called as oxysomes or elementary particles or F particles or subunits of Fernandez Moran. Each F particle consists of three parts - Basal plate, Stalk and Head (Figure 2.2). ATP synthesis occurs in head region of oxysome because here ATPase enzyme is present. This factor also termed as Oligomycin Sensitivity Conferring Protein (OSCP). Oxysomes composed of ATPase enzymes and concerned with Oxidative phosphorylation.

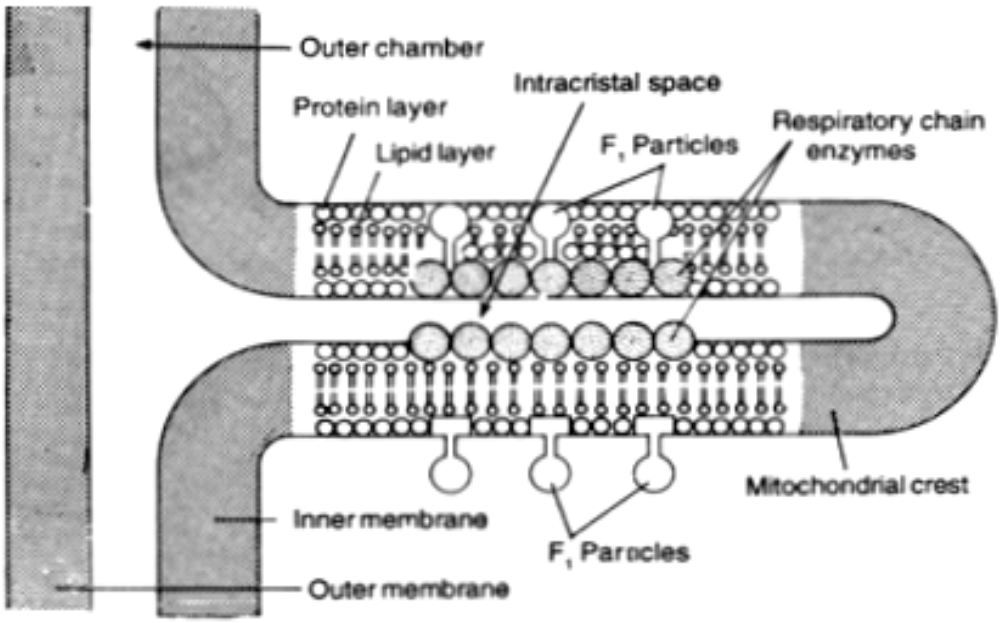

Figure-2.2: Ultrastructure of F1 Particle

Chemical Composition of Mitochondria

The gross chemical composition of the mitochondria varies in animal and plant cells. However, the mitochondria are found to contain 65 to 70 per cent proteins, 25 to 30 per cent lipids, 0.5 per cent RNA and small amount of the DNA. The lipid contents of the mitochondria are composed of 90 per cent phospholipids (lecithin and cephalin), 5 per cent or less cholesterol and 5 per cent free fatty acids and triglycerides. The inner membrane is rich in phospholipid **cardiolipin** which makes this membrane impermeable to a variety of ions and small molecules e.g., Na+, K+, NAD+, AMP, GTP, CoA etc. The outer mitochondrial membrane has typical ratio of 50 per cent proteins and 50 per cent phospholipids of 'unit membrane'. However, it contains more unsaturated fatty acids and less cholesterol. The mitochondrial regions contain a special set of proteins that mediate distinct functions.

1) **Enzymes of outer membrane:** Besides porins, other proteins of this membrane include enzymes involved in mitochondrial lipid synthesis and those enzymes that convert lipid substrates into forms that are subsequently metabolized in the matrix. Certain important enzymes of this membrane are monoamine oxidase, rotenone-insensitive NADH-cytochrome-C-reductase, kynurenine hydroxyalase, and fatty acid CoA ligase.

2) **Enzymes of intermembrane space:** This space contains several enzymes that use the ATP molecules passing out of the matrix to phosphorylate other nucleotides. The main enzymes of this part are adenylate kinase and nucleoside diphosphokinase.

3) **Enzymes of inner membrane:** This membrane contains proteins with three types of functions: 1. those that carry out the oxidation reactions of the respiratory chain; 2. an enzyme complex, called ATP synthetase that makes ATP in matrix; and 3. specific transport proteins (see Table 10-1) that regulate the passage of metabolites into and out of the matrix. Since an electrochemical gradient, that drives ATP synthetase, is established across this membrane by the respiratory chain, it is important that the membrane be impermeable to small ions. The significant enzymes of inner membrane are enzymes of electron transport pathways, *viz.*, nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), diphosphopyridine nucleotide (DPN) dehydrogenase, four cytochromes (Cyt. b, Cyt. c, Cyt.c1, Cyt. a and Cyt. a3), ubiquinone or coenzyme Q10, non-heme copper and iron, ATP synthetase, succinate dehydrogenase; β-hydroxybutyrate dehydrogenase; carnitive fatty acid acyl transferase.

4) **Enzymes of mitochondrial matrix.** The mitochondrial matrix contains a highly concentrated mixture of hundreds of enzymes i.e. malate dehydrogenase, isocitrate dehydrogenase, fumarase, aconitase, citrate synthetase, α-keto acid dehydrogenase, β-oxidation enzymes. These enzymes further participate in oxidation of pyruvate and fatty acids and the citric acid cycle. The matrix also contains several identical copies of the mitochondrial DNA, special 55S mitochondrial ribosomes, tRNAs and various enzymes required for the expression of mitochondrial genes.

Functions of Mitochondria:

The major functions carried by Mitochondria are:

1) **ATP production (Cellular Respiration):** Mitochondria generate adenosine triphosphate (ATP), the energy currency of the cell, through oxidative phosphorylation.

2) **Regulation of Cell metabolism:** They play a role in metabolic pathways like the citric acid cycle (Krebs cycle) and fatty acid oxidation.

3) **Calcium storage and Regulation:** Mitochondria help maintain calcium ion levels, which are crucial for cell signaling and muscle contraction.

4) **Apoptosis (Programmed Cell Death):** They release cytochrome c and other factors that trigger apoptosis, helping remove damaged or unnecessary cells.

5) **Heat production:** In specialized cells, mitochondria generate heat instead of ATP, a process known as non-shivering thermogenesis (important in brown fat tissue).

6) **Hormone synthesis:** They contribute to the production of steroid hormones (e.g., estrogen, testosterone) in endocrine tissues.

7) **Reactive Oxygen Species (ROS) Management:** Mitochondria produce ROS as a byproduct of respiration and help regulate oxidative stress.

8) **DNA and Protein synthesis:** Mitochondria have their own DNA (mtDNA) and ribosomes, allowing them to produce some of their own proteins independently of the cell nucleus.

2.3. CHLOROPLASTS:

Types of Plastids:

The term 'plastid' is derived from the Greek word "*plastikas*" (= formed or moulded) and was used by **A.F.W. Schimper** in 1885. **Schimper** classified the plastids into following types according to their structure, pigments and the functions.

1) **Leucoplasts:** The leucoplasts (Gr., *leuco* = white; *plast* = living) are the colourless plastids which are found in embryonic and germ cells. They are also found in meristematic cells and in those regions of the plant which are not receiving light. They never become green and photosynthetic. True leucoplasts do not contain thylakoids and even ribosomes.

   They store the food materials as carbohydrates, lipids and proteins and these are of following types:

   i) **Amyloplasts:** The amyloplasts (L., *amyl*=starch; Gr., *plast*=living) are those leucoplasts which synthesize and store the starch. The outer membrane of the amyloplst encloses the stroma and contains one to eight starch granules. Starch granules of amyloplasts are typically composed of concentric layers of starch.

   ii) **Elaioplasts:** The elaioplasts store the lipids (oils) and occur in seeds of monocotyledons and dicotyledons. They also include sterol-rich **sterinochloroplast**.

   iii) **Proteinoplasts:** The proteinoplasts are the protein storing plastids which mostly occur in seeds and contain few thylakoids.

2) **Chromoplasts:** The chromoplasts (Gr., *chroma*=colour; *plast*=living) are the coloured plastids containing **carotenoids** and other pigments. They impart colour (e.g., yellow, orange and red) to certain portions of plants such as flower petals (e.g., daffodils, rose), fruits (e.g., tomatoes) and some roots (e.g., carrots). Chromoplast structure is quite diverse; they may be round, ellipsoidal, or even needle-shaped, and the carotenoids that they contain may be localized in droplets or in crystalline structures. The function of chromoplasts is not clear but in many cases (e.g., flowers and fruits) the colour they produce probably plays a role in attracting insects and other animals for pollination or seed dispersal. In general, chromoplasts have a reduced chlorophyll content and are, thus, less active photosynthetically. The red colour of ripe tomatoes is the result of chromoplasts that contain the red pigment **lycopene** which is a member of carotenoid family. Chromoplasts of blue-green algae or cyanobacteria contain various pigments such as **phycoerythrin**, **phycocyanin**, **chlorophyll a** and **carotenoids**.

Chromoplasts are of following two types:

i) **Phaeoplast:** The phaeoplast (Gr., *phaeo*=dark or brown; *plast*=living) contains the pigment **fucoxanthin** which absorbs the light. The phaeoplasts occur in the diatoms, dinoflagellates and brown algae.

ii) **Rhodoplast:** The rhodoplast (Gr., *rhode*= red; *plast*=living) contains the pigment **phaeoerythrin** which absorbs the light. The rhodoplasts occur in the red algae.

3) **Chloroplasts:** The chloroplast (Gr., *chlor*=green; *plast*=living) is most widely occurring chromoplast of the plants. It occurs mostly in the green algae and higher plants. The chloroplast contains the pigment chlorophyll a and chlorophyll b and DNA and RNA.

Distribution:

The chloroplasts remain distributed homogeneously in the cytoplasm of plant cells. But in certain cells, the chloroplasts become concentrated around the nucleus or just beneath the plasma membrane. The chloroplasts have a definite orientation in the cell cytoplasm. Since chloroplasts are motile organelles, they show passive and active movements.

Structure:

Shape: Higher plant chloroplasts are generally biconvex or plano-convex. However, in different plant cells, chloroplasts may have various shapes, *viz*., filamentous, saucer-shaped, spheroid, ovoid, discoid or club-shaped. They are vesicular and have a colourless centre.

Size: The size of the chloroplasts varies from species to species. The chloroplasts generally measure 2–3 µm in thickness and 5–10 µm in diameter (e.g., *Chlamydomonas*). The chloroplasts of polyploid plant cells are comparatively larger than the chloroplasts of the diploid plant cells. Generally, chloroplasts of plants grown in the shade are larger and contain more chlorophyll than those of plants grown in sunlight.

Number: The number of the chloroplasts varies from cell to cell and from species to species and is related with the physiological state of the cell, but it usually remains constant for a particular plant cell. The algae usually have a single huge chloroplast. The cells of the higher plants have 20 to 40 chloroplasts.

A chloroplast comprises the following three main components (Figure 2.3).

1) **Envelope:** The entire chloroplast is bounded by an **envelope** which is made of double unit membranes. Across this double membrane envelope occurs exchange of molecules between chloroplast and cytosol.

2) **Stroma:** The matrix or stroma fills most of the volume of the chloroplasts and is a kind of gel-fluid phase that surrounds the grana thylakoids. It contains about 50 per cent of the proteins of the chloroplast, ribosomes and DNA molecules. The stroma is the place where CO_2 fixation occurs and where the synthesis of sugars, starch, fatty acids and some proteins takes place.

3) **Thylakoids:** The thylakoids (thylakoid = sac-like) consists of flattened and closed vesicles arranged as a membranous network. The outer surface of the thylakoid is in contact with the stroma, and its inner surface encloses an **intrathylakoid space**. Thylakoids may be stacked like a neat pile of coins, forming **grana** or they may be unstacked **stromal thylakoids.** There may be 40 to 80 grana in the matrix of a chloroplast. The number of thylakoids per granum may vary from 1 to 50 or more.

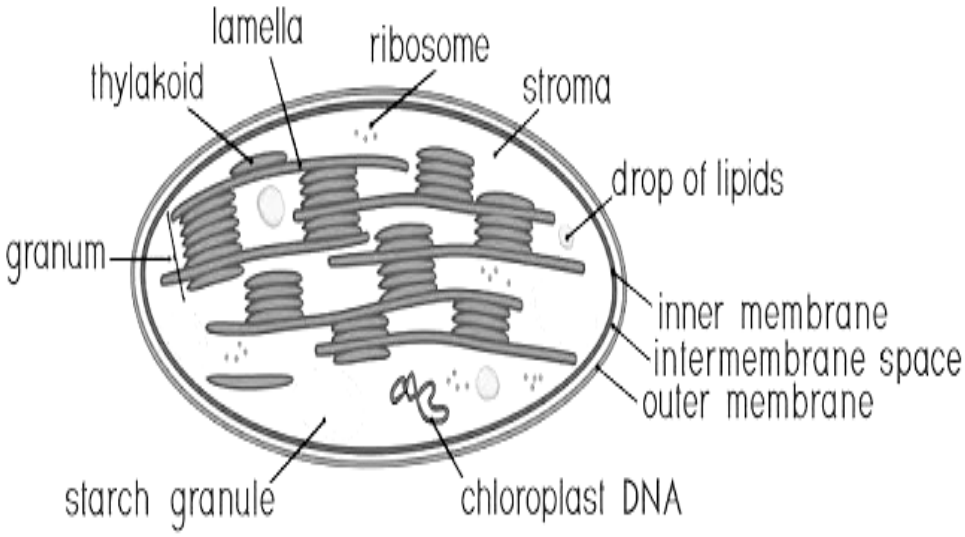

Figure-2.3: Structure of Chloroplast

Molecular Organization of Thylakoids:

Molecular organization of the membrane of thylakoids is based on the fluid-mosaic model of the membrane which represents fluidity, asymmetry and economy. Lipids represent about 50 per cent of the thylakoid membrane and these directly involved in photosynthesis E.g.: **chlorophylls**, **carotenoids** and **plastoquinones**. Structural lipids of thylakoids include glycolipids, sulpholipids and a few phospholipids. Most of these structural lipids are highly unsaturated which confer to the fluidity of thylakoid. The protein components of thylakoid membrane are represented by 30 to 50 polypeptides which are disposed in the five major supramolecular complexes. Molecular organization of thylakoids is given in figure 2.4.

1) **Photosystem I (PS I):** This complex contains a reactive centre composed of P700, several polypeptides, a lower chlorophyll *a/b* ratio and β-carotene. It acts as a light trap and is present in unstacked thylakoid membranes. In it light induced reduction of $NADP^+$ takes place.

2) **Photosystem II (PS II):** This complex comprises two intrinsic proteins that bind to the reaction centre of chlorophyll P680. It contains a high ratio of chlorophyll *a/b* and β- carotene. Frequently, the PS IIs are associated with the light harvesting complex and are involved in light induced release of O_2 from H_2O. PS II works as a light trap in photosynthesis and is mainly present in the stacked thylakoid membranes of grana.

3) **Cytochrome b/f.** This complex contains one cytochrome F, two cytochromes of b 563, one FeS centre and a polypeptide. It is uniformly distributed in the grana and acts as the electron carrier. These three complexes are related to the electron transport and are linked by mobile electron carriers i.e., plastoquinone, plastocyanin and ferredoxin. Electron transport through PS II and PS I finally results in the reduction of the coenzyme $NADP^+$. Simultaneously, the transfer of protons from the outside to the inside of the thylakoid membrane occurs.

4) **ATP synthetase:** As in mitochondria, this complex consists of a **CF0** hydrophobic portion, a proteolipid that makes a proton channel and a coupling factor one **(CF1**) that synthesizes ATP from ADP and Pi, using the proton gradient provided by the electron transport. ATP synthetase complexes are located in stacked membrane grana.

5) **Light harvesting complex (LHC):** The main function of LH complex is to capture solar energy. It contains two main polypeptides and both cholorophyll *a* and *b*. LH complex is mainly associated with PS II, but may also be associated with PS I. LHC is localized in stacked membranes and lacks photochemical activity.

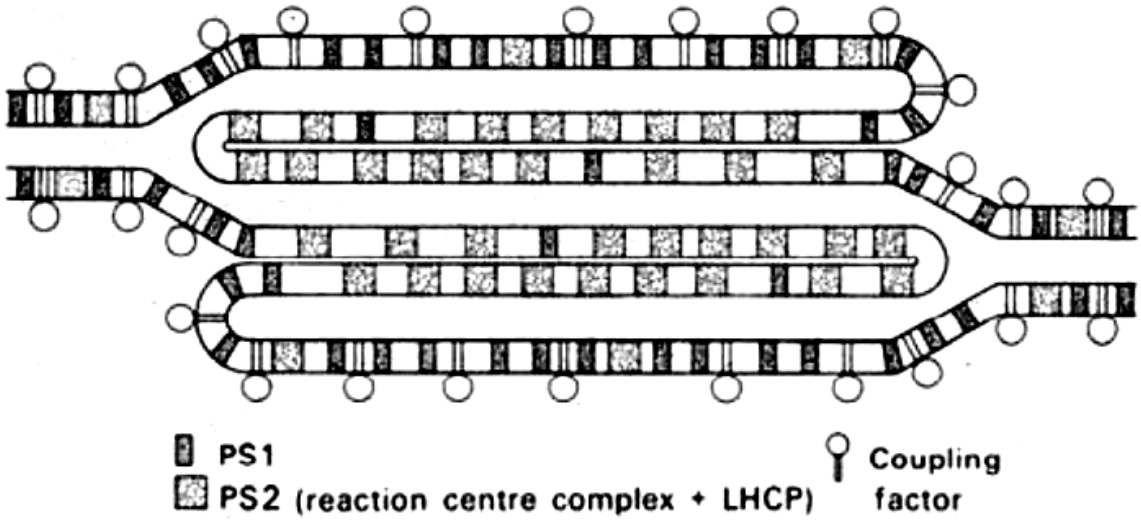

Figure-2.4: Molecular Organization of Thylakoids

Functions of the Chloroplast:

Chloroplasts role in plant growth and development is very crucial and here are some the functions performed by chloroplasts.

1) **Photosynthesis:** Chloroplasts carry out photosynthesis, converting light energy into chemical energy in the form of glucose. This process occurs in two main stages:

   Light-dependent reactions (Thylakoid Membranes): Takes place in the thylakoid membranes. Uses sunlight to excite electrons in chlorophyll. Splits water molecules (photolysis), producing oxygen (O_2) as a byproduct. Generates ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate), which are energy carriers.

   Light-independent reactions / Calvin Cycle (Stroma): Takes place in the stroma. Uses ATP and NADPH to convert carbon dioxide (CO_2) into glucose through a series of enzyme-controlled reactions.

2) **Production of ATP and NADPH:** The chloroplast's thylakoid membranes contain ATP synthase, which produces ATP during the light reactions. NADP+ is reduced to NADPH, which is used in the Calvin cycle to fix CO_2 into carbohydrates.

3) **Oxygen Production:** During photolysis in the light-dependent reactions, water molecules split to release oxygen. This oxygen is essential for the survival of aerobic organisms, including plants themselves.

4) **Carbon Fixation (Calvin Cycle):** Uses ATP and NADPH to convert CO_2 into glucose. Involves enzyme RuBisCO (Ribulose-1,5-bisphosphate carboxylase-oxygenase), which helps fix CO_2.

5) **Storage of Starch and Lipids:** Excess glucose produced in photosynthesis is stored as starch in the stroma. Chloroplasts can also store lipids, which can be converted into energy when needed.
6) **Synthesis of Amino Acids and Fatty Acids:** Chloroplasts play a role in producing some amino acids needed for protein synthesis. They also contribute to the synthesis of fatty acids, essential for membrane formation.
7) **Regulation of Plant Metabolism:** Chloroplasts help regulate metabolic pathways by interacting with mitochondria and peroxisomes. They participate in photorespiration, a process where oxygen is consumed and CO_2 is released, balancing plant metabolism.
8) **Defence against Oxidative Stress:** Chloroplasts contain antioxidants like carotenoids and ascorbate (vitamin C), which protect the plant cell from damage caused by reactive oxygen species (ROS).
9) **Chlorophyll and Pigment Storage:** Chloroplasts store chlorophyll, which captures light energy. They also contain accessory pigments like carotenoids and xanthophylls, which help absorb additional light wavelengths and protect against photo damage.
10) **Intercellular Communication and Signalling:** Chloroplasts communicate with the nucleus to regulate gene expression based on environmental conditions. They send signals when under stress, helping the plant adapt to changes like drought or excessive light.
11) **Role in Senescence (Aging of Leaves):** When leaves age, chloroplasts break down chlorophyll and transfer nutrients to other parts of the plant before the leaf falls off.
12) **Adaptation to Environmental Changes:** Chloroplasts can adjust their position within a cell in response to light intensity. They regulate stomata movement by interacting with guard cells to control water loss.

2.4. RIBOSOMES:

Ribosomes are the remarkable organelles of the cell and they were studied before their discovery. These ribosomes were first observed in mid 1950s by George Emil Palade, Romanian-American cell biologist as dense particles or granules (Palade particles) by using electron microscope. The term ribosome was coined by Richard B. Roberts at the end of 1950s. But the first isolation was done by Tissieres and J.D. Watson in 1958 from bacterium, *Escherichia coli*. However, the detailed structure and mechanism of the ribosome was given by Venkatraman Ramakrishnan, Thomas A.Steitz and AdaE.Yonath who shared the noble prize in chemistry in 2009 for their work.

The ribosomes occur both in prokaryotic and eukaryotic cells. The presence of ribosomes in both in free state and membrane attached form was confirmed by Palade and Siekevitz through electron microscopy. Often the ribosomes occur freely in cytoplasm in prokaryotic cells. However, in eukaryotic cells, ribosomes occur freely in cytoplasm or found attached to the outer membrane surface of endoplasmic reticulum. The yeast cells,

lymphocytes, meristematic plant cells, embryonic nerve cells and cancerous cells usually consists large number of ribosomes which occur freely in cytosol. In case of pancreatic cells, plasma cells, hepatic parenchymal cells, osteoblasts, thyroid cells and mammary gland cells wherein active protein synthesis takes place, ribosomes are found attached with ER. In erythroblasts, developing muscle cells, skin and hair that synthesize specific proteins for intracellular utilization and storage may contain larger number of ribosomes and in free state.

Ribosomes in the members of eubacteria, archaea and eukaryotes of three-domain classification system resembles with each other to a remarkable degree giving an evidence for common origin. However, they differ in their size, structure, sequence, and in ratio of protein to RNA. The ribosome is a complex cellular machine and made up of with specialized RNAs namely ribosomal RNA (rRNA) and some distinct proteins whose number may vary between prokaryotic and eukaryotic cells. The ribosomal proteins and RNAs are arranged into two distinct units of different sizes, larger subunit and smaller subunit. Structurally, the ribosomes are slightly longer in axis than in diameter as are formed from two unequal size subunits. During the protein synthesis, the two subunits fit together and work as one to translate the mRNA into a polypeptide chain. There are two types of ribosomes, based on their sedimentation coefficient rate which is measured in terms of Svedberg units, namely 70S ribosomes (present in prokaryotes and in mitochondria and chloroplast of eukaryotic cells) and 80S ribosomes (occur in eukaryotic cells).

70S ribosomes – compared to 80S ribosomes, these are smaller in size with sedimentation coefficient of 70S and molecular weight of 2.7 x 10^6 daltons. They show a dimension of 170 x 170 x 200 A^o units. They are around 20 nm in diameter and composed of 65% rRNA and 35% ribosomal proteins. This 70S ribosome consists two subunits viz., 50S larger subunit and 30S smaller subunit. The 30S subunit consists of 16S rRNA with 1540 nucleotides that is bound with 21 proteins. These proteins associated with smaller subunit are designated as S1 to S21. The 50S subunit contains one 5S rRNA (120 nucleotides), one 23S rRNA (2900 nucleotides) and 32 to 34 proteins which are labelled as L1 to L34. In bacterial cells, several ribosomes work simultaneously on a single mRNA during protein synthesis forming a structure called as polysome or polyribosome.

80S ribosomes – in diameter these ribosomes are between 25 and 30 nm with rRNA to protein ratio is close to 1 or 50:50. In eukaryotic cells, these 80S ribosomes located in cytosol consist a smaller subunit of 40S and a larger one of 60S. The 40S subunit has 18S rRNA (1900 nucleotides) and associated with 33 proteins. The 60S larger subunit is composed of one 5S rRNA (120 nucleotides), one 28S rRNA (4700 nucleotides) and one 5.8S rRNA (160 nucleotides) with 46 proteins.

Molecular Organization of Ribosomes:

The molecular organization and function of ribosomes have been studied extensively in prokaryotes than in eukaryotes. In both smaller and larger subunits, the rRNA and proteins are intertwined and arranged in a complex manner. To explain the three-dimensional structure of 70S prokaryotic ribosome, two models have been suggested – 1) Quasi-symmetrical model or Stoffler and Wittmann's model 2) Asymmetrical model or Lake's model.

Quasi-Symmetrical Model (Figure-2.5) – according to this, the 30S subunit has an elongated, slightly bent prolate shape and is a bipartite structure. A transverse hollow or cleft divides the 30S subunit into two parts, a smaller **head** and larger **body**. The 50S ribosomal

subunit showed variations in shape basing on the different observed angles. In frontal view, 50S subunit is bilaterally symmetrical and shows three protuberances arising from a rounded base resembling maple leaf structure wherein the central protuberance being the most prominent one. During the formation of 70S ribosome by the association of 30S and 50S subunits, the frontal face of 30S subunit with its hollow part faces the base of the 50S subunit. And the long axis of 30S subunit is oriented transversely to the central protuberance of the 50S subunit. As a result, a tunnel is formed between the hollow of the small subunit and round base of the larger subunit.

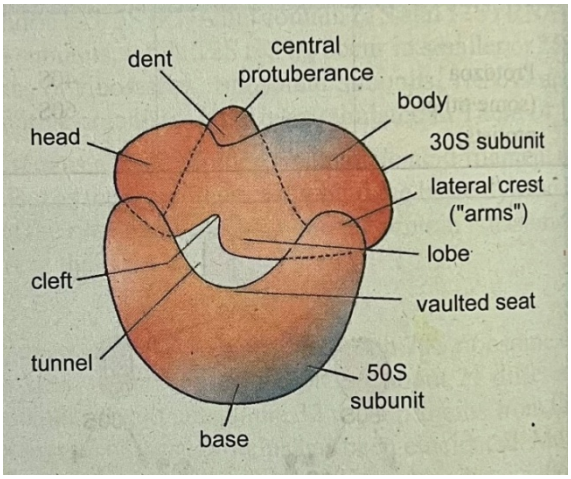

Figure-2.5: Quasi-symmetrical model of 70S ribosome

Asymmetrical model (Figure-2.6) – this model has been suggested by James A. Lake in 1981 and is completely asymmetrical one. The smaller subunit shows a head, a base, and a platform. The head and base are separated by platform through a cleft. This cleft is considered as an important functional region and assumed to be the site of codon-anticode interaction and also as a part of binding site for initiation factors of protein synthesis. The larger subunit contains a **ridge**, a **central protuberance** and a **stalk**. The ridge and the central protuberance are separated with the help of a valley.

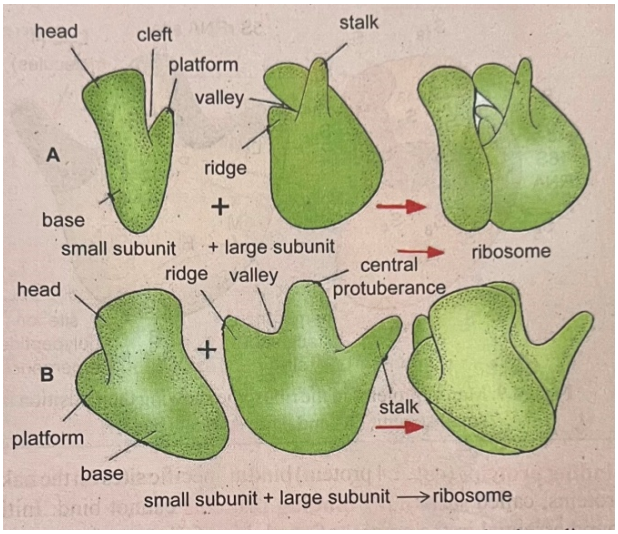

Figure-2.6: Asymmetrical model of 70S ribosome in two different orientations

Three-dimensional model of 80S ribosome

The cytoplasmic 80S ribosomes of eukaryotes are remarkably similar in morphology to those of prokaryotes except the differences in molecular weights, sedimentation constants, size, number of rRNAs and proteins. The 40S subunit of eukaryotic ribosome is divided into **head** and **base** segments by a transverse groove. The 60S ribosomal subunit is generally round shaped than the small unit and flattened at one side. This flat side becomes confluent with the small subunit during the formation of 80S ribosome. In addition to the cytoplasmic 80S ribosomes in eukaryotic cells, the organelles viz., mitochondria and chloroplast contains their own ribosomes but are of 70S. The ribosomes present in mitochondria are called as **mitoribosomes** and that of chloroplast are as **plastoribosomes**. Of the two, chloroplastic ribosomes are closely similar to prokaryotic ribosomes than that of mitochondrial ones.

Functional Sites on Ribosomes:

Each ribosome has different functional sites viz.,

1. A-site, **2**. P-site, **3**. mRNA site, **4**. Peptidyl transferase site, **5**. EF-Tu site, **6**. EF-G site, **7**. 5S rRNA site and **8**. Exit site (Figure-2.7). The ribosome may be divided longitudinally into two functional domains namely Exit domain, wherein exit site is located and Translational domain that covers 2/3 of ribosome structure and contains all the remaining 7 sites.

A-site and **P-site** – these are the two distinct and adjacent sites for the attachment of aminoacyl tRNA. The tRNA first attached to A-site and then transferred to P-site, making the A-site available for the next incoming aminoacyl tRNA. Both the sites are usually located in 30S subunit.

mRNA site – is located in 30S subunit for binding of mRNA which requires protein S1.

Peptidyl transferase site – this site is localized in the central protuberance of the 50S subunit.

EF-Tu and **EF-G sites** – the EF-Tu binding site is located in the 30S subunit and close to this site in 50S subunit the EF-G binding site is located for the binding of EF-Tu and EF-G proteins.

5S rRNA site – this site is located in central protuberance of 50S subunit.

Exit site – the growing polypeptide chain is extruded through this exit site of exit domain in 50S subunit of the ribosome.

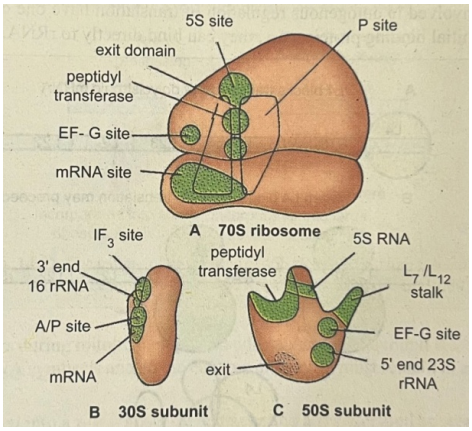

Figure-2.7: Functional sites on 70S ribosome

Comparison of 70S and 80S ribosomes

Eukaryotic 80S ribosomes differ from that of prokaryotic 70S ribosomes in means of –

1) Larger in size.
2) Containing more number of proteins associated.
3) Having four types of rRNA molecules instead of three types.
4) Large-sized proteins and nucleic acids.
5) rRNA –protein ratio is 1:1 instead of 2:1 in 70S ribosomes.
6) Resistant to antibiotic chloramphenicol whereas 70S ribosome is susceptible. But 80S is sensitive to cycloheximide and protein synthesis is inhibited.

The prime and important function of both prokaryotic and eukaryotic ribosomes is the assembly of amino acids to form polypeptide proteins that are essential to perform various cellular functions. The proteins synthesized by free located ribosomes are usually utilized in the cytoplasm itself. But the proteins that are synthesized by bound form of ribosomes are transported to outside of the cell.

2.5. SUMMARY:

Mitochondria, the cell's energy producers, possess a highly specialized internal structure optimized for ATP synthesis through cellular respiration. It is covered by double membrane i.e. Outer Membrane - Smooth and permeable due to porins, allowing small molecules and ions to pass freely. Inner Membrane - Highly folded into cristae, dramatically increasing surface area to host electron transport chain (ETC) complexes and ATP synthase. Mitochondria are rich in proteins and cardiolipin, making it impermeable to ions, crucial for maintaining the proton gradient. The compartment between the two membranes, where protons accumulate during the ETC, creating a electrochemical gradient used to drive ATP synthesis. The mitochondrial matrix contains enzymes for the Krebs cycle, fatty acid oxidation, and amino acid breakdown. It houses mitochondrial DNA (mtDNA), which is circular and bacterial-like, supporting the endosymbiotic theory, contains 70S ribosomes for synthesizing a subset of mitochondrial proteins. Includes nucleoids (mtDNA clusters) and granules storing ions/metabolites. Cristae maximize efficiency of oxidative phosphorylation by expanding the surface area for ETC enzymes and ATP synthase. The proton gradient across the inner membrane powers ATP synthase, converting ADP to ATP as protons flow back into the matrix. Semi-autonomous nature due to mtDNA and ribosomes enables limited protein synthesis, though most proteins are nuclear-encoded. This compartmentalized structure ensures efficient ATP production, linking biochemical pathways (Krebs cycle, ETC) to structural features (cristae, membranes), and exemplifying form-function specialization.

Chloroplasts, the photosynthetic organelles in plants, possess a complex internal structure optimized for light-driven ATP and NADPH production. Enclosed by a double membrane, their interior comprises the stroma (a protein-rich fluid) and an extensive thylakoid membrane system. Thylakoids are flattened, membrane-bound sacs organized into stacked grana (singular: granum) interconnected by unstacked stroma lamellae, maximizing surface area for light absorption and electron transport. The thylakoid membrane is a lipid bilayer rich in galactolipids (monogalactosyldiacylglycerol, MGDG) and sulfolipids, enhancing fluidity and protein integration. It houses key photosynthetic

complexes: Photosystem II (PSII) - Primarily located in grana regions, PSII contains chlorophyll and accessory pigments. It houses the oxygen-evolving complex that splits water, releasing oxygen, protons, and electrons. Cytochrome b6f Complex: Embedded in the membrane, it mediates electron transfer between PSII and PSI via plastoquinone and plastocyanin, contributing to the proton gradient across the membrane. Photosystem I (PSI) - enriched in stroma lamellae, PSI accepts electrons from plastocyanin, ultimately reducing NADP+ to NADPH. ATP Synthase - situated in stroma-exposed regions, it utilizes the proton gradient (lumen pH ~4 vs. stroma pH ~8) to synthesize ATP. The thylakoid's compartmentalization separates photochemical reactions (thylakoid membrane) from carbon fixation (stroma). The spatial segregation of complexes ensures efficient energy transfer, directional electron flow, and optimal proton gradient utilization, underpinning photosynthesis efficiency.

The ribosomes of both 70S (prokaryotic ribosomes) and 80S (eukaryotic ribosomes) types referred to as work benches for making the proteins. A ribosome is a small dense and granular ribonucleoprotein particle which serves as the site for protein synthesis in the cell. The ribosome reads the sequence of the mRNA and, using the genetic code, translates the sequence of RNA bases into a sequence of amino acids.

2.6. TECHNICAL TERMS:

Mitochondria, Chloroplast, Ribosomes, F1 particles, Thylakoids, Cristae, Plastids, Leucoplasts, Chromoplasts, Chloroplasts, Quasi-symmetrical model, Asymmetrical model, functional sites.

2.7. SELF ASSESSMENT QUESTIONS:

1) Write an account on eukaryotic mitochondria and its functions.
2) Give an account on eukaryotic chloroplast and its functions.
3) Describe the structure and functions of ribosomes.

2.8. SUGGESTED READINGS:

1) Cell Biology, Genetics, Molecular Biology, Evolution and Ecology – P.S.Verma and V.K. Agarwal, 2022. S.Chand and Company limited, New Delhi.
2) Cell Biology - C.B. Powar. 2010. Himalaya Publishing House, Mumbai – 400004.
3) Cell and Molecular Biology (8^{th} Edition), De Roberties E.D.P & De Roberties (Jr.) 2017.
4) Wolters Kluwer (India0 Pvt Ltd., New Delhi.

Dr. YRKV Tirupati Rao

LESSON-3

EUKARYOTIC ENDOPLASMIC RETICULUM, GOLGI APPARATUS, PEROXISOMES, LYSOSOMES AND CYTOSKELETON

3.0 OBJECTIVE:

- To understand the features and functions of endoplasmic reticulum, Golgi apparatus and Cytoskeleton of the eukaryotic cell.

STRUCTURE:

3.1 Introduction

3.2 Endoplamic Reticulum

3.2.1. Rough Endoplasmic Reticulum

3.2.2. Smooth Endoplasmic Reticulum

3.3 Golgi Apparatus

3.4 Peroxisomes

3.5 Lysosomes

3.6 Summary

3.7 Technical Terms

3.8 Self-Assessment Questions

3.9 Suggested Readings

3.1. INTRODUCTION:

The cytoplasm of the eukaryotic cell is coursed with a multitude of internal membranous systems and different organelles. Some of the important components of the cell are endoplasmic reticulum, Golgi apparatus, ribosomes and lysosomes. These structures perform specific functions in the cell. An extensive network of membrane limited channels occurs in cytoplasm of most of the animal cells which is referred as endoplasmic reticulum. Two types of endoplasmic reticulum viz., rough endoplasmic reticulum performs protein synthesis and smooth endoplasmic reticulum is tasked with lipid synthesis. The Golgi apparatus, a cup shaped organelle involves in the secretion and transportation of secreted products. Whereas the degradation of several macromolecules occurs in lysosomes, the tiny spheroid or irregular shaped membrane bound vesicles.

3.2. ENDOPLASMIC RETICULUM:

Endoplasmic reticulum (ER) was discovered by Keith R. Porter, A. Claude and Thompson in 1945 and the term ER was coined by Porter and Kallman in 1952. The ER found in almost all animal and plant cells except in mature erythrocytes, ova and embryonic

cells. It is an extensive network of membrane limited channels that occurs abundantly in metabolically active cells and in simple form in storage cells. In spermatocytes, it is present in much reduced form. In eukaryotic cells, the ER is a part of a transportation system and has many other important functions in the cell. Some part or potion of the ER membrane remains continuous with the plasma membrane of the cell and also with the nuclear envelope. ER occupies 10% of total cell volume and its membrane accounts to 30-60% of all cellular membranes. When the cells are disrupted during fractionation, the ER breaks up into small vesicles and microsomes.

Endoplasmic reticulum membranes may assume different forms viz., cisternae, tubules and vesicles and all are filled with endoplasmic matrix. **Cisternae** are unbranched, broad, flat and membrane bound spaces arranged in parallel to each other to form into lamellae. They are interconnected with each other and present cells having active synthetic roles. **Tubules** are irregularly branched tube-like structures with a size range of 50 – 100 A^o units in diameter. These forms of tubules are very common in cells that are engaged in the synthesis of lipids and steroids. **Vesicles** or sacs appear as membrane bound and isolated globose cavities. They are round, spherical or ovoid in shape and range in size of 25 – 500 µm in diameter. They are found dispersed in cytoplasm and rich in pancreatic cells.

In eukaryotic cells, the ER exists in two morphological types namely Rough Endoplasmic Reticulum (RER) and Smooth Endoplasmic Reticulum (SER) (Figure 3.1). These two types share many of the same proteins and involve in some common activities viz., synthesis of certain lipids and cholesterol. Basing on the activities of the cell, these two types of ER occur in different ratios in different types of cells. Depending on the metabolic requirements of the cell, RER and SER are inter-convertible and RER is more stable than SER.

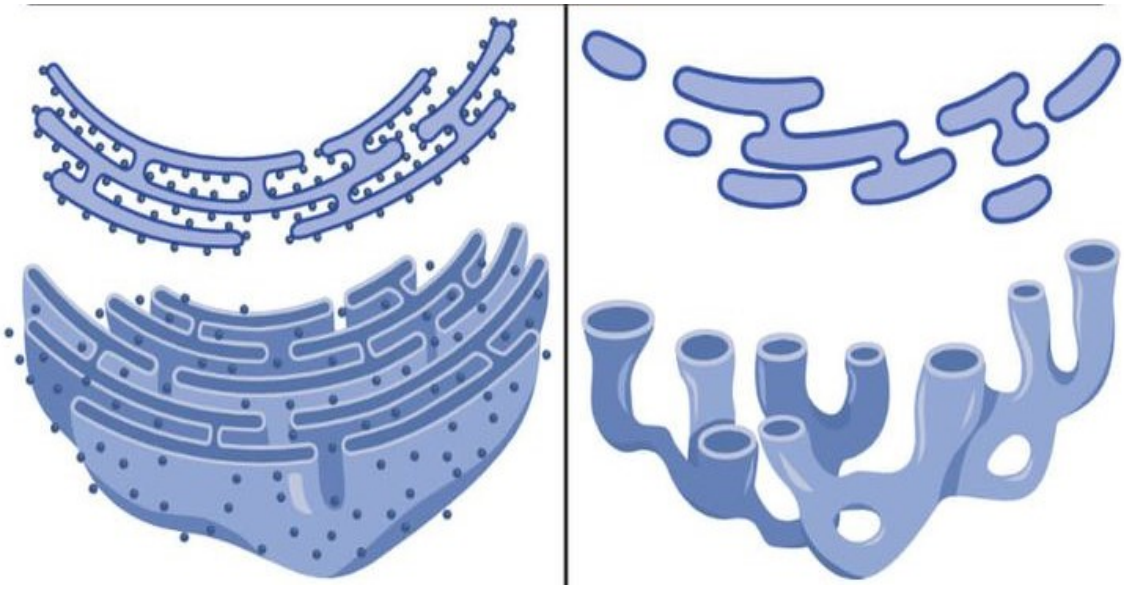

Figure-3.1: Structures of Rough Endoplasmic Reticulum and Smooth Endoplasmic Reticulum

Modifications of ER:

1) **Annulate ER or Annulate lamellae:** This type of ER may be smooth or rough. They form usually from blebbing of nuclear envelope. This ER consists some pores like nuclear pores.
2) **Sarcoplasmic reticulum:** This is a modified type of SER found in striated muscle cells like skeletal and cardiac cells. It helps in intracellular impulse transmission and contraction of muscle cells. It also supplies Ca^{2+} to muscle cell cytoplasm.
3) **Myeloid body:** This is also a modified SER and mainly present in pigmented epithelial cells of frog eye retina and helps in photoreception.
4) **Nissl body:** Nissl bodies are also called as Nissl granules or tigroid body. These are the discrete granular structures present in neurons that consist of rough endoplasmic reticulum, a collection of parallel, membrane-bound cisternae and cluster of free ribosomes in nerve cells.

Some Common Functions of the ER includes:

- Provides mechanical support to the cytoplasmic matrix.
- Provides large surface area for the synthesis of various materials.
- Helps in keeping the various cell organelles in their respective positions.
- Facilitates quick intracellular transport by forming the circulatory system in the cell.
- Controls the movement of materials between the adjacent cells by extending itself through plasmodesmata as desmotubules.

3.2.1 Rough Endoplasmic Reticulum – found mainly towards the nucleus of the cell and granular in form. It shows somewhat rough appearance due to the attachment of ribosomes on its surface. RER is predominant in cells which actively synthesize the proteins like enzyme secreting cells especially hepatocytes. RER accounts to 2/3 of the total ER content in the cell. RER is mainly composed of cisternae and tubules are few. On surface, RER contains two types of glycoproteins namely ribophorin I and ribophorin II for the purpose of attachment of ribosomes. Due to the presence of ribosomes on its surface, RER shows basophilic staining property.

Growing secretory polypeptide emerges from ribosome, it passes through the RER membrane and gets accumulated in lumen of RER. There, these polypeptide chains undergo tailoring, maturation, and molecular folding to form functional secondary or tertiary protein molecules. RER pinches off certain tiny protein-filled vesicles which ultimately get fused to cis-Golgi apparatus.

RER Specific Functions:

1) Helps in the formation of nuclear envelope, plasma membrane and smooth endoplasmic reticulum.
2) Holding of ribosomes by ribophores that present on RER surface.
3) Synthesizes the proteins destined for secretions, lysosomes and plasma membrane.

3.2.2 Smooth Endoplasmic Reticulum -- found towards the cell membrane or plasma membrane. SER lacks the attached ribosomes and especially abundant in mammalian liver, gonad cells and sebaceous glands. SER is agranular in nature and occurs in the form of tubules and vesicles but very rarely occurs in cisternae form. SER is characteristic of cells in which synthesis of non-protein substances like phospholipids, glycolipids and steroid hormones takes place. It is only 1/3 of the total ER content of the cell.

SER Specific Functions:

1) Helps in the synthesis of phospholipids, cholesterol, steroids etc. by involving in lipid metabolism.
2) In liver cells, SER possess some enzyme bodies called glycosomes for glycogen metabolism.
3) Helps in detoxification of toxins using cytochrome P-450.
4) Provides visual pigments from Vit-A in retinal cells.
5) In muscle cells, SER modifies into sarcoplasmic reticulum which store and release Ca^{2+} for muscle contraction.
6) Involves in production of cell organelles like Golgi apparatus, lysosomes, sphaerosomes and vacuoles.

3.3. GOLGI APPARATUS:

Golgi apparatus also known as Golgi body or Golgi complex was discovered in 1898 by an Italian physician Camillo Golgi for the first time in nerve cells of owl. The Golgi apparatus occurs in all cells except the prokaryotic cells and eukaryotic cells 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 bodies per plant cell may vary from several hundred (eg. corn root tissues) to a single organelle in some algal members. Largest and most complicated Golgi bodies are found in certain algal cells such as *Pinnularia* and *Microsteras*. Golgi apparatuses are more common in secretory cells and young rapidly growing cells of higher plants. In animal cells, usually a single Golgi apparatus occurs, but the number may vary from animal to animal and cell to cell types. The *Paramoeba* species shows two Golgi apparatuses, whereas the liver cells, nerve cells and chordate oocytes have many Golgi bodies.

In higher plants, Golgi bodies or dictyosomes are generally found scattered throughout the cytoplasm and not seem to be localized in any specific pattern. However, in animal cells the Golgi apparatus are polar in nature occupying a position between nucleus and periphery in case of cells of ectodermal and endodermal origin. But in nerve cells it occurs at circum-nuclear position. Morphologically, the Golgi apparatus is very similar in both animal and plant cells. However, it is pleomorphic and is compact and limited in some cell types or spread out and reticular in some other cells. The shape and form of Golgi apparatus may vary from one cell type to another cell type. However, the Golgi apparatus typically appears as a complex array of interconnecting tubules, vesicles and cisternae. The structure of the Golgi apparatus is given in Fig. 3.2.

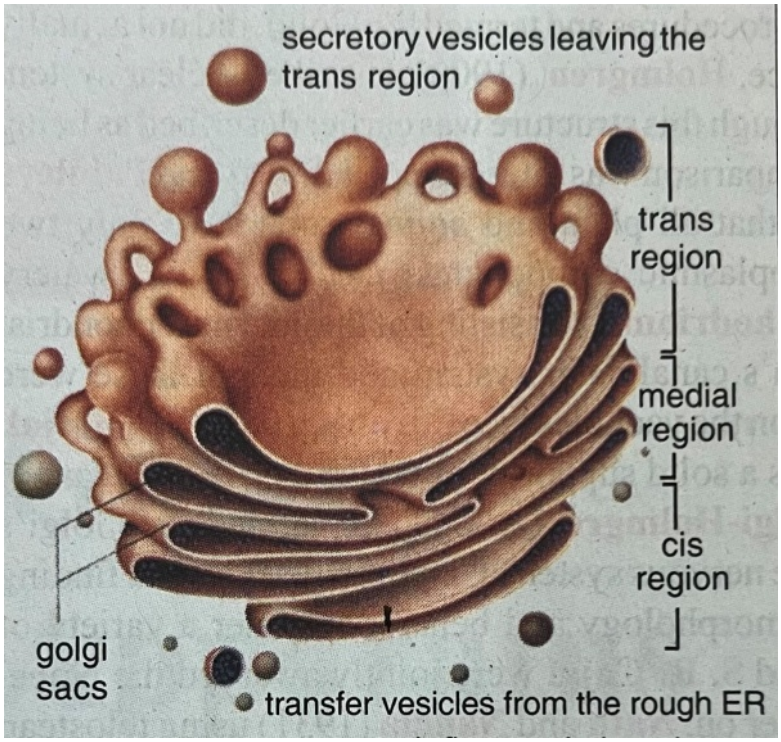

Figure-3.2: Structure of the Golgi Apparatus

Cisternae – are the elongated flattened sacs filled with fluids and piled one upon the other to form a stack like arrangement. In a stack, number of cisternae varies in general from 3 to 7 in animal cells and 20 or more in plant cells. Each cisterna is bounded by smooth unit membrane with a thickness of 7.5nm with lumen width varying from 500 nm to 1000 nm. The cisternae are slightly curved and so have convex and concave surfaces. The convex surface of the cisternae is referred to as forming face or cis-face and the concave surface is called as maturing face or trans-face. The cis-face is located towards nucleus or ER and trans-face is positioned near the plasma membrane. This type of polarization of Golgi apparatus is called as cis-trans axis. Always the new lamellae are received by cisternae from endoplasmic reticulum on the forming face and the losing membranes on the maturing face through the formation of secretory vesicles. The formation of new cisternae that results in Golgi apparatus may occur by any one of the two methods – 1. Individual stacks of cisternae may arise from the pre-existing stacks by division or fragmentation. 2. Alternatively by *de novo* formation which means the creation of something entirely new without relying on any pre-existing structure. Infact, the Golgi apparatus forms from the membranes of smooth ER which in turn originate from rough ER.

Tubules – a complex array of associated vesicles and anastomosing tubules (30 nm to 50 nm dia) surround the Golgi apparatus and radiate from it.

Vesicles – are the small droplet-like structures and closely associated with the periphery of the cisternae. They develop either by budding or by constriction of the ends of cisternae. These vesicles are of 3 types (i) **Transitional vesicles** – small, membrane limited vesicles which are assumed to form as blebs from the transitional ER to migrate and converge to cis-

face of Golgi and coalesce to form new cisternae. (ii) **Secretory vesicles** – varied sized and membrane limited vesicles which discharge from cisternae margins. Often, they occur between maturing face of Golgi and plasma membrane. (iii) **Clathrin-coated vesicles** – spherical protuberances with rough surface. They are morphologically quite different from secretary vesicles and usually found at the periphery of the Golgi apparatus. These are known to perform a role in intra-cellular traffic of membranes and of secretory products i.e., between ER and Golgi.

The Golgi apparatus is surrounded by a clear and differentiated region of cytoplasm wherein ribosomes, glycogen, mitochondria and chloroplasts of absent. This region is called as zone of exclusion and the ER within this zone of exclusion has a smooth surface. The synthesized proteins appear to move in the cytoplasm in the pathway direction of – RER —› cis-Golgi—› median Golgi —› trans-Golgi —› secretory vesicles/cortical granules of egg/lysosomes.

Functions of Golgi Apparatus:

1) Packaging of secretory materials like enzymes, mucin, lactoprotein of milk, melanin pigment etc. that are to be discharged from the cell.
2) Processing of proteins i.e., glycosylation, phosphorylation, sulphation and selective proteolysis.
3) Synthesis of certain polysaccharides and glycolipids.
4) Sorting of proteins destined for various locations in the cell.
5) Formation of acrosome of the spermatozoa during spermatogenesis.
6) Proliferation of membranous element for plasma membrane as secretory vesicles formed from Golgi fuse with plasma membrane.
7) Biosynthesis of lysosomes as Golgi cisternae bud off small vesicles that forms into primary lysosomes.
8) Membrane trafficking – involve in intracellular and intercellular transport of the biosynthetic products.
9) Cell wall formation – during cytokinesis, Golgi vesicles accumulate in equatorial plane and helps in cell plate formation.

3.4. PEROXISOMES:

Peroxisomes are granular micro bodies containing peroxidases. They are tiny, 0.2 to 1.5 mm diameter, single membrane bound organelles. They are found in protozoa, yeasts, higher plants, liver and kidney cells. They are formed as dilations of endoplasmic reticulum. They contain a crystal core of enzymes viz., urate oxidase, peroxidase, D-amino oxidase and catalase (e.g. liver and cells). These enzymes are required by peroxisomes in detoxification activity. Peroxisomes are also related with β-oxidation of fatty acids and thrermogenesis like mitochondria and also in degradation of amino acids. Peroxisomes are self-regulating organelles which selectively import proteins and enzymes from cytosol. Leaf peroxisomes are associated with endoplasmic reticulum, chloroplasts and mitochondria and are involved in photorespiration. Peroxisomes show a relationship with steroid synthesis and generation of

NAD and NADH. Peroxisomes oxidize a variety of substrates in a two-step reaction. In the first step, substrates like amino acids, uric acid, and lactic acid are oxidized by molecular O_2 to form H_2O_2 which is catalysed by oxidases. In second step, substrates like ethyl alcohol, methyl alcohol, nitrites, formic acid are oxidized by H_2O_2 formed in the first step. This step is catalysed by the enzyme catalase.

3.5. LYSOSOMES:

Lysosomes are the tiny membrane-bound vesicles involves in intracellular digestion. The term lysosome is derived from Greek language *lyso*=digestive and *soma*=body. In 1955, C. de Duve discovered and named these organelles as lysosomes. For the work on lysosomes, he shared the noble prize in 1974 along with Palade and Claude in the field of physiology. They consists a variety of hydrolytic enzymes which remain active in acidic conditions. These organelles are important in digestion of a variety of biological materials and also cause aging and death of animal cells. The lysosomes occur in most of the animal cells and few plant cells but totally absent in bacteria and mature mammalian erythrocytes. Few lysosomes occur in muscle cells but rich and abundant in granulocytes, phagocytic cells, and epithelial cells of absorptive, secretory and excretory organs, lungs and uterus.

Lysosomes are the round vacuolar structures bounded with single unit membrane and filled with dense material. Lysosomes vary greatly in shape and density with a size range of 0.2 to 0.5µm. The size and shape of lysosomes vary from cell to cell and time to time. The lysosome may consist up to 40 types of hydrolytic enzymes that include proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases and sulphatases. All the lysosomal enzymes are acid hydrolases and optimally active at pH 5.0 within the organelle. The lysosomal membrane contains substantial amounts of carbohydrate material, particularly sialic acid. Most of the lysosomal membrane proteins are usually highly glycosylated and the membrane shows an unique property of fusing with other membranes of the cell. Lysosomal membrane can be destabilized and ruptured which result in the release of lysosomal enzymes by surface active agents such as liposoluble vitamins and steroid sex hormones. However, the cortisone, hydrocortisone and other drugs tend to stabilize the lysosomal membrane. The entire digestion process occurs within the lysosome and acidification of lysosomal content depends on an ATP-dependent proton pump that present in lysosomal membrane. The lysosomal membrane also contains transport proteins which allow the digested macromolecules to exit so that they can be excreted or reutilized by the cell.

Polymorphism in Lysosomes:

Lysosomes are extremely dynamic organelles and exhibit polymorphism in their morphology. Totally, four types of lysosomes have been recognized in different types of cells or at different times in the same cell. Of the four lysosomes, first one is referred as Primary lysosome and the remaining three viz., Heterophagosomes, Autophagosomes, and Residual bodies are grouped together and considered as Secondary lysosomes.

Primary Lysosomes - also called as storage granules, protolysosomes or virgin lysosomes. These are the newly formed, single membrane bounded organelles with a typical diameter of 100 nm. They contain degradative enzymes which will not involve in any digestive process. Each primary lysosome contains one or another type of enzyme and only exhibit hydrolyzing activity.

Heterophagosomes – also be referred as heterophagic vacuoles, heterolysosomes or phagolysosomes. These are formed by the fusion of primary lysosomes with cytoplasmic vacuoles containing extracellular substances that are carried into the cell by a variety of endocytic processes namely pinocytosis, phagocytosis or receptor-mediated endocytosis. The engulfed substances are digested by the activity of hydrolytic enzymes present in secondary lysosomes. The digested low molecular material can readily passes through the lysosomal membrane and become the part of the cell matrix.

Autophagosomes – these are also be called as autophagic vacuoles, cytolysosomes or autolysosomes. Primary lysosomes are also able to digest the intracellular structures including mitochondria, ribosomes, peroxisomes and glycogen granules of the cell. This type of autodigestion is a normal event that occurs during cell growth and repair which may be prevalent in cells undergoing programmed cell death in the process of metamorphosis or regeneration and also in the tissues under stress. This autophagy may occur in different modes. Sometimes, the lysosome appears to move around the cell organelle and fuse with and enclose it in a double membrane sac. Then, the inner membrane breaks down and the lysosomal enzymes penetrate into the enclosed organelle. In other cases, the organelle to be digested is first fenced by smooth endoplasmic reticulum forming a vesicle which then fuses with a primary lysosome. Lysosomes can also regularly engulf small portions of cytoplasmic matrix and is degraded by a process which is referred to as microautophagy.

Residual Bodies – also called as telolysosomes or dense bodies. These are formed due to the incomplete digestion in food vacuole because of the absence of some lysosomal enzymes. The residual bodies are generally large, irregular in shape and electron-dense. In some cells of A*moeba* and other protozoa, these residual bodies are eliminated by defecation process. In other cases, residual bodies may remain for longer periods in cells and result in their aging. For example, the presence of aging pigment inclusions in some nerve cells, liver cells, heart cells and muscle cells of old animals is due to the accumulation of these residual bodies.

Functions of lysosomes:

1) Digestion of large extracellular particles i.e. food contents of phagosomes and pinosomes.
2) Digestion of intracellular substances – during starvation, lysosomes digest the stored food contents viz., proteins, lipids, some carbohydrates of the cell cytoplasm and supply the needed energy to the cell.
3) Autolysis – in certain pathological and disaster conditions, lysosome digest the various organelles of the cells particularly of dead ones.
4) In case of sperm cells, during fertilization, lysosomes discharge their enzymes to outside the cell and digest the limiting membranes of the ovum thereby facilitate the penetration of sperm into the ovum.

Lysosomes in Plants

Several of the hydrolases in plant cells are not always neatly compartmentalized and not necessarily present in membrane bound vacuoles as in animal cells. Instead, many types of vacuoles and storage granules in plant cells are found to contain certain digestive enzymes and thereby these granules are considered as lysosomes of plant cell in 1972 by Gahan. Matil in 1975 has divided these structures into three types viz., Vacuoles, Spherosomes and Aleurone grain.

1) **Vacuoles** – the vacuole of a mature plant cell forms from the enlargement and fusion of smaller vacuoles present in meristematic cells. These provacuoles are believed to be derived from the ER and possibly the Golgi and contain acid hydrolases. Sometimes, mitochondria and plastids are observed inside the vacuoles which suggest the autophagy in plants.
2) **Spherosomes** – these are the membrane-bound, spherical structures with a size of 0.5 to 2.5 µm diameter and occur in most plant cells. They originate from the ER and during this process, oil accumulates at the end of a strand of ER and then a small vesicle is cut off to form particles known as prospherosomes. These prospherosomes grow in size to form spherosomes. The basic functions of spherosomes are lipid synthesis and storage. But in maize root tips and tobacco endosperm tissue, the spherosomes are found rich in hydrolytic digestive enzymes and so considered as lyosomes.
3) **Aleurone Grain** – also called as protein bodies which are spherical membrane-bound storage particles that generally occur in cells of endosperm and cotyledons of seeds. Usually, they form during the later stages of seed ripening and disappear in the early stages of germination. They store proteins and phosphate in the form of phytin. Like spherosomes, aleurone grains store reserve materials, mobilize them during germination and in addition form a compartment for the digestion of other cell components.

3.6. CYTOSKELETON:

The cytoskeleton, a network of filamentous proteins, allows a cell to keep its shape, transport its cargo, and move. The cytoskeleton consists of tiny filamentous proteins. The cytoskeleton is responsible for eukaryotic cells' capacity to change shape and perform coordinated and controlled movements. Koltzoff proposed the existence of an ordered fibrous array or cytoskeleton in the protoplasm in 1928. The cytoskeleton extends into the cytoplasm and is a complicated network comprising three types of protein filaments. 1. Microtubules 2. Microfilaments (actin filaments) and 3. Intermediate filaments (IFs). The cytoskeleton contains the proteins like tubulin in microtubules, actin, myosin, tropomyosin in microfilaments and keratins, vimentin, desmin, and lamin in intermediate filaments.

Microtubules:

Microtubules were first observed by Robertisand Franchi (1953). The exact nature of microtubules was brought by Sabatini, Bensch and Barnett (1963). Microtubules of plant cells were first described in detail by Ledbetter and Porter (1963).

Occurrence:

All eukaryotic cells include microtubules, either free in the cytoplasm or as part of centrioles, cilia, and flagella. Microtubules are found in the cytoplasm of animal and plant cells, specifically in 1. Cilia and flagella. 2. Centrioles and basal bodies. 3. Neurons 4. The mitotic apparatus 5. The cortex of meristematic plant cells 6. Cells that elongate, such as during lens development or spermatogenesis in certain insects 7. Few structures in Protozoa.

Structure:

Microtubules are a group of morphologically and chemically similar filamentous rods found in both plant and animal cells. A microtubule is a long, unbranched, hollow tube 24-25 nm in diameter, several micrometers long, with a 6 nm thick wall and 13 subunits or protofilaments (Figure 3.3). Tubulin is a protein that forms the protofilament of microtubules. Tubulin comes in two forms: α-tubulin and β-tubulin, both containing around 450 amino acids. Tubulin in the form of dimers polymerizes into microtubules. Several proteins have recently been identified as interacting with the surface of microtubules. These proteins are known as microtubule-associated proteins, or MAPs.

The following two primary types of MAPs have been isolated

1) HMW proteins with molecular weights between 2,00,000 and 3,00,000,
2) Tau proteins have molecular weights ranging from 40,000 to 60,000.

Cytoplasmic microtubules are extremely dynamic structures that constantly emerge and disappear in response to cellular processes. The polymerization (assembly) and depolymerisation (disassembly) of microtubules appear to be a type of self-assembly. The assembly of microtubules from tubulin dimers is a carefully planned and directed process. The amount of polymerized tubulin is highest in interphase (cytoplasmic microtubules) and metaphase (spindle microtubules), but lowest in prophase and anaphase. Within the cell, microtubules and free tubulin are in equilibrium. Phosphorylation of tubulin monomers by a cyclic AMP-dependent kinase promotes polymerization. A significant association has been discovered between cell shape, the number and direction of microtubules, and cAMP. Tubulin assembly and disassembly are polarizing phenomena. Tubulin dimers are assembled at one end of a microtubule, and disintegration occurs at the other. Certain medications, including as colchicine, vincristine, and vinblastine, block microtubule assembly. Furthermore, the assembly is accompanied by the hydrolysis of guanosine triphosphate (GTP) to guanosinediphosphate (GDP), and the assembly is terminated when GTP is not present.

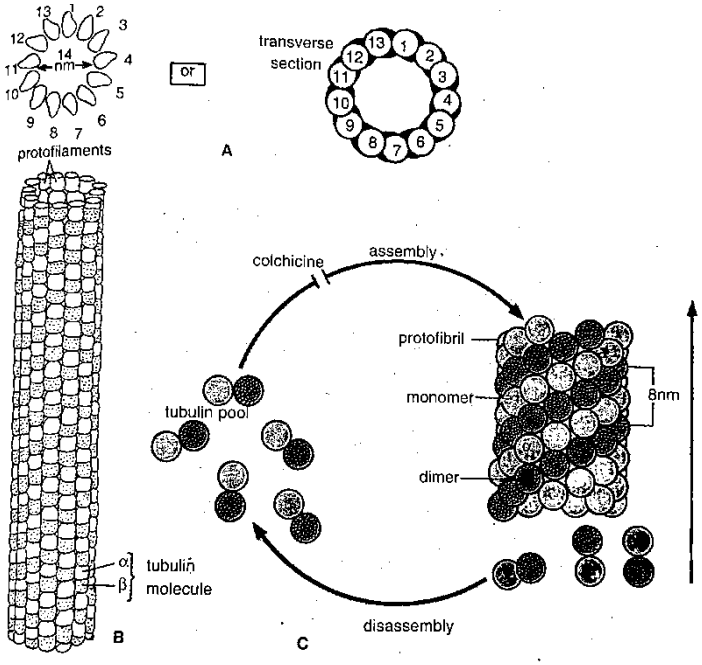

Figure-3.3: Schematic Diagrams of a Microtubule

A. Tubulin molecules (subunits of protofilaments) in cross section.

B. Side view of a short section of a microtubule.

C. Assembly and disassembly of the microtubule

Functions of Cytoplasmic Microtubules:

Microtubules have several functions in the eukaryotic cells such as follows:

1) **Mechanical function:** The shape of the cell and some cell processes or protuberances such as axons and dendrites of neurons, microvilli, etc., have been correlated to the orientation and distribution of microtubules.
2) **Morphogenesis:** During cell differentiation, the mechanical function of microtubules is used to determine the shape of nucleus of the spermatid during spermiogenesis, the elongation of the cells during induction of the lens placode in the eye.
3) **Cellular polarity and motility:** The determination of the intrinsic polarity of certain cells is also related to the microtubules. Directional gliding of cultured cells is found to depend on the microtubules.
4) **Contraction:** Microtubules play a role in the contraction of the spindle and movement of chromosomes and centrioles as well as in ciliary and flagellar motion.
5) **Circulation and transport:** Microtubules are involved in the transport of macromolecules, granules and vesicles within the cell.

Microfilaments:

Microfilaments are actin-based cytoskeleton fibres (Figure 3.4). Actin is one of the most abundant proteins in eukaryotic cells, accounting for 20% of all cellular protein by weight in muscle cells. Microfilaments are typically found in the cortical areas of the cell, immediately beneath the plasma membrane. Microfilaments also penetrate cell processes, particularly where there is mobility. As an example, consider microvilli. Actin is the primary structural protein in microfilaments. There are three types of actins namely α, β, and γ. Fully matured muscular tissue contains α-actin. The other two kinds are more typical of non-muscle cells. Actin amino acid sequences are largely conserved in eukaryotic cells. Actin can exist as a free monomer called G-actin (globular) or as a polymer microfilament termed F-actin ("F" for filamentous). Actin must be coupled to ATP in order to assemble into filaments and retain their structural integrity. The actin filament possesses structural polarity. The term "polarity" refers to the filament's two different ends. These ends are referred to as the "(-)" and "(+)" ends. Actin subunits are added to the elongating filament at the "(+)" end; whereas they are disassembled or fall off at the "(-)" end. The ATP to ADP conversion controls the process of assembly and disassembly.

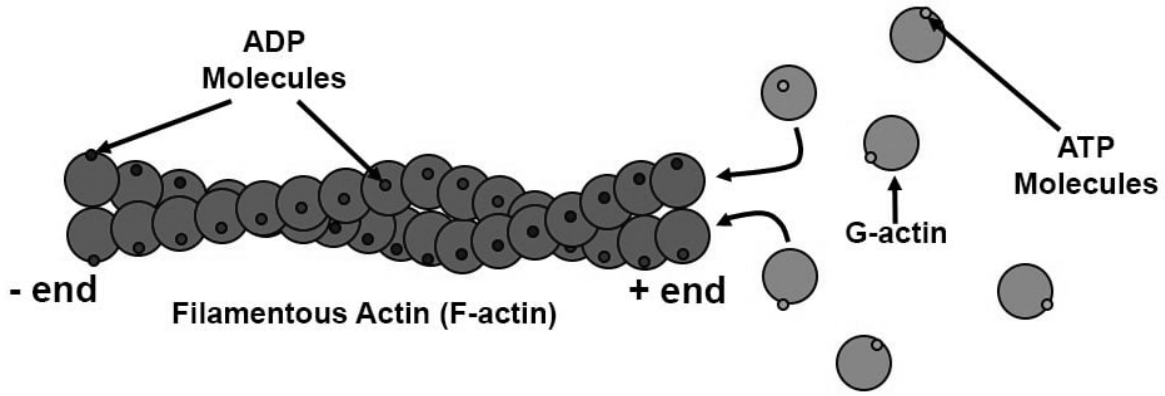

Figure-3.4: Microfilament Structure

Functions:

1) Microfilaments are the part of muscle cells and allow these cells to contract, along with myosin.
2) Actin and myosin are the two main components help in the contraction of muscles.
3) They play role in cell migration via lamellipodia and filopodia, amoeboid movement, cytoplasmic streaming.
4) The parallel bundles of microfilament form the microvilli.
5) They produce cleavage furrows that divide the cytoplasm of cell during cytokinesis. Help to maintain the cell shape.

Intermediate Filaments:

Most of the eukaryotic cells cytoplasm contains durable and resilient protein fibers known as intermediate filaments (IFs). They are usually between 8 and 10 nm in diameter, which is "intermediate" between thin and thick filaments. IFs are resistant to colchicine and cytochalasin B, but are vulnerable to proteolysis. The intermediate filaments have been given several names, which are based on the cell type in which they are detected. Thus, IFs in epidermal cells are known as tonofilaments, in nerve cells as neurofilaments, and in neuroglial cells as glial filaments. In cross-section, intermediate filaments seem tubular. Each tubule appears to be composed of four or five protofilaments stacked in parallel. IFs are composed of polypeptides of about 40,000 to 130,000 daltons.

Types of Intermediate Filaments:

The intermediate filaments are grouped into following four main types based on their morphology and localization.

1) **Type I IF Proteins:** They are found mostly in epithelial cells and include two keratin subfamilies (cytokeratin). 1. Acidic keratin; 2. Neutral or Basic keratin. Keratin filaments are always heteropolymers composed of an equal number of subunits from both of these keratin subfamilies. Keratins are the most complicated class of IF proteins, with 19 different forms in human epithelia and another 8 in hair and nail keratins.

2) **Type II IF Proteins:** These polypeptides are classified into four different categories. 1. Videtin, 2. Desmin, 3. Synemin and 4. Glial filaments also known as glial fibrillary acidic proteins. Fibroblasts, blood vessel endothelial cells, and white blood cells are all rich in vimentin. Desmin exists in both striated and smooth muscle cells. Astrocytes and Schwann cells both have glial filaments. Synemin, desmin, and vimentin are all found in muscle intermediate filaments.
3) **Type III IF Proteins:** The IF proteins are known as neurofilament proteins because they form neurofilaments, which are an important cytoskeletal component of nerve axons and dendrites. The three different polypeptides that make up Type III IFs in vertebrates are referred to as the neurofilament triplet.
4) **Type IVIF Proteins:** They are the **nuclear lamins** which form highly organized two dimensional sheets of filaments. These filaments rapidly disassemble and reassemble at specific stage of mitosis.

General Structure of Ifs:

All cytoplasmic IF proteins are encoded by members of the same multigene family, despite the wide variations in size. A similar central section consisting of approximately 310 amino acid residues forms a prolonged α helix with three brief α-helical interruptions, according to their amino acid sequences.

Assembly of IFs

The following steps are included in a current model of intermediate filament assembly: 1. A dimer is formed by the pairing of two identical monomers, with the conserved helical core sections oriented in parallel and twisted into a coiled coil. 2. Two dimers then align themselves side by side to create a protofilament with four polypeptide chains that measures 48 nm by 3 nm. 3. Subsequently, these protofilaments form progressively larger structures by staggered association. 4. The intermediate filament's final 10 nm diameter is believed to be made up of eight protofilaments that are linked end on end to their neighbours by staggered overlap to create the long, rope-like filaments (Figure 3.5).

IFs during Mitosis:

Vimentin and cytokeratin intermediate filaments undergo dramatic alterations throughout the mitosis of cultured epithelial cells. The 10 nm filaments unwind into 2–4 nm threads and spheroidal aggregates with both kinds of proteins during prophase. The filamentous cytoskeleton gradually re-establishes during telophase, whereas the majority of vimentin and cytokeratin emerge as spheroid entities during metaphase and anaphase.

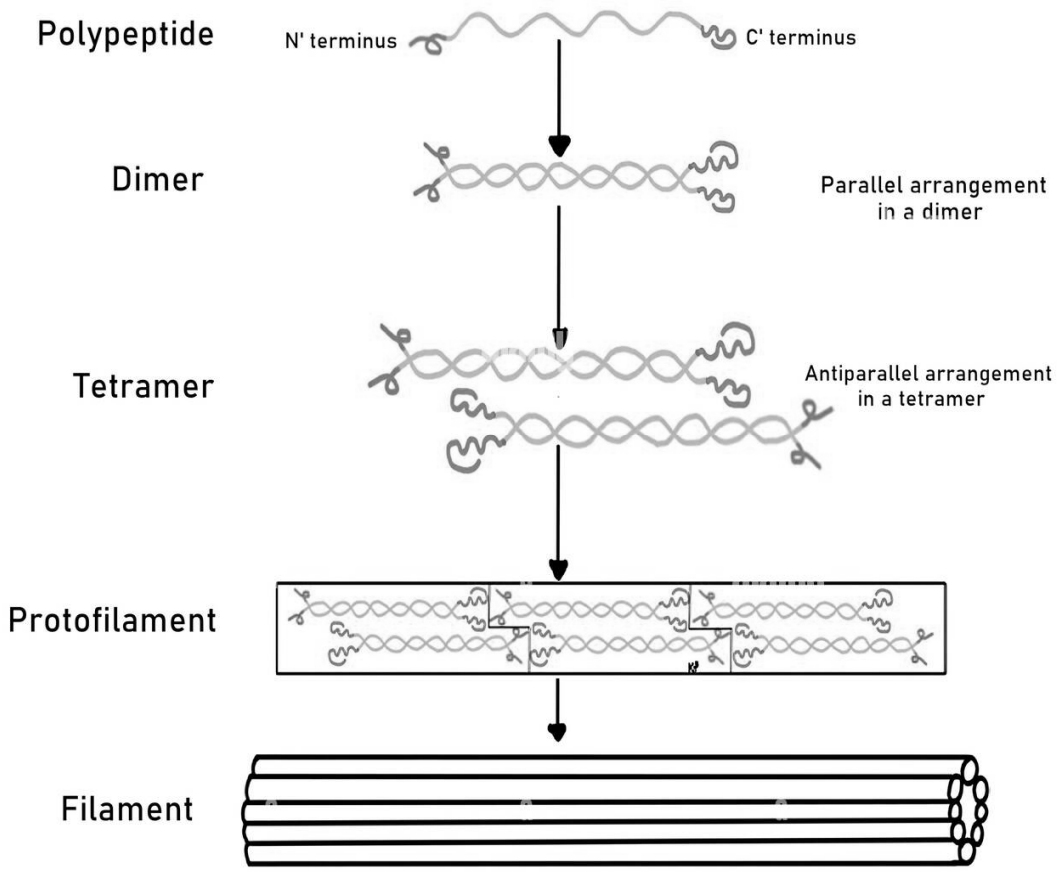

Figure-3.5: Assembly of Intermediate Filaments

Functions of Ifs:

1) The intermediate filaments in the cytoplasm maintain the cell's shape, bear tension, and provide structural support to the cell.
2) Fix the organization of certain cell organelles.
3) Intermediate filaments organize the internal tridimensional structure of the cell, anchoring organelles and serving as structural components of the nuclear lamina.
4) Keratin intermediate filaments in epithelial cells provide protection for different mechanical stresses that skin may endure.
5) They also provide protection for organs against metabolic, oxidative, and chemical stresses.
6) Strengthening of epithelial cells with these intermediate filaments may prevent onset of apoptosis, or cell death, by reducing the probability of stress.
7) In combination with proteins and desmosomes, the intermediate filaments form cell-cell connections and anchor the cell-matrix junctions that are used in messaging between cells.

The Arrangement of cytoskeleton filaments at cellular level was given in figure 3.6.

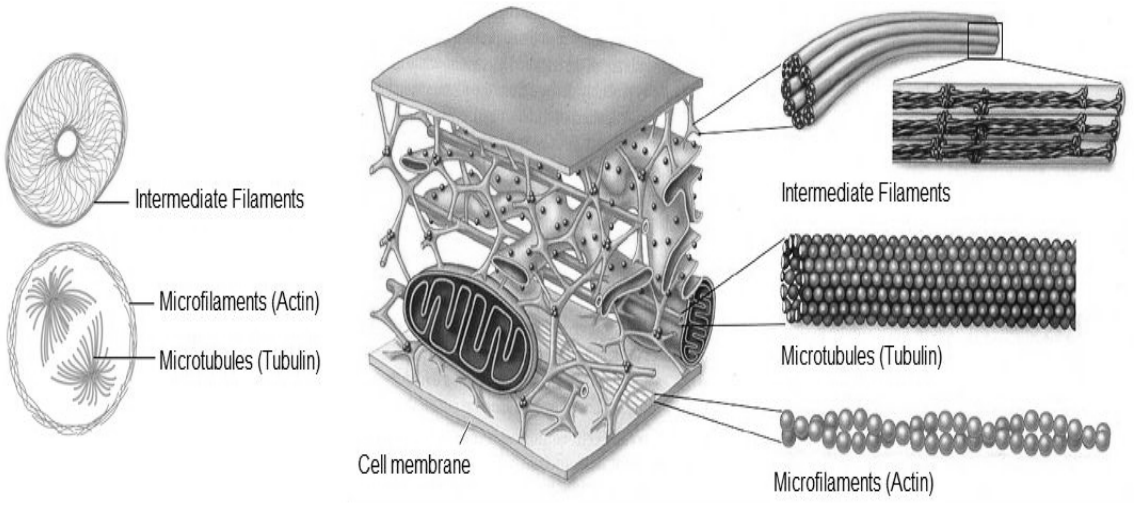

Figure-3.6: Arrangement of Cytoskeleton Filaments at Cellular Level

3.6. SUMMARY:

The cytoplasmic matrix of a eukaryotic cell is traversed by a complex network of inter-connecting membrane bound vacuoles or cavities which often remain concentrated in the endoplasmic portion of cytoplasm and referred as endoplasmic reticulum. The rough endoplasmic reticulum that is associated with ribosomes on its surface function mainly in protein synthesis. Whereas, the smooth endoplasmic reticulum devoid of attached ribosomes involves in lipid synthesis. The Golgi apparatus carry out certain cellular functions like the biosynthesis of polysaccharides, compartmentalization of cellular synthetic products, production of exocytotic secretory vesicles and differentiation of cellular membranes. Golgi apparatus is a system of sacs, with parallely arranged, flattened, membrane bounded vesicles. Lysosomes are the tiny, membrane-bound vesicles containing a variety of hydrolytic enzymes and involve in the digestion of various biological materials in the cells. Cytoskeleton provides the architecture or framework for which the entire cell is able to support itself and to quickly revise three subclass of Cytoskeleton. They are (A) Microtubule (B) Microfilament (C) Intermediate Filament. The microtubules are made up of α- tubulin and β- tubulin arranged alternately to form a protofilament. The microtubule is to promote scaffold formation, gives shape to the cell and this is what contributes to the various shapes of the eukaryotic cells. Microfilament or Actin filaments are chiefly found in the skeletal muscle cells. The microtubule is to promote scaffold formation, gives shape to the cell and this is what contributes to the various shapes of the eukaryotic cells. Microfilament or Actin filaments are chiefly found in the skeletal muscle cells.

3.7. TECHNICAL TERMS:

Rough endoplasmic reticulum, Smooth endoplasmic reticulum, Golgi apparatus, Cisternae, Vesicles, 70S ribosomes, 80S ribosomes, Primary lysosomes, Heterophagosomes, Autophagosomes, Vacuoles, Spherosomes, Aleurone grain.

3.8. SELF ASSESSMENT QUESTIONS:

1) Explain in detail about the structure and functions of endoplasmic reticulum.
2) Write an account on the Golgi apparatus.
3) Give an account on lysosomes of animal and plant cells.
4) Describe the components of cytoskeleton of eukaryotic cell and its functions.

3.9. SUGGESTED READINGS:

1) Cell Biology, Genetics, Molecular Biology, Evolution and Ecology – P. S. Verma and V. K. Agarwal, 2022. S. Chand and Company limited, New Delhi.
2) Cell Biology - C.B. Powar. 2010. Himalaya Publishing House, Mumbai – 400004.
3) Cell and Molecular Biology (8^{th} Edition), De Roberties E.D.P & De Roberties (Jr.) 2017.
4) Wolters Kluwer (India0 Pvt Ltd., New Delhi.

Dr. YRKV Tirupati Rao

LESSON-4

THE CELL NUCLEUS

4.0 OBJECTIVE:

- Students are able to understand how genetic material is organized, regulated, and transmitted, and how these processes control cellular structure and function.

STRUCTURE:

4.1 Introduction

4.2 Structure and Functions of Nucleus

4.3 Summary

4.4 Technical Terms

4.5 Self-Assessment Questions

4.6 Suggested Readings

4.1. INTRODUCTION:

The nucleus is a membrane-bound organelle present in all eukaryotic cells, housing the cell's hereditary material and regulating cellular growth and reproduction. Functioning as the regulatory centre of the eukaryotic cell, it is the site where nearly all of the cell's DNA is localized, replicated, and transcribed. The nucleus was first observed and named by Robert Brown in 1831 in plant cells. It is separated from the cytoplasm by the nuclear membrane. The position of the nucleus varies depending on the cell type and its metabolic activity; it is typically centrally located, but may shift—for example, it is centrally positioned in embryonic cells, whereas in glandular cells it may be found toward the basal region. Mitochondria are vital organelles present in almost all eukaryotic cells, commonly referred to as the "powerhouses" of the cell because of their key role in ATP production. Derived from the Greek *mito* (thread) and *chondrion* (granule), mitochondria occur as filamentous or granular structures in the cytoplasm of all aerobic cells of higher plants, animals, and various microorganisms including algae, protozoa, and fungi; however, they are absent in bacteria. Their internal lipoprotein framework contains numerous enzymes and coenzymes essential for cellular energy metabolism. Mitochondria also possess their own DNA, which enables cytoplasmic inheritance, and contain ribosomes necessary for protein synthesis. Chloroplasts were first described by early microscopists such as Nehemiah Grew and Antonie van Leeuwenhoek. The term plastid was introduced by Schimper in 1885. Later, in 1918, Wilstätter and Stoll successfully isolated and characterized the green pigments chlorophyll *a* and *b*. In 1947, K. Porter and S. Granick elucidated the ultrastructure of chloroplast grana. Pioneering studies by Julius Sachs demonstrated that chloroplasts absorb carbon dioxide in the presence of sunlight. Dutrochet (1837) established that chlorophyll is essential for the release of oxygen by plants. In 1932, Emerson and Arnold conducted the famous flashing light experiment, distinguishing the light and dark reactions of photosynthesis and proposing the concept of the photosynthetic unit (PS I).

4.2. STRUCTURE AND FUNCTIONS OF NUCLEUS:

Morphology:

The structure of a cell nucleus consists of a) Nuclear membrane / Nuclear envelope b) Nucleoplasm c) Nucleolus and d) Chromosomes.

a) Nuclear Membrane: The presence of the nuclear membrane was first confirmed by O. Hertwig in 1893. The ultrastructure of the nuclear envelope, nuclear pore complexes, and the nuclear lamina was later described in detail by Kirschner and colleagues in 1977 and independently by Schatten and Thoman in 1978. A fundamental distinction between prokaryotic and eukaryotic cells lies in the absence or presence of this nuclear envelope. The nuclear envelope, also referred to as the nuclear membrane, is composed of two parallel membranes an inner and an outer membrane each measuring about 5–10 nm in thickness, and separated by a perinuclear space of 10–50 nm (Figure 4.1). The envelope fully surrounds the nucleus, effectively isolating the cell's genetic material from the cytoplasm and preventing the unregulated exchange of macromolecules between the nucleoplasm and the cytoplasmic compartment. The outer nuclear membrane is continuous with the membrane of the rough endoplasmic reticulum (RER) and bears ribosomes in a similar manner. Consequently, the perinuclear space is continuous with the lumen of the RER. The inner nuclear membrane is supported by the nuclear lamina, a dense meshwork of lamin proteins. This fibrous layer, typically 50–80 nm or sometimes 10–20 nm thick, is formed by intermediate filament proteins called lamins, which are synthesized in the cytoplasm and subsequently imported into the nucleus for assembly into the lamina. The nuclear lamina underlies the inner membrane except at regions occupied by nuclear pores and exhibits a square lattice-like organization. In mammals, the lamins belong to three main types lamin A, lamin B, and lamin C with molecular weights of approximately 74,000, 72,000, and 62,000 daltons, respectively.

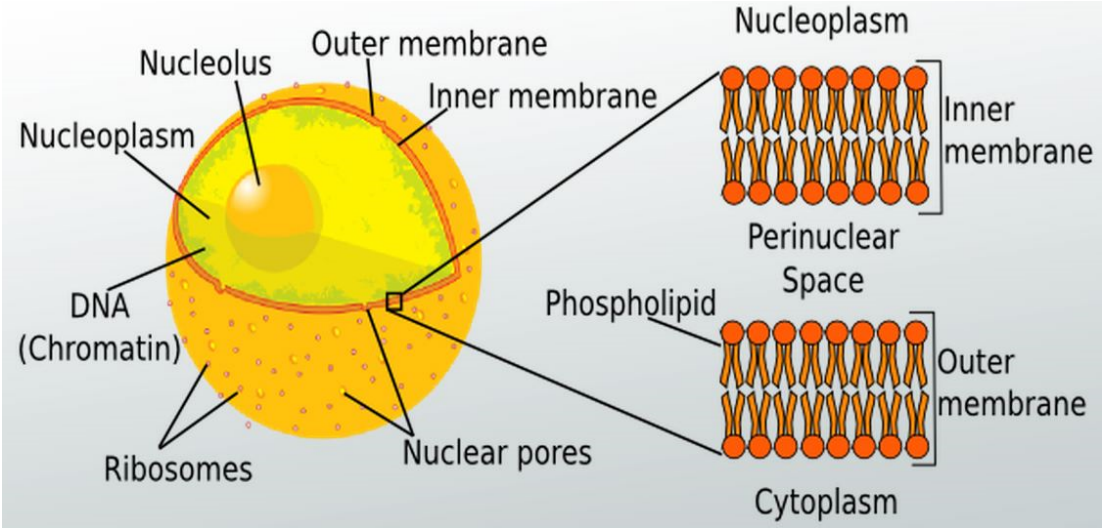

Figure 4.1 Intenral Structure of Nuclear Membrane

Nuclear Pore Complex:

The materials exchanged between nucleus and cytoplasm must traverse the nuclear pore complexes. This exchange is very selective and allows passage of only certain molecules, of either low or very high molecular weight. The nuclear envelope in all

eukaryotic forms, from yeasts to humans, is perforated by nuclear pores. In 1950, Callan and Tomlin observed the nucleo-pores in the nuclei of amphibian oocytes. The pores are octagonal orifices and 60 nm in diameter while, freely diffusible size is only about 9 nm wide, due to the presence of regulatory systems within the center of the pore. This size allows the free passage of small water-soluble molecules and preventing larger molecules, such as nucleic acids and larger proteins, from incorrectly entering or exiting the nucleus (Figure 4.2). Each pore complex has an estimated molecular weight of 50-100 million daltons. Particles (P) are anchored to cytoplasmic ring and are thought to be inactive ribosomes. This hole often appears to be plugged by a large central granule or plug which consist of newly made ribosomes. The pore complex perforates the nuclear envelope bringing the lipid bilayers of the inner and outer nuclear membrane together around the margins of each pore.

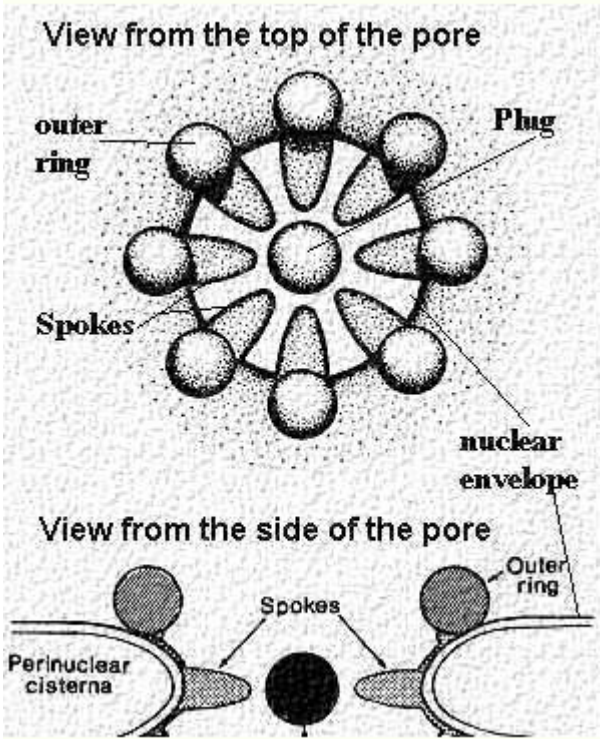

Figure 4.2 Structure of Nuclear Pore Complex

Functions of Nuclear Membrane:

1. Protection of DNA 2. Nucleo-cytoplasmic material exchange 3. Attachment of structural elements in the cytoplasm 4. Attachment of nuclear component during interphase 5. Contribution to formation of other cell membranes 6. Protein synthesis 7. Antibody production 8. Synthesis of chromosomal enzymes.

b) Nucleoplasm: The space between the nuclear envelope and the nucleolus is filled by a transparent, semi-solid, granular and slightly acidophilic ground substance or the matrix known as the nuclear sap or nucleoplasm. The nuclear components such as the chromatin threads and the nucleolus remain suspended in the nucleoplasm. It is composed of mainly the nucleoproteins but it also contains other inorganic and organic substances, *viz.*, nucleic acids, proteins, enzymes and minerals.

Nucleic Acids:

The most common nucleic acids of the nucleoplasm are the DNA and RNA. Both may occur in the macromolecular state or in the form of their monomer nucleotides.

Proteins:

The nucleoplasm contains many types of complex proteins. The nucleoproteins can be categorized into following two types:

i) **Basic Proteins:** the proteins, which take basic stain (Feulgen – Robert Feulgen - 1924, Acridine orange- Benda - 1889), are known as the basic proteins. The most important basic proteins of the nucleus are nucleoprotamines and the nucleohistones. The nucleoprotamines are simple and basic proteins having very low molecular weight (about 4000 daltons). The most abundant amino acid of these proteins is arginine (pH 10 to 11). The protamines usually remain bounded with the DNA molecules by the salt linkage. The nucleohistones have high molecular weight, e.g., 10,000 to 18,000 daltons. The histones are composed of basic amino acids such as arginine, lysine and histidine. The histone proteins remain associated with the DNA by the ionic bonds and they occur in the nuclei of most organisms. According to the composition of the amino acids following types of histone proteins have been recognised, *e.g., histones* rich in lysine, histones with arginine and histones with poor amount of the lysine.

ii) **Non-Histone or Acidic Proteins:** The acidic proteins either occur in the nucleoplasm or in the chromatin. The most abundant acidic proteins of the euchromatin (a type ofchromatin) are the phosphoproteins.

iii) **Enzymes:** The nucleoplasm contains many enzymes which are necessary for the synthesis of the DNA and RNA. Most of the nuclear enzymes are composed of non-histone (acidic) proteins. The most important nuclear enzymes are the DNA polymerase, RNA polymerase, NAD synthetase, nucleoside triphosphatase, adenosine diaminase, nucleoside phosphorylase, guanase, aldolase, enolase, 3-phosphoglyceraldehyde dehydrogenase and pyruvate kinase. The nucleoplasm also contains certain cofactors and coenzymes such as ATP and acetyl Co-A.

Lipids:

According to Stoneburg (1937) and Dounce (1955), the nucleoplasm contains small lipid content.

Minerals:

The nucleoplasm also contains several inorganic compounds such as phosphorus, potassium, sodium, calcium and magnesium.

Sub-Nuclear Structures:

Apart from these, the nucleoplasm also contains various sub-nuclear structures such as nuclear bodies, nuclear speckles, cajal bodies and promyelocytic leukemia (PML) nuclear bodies (Figure 4.3).

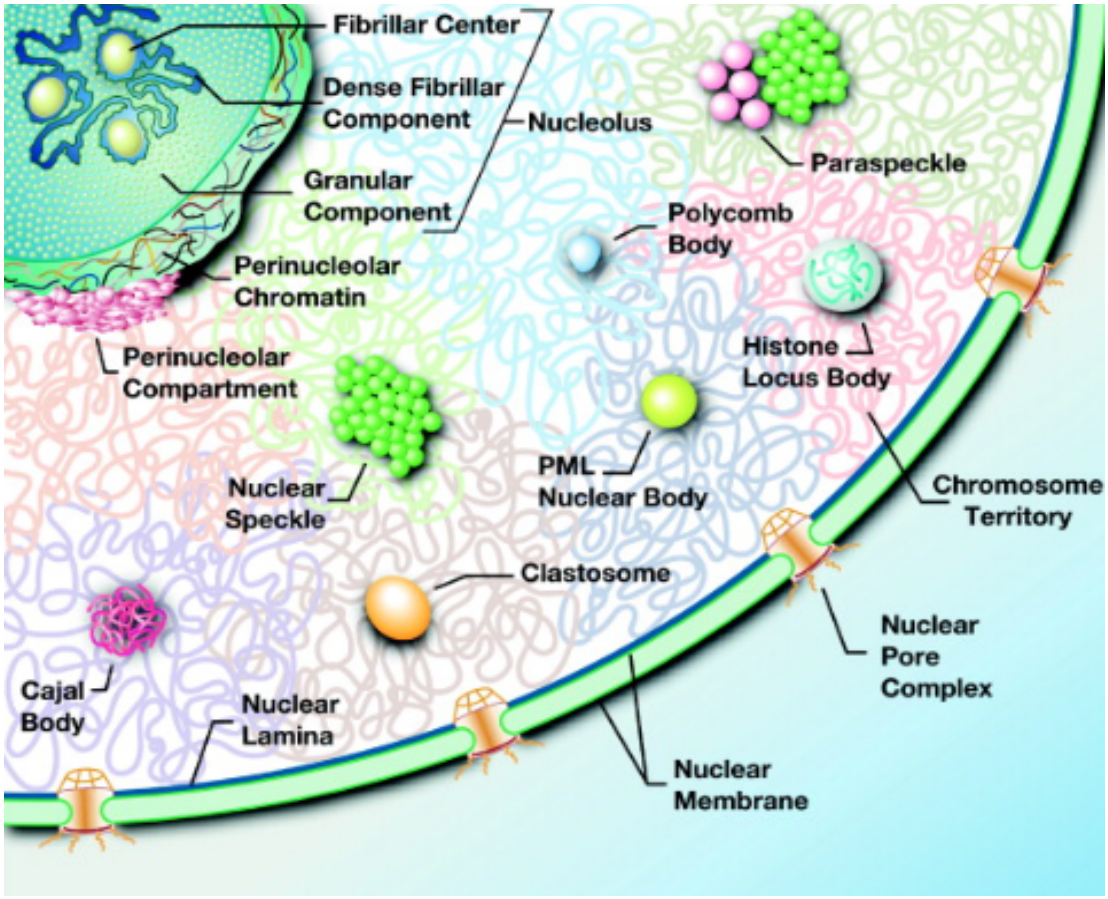

Figure 4.3 Structure of Nuclear Bodies

Nuclear Bodies:

The nucleus is compartmentalized and contains numerous sub-nuclear structures called nuclear bodies. The nuclear bodies present in nucleus are nucleoli, splicing speckles, Cajal bodies (CB), gems, and promyelocytic leukemia (PML) nuclear bodies. In contrast to cytoplasmic compartments, the sub-nuclear bodies lack a membrane separating them from the nucleoplasm. These sub-nuclear bodies may serve to enhance the efficiency of specific nuclear processes.

Nuclear Speckles:

Nuclear Speckles, also known as interchromatin granule clusters, are irregular shaped structures of varied size and the nucleus typically contains 25-50 of these sub-nuclear bodies. Nuclear speckles are enriched in pre-mRNA splicing factors including small nuclear ribonucleoprotein particles (snRNPs) and non-snRNP protein splicing factors eg: splicing factor SC35. Speckles are often found close to actively transcribed genes and act as a reservoir for the splicing of nascent pre-mRNA at nearby genes.

Cajal Bodies:

These are discovered by Santiago Ramón y Cajal (1903). Cajal bodies are roughly spherical structures numbering one to five per nucleus, varying in number and size. These structures appear in the form of coiled threads and are characterized by the presence of the

coilin protein. The cajal bodies are not seen in all cells or tissues, but are especially prominent in highly proliferative cells such as cancer cells or metabolically active cells. Cajal bodies are thought to play a role in snRNP biogenesis and in the trafficking of snRNPs and small nucleolar RNPs (snoRNPs).

Promyelocytic Leukemia (PML) Nuclear Bodies:

PML bodies are characterized by the presence of PML protein. PML bodies vary in size from 0.3-1micron meter in diameter and a nucleus typically contains 10-30 of these structures. PML nuclear bodies have emerged as important regulators of cell cycle, defense against viral infection, induction of apoptosis (tumour suppressor) and cellular senescence.

Significance of Nuclear Bodies:

Nuclear bodies help in splicing (removal of non-coding introns). Some nuclear bodies eg: cajal bodies contains some molecules called small nuclear RNPs (sn RNPs) and small nucleolar RNPs (Sno RNPs), which helps in the splicing of some non-coding sequences from mRNA and rRNA. Nuclear bodies helps in organization and formation of ribosomal subunits. Some nuclear bodies acts as transcriptional coactivator and thus enhance gene expression. Nuclear bodies such as PML nuclear bodies acts as tumor suppressor protein, provide defense against viral infection and induce apoptosis.

c) Nucleolus: Nucleolus was first observed by Fontana in the year 1781 but was described by M. J. Schleiden in 1838. The term nucleolus was coined by Bowman in 1840. It is a discrete densely stained, acidophilic body found in the nucleus. It is not surrounded by a membrane, and is sometimes called a *suborganelle*. Cells of bacteria and yeast lack nucleolus. The size of the nucleolus is found to be related with the synthetic activity of the cell. Therefore, the cells with little or no synthetic activities, *e.g., sperm cells, blastomeres, muscle cell, etc., are* found to contain smaller or no nucleoli, while the oocytes, neurons and secretory cells which synthesize the proteins or other substances contain comparatively large-sized nucleoli. The number of the nucleoli in the nucleus depends on the species and the number of the chromosomes. The number of the nucleoli in the cells may be one, two or four. The position of the nucleolus in the nucleus is eccentric. It stains with basophilic dyes like pyronine and absorbs ultraviolet light at 260 nm.

A nucleolus is often associated with the nucleolar organizer regions (NOR) which represents the secondary constriction of the nucleolar organizing chromosomes, and are 10 in number in human beings (Figure 4.4). In 1934, Barbara Mc Clintock recognized and named nucleolar organizers in the chromosomes. It forms around tandem repeats of rDNA and DNA coding for ribosomal RNA (rRNA). Nucleolar organizer consists of the genes for 18S, 5.8S and 28S rRNAs. The genes for fourth type of r RNA, *i.e., 5S rRNA* occur outside the nucleolar organizer. The main roles of the nucleolus are to synthesize rRNA and assemble ribosomes.

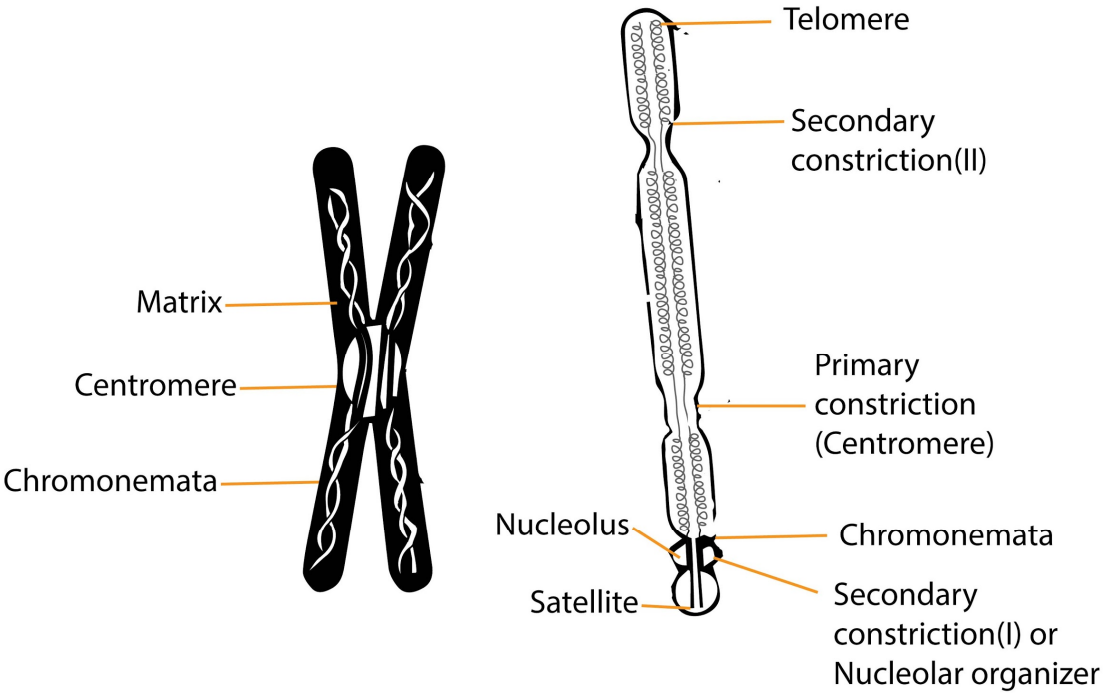

Figure 4.4 Nucleolus and Nucleolus Organizing Region

I. Chemical Composition of Nucleolus:

Nucleolus is not bounded by any limiting membrane; calcium ions are supposed to maintain its intact organization. Chemically, nucleolus contains DNA of nucleolar organizer, four types of rRNAs, 70 types of ribosomal proteins, RNA binding proteins (*e.g.*, nucleolin) and RNA splicing nucleoproteins (U1, U2, …, U12). It also contains phospholipids, orthophosphates and Ca2+ ions. Nucleolus also contains enzymes such as acid phosphatase, nucleoside phosphorylase and NAD+ synthesizing enzymes for the synthesis of some coenzymes, nucleotides and ribosomal RNA. RNA methylase enzyme, which transfers methyl groups to the nitrogen bases, is found in the nucleolus of some cells.

II. Ultrastructure and Function of Nucleolus:

This nucleolus is believed to contain 3 different regions (Figure 4.5).

A. Fibrillar Centre: This pale-staining part represents the innermost region of nucleolus. The RNA genes of nucleolar organizer of chromosomes are located in this region. It is the place for initializing the transcription *i.e., ribosomal RNA* synthesis of these genes.

B. Dense Fibrillar Component: The dense fibrillar component surrounds the fibrillar centre and RNA synthesis progresses in this region. Binding of 70s ribosomal proteins (rps) to the transcripts takes place in this region.

C. Cortical Granular Components: This is the outermost region of the nucleolus where processing and maturation of pre-ribosomal particles takes place.

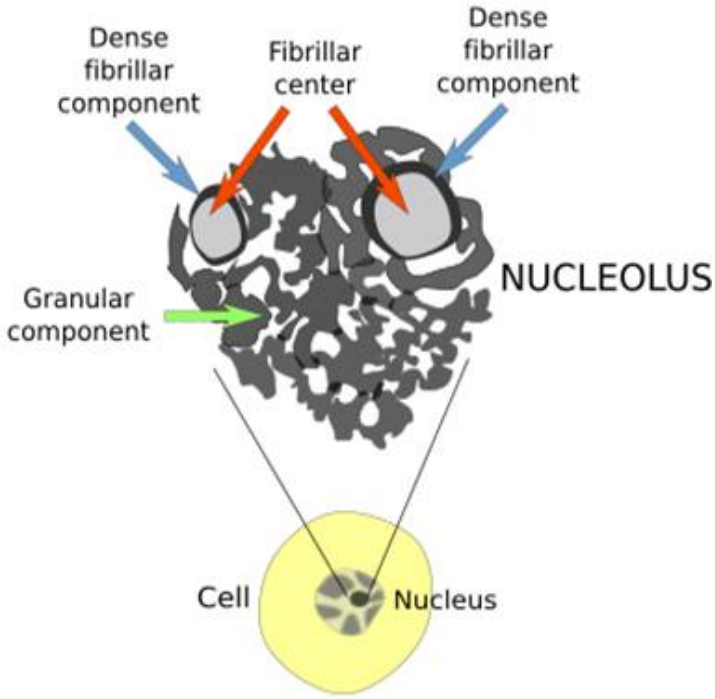

Figure 4.5. Regions of Nucleolus

(III) Mitotic Cycle and Nucleolus: The appearance of nucleolus changes dramatically during the cell cycle. During mitosis, the nucleoli undergo cyclic changes. The nucleoli are formed around the DNA loop that extends from nucleolar organizer and disappears in prophase stage. As the cell approaches mitosis, the nucleolus first decrease in size and then disappears as the chromosomes condense and all RNA synthesis stops, so that generally there is no nucleolus in a metaphase cell. During late prophase, the DNA loop containing the rRNA genes gradually retracts and coils into the nucleolar organizer of the corresponding chromosome. Since the DNA is very extended as a consequence of intense RNA synthesis, the nucleolar organizer region is one of the last to undergo condensation, hence producing a secondary constriction on the chromosome. After the cell divides, during telophase, the nucleolar organizer DNA uncoils and the nucleolus is reassembled.

Functions of Nucleolus:

1) **Ribosome Biogenesis:** The nucleolus is primarily responsible for the production and assembly of ribosomal subunits. It synthesizes ribosomal RNA (rRNA), which is a critical component of ribosomes. The nucleolus also assembles rRNA with ribosomal proteins to form the large and small subunits of ribosomes, which are then exported to the cytoplasm for protein synthesis.

2) **rRNA Transcription and Processing:** The nucleolus contains the genes for rRNA (rDNA), which are transcribed into precursor rRNA (pre-rRNA). It processes pre-rRNA into mature rRNA through cleavage and chemical modifications.

3) **Assembly of Ribosomal Proteins:** The nucleolus facilitates the assembly of ribosomal proteins, which are imported from the cytoplasm, with rRNA to form functional ribosomes.

4) **Cell Cycle Regulation:** The nucleolus plays a role in regulating the cell cycle by interacting with proteins involved in cell division and growth. It can act as a sensor for cellular stress, influencing cell cycle progression or arrest.

5) **Stress Response:** Under stress conditions (e.g., heat shock, nutrient deprivation), the nucleolus can undergo structural changes and participate in stress response pathways. It can sequester or release specific proteins to modulate cellular responses.

6) **Regulation of Telomerase:** The nucleolus is involved in the assembly and regulation of telomerase, an enzyme that maintains telomere length and chromosome stability.

7) **Biogenesis of Other Ribonucleoprotein (RNP) Particles:** In addition to ribosomes, the nucleolus is involved in the assembly of other RNP particles, such as signal recognition particles (SRPs), which are essential for protein targeting to the endoplasmic reticulum.

8) **Quality Control:** The nucleolus ensures the proper assembly and function of ribosomes by monitoring and degrading defective rRNA or ribosomal subunits.

9) **Regulation of Gene Expression:** The nucleolus can influence gene expression by sequestering or releasing transcription factors and other regulatory proteins.

10) **Viral Defense:** The nucleolus plays a role in the cellular response to viral infections by interacting with viral components and participating in antiviral defense mechanisms.

d) Chromosomes or Chromatin Fibres:

W. Flemming (1879) coined the term chromatin for chromosomal meshwork. The cell nucleus contains the majority of the cell's genetic material in the form of multiple linear DNA molecules organized into structures called chromosomes. Each human cell contains 2 m of DNA. Most of the protein of chromatin is histone, but “non-histone” proteins are also present. The protein and DNA weight ratio averages about 1:1. Histones are constituents of the chromatin of all eukaryotes except fungi, which, therefore, resemble prokaryotes in this respect. During most of the cell cycle these are organized in a DNA-protein complex known as chromatin, but during the cell division, the chromatin can be seen to form the well-defined chromosomes. There are two types of chromatin found in the nucleus, (I) Euchromatin (II) Heterochromatin (Figure 4.6).

I. Euchromatin: It is the less compact DNA form, and contains genes that are frequently expressed by the cell. This region can be visualized in the condensed chromosomes as the regions that stain very lightly. Euchromatin is rich in gene concentration, and is often under active transcription. Euchromatin comprises the most active portion of the genome within the cell nucleus. About 92% of the total human genome is euchromatic.

II. Heterochromatin: It is the more compact form, and contains DNA that is infrequently transcribed. This region is stained darkly. This structure is further categorized into *facultative* heterochromatin, consisting of genes that are organized as heterochromatin only in certain cell types or at certain stages of development, and *constitutive* heterochromatin that consists of chromosome structural components such as telomeres and centromeres and is permanently condensed in all types of cells.

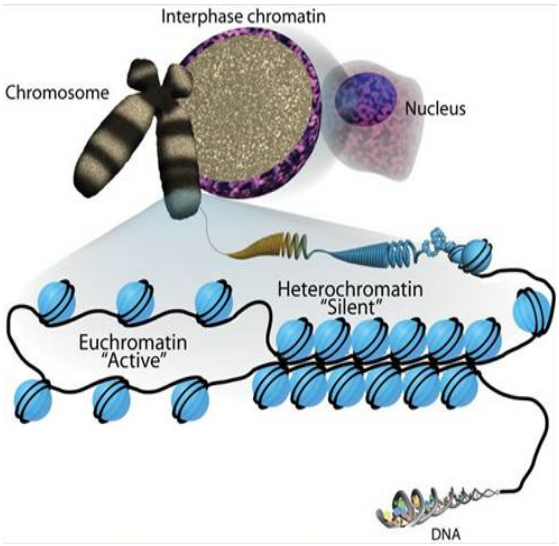

Figure 4.6: Euchromatin and Heterochromatin

Cell Nucleus Functions:

1) It controls the hereditary characteristics of an organism and is responsible for the protein synthesis, cell division, growth and differentiation.
2) Stores the hereditary material, referred to as chromatins.
3) Storage of proteins and RNA (ribonucleic acid) in the nucleolus.
4) Nucleus is a site for transcription in which messenger RNA (mRNA) are produced for the protein synthesis.
5) Exchange of hereditary molecules (DNA and RNA) between the nucleus and rest of the cell.
6) Production of ribosomes (protein factories) in the nucleolus.
7) Selective transportation of regulatory factors and energy molecules through nuclear pores.

4.3. SUMMARY:

The cell nucleus is a large, membrane-bound organelle that acts as the central regulatory hub of all eukaryotic cells. It houses the organism's genetic material in the form of DNA, which is organized with histone proteins into chromatin. This genetic material controls essential cellular processes such as growth, differentiation, metabolism, reproduction, and gene expression. The nucleus is enclosed by a double-layered nuclear envelope, which separates nuclear contents from the cytoplasm and contains numerous nuclear pores that regulate the selective transport of RNA, proteins, and other molecules. Within the nucleus, the nucleoplasm provides a supportive medium for chromatin, enzymes, and nuclear bodies. A prominent structure inside the nucleus is the nucleolus, responsible for synthesizing ribosomal RNA and assembling ribosomal subunits. Chromatin exists in two forms euchromatin, which is lightly packed and transcriptionally active, and heterochromatin, which is densely packed and generally inactive. The nuclear envelope is supported internally by the nuclear lamina, a protein meshwork that maintains nuclear shape and organizes

chromatin. Overall, the nucleus plays a vital role in safeguarding genetic information, coordinating gene activity, and ensuring accurate transmission of hereditary material during cell division.

4.4. TECHNICAL TERMS:

Nucleus, Nucleolus, Chromatin, Nucleoplasm**,** Nuclear Pore Complex (NPC)**,** Nuclear Lamina, Nucleolus, Euchromatin, Heterochromatin.

4.5. SELF-ASSESSMENT QUESTIONS:

1) What is the nuclear envelope? Mention its major components.
2) Differentiate between euchromatin and heterochromatin.
3) Name the components of the nucleolus and their specific functions.
4) Describe the structure and functions of the cell nucleus in detail.
5) Explain the nuclear envelope with reference to its structure, composition, and role in nucleocytoplasmic transport.
6) Give a detailed account of the nucleolus—its structure, components, and role in ribosome biogenesis.
7) Write an essay on the chemical composition of the nucleus and its importance in cellular functioning.

4.6. SUGGESTED READINGS:

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2) De Roberties E.D.P & De Roberties (Jr.) 2017. Cell and Molecular Biology (8^{th} Edition).
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4) Alberts, B. et. al. (Molecular Biology of the Cell) – Garland Science
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8) Watson et al., Molecular Biology of the Gene – Pearson
9) P.S. Verma & V.K. Agarwal, 2021. Cell Biology, Genetics, Molecular Biology, Evolution and Ecology. S.Chand and Company Limited, New Delhi – 110044.
10) Sara Assem, Praxi Labs (https://praxilabs.com).

Dr. Kakumanu Babu

LESSON-5

ULTRASTRUCTURE OF PROKARYOTIC AND EUKARYOTIC CHROMOSOMES

5.0 OBJECTIVE:

- Students are able to understand how DNA is organized and packaged to regulate gene expression, ensure accurate replication, and maintain genetic stability.

STRUCTURE:

5.1 Introduction

5.2 Ultrastructure of Prokaryotic Chromosome

5.3 Ultra Structure of Eukaryotic Chromosome

5.4 Summary

5.5 Technical Terms

5.6 Self-Assessment Questions

5.7 Suggested Readings

5.1. INTRODUCTION:

Chromosomes are the fundamental units of genetic organization in all living cells, responsible for storing, transmitting, and regulating hereditary information. Although prokaryotes and eukaryotes both possess chromosomes composed of DNA, their structural organization, packaging mechanisms, and cellular localization vary significantly due to differences in genome size, complexity, and evolutionary lineage. These differences reflect how each cell type has adapted to efficiently maintain and express its genetic material. Prokaryotic chromosomes are generally simple in architecture. Most prokaryotes, such as bacteria, possess a single, circular, double-stranded DNA molecule located in an unenclosed region of the cytoplasm called the nucleoid. They lack a nuclear membrane, allowing transcription and translation to occur simultaneously. Unlike eukaryotic chromosomes, prokaryotic chromosomes do not bind true histone proteins instead, they are compacted by nucleoid-associated proteins (NAPs) such as HU, IHF, and FIS, which help organize DNA into supercoiled loops. Some bacteria may contain plasmids, which are small, extrachromosomal DNA molecules that provide additional adaptive traits like antibiotic resistance. The relatively small genome size and simpler internal structure of prokaryotic chromosomes enable rapid replication and cell division, supporting their fast growth and adaptability. Eukaryotic chromosomes, in contrast, are far more complex, reflecting the larger genome sizes and functional diversity of eukaryotic organisms. These chromosomes are linear DNA molecules located inside a well-defined nucleus and are extensively associated with histone proteins to form chromatin. Chromatin shows a hierarchical structure. DNA wraps around histone octamers to form nucleosomes, which coil into 30-nm fibers and further fold into higher-order structures during cell division. Eukaryotic chromosomes possess specialized internal regions such as centromeres (for spindle attachment during mitosis),

telomeres (which protect chromosome ends), and multiple origins of replication that ensure timely and accurate DNA duplication. This sophisticated organization not only compacts the long DNA molecules but also allows precise control over gene expression, DNA repair, and genome stability. Together, the highly ordered internal structure and functional specialization of eukaryotic chromosomes underpin the complex developmental and physiological processes characteristic of higher organisms.

5.2. ULTRASTRUCTURE OF PROKARYOTIC CHROMOSOME:

Prokaryotes are simple, mostly unicellular (Figure 5.1) and some forms colonies and behaves as multicellular organisms. These organisms reproduce asexually most often and are haploid (there is always only one copy of the gene). Prokaryotes often contain several plasmids (extra chromosomal DNA molecules, which are linear or circular). Unlike chromosomal DNA, plasmid DNA is typically smaller and encodes genes that are not necessary for survival. Often, however, give to the cell some advantage (e.g. resistance to antibiotics). Replication of plasmids is independent of chromosomal replication. Prokaryotes need to cram all their genes within one chromosome, so it doesn´t remain too much space for the non–coding sequences. While in eukaryotes, the share of non–coding parts of DNA is about 98%, in prokaryotes it is only 12%.

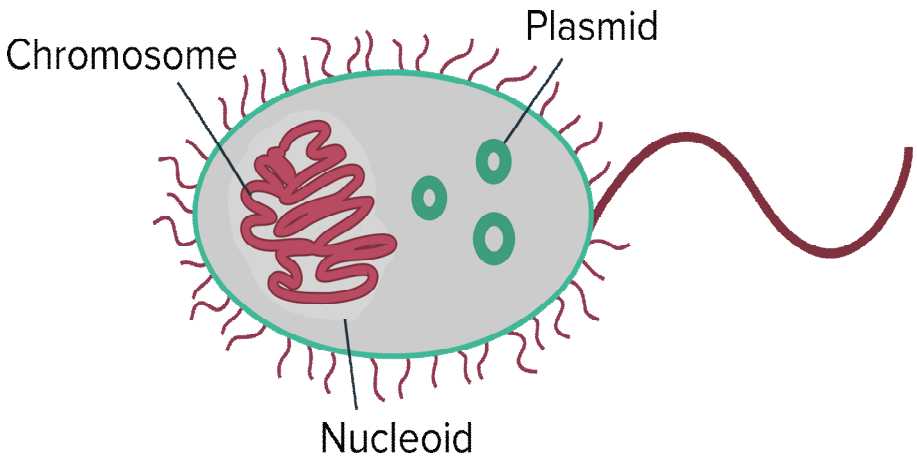


Figure-5.1: Simple Prokaryote Cell

DNA communicates with the cytoplasm, so it allows direct connection to transcription and translation. The genome of prokaryotes is usually made up of one "chromosome" and *plasmids*. Eukaryota however, contain a larger number of chromosomes - we distinguish two types of eukaryota´s chromosomes (nuclear and mitochondrial) and sometimes even plasmids. Most of the information about chromosomes of prokaryotes have been obtained from studies of *E. coli*. It is the organism of choice for such research of prokaryotes. Chromosome consists of double stranded circular DNA. Prokaryotes do not contain nucleus or other membrane bounded organelles. The term "prokaryotes" actually means "before nucleus". Chromosome is stored in a special area called nucleoid. The genome of prokaryotes is often significantly larger than the cell itself. Eukaryota solves this problem by *wrapping DNA* around the histones. However, prokaryotes do not contain histones (with a few exceptions). Prokaryotes to compress their DNA using

fiber rolled into small rolls supercoiling. The fibers are twisted so tightly that the final consequences loops overlap to form one big ball. Distinguishes two types of collapse – positive (DNA turns are in the same direction as the helix) or negative (DNA is coiled in the opposite direction than the helix) (Figure 5.2).

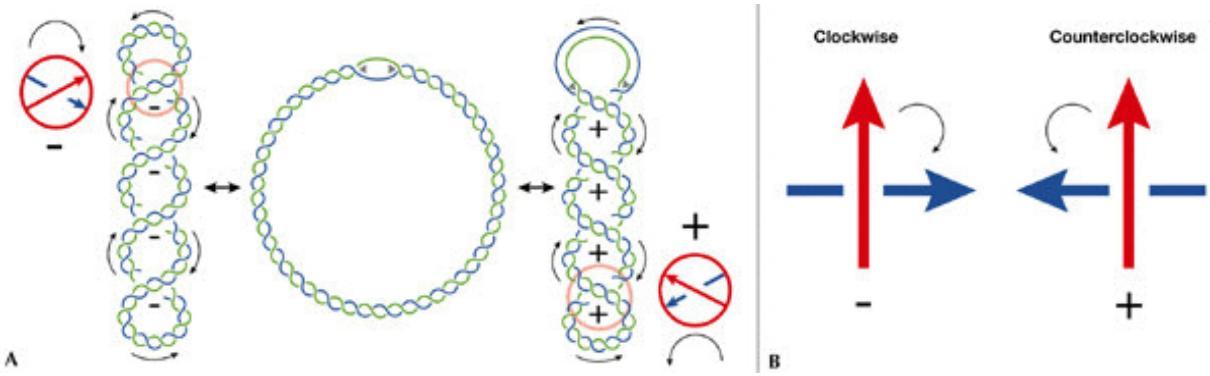


Figure-5.2: Positive and Negative Prokaryotic DNA

E. coli: A Model Prokaryote

Much of what is known about prokaryotic chromosome structure was derived from studies of *Escherichia coli*, a bacterium that lives in the human colon and is commonly used in laboratory cloning experiments. In the 1950s and 1960s, this bacterium became the model organism after discovery of phase-contrast microscopy and autoradiography. These techniques revealed that the essential genes of *E. coli* are encoded on a single circular chromosome packaged within the cell nucleoid (Figure 5.3).

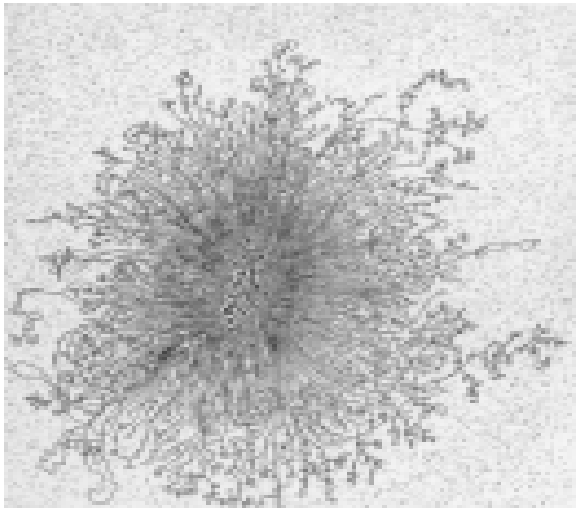


Figure-5.3: Scanning Electron Microscopy Photograph of Prokaryote Nucleoid

Proteins Involved in Supercoiling:

During the 1980s and 1990s, researchers discovered that multiple proteins act together to fold and condense prokaryotic DNA (In particular, one protein called HU (Heat Unstable), which is the most abundant protein in the nucleoid. HU works with an enzyme called

topoisomerase I to bind DNA and introduce sharp bends in the chromosome, generating the tension necessary for negative supercoiling. Integration host factor (IHF), can bind to specific sequences within the genome and introduce additional bends. H-NS (Histone like Nucleoid Protein), plays an active role in transcription by modulating the expression of the genes involved in the response to environmental stimuli. Factor for inversion stimulation (FIS) protein is abundant during exponential growth and regulates the expression of more than 231 genes, including DNA topoisomerase I. Once the prokaryotic genome has been condensed, DNA topoisomerase I, DNA gyrase, and other proteins help maintain the supercoils.

Accessing Supercoiled Genes:

It has been determined that prokaryotic DNA replication occurs at a rate of 1,000 nucleotides per second, and prokaryotic transcription occurs at a rate of about 40 nucleotides per second. During transcription, small regions of the chromosome can be seen to project from the nucleoid into the cytoplasm (i.e., the interior of the cell), where they unwind and associate with ribosomes, thus allowing easy access by various transcriptional proteins. These projections are thought to explain the mysterious shape of nucleoids during active growth. When transcription is inhibited, however, the projections retreat into the nucleoid, forming the aforementioned spherical shape. Because there is no nuclear membrane to separate prokaryotic DNA from the ribosomes within the cytoplasm, transcription and translation occur simultaneously in these organisms.

Variations in Prokaryotic Genome Structure:

Recent studies have indicated that some prokaryotes contain as many as four linear or circular chromosomes. For example, *Vibrio cholerae*, the bacteria that causes cholera, contains two circular chromosomes. One of these chromosomes contains the genes involved in metabolism and virulence, while the other contains the remaining essential genes. An even more extreme example is provided by *Borrelia burgdorferi*, the bacterium that causes Lyme disease. This organism is transmitted through the bite of deer ticks, and it contains up to 11 copies of a single linear chromosome. Unlike *E. coli*, *Borrelia* cannot supercoil its linear chromosomes into a tight ball within the nucleoid; rather, these strands are diffused throughout the cell. Other organisms, such as *Bacillus subtilis*, form nucleoids that closely resemble those of *E. coli*, but they use different architectural proteins. DNA molecules of Archaea, are the only group of prokaryotes that use eukaryote-like histones.

Plasmids:

A plasmid is a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently (Figure 2.4). The term was coined by Joshua Lederberg and Hays, discovered by Tatum. They are most commonly found in bacteria as small circular, double-stranded DNA molecules; however, plasmids are sometimes present in archaea and eukaryotic organisms. Plasmids carry genes that may benefit the survival of the organism, for example antibiotic resistance. Chromosomes are big and contain all the essential genetic information for living under normal conditions, plasmids usually are very small and contain only additional genes that may be useful to the organism under certain situations or particular conditions.

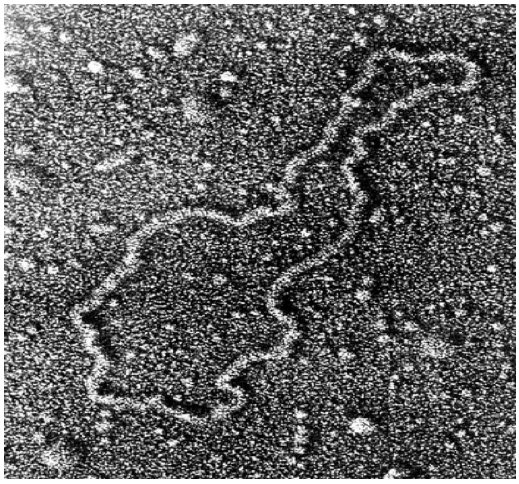


Figure-5.4: Scanning Electron Microscope Structure of Plasmid

Structure of Plasmids:

Every plasmid has certain essential elements such as 1. Origin of replicon 2. Selectable marker site 3. Promoter region 4. Primer binding site 5. Multiple cloning sites (Figure 5.5).

1) **Origin of replication (OR) –** This refers to a specific location in the strand where the replication process begins. In plasmids, this region is A=T rich region as it is easier to separate the strands during replication.
2) **Selectable marker site –** This region consists of Antibiotic resistance genes which are useful in the identification and selection of bacteria that contain plasmids.
3) **Promoter region –** this is the region where the transcriptional machinery is loaded.
4) **Primer binding site –** this is the short sequence of single-strand DNA which is useful in DNA amplification and DNA sequencing.
5) **Multiple cloning sites –** This site contains various sequences where the restriction enzymes can bind and cleave the double stranded structure.

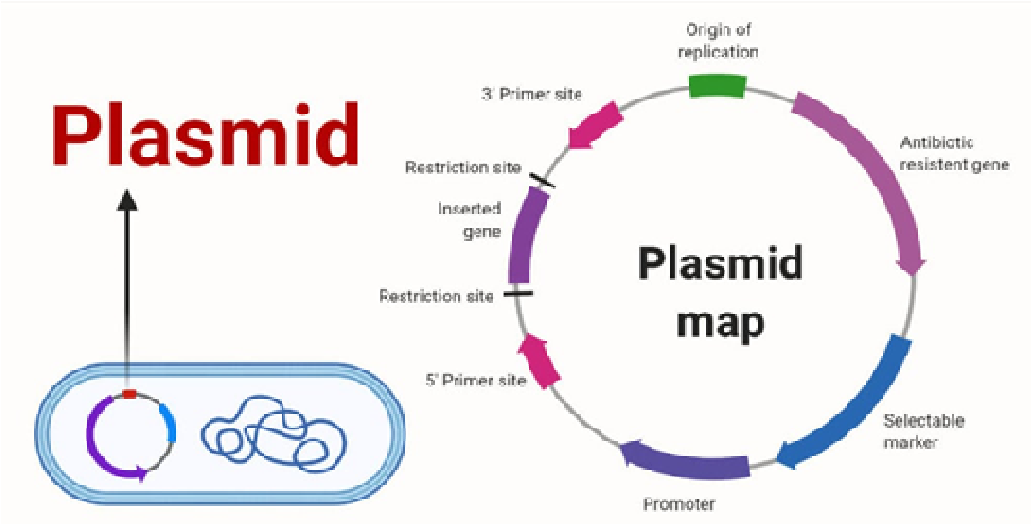


Figure-5.5: Structure of Plasmid

Most of the plasmids contain the TRA gene, which is the transferred gene and is essential in transferring the plasmid from one cell to another. Plasmids are considered *replicons*, a unit of DNA capable of replicating autonomously within a suitable host. Plasmids can be transmitted from one bacterium to another via three main mechanisms such as transformation, transduction, and conjugation. This host-to-host transfer of genetic material is called horizontal gene transfer, and plasmids can be considered part of the mobilome. Unlike viruses (which encase their genetic material in a protective protein coat called a capsid), plasmids are "naked" DNA. Some classes of plasmids encode the conjugative "sex" pilus necessary for their own transfer. The size of the plasmid varies from 1 to over 200 kbp, and the number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances.

Properties and Characteristics of Plasmid:

The self-replicating unit, in this case the plasmid, is called a replicon. A typical bacterial replicon may consist of a number of elements, such as the gene for plasmid-specific replication initiation protein (Rep), repeating units called iterons, DnaA boxes, and an adjacent AT-rich region. Smaller plasmids make use of the host replicative enzymes to make copies of themselves, while larger plasmids may carry genes specific for the replication of those plasmids. A few types of plasmids can also insert into the host chromosome, and these integrative plasmids are sometimes referred to as episomes in prokaryotes. Plasmids almost always carry at least one gene. Some of these genes encode traits for antibiotic resistance or resistance to heavy metal, while others may produce virulence factors that enable a bacterium to colonize a host and overcome its defences, or have specific metabolic functions that allow the bacterium to utilize a particular nutrient, including the ability to degrade recalcitrant or toxic organic compounds. Plasmids can also provide bacteria with the ability to fix nitrogen. Some plasmids, have no observable effect on the phenotype of the host cell or its benefit to the host cells cannot be determined, and these plasmids are called cryptic plasmids. Plasmids are generally circular, however examples of linear plasmids are also known. Plasmids may be present in an individual cell in varying number, ranging from one to several hundreds. The normal number of copies of plasmid that may be found in a single cell is called the copy number. Larger plasmids tend to have lower copy numbers.

Classification of Plasmids:

Plasmids may be classified in a number of ways. Plasmids can be broadly classified into a) Conjugative plasmids and b) Non-conjugative plasmids.

a) **Conjugative Plasmids:** Conjugative plasmids contain a set of transfer or *tra* genes which promote sexual conjugation between different cells. In the complex process of conjugation, plasmid may be transferred from one bacterium to another via sex pili encoded by some of the *tra* genes (Figure 5.6).

b) **Non-conjugative Plasmids:** Non-conjugative plasmids are incapable of initiating conjugation; hence they can be transferred only with the assistance of conjugative plasmids. An intermediate class of plasmids is mobilizable, and carries only a subset of the genes required for transfer. They can parasitize a conjugative plasmid, transferring at high frequency only in its presence.

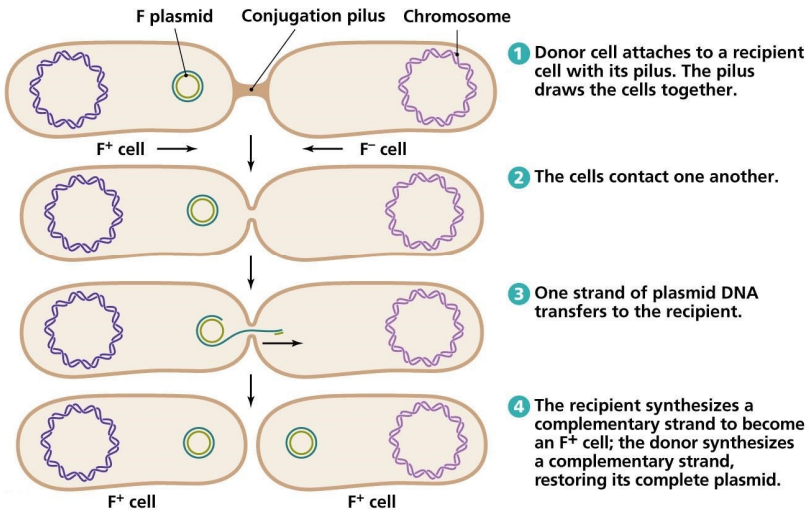


Figure-5.6: Conjugative Plasmid

Incompatibility Groups:

Plasmids can also be classified into incompatibility groups. A microbe can harbor different types of plasmids; however, different plasmids can only exist in a single bacterial cell if they are compatible. If two plasmids are not compatible, one or the other will be rapidly lost from the cell. Incompatible plasmids normally share the same replication or partition mechanisms and can thus not be kept together in a single cell.

Based on Function: Basing on the function plasmids classified into five main classes:

1) Fertility F-plasmids
2) Resistance (R) plasmids
3) Col plasmids
4) Degradative plasmids
5) Virulence plasmids

1) Fertility Plasmids: F-plasmid which contain *tra* genes. They are capable of conjugation and result in the expression of sex pili. The Fertility factor allows genes to be transferred from one bacterium carrying the factor to another bacterium lacking the factor by conjugation. The F plasmid belongs to a class of conjugative plasmids that control sexual functions of bacteria with a fertility inhibition (Fin) system. It includes three main regions (Figure 5.7).

i) OriT (Origin of Transfer): The sequence which marks the starting point of conjugative transfer.

ii) OriC (Origin of Replication): The sequence starting with which the plasmid-DNA will be replicated in the recipient cell.

iii) Tra-Region (Transfer Genes): Genes coding the F-Pilus and DNA transfer process.

Apart from these IS (Insertion Elements) composed of one copy of IS2, two copies of IS3, so-called "selfish genes" (sequence fragments which can integrate copies of themselves at different locations).

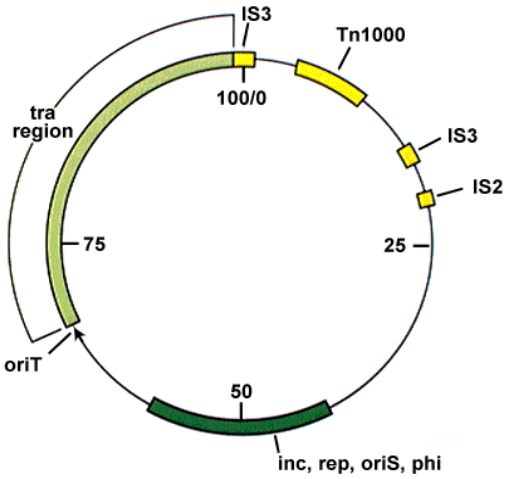


Figure-5.7: Fertility Plasmids (F-Plasmid)

2) R-Plasmids:

Resistance (R) plasmids, which contain genes that provide resistance against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood. R-factor was first demonstrated in *Shigella* in 1959 by Japanese scientists. Often, R-factors code for more than one antibiotic resistance factor, genes that encode resistance to unrelated antibiotics may be carried on a single R-factor, sometimes up to 8 different resistances (Figure 5.8). Many R-factors can pass from one bacterium to another through bacterial conjugation and are a common means by which antibiotic resistance spreads between bacterial species, genera and even families. For example, RP1, a plasmid that encodes resistance to ampicillin, tetracycline and kanamycin originated in a species of *Pseudomonas*, from the Family Pseudomonadaceae, but can also be maintained in bacteria belonging to the family Enterobacteriaceae, such as *Escherichia coli*.

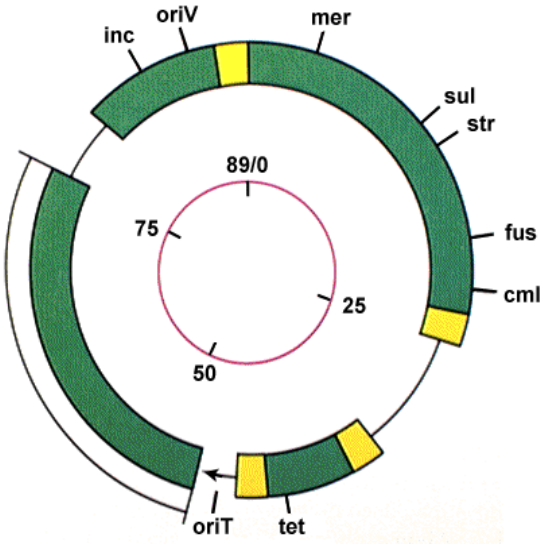


Figure-5.8: Resistance Plasmid (R-Plasmid)

3) Col Plasmids:

Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria. Col plasmids (colicinogenic plasmids) are extrachromosomal, self-replicating DNA molecules found mainly in *Escherichia coli* and some other Gram-negative bacteria. They carry genes that encode colicins, which are antibacterial proteins capable of killing or inhibiting closely related bacterial strains. These plasmids often include genes for colicin production, immunity proteins that protect the host cell from its own colicin, and systems for plasmid maintenance and transfer. Col plasmids play an important ecological role by giving the host bacterium a competitive advantage in microbial communities and may be transferred between cells through conjugation, enhancing their spread and persistence. (Figure 5.9).

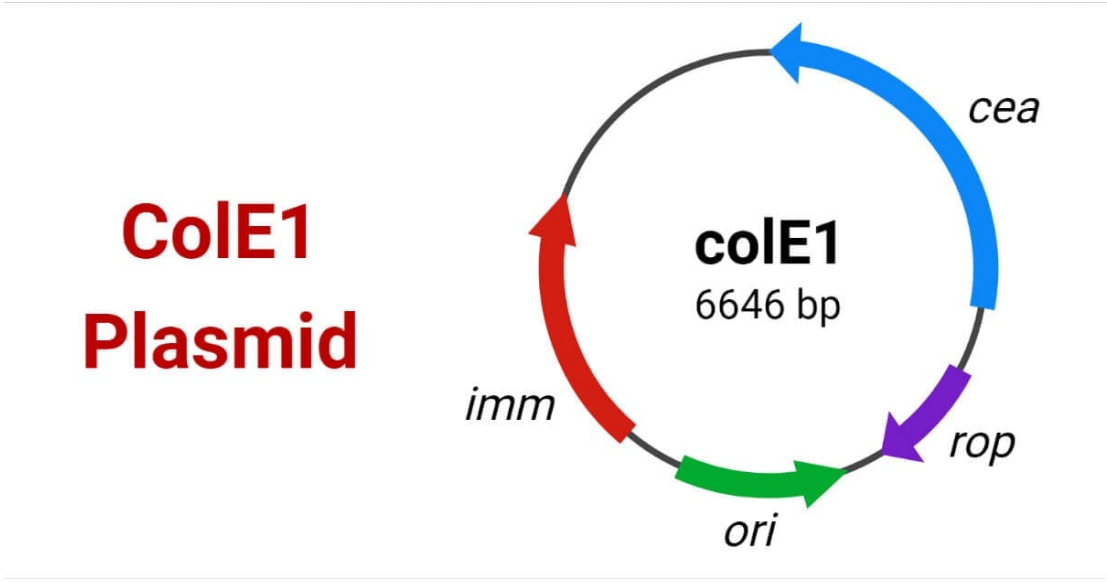


Figure-5.9 Col Plasmid

4) Degradative Plasmids:

Degradative plasmids are specialized extrachromosomal DNA molecules (Figure 5.10) found in certain bacteria that carry genes enabling the host cell to metabolize or break down unusual or complex organic compounds, such as toluene, camphor, naphthalene, and hydrocarbons. These plasmids encode specific enzymes and metabolic pathways that allow bacteria to use these compounds as carbon and energy sources, often giving them a significant ecological advantage in polluted or nutrient-limited environments. Degradative plasmids are frequently involved in biodegradation and play a key role in environmental processes such as bioremediation. They can also be transferred between bacteria through conjugation, spreading these metabolic capabilities within microbial communities. The presence of these plasmids in the organism enables the breakdown of various chemicals and substances.

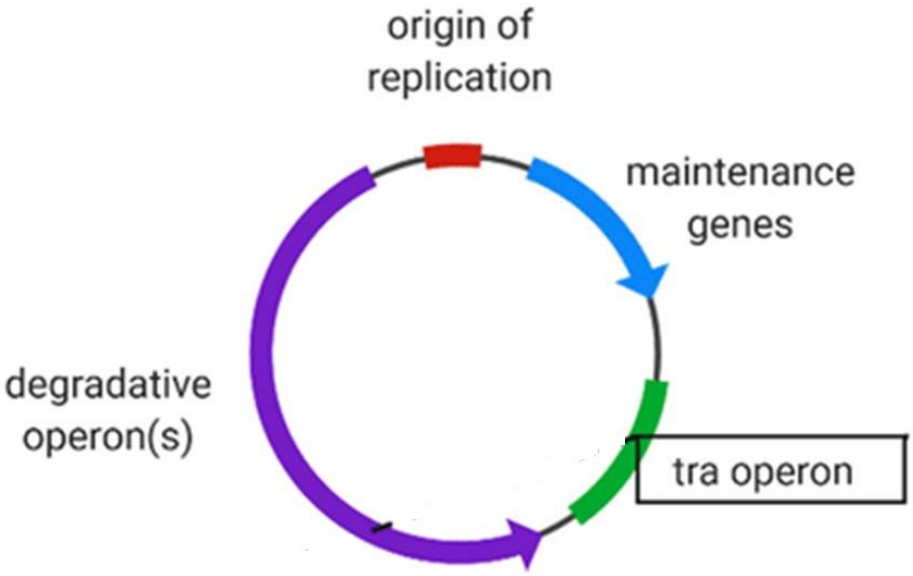


Figure-5.10: Degradative Plasmid

1) **Virulence Plasmids:** Virulence plasmids are extrachromosomal DNA molecules that carry genes enabling bacteria to infect hosts, evade immune responses, or produce toxins that enhance pathogenicity (Figure 5.11). These plasmids encode factors such as adhesins, invasins, toxins, iron-uptake systems, and secretion machinery that collectively increase the organism's ability to cause disease. Examples include the Ti plasmid in *Agrobacterium tumefaciens*, which induces tumor formation in plants, and virulence plasmids in *Salmonella* or *Yersinia* that facilitate host invasion. Virulence plasmids can be transmitted between bacteria through conjugation, contributing to the spread of pathogenic traits within microbial populations.

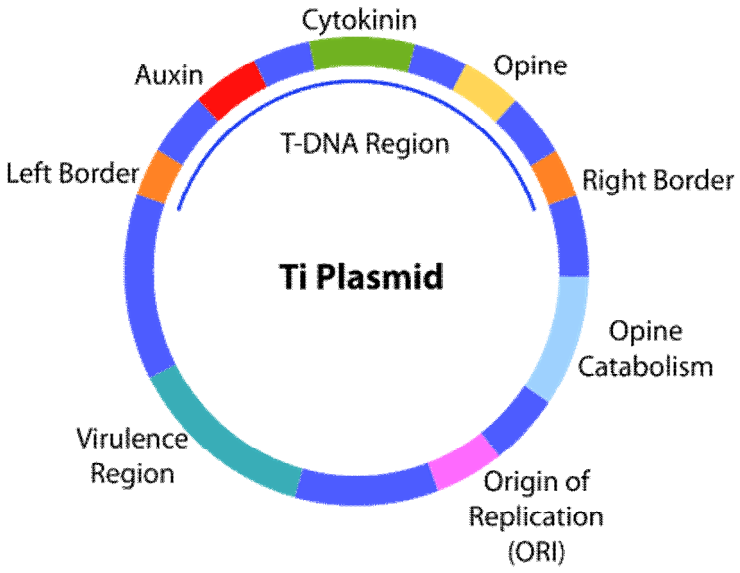


Figure-5.11: Ti Plasmid

Applications of Plasmids:

Vectors:

1) Artificially constructed plasmids may be used as vectors in genetic engineering.
2) These plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to clone and amplify (make many copies of) or express particular genes.
3) These include a gene that confers resistance to particular antibiotics (ampicillin is most frequently used for bacterial strains), an origin of replication to allow the bacterial cells to replicate the plasmid DNA, and a suitable site for cloning.

Cloning:

1) Plasmids are the most-commonly used bacterial cloning vectors.
2) These cloning vectors contain a site that allows DNA fragments to be inserted, for example a multiple cloning site or polylinker which has several commonly used restriction sites to which DNA fragments may be ligated.
3) After the gene of interest is inserted, the plasmids are introduced into bacteria by a process called transformation.
4) These plasmids contain a selectable marker, usually an antibiotic resistance gene.
5) The cells after transformation are exposed to the selective media, and only cells containing the plasmid may survive.
6) In this way, the antibiotics act as a filter to select only the bacteria containing the plasmid DNA.

Protein Production:

1) Another major use of plasmids is to make large amounts of proteins.
2) In this case, researchers grow bacteria containing a plasmid harboring the gene of interest.
3) Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene.
4) This is a cheap and easy way of mass-producing the protein the gene codes for, for example, insulin.

Gene Therapy:

1) Plasmid may also be used for gene transfer into human cells as potential treatment in gene therapy so that it may express the protein that is lacking in the cells.
2) Some strategies of gene therapy require the insertion of therapeutic genes at pre-selected chromosomal target sites within the human genome.
3) Plasmid vectors are one of many approaches that could be used for this purpose.

5.3. EUKARYOTIC CHROMOSOMES:

Chromosome, the microscopic threadlike part (chromatin) of the cell that carries hereditary information in the form of genes. That genetic material, which determines how an organism develops, is a molecule of deoxyribonucleic acid (DNA). Chromosomes were first described by Strasburger in 1815. In 1842, Karl Wilhelm von Nageli, a Swiss Botanist discovered in chromosomes in plat cells. Term 'chromosome' was first used by Waldeyer in 1888. Different species have a different number of chromosomes based on their genome size. A molecule of DNA is a very long, coiled structure. In prokaryotes, or cells without a nucleus, the chromosome is merely a simple structure. In eukaryotes, or cells with a distinct nucleus, chromosomes are much more complex in structure.

A molecule of DNA, in turn, consists of thousands and thousands of subunits, known as nucleotides, joined to each other in very long chains. A single molecule of DNA within a chromosome may be as long as 8.5 centimeters (3.3 inches). To fit within a chromosome, the DNA molecule has to be twisted and folded into a very complex shape. A chromosome is an organized structure of DNA and protein that is found in cells. A chromosome is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve to package the DNA and control its functions. The word chromosome comes from the Greek chroma - color and soma - body due to their property of being very strongly stained by particular dyes. Chromosome= chroma (colour) + some (body). The DNA molecule be composed of 10,000 to 1,000,000,000 nucleotides in a long chain. Typically eukaryotic cells (cells with nuclei) have large linear chromosomes and prokaryotic cells (cells without defined nuclei) have smaller circular chromosomes, although there are many exceptions to this rule. Most of the organisms'chromosomes are arranged in pairs in the nucleus of the cell. A human cell contains 3 X 109 bp per haploid set of chromosomes. The average thickness of each base pair is 3.4 A˚. The estimated 25,000 genes in the human genome include an enormous amount of DNA that does not code for RNA or protein. If the DNA molecules in a haploid set of chromosomes were laid out end to end, the total length of DNA would be 1010A˚, or 1 m. For a diploid cell (as human cells typically are), this length is doubled to 2 m. Chromosomes are also associated with many proteins and RNA molecules required for the processes of gene expression, DNA replication, and DNA repair. The bacterial chromosome does not have the same structure as eukaryotic chromosomes. Each human cell contains two copies of each chromosome, one inherited from the mother and one from the father. The maternal and paternal chromosomes of a pair are called homologous chromosomes (homologs). The only nonhomologous chromosome pairs are the sex chromosomes in males, where a Y chromosome is inherited from the father and an X chromosome from the mother.

Chromosome Number:

There are normally two copies of each chromosome present in every somatic cell. The number of chromosomes (N) in such a cell is known as its haploid number, and the total number of chromosomes (2N) is its diploid number. The suffix 'ploid' refers to chromosome 'sets'. The haploid set of the chromosome is also known as the genome. In Eukaryotes other than the nucleus chromosomes are also present in mitochondria and chloroplast. The number of chromosomes in each somatic cell is same for all members of a given species. The organism with lowest number of chromosomes is the nematode, *Ascaris megalocephalus univalens* which has only two chromosomes in the somatic cells (2n=2). In the radiolarian protozoan *Aulacantha* diploid number of chromosomes (2N) is 1600. In plants chromosome

number varies from 2N=4 as in *Haplopappus gracilis* (a flowering plant of family Asteraceae) to 2N=>1200 as in *Ophioglossum reticulatum* (a fern).

In mitotic metaphase chromosomes, the following structural feature (except chromomere) can be seen under the light microscope i.e. (1) Chromatid (2) Chromonema (3) Chromomeres (4) Centromere (5) Secondary constriction or Nucleolar organizer (6) Telomere and (7) Satellite (Figure 5.12).

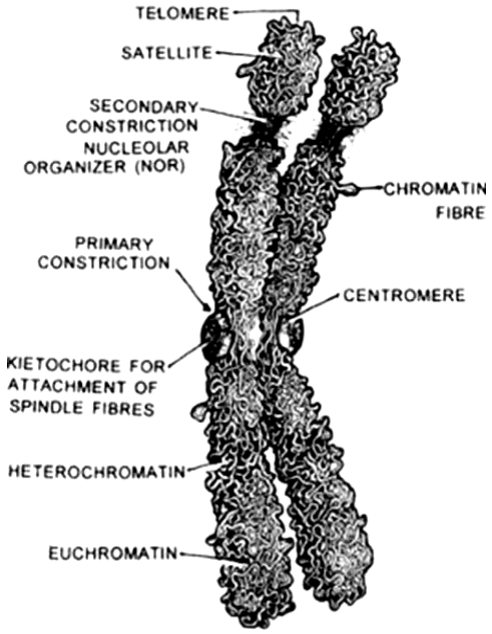


Figure-5.12: Metaphase Chromosomes

Structure of Chromosome divided into three types:

1) Morphological structure
2) Chemical structure
3) Molecular structure

1) Morphological Structure:

The eukaryotic chromosome generally contain following parts. Structurally, each chromosome is differentiated into three parts a) Pellicle b) Matrix c) Chromonemata.

a) Pellicle: It is the outer envelope around the substance of chromosome. It is very thin and is formed of achromatic substances.

b) Matrix: It is the ground substance of chromosome which contains the Chromonemata. It is also formed of non-genic materials.

c) Chromonemata: Embedded in the matrix of each chromosome are two identical, spirally coiled threads, the chromonemata (Chromatids/sister chromatids), normally seen at Metaphase stage. It is the structural and functional unit of chromosomes. The two chromonemata are also tightly coiled together that they appear as single thread of about 800 A° thickness. Each chromonemata consists of about 8 microfibrils, each of which is formed of a double helix of DNA. Chromonemata contains the following structures.

Telomeres:

The two ends of a chromosome are known as 'Telomeres' are the region of repetitive nucleotide sequences at each end of a chromosome (Figure 5.13). These protect the end of the chromosome from deterioration or from fusion with neighboring chromosomes.

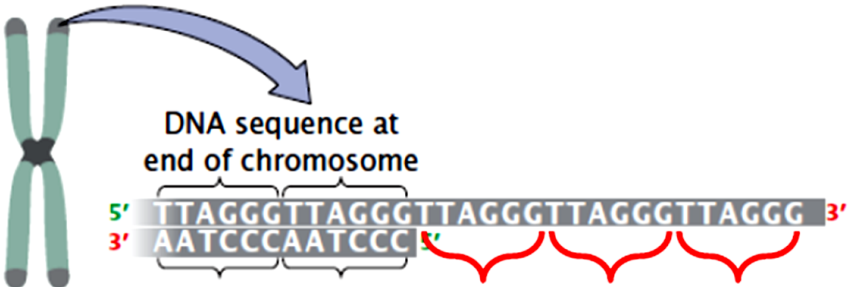


Figure 2.13 Repetitive Sequences of Telomers

Kinetochores:

These are the attachment point for spindle fibers which helps to pull apart the sister chromatids as the mitosis process proceeds to anaphase stage. The kinetochore is a large protein complex built on the centromere of each sister chromatid (chromosome). The kinetochores attach to microtubules emanating from each opposing spindle pole, segregating chromatids. Electron microscopy studies revealed that the kinetochore has three layers in its structure i.e. electron-dense inner and outer plates, and an electron-translucent middle layer (Figure 5.14).

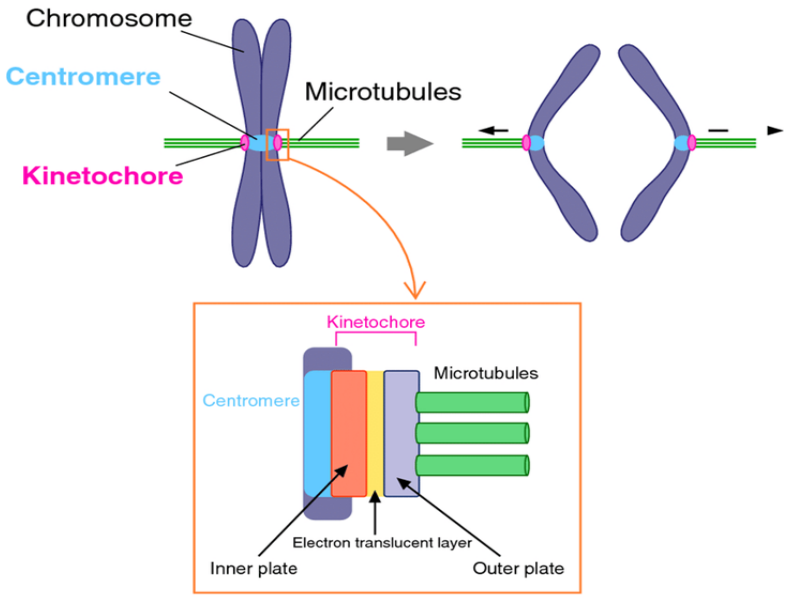


Figure-5.14: Kinetochore of the Chromosomes

A Model of Basic Kinetochore Structure:

The main structure of the kinetochore is formed of constitutive centromere-associated network (CCAN) composed of 16 protein subunits and the KMN (Knl1, Mis12, and Ndc80 complexes: Knl1C, Mis12C, Ndc80C) network (KMN). CCAN interacts with the centromeric chromatin epigenetically marked with the CENP-A nucleosome during the entire cell cycle (Figure 5.15). CCAN recruits KMN, which directly binds to microtubules, in M-phase, forming a linkage between the centromere and microtubules.

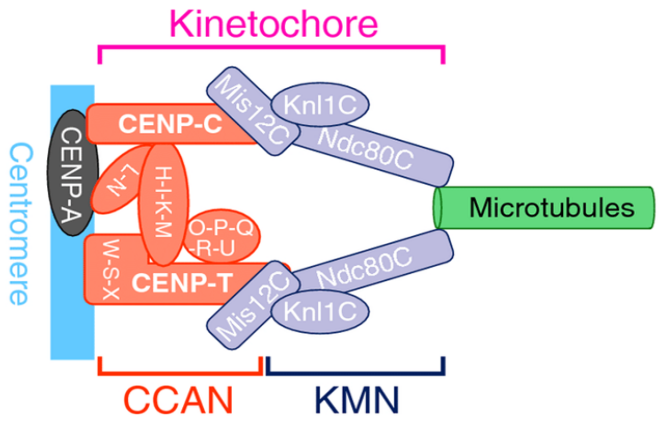


Figure-5.15: Kinetochore Protein Structure

Centromere:

The region where two sister chromatids appear to be joined during mitotic metaphase is known as centromere. It generally appears as constriction and hence called primary constriction. Helps in the movement of the chromosomes to opposite poles during anaphase of cell division. The centromere consists of two disk shaped bodies called kinetochores. Normally chromosomes are monocentric having one centromere each. The pericentromere provides cohesion between sister chromatids and acts as a foundation for the centromere core, which assembles the kinetochore complexes for the attachment of microtubules. Chromatin of the centromere core is folded to expose the CENP-A nucleosomes to the surface of the primary constriction. Within the centromere region, most species have several locations where spindle fibers attach, and these sites consist of DNA as well as protein. The actual location where the attachment occurs is called the kinetochore and is composed of both DNA and protein. The DNA sequence within these regions is called *CEN DNA*. *CEN DNA* can be moved from one chromosome to another and still provide the chromosome with the ability to segregate. Typically CEN DNA is about 120 base pairs long and consists of several subdomains, CDEI, CDEII and CDEIII. Mutations in the first two subdomains have no effect upon segregation, but a point mutation in the CDEIII subdomain completely eliminates the ability of the centromere to function during chromosome segregation. Therefore, CDEIII must be actively involved in the binding of the spindle fibers to the centromere. A complex of three proteins called CbfIII binds to normal CDEIII regions but cannot bind to a CDEIII region with a point mutation that prevents mitotic segregation. Furthermore, mutants of the genes encoding the CbfIII proteins also eliminate the ability for chromosomes to segregate during mitosis.

Depending on position of the centromere, chromosomes can be grouped as a) Metacentric b) Sub-metacentric c) Acrocentric d) Telocentric (Figure 5.16).

a) Metacentric: Centromere is in middle, meaning p and q arms are of comparable length (e.g. chromosomes 1, 3, 16, 19, 20). Appear as 'V' shape at anaphase.

b) Submetacentric: Centromere is on one side of center point, leading to shorter p arm relative to q arm (e.g. chromosomes 2, 4 - 12, 17, 18, X). One arm is longer than other arm. These chromosomes appear as 'J' or 'L' at anaphase.

c) Acrocentric: Centromeres located close to one end of the chromosome and thus giving very long arm and very short arm. These chromosomes appear 'J' or rod shape during anaphase. Centromere severely off-set from center, leading to much shorter p arm (e.g. chromosomes 13 - 15, 21, 22, Y).

d) Telocentric: Centromere found at end of chromosome, so, that chromosome is having only one arm, meaning no p arm exists. Chromosomes appear 'I' shape or rod shape during anaphase.

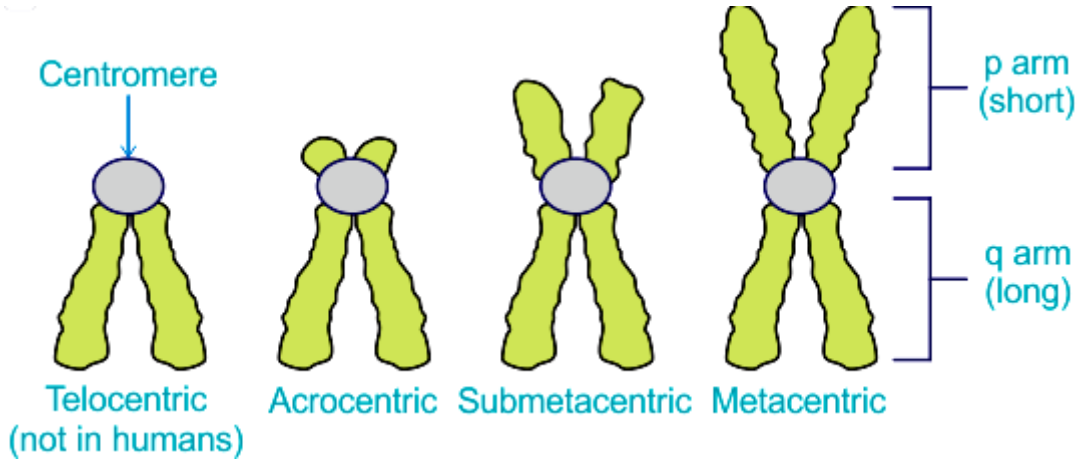


Figure-5.16 Types of chromosomes based on centromere position

Depending upon the number of centromeres, the chromosomes may be:

1) Monocentric with one centromere.
2) Dicentric with two centromeres.
3) Polycentric with more than two centromeres.
4) Acentric without centromere. Such chromosomes represent freshly broken segments of chromosomes which do not survive for long.
5) Diffused or non-located with indistinct centromere diffused throughout the length of chromosome (Figure 5.17).

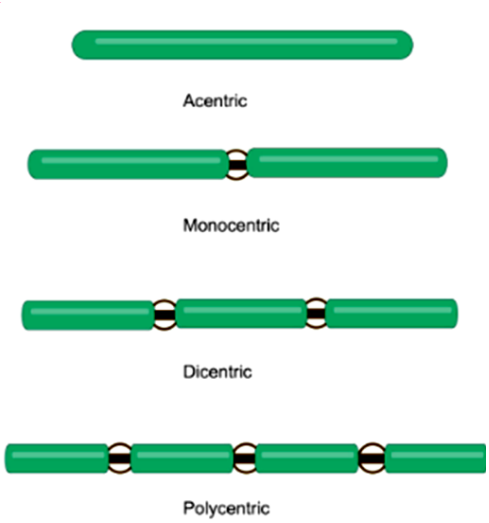


Figure-5.17: Types of Chromosomes based on Centromere Number

Secondary Constriction:

The constricted or narrow region other than that of centromere is called secondary constriction (Figure 5.18). The chromosomes having secondary constriction are known as satellite chromosomes or sat chromosomes. Chromosome may possess secondary constriction in one or both arms of it. Chromosomal end distal to the secondary constriction is known as satellite. It is present in short arm near one end, or in many chromosomes they are located in the long arm nearer to the centromere. Production of nucleolus is associated with secondary constriction and therefore it is also called nucleolus organizer region. Satellite organizer chromosomes are often referred to as nucleolus organizer chromosomes.

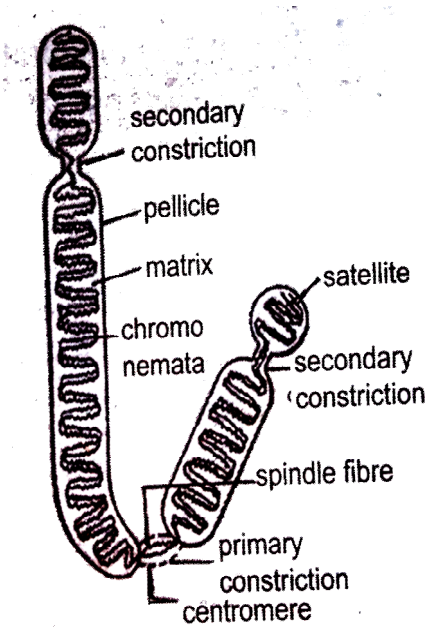


Figure-5.18: Secondary Constriction of Chromosome

Euchromatin:

Chromosome material which does not stain strongly except during cell division. It represents the major genes and is involved in transcription.

Heterochromatin:

Heterochromatin is such part of the chromosomes, which is a firmly packed form and is genetically inactive.

2) **Chemical structure:** Chemically, the eukaryotic chromosomes are composed of deoxyribonucleic acid (DNA) – 30-40%, ribonucleic acid (RNA) – 1-10%, histone and non-histone proteins (50-65%) and certain metallic ions.

Enzymatic Proteins:

The most important enzymatic proteins of chromosomes are phosphoproteins, DNA polymerase, RNA-polymerase, DPN pyrophosphorylase, and nucleoside triphosphatase.

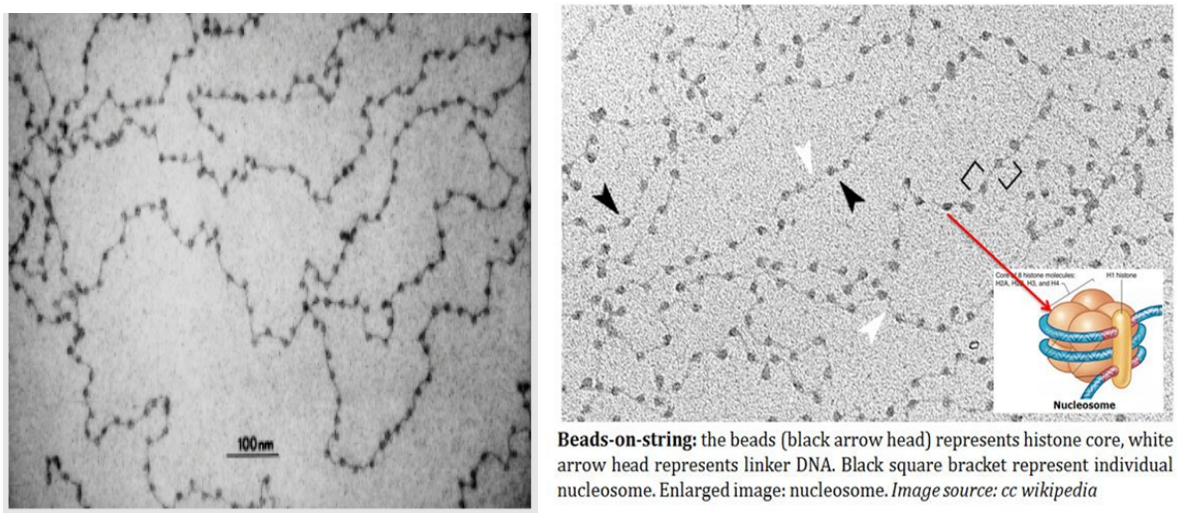
Metal Ions:

The metal ions as Ca+ and Mg+ are supposed to maintain the organization of chromosomes intact.

3) **Molecular structure of chromosome:** Molecular structure of chromosomes consists of Chromatin.

Chromatin:

Chromatin consists of DNA, RNA and protein. During interphase the highly condensed and darkly stained chromatin called as heterochromatin. The chromatin which is lightly stained and which remains extended was called euchromatin (Figure 5.19).



Beads-on-string: the beads (black arrow head) represents histone core, white arrow head represents linker DNA. Black square bracket represent individual nucleosome. Enlarged image: nucleosome. *Image source: cc wikipedia*

Figure-5.19: Electron Microscopy of Chromatin

The Proteins of Chromatin are of two types a) Histones and b) Non-Histones.

a) Histones: Histones were discovered in 1884 by Albrecht Kossel. They are a group of highly alkaline relatively small proteins, and they have a strong positive charge because they have a high content of amino acids, for example, Lysine and Arginine, and are present in the eukaryotic cell nuclei. The DNA is negatively charged and the binding of DNA with the histones is stabilized by the ionic bonding. These opposite charges help the binding of the DNA with histone proteins, known as Nucleosomes. It is very important to maintain the pH of the histone because at below pH 4, they lose their specific secondary and tertiary structure, undergo non-specific aggregation, and become partially unfolded. Histones play an important role in maintaining the structure of chromosomes. To fit into the cell nucleus and for the purpose of giving the chromosomes a more compact shape, DNA wraps itself around the complexes of histone proteins.

Types of Histones:

There are five main types of histones. H1, H2A, H2B, H3, H4 (Lewin, 1975). These types are divided into two main classes (Figure 5.20). The core histones are H2A, H2B, H3, and H4. The linker histones are H1 and H5 (highest Lysine and Arginine ratio). The linker histones are involved in the highly ordered structure of chromatin. For the formation of a highly ordered structure of DNA, the linker histone protein H1 locks the DNA into place by binding with the nucleosomes at the starting and ending sites of DNA. H5 histones are individual proteins and play an important role in the packaging of a specific region of DNA. The mass of the histones in chromatin is approximately equal to the mass of the DNA in most cells. An equal volume of H2A, H2B, H3, and H4 molecules are present in chromatin but about half of that number of H1 molecules. H1, H2A H2B, H3, and H4-which are very similar among different species of eukaryotes and have been highly conserved during evolution. H1 is the least conserved among all and is also loosely bound with DNA.

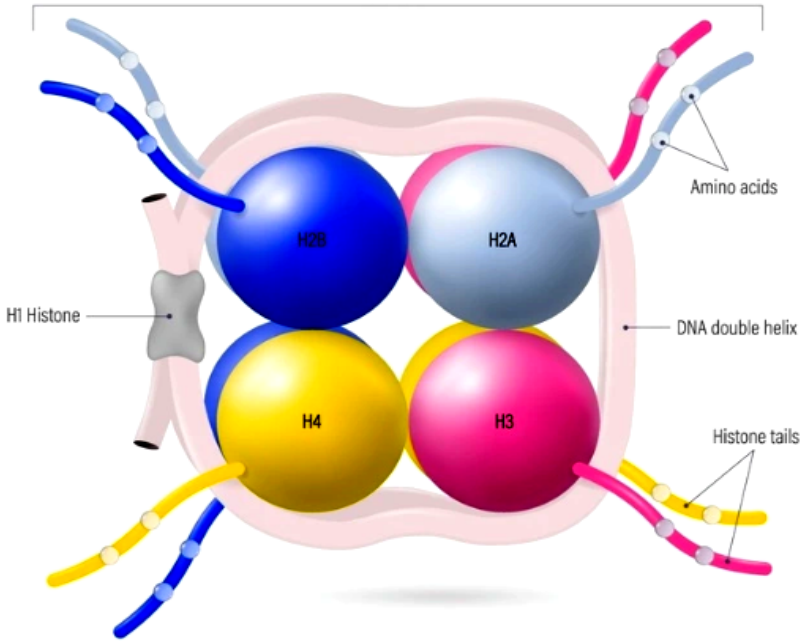


Figure-5.20: Types of Histone Proteins

Non-Histones:

The non-histone proteins are mostly acidic and have been considered more important than histones as regulatory molecules. Some non-histone proteins also have enzymatic activities. Non-histone proteins, which are involved in DNA replication and gene expression. They display more diversity or are not conserved. They may also differ between different tissues of same organism.

Chromosome Model:

Several models of chromosome structures have been proposed. These models are based on biochemical and electron microscope studies. These models include

1) Folded fibre model
2) Solenoid model
3) Zig-Zag model

1) **Folded Fibre Model of Chromosomes:** This model was proposed by Du Praw in 1965 and is widely accepted. According to this model, chromosomes are made up of chromatin fibers of about 230A° diameter. Each chromatin fibre contains only one DNA double helix which is in a coiled state, this DNA coil is coated with histone and non-histone proteins. The coils of which are stabilized by proteins and divalent cations (Ca++ and Mg++).

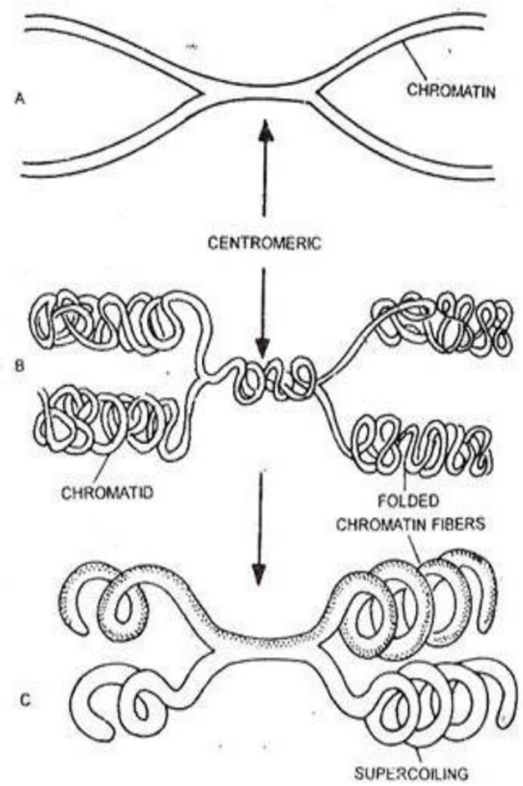


Figure-5.21: Folded Fibre Model of Chromosomes

Each chromatid contains single long chromatin fibers. The DNA of this fibre replicates during interphase producing two sister chromatin fibers, it remains unreplicated in the centromeric region so that the two sister fibers remain joined in the region (Figure 5.21). Subsequently, the chromatin fibre undergoes replication in the centromeric region. During cell division the two sister chromatin fibers undergo extensive folding separately in an irregular manner to give rise to two sister chromatids. This folded structure normally undergoes supercoiling which further increases the thickness of chromosomes and reduces the length. Most of the available evidence supports this model. Each chromatid contains a single giant DNA molecule. The strongest evidence in the support of the unineme model (single stranded chromatid) is provided by studies on lamp-brush chromosomes.

2) Nucleosome or Solenoid Model:

Roger Kornberg in 1974 described the basic structural unit of chromatin known as the nucleosome. The solenoid model is one of the classical models proposed to explain the higher-order folding of chromatin within eukaryotic chromosomes. While the nucleosome represents the fundamental repeating unit of chromatin, packaging DNA into a 10 nm "beads-on-a-string" structure, further compaction is essential to fit the large eukaryotic genome into the nucleus (Figure 5.22). The solenoid model describes how these nucleosomes are arranged into a 30 nm chromatin fibre, a critical level of organization that contributes to chromosome stability, regulation, and mitotic condensation. According to the solenoid model, nucleosomes are arranged in a helical, coiled structure resembling a solenoid. Typically, about six nucleosomes are packed per turn of the solenoid. The nucleosomes interact with each other through their histone tails. The linker histone H1 plays a crucial role by binding at the entry and exit points of DNA on the nucleosome, bringing adjacent nucleosomes closer and promoting the formation of the solenoid structure. This helical arrangement results in the formation of a compact 30 nm fibre (also called the solenoid fibre), significantly increasing the degree of chromatin compaction compared to the 10 nm fibre. Histone H1 is essential for the stability of the solenoid model. It binds to the linker DNA and nucleosome core. It facilitates the bending of DNA and helps nucleosomes coil into a tight super helix. In the absence of H1, chromatin tends to remain in the extended 10 nm fibre form. The solenoid fibre represents an intermediate level of chromatin organization between the nucleosome and the fully condensed metaphase chromosome. The formation of the solenoid greatly increases DNA compaction. Allows efficient organization of chromatin loops that attach to a protein scaffold. Serves as a template for even higher levels of folding, especially during mitosis and meiosis.

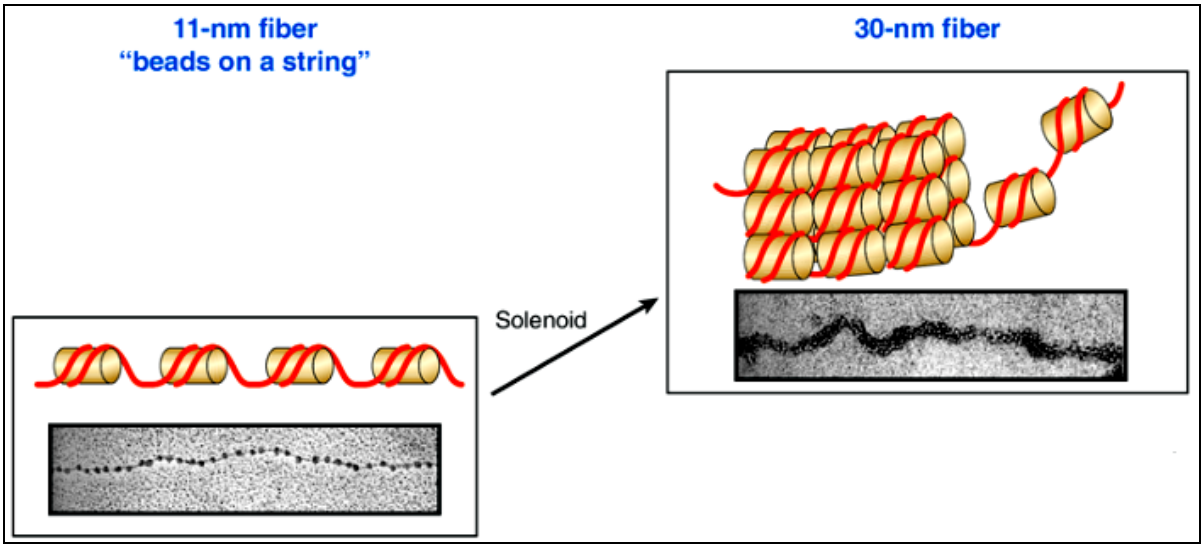


Figure-5.22: Solenoid Structure of Chromosome

3) Zig-Zag model:

The zig-zag model is one of the major models proposed to explain the organization of nucleosomes into the 30 nm chromatin fibre, a higher-order structure crucial for efficient DNA packaging inside eukaryotic nuclei (Figure 5.23). This model serves as an alternative to the classical solenoid model and is supported by modern structural and biophysical studies. In the zig-zag model, Nucleosomes are arranged in a back-and-forth, zig-zag pattern rather than forming a tight helix. Linker DNA passes straight between nucleosomes that are two units apart (n and n+2), instead of bending to connect adjacent ones, as seen in the solenoid model. This gives the chromatin fibre a more open, extended, and angular configuration. Linker DNA in the zig-zag model is longer and straighter. Nucleosomes alternate from left to right, creating a criss-cross pattern. This architecture is particularly favoured when linker DNA length is around 40–60 bp. Histone H1 still participates in stabilizing the 30 nm fibre. However, its role is less about compacting adjacent nucleosomes and more about organizing the entry exit path of DNA to maintain the zig-zag arrangement. The resulting 30 nm fibre appears more linear and less compact than the solenoid. Nucleosome cores are farther apart and do not interact closely with immediate neighbours. The fibre can easily transition between open and compact states depending on ionic concentration, histone modifications and chromatin-binding proteins. Cryo-electron microscopy (cryo-EM), X-ray scattering and Computational modelling have provided strong evidence that chromatin often adopts a zig-zag configuration under physiological conditions. This model is considered more accurate for chromatin with longer linker DNA, more dynamic regulatory regions and euchromatin-like environments

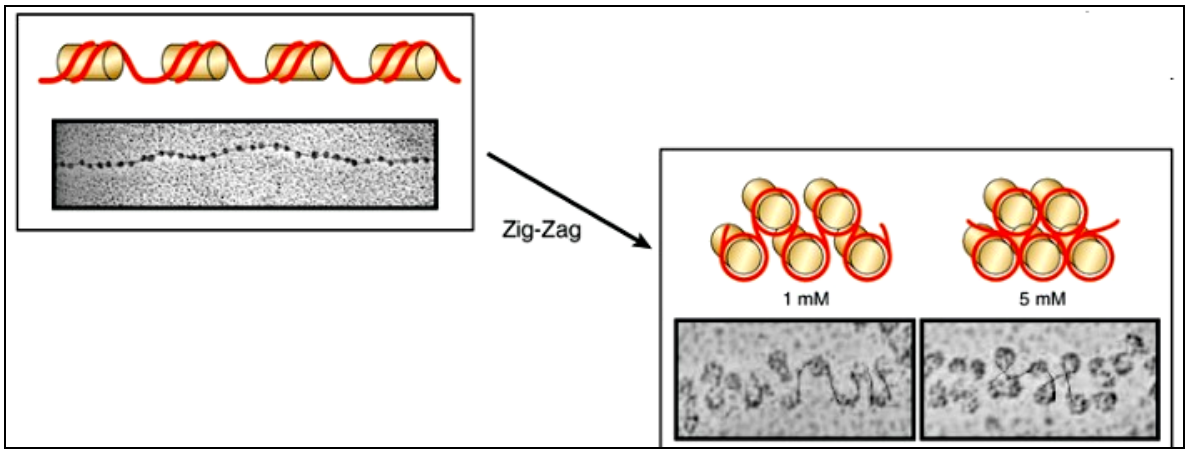


Figure-5.23: Zig-Zag Model of Chromosome

Functions of Chromosomes:

The role of chromosomes in heredity was suggested independently by Sutton and Bover in 1902.

1) It is universally accepted that DNA is the genetic material.
2) The most important function of chromosomes is to provide the genetic information for various cellular functions essential for growth, survival, development, reproduction, etc., of organisms.
3) Chromosomes protect the genetic material (DNA) from being damaged during cell division.
4) Chromosomes are coated with histones and other proteins which protect it from both chemical (e.g., enzymes) and physical forces.
5) The properties of chromosomes ensure a precise distribution of DNA (genetic material) to the daughter nuclei during cell division.
6) Centromeres of chromosomes perform an important function in chromosome movements during cell division.
7) Gene action in eukaryotes is believed to be regulated through histone and non-histone proteins associated with chromosomes.

5.4. SUMMARY:

Prokaryotic chromosomes are generally single, circular, double-stranded DNA molecules located in a region called the nucleoid. They lack histone proteins (except in Archaea, which have histone-like proteins) and do not form true chromatin. Their DNA is compacted through supercoiling and nucleoid-associated proteins. Prokaryotes may also contain small, extra-chromosomal DNA elements known as plasmids**.** Eukaryotic chromosomes are multiple, linear, double-stranded DNA molecules housed inside a membrane-bound nucleus. They are organized with histone proteins into chromatin, which exists in two forms: euchromatin (less condensed, active) and heterochromatin (highly condensed, inactive). Eukaryotic chromosomes contain structural features such as centromeres, telomeres, and multiple origins of replication, enabling controlled cell division and complex gene regulation. Chromosome models were proposed to explain how the long

DNA molecule fits into the nucleus and how chromatin is structurally organized. The major chromosome models include 1. Nucleosome Model (Core Chromatin Organization): This universally accepted model states that DNA is wrapped around histone octamers forming bead-like units called nucleosomes. These nucleosomes are linked by "linker DNA," forming a structure that resembles beads-on-a-string. It explains the first level of DNA compaction. 2. Solenoid Model: Proposed by Finch and Klug, it suggests that nucleosomes coil into a helical, solenoid-like structure with about six nucleosomes per turn. This model describes the 30 nm fibre as a compact, uniform coil and explains higher-order chromatin folding. 3. Zig-Zag Model: This model proposes that nucleosomes are connected in a back-and-forth, zig-zag arrangement with linker DNA crossing the central axis. It explains the 30 nm fibre with a more open and irregular geometry, accounting for variability in linker DNA length.

5.5. TECHNICAL TERMS:

Nucleoid, Plasmids, Chromatin, Nucleosome, Histone Octamer, Euchromatin, Heterochromatin, Centromere, Kinetochore,

5.6. SELF-ASSESSMENT QUESTIONS:

1) Distinguish between plasmids and chromosomal DNA.
2) Differentiate euchromatin from heterochromatin.
3) What are the solenoid and zig-zag model models of chromatin.
4) Describe the structure, organization, and replication of prokaryotic chromosomes.
5) Write an essay on types and significance of plasmids in bacterial genetics.
6) Explain the detailed structure of eukaryotic chromosomes with emphasis on nucleosome organization and chromatin states.
7) Describe the structural features of eukaryotic chromosomes (centromere, telomere, origin of replication) and their functional significance.

5.7. SUGGESTED READINGS:

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Dr. Kakumanu Babu

LESSON-6

CHROMOSOME BANDING, KARYOTYPE, EUCHROMATIN AND HETEROCHROMATIN

6.0 OBJECTIVE:

- To acquaint the students about chromosome banding, karyotype and two types of chromatin materials.

STRUCTURE:

6.1 Introduction
6.2 Chromosomal Banding
6.3 Karyotype
6.4 Euchromatin and Heterochromatin
6.5 Summary
6.6 Technical Terms
6.7 Self-Assessment Questions
6.8 Suggested Readings

6.1. INTRODUCTION:

Chromosomal banding refers to a set of staining techniques used in cytogenetics to produce characteristic patterns of light and dark bands along the length of chromosomes. These bands help scientists identify individual chromosomes, detect structural abnormalities, and analyse chromosomal organization. Various stains such as Giemsa, Quinacrine, and Reverse banding reagents highlight differences in DNA composition, heterochromatin content, and gene density, allowing accurate chromosomal mapping and diagnosis of genetic disorders. A karyotype is the complete set of chromosomes of an organism, arranged systematically based on size, centromere position, and banding pattern. It provides a visual profile of all chromosomes, typically displayed as homologous pairs, enabling the study of chromosomal number and structural features. Karyotyping is essential for identifying numerical abnormalities like aneuploidy, structural changes such as translocations or deletions, and for understanding evolutionary relationships, species differentiation, and genetic health. Euchromatin is the lightly stained, loosely packed region of chromatin that remains transcriptionally active and accessible to transcription factors. Rich in genes and less compact than heterochromatin, euchromatin allows rapid DNA replication and active gene expression. Its open structure facilitates essential cellular processes such as transcription, recombination, and repair, making it a crucial component of genome function and regulation. Heterochromatin is the densely packed, darkly stained region of chromatin that is typically transcriptionally inactive and highly condensed. It contains repetitive DNA sequences, structural genes, and centromeric and telomeric regions important for chromosome stability and segregation. Classified into constitutive and facultative types, heterochromatin plays vital roles in genome organization, gene silencing, protection of chromosome ends, and maintenance of genomic integrity.

6.2. CHROMOSOME BANDING

This is a technique for the identification of chromosomes and its structural abnormalities in the chromosome complement. Chromosome identification depends on their morphological characteristics such as relative length, arm ratio, presence and absence of secondary constrictions on the chromosome arms. It could be used for identification of chromosome segments that predominantly consist of either GC or AT rich regions or constitutive heterochromatin. On banded chromosome, darkly stained or brightly fluorescent transverse bands (positive bands) alternate with the lightly stained or less fluorescent (negative bands). The bands are consistent, reproducible and are specific for each species and each pair of homologous chromosomes. Banding techniques also revealed the extensive genetic polymorphism.

Study of chromosome number and structure by staining the dividing cells with certain dyes for cytogenetic analysis is called chromosome banding. Most cytological and cytogenetic analysis is performed on dividing cells (metaphase of mitosis). E.g.: Meristematic cells of plant, embryo cell of animals. The development of cell-culturing techniques has made it possible to study chromosomes in other types of cells. E.g.: Human white blood cells. The stains such as Feulgen's reagent or aceto-carmine stain the chromosome uniformly making difficult to distinguish one chromosome from other. Initially four basic types of banding techniques were used to identify of Human chromosomes (Q, C, G and R, N and T bands) for complete identification of the chromosome complement (Figure 6.1).

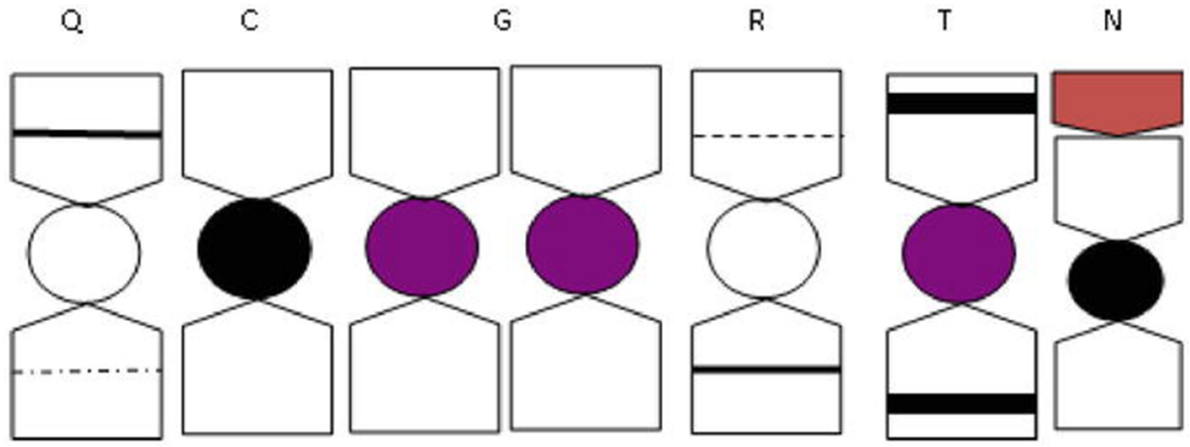

Figure-6.1: Code System for Banding Pattern

Code System for Banding Pattern:

There were 3 letter coding system for the banding procedure, for example, first letter codes for the type of banding to be done; second letter codes for the general technique to be used and third letter codes for the stain to be used. For instance, code QFQ indicates the Q-band to be done, fluorescence technique to be used and quinacrin mustard stain to be used during banding procedure. Similarly, other codes may be QFH, QFA, GTG, GTL, GAG, CBG, RFA, RHG, RBG, RBA, THG and THA depending on the bands, techniques and stains.

G (Giemsa) Stain:

This is the most commonly used banding method for cytogenetic analysis using Giemsa stain. The technique was first developed by Dr. Marina Seabright in 1971. The banding could also be recognized as the modification of C banding procedure. Giemsa is a visible light dye, which binds DNA through intercalation. Visible light dyes are more stable and capable of producing clearer bands than fluorochromes. Giemsa stain is a mixture of cationic thiazine dyes and anionic eosin dyes such as eosin Y. Positive thiazine dye molecules are smaller and two molecules of the same quickly intercalate into the negative DNA molecule, and stains it blue. The anionic eosin molecule then binds the two thiazine molecule and stains the DNA purple. The technique permits the accurate identification of each pair of the chromosomal complement. The preparations are permanent after staining with giemsa.

Chromosome Bands:

1) **Chromosome bands based on Giesma stain:** Giemsa stains the hydrophobic regions better. There are four different types of banding techniques, which can be done using Giemsa i) Gbands ii) R-bands iii) C-bands iv) T–banding.

   i) **Giesma banding (G-Banding):** Chromosomes are treated with **trypsin** (partially digests proteins) and stained with **Giemsa**. A number of modifications for G bands have been developed and proposed such as pre-treatment with trypsin, urea, enzymes and salts. Produces a pattern of a) **dark (G-positive)** and b) **light (G-negative)** bands. The two types of bands which are observed are

      a) **Positive G-bands:** Positive G-bands are the darkly stained bands. These regions are hydrophobic, and favour the formation of the thiazine-eosin precipitate. The hydrophobicity is due to the hydrophobic proteins.

      b) **Negative G-bands:** The lightly stained bands are called negative G-bands. These regions are rich in GC base pairs. These regions are less hydrophobic and less favorable for the formation of the thiazine-eosin precipitate.

   ii) **R (Reverse) Banding:** R banding patterns are based on the thermal treatment of chromosomes proposed by Dutrillaux and Lejeune. The technique is performed on a fixed chromosomal preparation and is based on heat denaturation of chromosomal DNA. The centromeric regions are easily distinguished. This permits the observation of minor abnormalities in the terminal regions of chromosomes and the precise determination of chromosomal lengths. This banding technique reveals the GC-rich euchromatin and produces positive bands. Giemsa stained R bands can be observed under phase contrast microscope while acridine orange stained R bands require fluorescence microscope.

   iii) **C (constitutive heterochromatin) banding:** The C banding technique is based on the denaturation and renaturation of DNA and the regions containing constitutive heterochromatin stain dark (C band) and could be visible near the centromere of each chromosome. The C bands are polymorphic in size. C banding allows precise analysis of abnormalities in the centromeric regions and detection of isochromosomes. Sometimes, C banding also permits to ascertain the parental origin of foetal chromosomes.

iv) **T (Telomeric) banding:** It may be regarded as the modifications of the R banding technique. The clear marking of telomeric regions of chromosome with T banding enables the detailed analyses of the structural rearrangements at the ends of chromosomes. It also allows the detection of human chromosome 22 and its involvement in translocation. The usefulness of this method is for the detection of dicentric rings. T bands can be observed either after giemsa or acridine orange staining.

2) Q (Quinacrine) Banding:

The band stains the chromosome with fluorochromequinacrine mustard or quinacrinedihydrochloride (atebrin). The fluorescence intensity is determined by the distribution of DNA bases. The AT-rich regions enhance the fluorescence while GC-rich regions quench the fluorescence. The brightly fluorescent Q bands show high degree of genetic polymorphism. The fluorescence of Q band is not permanent and fades rapidly, banding must be observed on fresh preparation and selected metaphases. The disadvantage of the technique is the application of an expensive fluorescent microscope. Q banding could also be achieved by fluorochromes e.g. daunomycin, hoechst33258, BrdU etc., which enhances AT-rich regions and quenches GC-rich regions. Acridine ornage stains AT-rich regions red and GC rich regions green.

3) N (Nucleolar Organizing Regions) Banding:

The NOR regions could be selectively stained by techniques involving either giemsa or silver staining. The giemsa technique developed by Matsui and Sasaki (1973) allows the staining NOR after extraction of nucleic acids and histones. The silver staining technique N banding was improved by Bloom and Goodpasture in 1976. The silver staining technique falls into two categories.

a) Ag-As method: The method is based on the staining with combined silver nitrate and ammonical silver solutions.

b) Ag method: In this method, staining with ammonical silver is omitted.

4) DAPI/Distamycin A Staining:

This is a fluorescent staining technique for labelling a specific subset of C bands. DAPI/Distamycin A staining is used in identification of peri-centromeric breakpoints in chromosomal rearrangements and chromosomes that are too small for standard banding techniques. 4′-6-diamidino2-phenylindole (DAPI) is a DNA-binding AT-specific fluorochrome, which gives blue fluorocence. Distamycin A is an DNA-binding AT-specific oligopeptide antibiotic. Distamycin A pretreatment, allowing only some specific regions of constitutive heterochromatin to brightly fluoresce. These regions can be compared with that in homologous chromosomes or chromosomes of different individuals or species to obtain information on diseases, evolution and parentage.

5) High Resolution Banding:

Elongated chromosomes (earlier stages of mitotic divisions before reaching metaphase stage) are standardized for banding patterns and prediction of aberrations

or arrangements called high resolution banding. Elongated chromosome standardization and preparation could be obtained through cell cycle synchronization technique. Cell synchronization is a process by which mitotic cells at different stages of cell cycle in culture are brought to the same phase through physical fractionation or chemical blockage or inhibition of DNA synthesis during S-phase. Cell synchrony may be defined as the progression of cells through cell cycle. The possible procedure for high resolution banding pattern:

Eg: Mitotic stages (cultured cells) ➔ cell synchronization by adding chemical reagents ➔ Amethopterin or methotrexate or thymidine or fluorouracil ➔ cultured cells ➔ Blocks DNA synthesis in cultured cells ➔accumulation of cells in S-phase of cell cycle ➔ block released ➔ cell synchrony (large quantities of cells continue their cycle from the same level) ➔ prophase to mid-metaphase range ➔ high number of bands (gives more information as compared to the compact metaphase banding).

This technique allows precise localization of break points in chromosomal rearrangements and detection of minute chromosomal alterations that are undetectable by the mid-metaphase banding techniques.

Banding Patterns:

Based on the type of banding procedures used, the banding patterns are of different types i) Sequential banding ii) Simultaneous banding

i) **Sequential Banding:** Single banding technique is usually sufficient for the detection of chromosomal abnormalities e.g. G banding or R banding. Complicated chromosomal rearrangements often require sequential staining by several banding techniques. The quality of chromosomes in sequential banding deteriorates with each staining therefore. It restricts the sequential banding up to 3 or 4 different staining techniques (Figure 6.2).

Eg: Single metaphase ➔ First procedure, Q banding ➔ Second procedure, G banding ➔ Third procedure, C banding ➔ deteriorates the chromosome quality ➔ therefore, restricts up to 3 or 4 staining procedures.

Figure-6.2: Sequential Staining/Banding

ii) **Simultaneous Banding:** This is the technique that produces simultaneously two types of banding on the same metaphase or on one slide but different metaphases (Figure 6.3).

E.g.: Single metaphase ➔ first procedure, G banding ➔ second procedure, C banding OR single slide with different metaphases ➔ first procedure, C banding ➔ second procedure, T banding.

Simultaneous banding restricts up to two staining procedures in different or single metaphase and results in precise estimation of chromosomal aberrations.

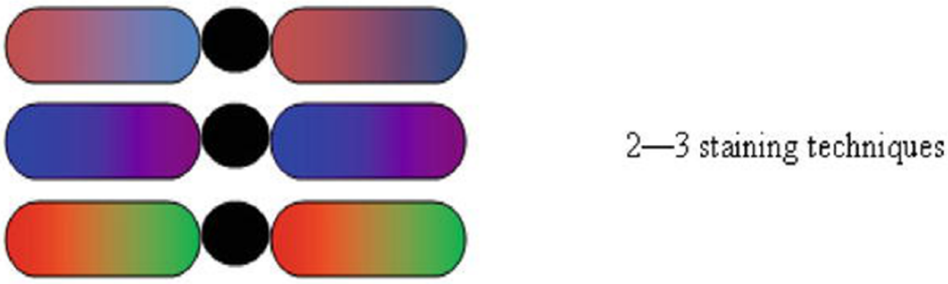

Figure-6.3: Simultaneous Staining/Banding

Computational Analysis of Chromosomal Banding

By using various softwares we can assess the clear and accurate bands of chromosomes. It is of two types i) Chromosome differentiation ii) Chromosome linearization.

i) Chromosome Differentiation:

Molecular banding techniques such as FISH (Fluoroscent in situ hybridization) and GISH (Genomic insitu hybridization) provide little more and specific information by causing more differentiation in banding pattern of a chromosome of a particular species. Now, computational analyses (software packages) of the chromosome bands provide maximum information on banding pattern by increasing number of bands which helps to predict the specific and precise result on chromosomal aberrations and arrangements. **E.g.:** Conventional chromosome bands ➔ enough bands for analysis ➔ molecular banding techniques ➔ more bands, more differentiation, more information ➔ computational techniques (software packages) ➔ still more bands, still more differentiation, still more information.

ii) Chromosome Linearization:

Chromosome linearization is an important tool under computational analyses of the chromosome bands. It suggests that linear chromosomes will provide maximum number of bands as well as information regarding aberrations or arrangements. The information obtained from the straight chromosomes will be larger or maximum in quantity and quality. The tool of image linearization enables a better visualization technique which ultimately extends and refines the information.

E.g.: Conventional chromosome bands ➔ enough bands for analysis ➔ molecular banding techniques ➔ more bands, more differentiation, more information ➔ computational techniques (software packages) ➔ better organization of cytogenetic data ➔ tool of image linearization ➔ still more bands, still more differentiation, still more information.

Application of Chromosome Banding:

1) **Karyotyping and Chromosome Identification:** Chromosomal banding is fundamental for karyotyping because each chromosome exhibits a unique and reproducible banding pattern that acts like a structural fingerprint. These distinct light and dark bands help accurately identify individual chromosomes, distinguish homologous pairs, and detect small morphological differences that cannot be seen with conventional staining. Banding patterns are used to construct standardized idiograms that serve as reference maps for humans, animals, and plants, making the technique indispensable for cytogenetic analysis and chromosome classification.
2) **Detection of Numerical Chromosomal Abnormalities:** Banding techniques enable precise identification of aneuploidies such as trisomies and monosomies, as well as polyploidy, by allowing cytogeneticists to count chromosomes accurately and identify which specific chromosome is duplicated or missing. This has major clinical importance in diagnosing disorders like Down syndrome (trisomy 21), Turner syndrome (45,X), and Klinefelter syndrome (47,XXY). In plants, where polyploidy is common, banding helps differentiate between euploid and aneuploid cytotypes, facilitating genetic and breeding studies.
3) **Detection of Structural Chromosomal Abnormalities:** Chromosomal banding plays a crucial role in detecting structural changes such as deletions, duplications, inversions, translocations, ring chromosomes, and isochromosomes. Changes in the normal banding pattern such as missing bands, extra bands, or rearranged band sequences allow precise identification of the type and extent of aberration. This is vital for diagnosing conditions like Cri-du-chat syndrome (5p deletion), identifying balanced or unbalanced translocations in families, and locating chromosomal breakpoints that lead to genetic disease or genomic instability.
4) **Prenatal Diagnosis:** Banding of fetal cells obtained through amniocentesis or chorionic villus sampling is widely used in prenatal diagnosis of chromosomal abnormalities. The ability to identify both numerical and structural changes provides early detection of conditions such as Down syndrome, Edwards syndrome, Patau syndrome, Turner syndrome, and major chromosomal rearrangements. This information helps clinicians counsel parents about fetal health, prognosis, and management, making chromosomal banding a cornerstone of prenatal genetic screening programs.
5) **Cancer Cytogenetics:** Chromosomal banding is extensively used in oncology to identify cancer-specific chromosomal abnormalities, many of which serve as diagnostic and prognostic markers. Banding techniques allow detection of characteristic translocations (t) such as the Philadelphia chromosome t(9;22) in chronic myeloid leukemia, t(14;18) in follicular lymphoma, and t(8;21) in acute myeloid leukemia. They also help monitor clonal evolution, assess treatment response, and guide targeted therapies, making banding a powerful tool in modern cancer diagnostics and management.
6) **Evolutionary and Comparative Genomics:** Banding patterns allow researchers to compare chromosomes across different species, revealing conserved regions, chromosomal rearrangements, and evolutionary relationships. By examining similarities and differences in band organization, scientists can trace chromosomal fusions, fissions, inversions, and translocations that occurred during evolution. This has been crucial in demonstrating, for example, that human chromosome 2 arose from the fusion of two ancestral ape chromosomes. Banding therefore contributes significantly to phylogenetic reconstruction and comparative genome organization studies.

7) **Gene Mapping and Physical Chromosome Mapping:** Chromosomal banding provides a structural framework for locating genes and markers on specific chromosomal sub-bands, forming the basis of early genetic mapping efforts. Regions such as 7q31 or Xp22 are described according to banding patterns, enabling precise gene position notation. Although molecular techniques now dominate mapping, banding remains essential for correlating genetic loci with their chromosomal environments, especially in identifying genes associated with structural abnormalities or locating breakpoints in chromosomal disorders.

8) **Study of Heterochromatin and Specialized Chromosomal Regions:** Specific banding methods, such as C-banding for constitutive heterochromatin and T-banding for telomeres, allow focused study of chromosomal regions with unique structural or functional properties. These techniques help analyse centromeric DNA, telomeric repeats, and other repetitive sequences that play critical roles in chromosome stability and segregation. They are also useful in identifying variations in heterochromatin distribution among individuals or species, aiding genetic, epigenetic, and structural studies of chromosome organization.

9) **Species Identification and Taxonomy:** Chromosomal banding is a valuable tool in taxonomy and systematics, helping distinguish closely related species, subspecies, and cytotypes based on differences in their banding patterns. Many organisms, including insects, fish, rodents, and plants, show distinct chromosomal polymorphisms that can be visualized through banding. These patterns help identify cryptic species, trace evolutionary divergence, and characterize natural populations, making banding indispensable in biodiversity studies and taxonomic classification.

10) **Crop Improvement and Plant Breeding:** In plant biotechnology and breeding, chromosomal banding is used to track alien chromosome introgressions, identify chromosomal rearrangements, and monitor the behaviour of added or substituted chromosomes in hybrid lines. Techniques like C-banding are especially useful in cereals such as wheat, rye, and barley, where breeders need to detect specific chromosomal segments transferred from wild relatives. Banding also aids in studying polyploid genomes, genome stability, and the chromosomal basis of agronomically important traits.

11) **Integration with Molecular Cytogenetics:** Even with modern methods such as FISH, CGH, and spectral karyotyping, chromosomal banding remains essential as a baseline structural reference. Molecular probes are often interpreted in relation to G- or R-band patterns to pinpoint their exact chromosomal location. Banding helps differentiate overlapping signals, verify chromosomal integrity before probe application, and correlate molecular findings with classical cytogenetic abnormalities. Thus, banding continues to serve as a bridge between classical and molecular cytogenetics.

12) **Research Applications in Chromosome Biology:** Banding techniques are widely used in chromosome research to study replication timing, chromatin condensation, fragile sites, DNA packaging, and the organization of active versus inactive chromatin regions. Differences between G-bands (late-replicating, AT-rich regions) and R-bands (early-replicating, GC-rich regions) help researchers investigate functional aspects of genome regulation. Banding also assists in analysing structural transitions during cell division and understanding mechanisms of chromosome stability and segregation.

6.3. KARYOTYPE:

Karyotype is a photographic arrangement of a complete set of chromosomes of a cell or organism (Figure 6.4). The concept of karyotype has been formulated by Navaschin. The study of karyotype is important since closely related species have similar karyotypes while distantly related ones have different karyotypes. Livitzky defined karyotype as the phenotypic appearance of the somatic chromosomes and the term idiogram is applied to the diagrammatic representation of the karyotype. The somatic chromosomes of different plant and animal species possess definite individuality in their number, size, and shape, position of primary and secondary constrictions or satellites. Karyotypes are usually studied from metaphase stages of root tip cells in plants or from pachytene stages of meiosis. Karyotypes describe the chromosome count of an organism. While studying karyotype attention is paid to their length, the position of the centromeres, banding pattern, any differences between the sex chromosomes and any other physical characteristics

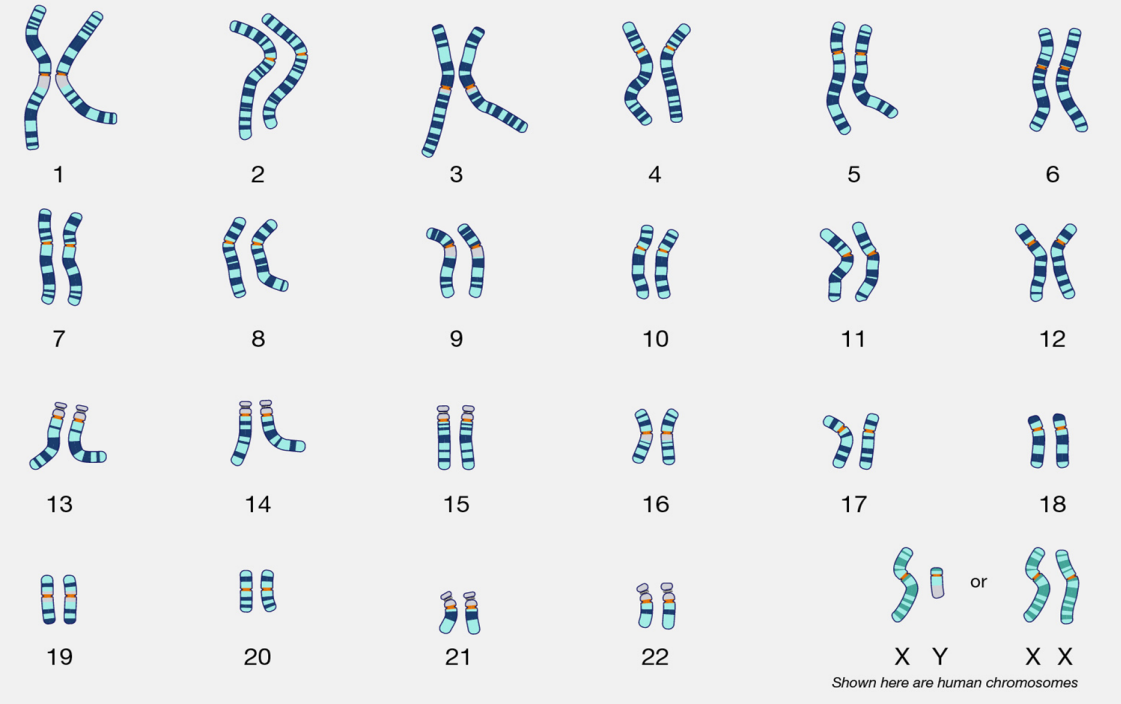

Figure-6.4: Human Chromosome Karyotype

We can distinguish different karyotypes based on

i) Variation in basic chromosome number

ii) Variation in form and relative size of the chromosomes

iii) Variation in absolute chromosome size

iv) Variation in number and size of satellites

v) Variation in degree and distribution of heterochromatic regions and repeated DNA segments

i) **Variations in Basic Chromosome Number:** In addition to polyploid and aneuploid alterations, variation in basic number can be achieved either by loss or gain of a chromosome by unequal translocations. *Crepis* is an example of reduction in basic number. There are different species of *Crepis* with basic numbers x=7, x=6, x=5, x=4 and x=3. The reduction in basic number is associated with certain trends of morphological specialization such as annual habit, deep tap roots, deeply pinnatified leaves, and small involucres, beaked and dimorphic achenes. Another type of variation in number is created by either progressive decrease or increase followed by amphidiploidy (Allotetraploids). Crytopolyploidy refers to an increase in chromosome size as a result of series of doublings. E.g.:- *Brassica*. The basic numbers in this genus are x=8, 9 and 10. The amphidiploid combinations possible are those with numbers n=16, 17, 18, 19, 24, 25, 26, 27, 28, 29, 30 and so on. In the genus *Carex*, the most extensive aneuploid series were reported ranging from x =6 to n=56 and every number from 12-43 is represented by one or more species. Polyploidy coupled with apomixis may also lead to extensive aneuploid series.

ii) **Variations in Form and Relative Size of The Chromosomes:** In many genera, though the chromosome number is same, the karyotypes differ. Basing on form and relative size karyotypes are of two types a) symmetrical and b) asymmetrical.

a) Symmetrical: In this karyotype, the chromosomes are more or less graded with many median and submetacentric chromosomes (Figure 6.5).

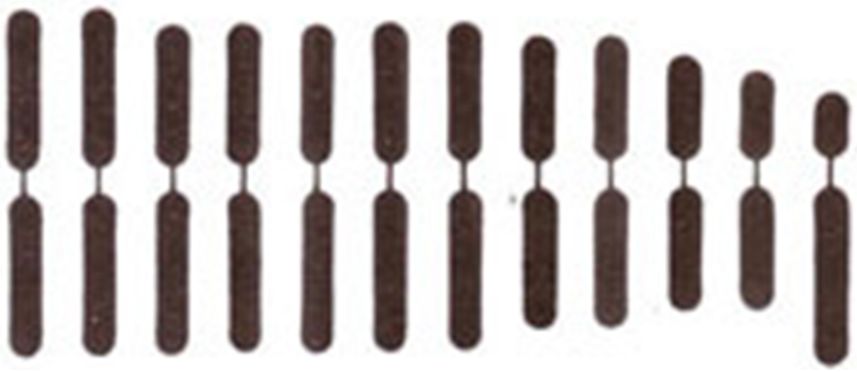

Figure-6.5: Symmetrical Karyotype

b) Asymmetrical: This karyotype possesses many chromosomes with subterminal chromosomes. The chromosomes exhibit great differences in size between the largest and smallest chromosomes (Figure 6.6).

Figure-6.6: Asymmetrical Karyotype

The evolution of karyotype is from symmetric to asymmetric types include a) Centric fusion and b) Centric fission play an important role in karyotype evolution (Robertsonian translocation).

Centric Fusion (Robertsonian Fusion):

Centric fusion, also known as Robertsonian fusion, is a chromosomal rearrangement in which two acrocentric chromosomes fuse at their centromeres to form a single metacentric or sub-metacentric chromosome (Figure 6.7). During this process, the short arms of the acrocentric chromosomes are usually lost, but because they contain repetitive, non-essential rDNA sequences, the loss is typically not harmful. Centric fusion results in a reduction in chromosome number without significant loss of genetic material. It is a major mechanism in karyotype evolution and is responsible for differences in chromosome numbers among related species (e.g., human chromosome 2 resulted from the fusion of two ancestral ape chromosomes). Robertsonian fusions can also cause clinical conditions such as Down syndrome when involving chromosome 21.

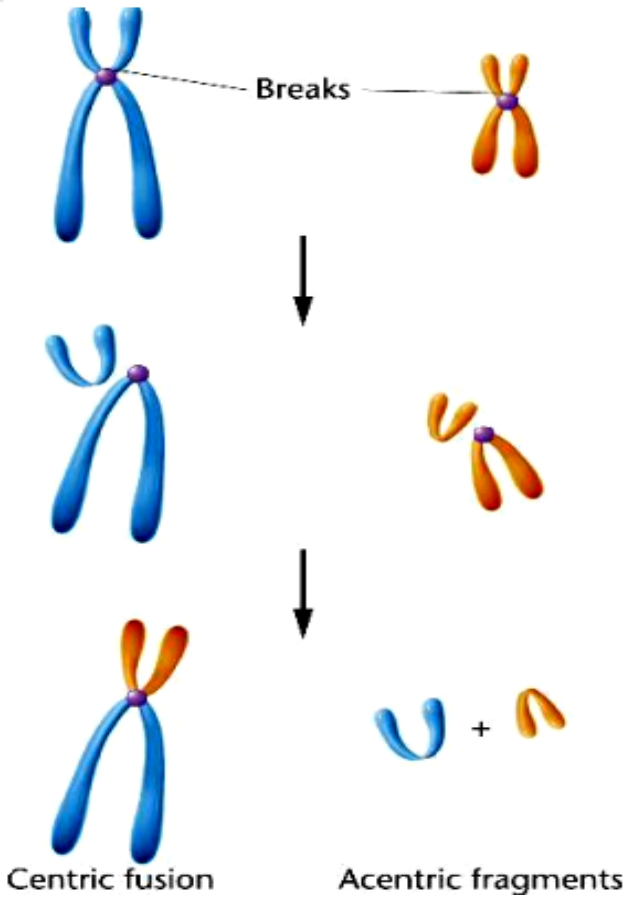

Figure-6.7: Centric Fusion

Centric Fission:

Centric fission is the opposite process of fusion, where a single metacentric chromosome splits at the centromere to give rise to two acrocentric chromosomes (Figure 6.8). This leads to an increase in chromosome number but generally preserves the overall genetic content. Centric fission is considered a mechanism of karyotype diversification and

speciation, especially in organisms with high chromosome numbers. Although less commonly observed compared to centric fusion, it plays a role in evolutionary cytogenetics by generating chromosome number variation within and between species. Centric fission can sometimes lead to instability unless the resulting chromosomes develop functional centromeres and telomeres.

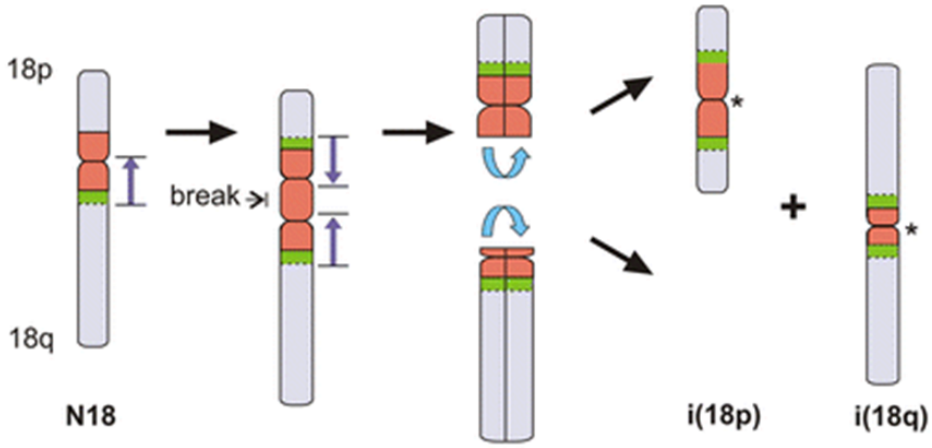

Figure-6.8: Centric Fission

Apart from the above a unique, bimodal type of karyotype is seen is Yucca and Agave. The haploid karyotype consists of 5 long and 25 dots like micro chromosomes.

Microchromosomes:

Microchromosomes are very small chromosomes that occur alongside larger (macro-) chromosomes in certain plant groups (Figure 6.9), including members of the Agavaceae such as *Yucca* and *Agave*. In these genera, the karyotype typically displays a bimodal pattern, meaning the chromosome set consists of a few large chromosomes and several tiny microchromosomes. These microchromosomes are often difficult to analyze due to their minute size, but they are believed to represent highly condensed, gene-poor, and repeat-rich regions of the genome. Their presence contributes to the large and complex genome size characteristic of agaves and yuccas. Microchromosomes may play a role in structural genome organization, species diversification, and adaptation.

Figure-6.9: Microchoromosomes of *Yucca*

iii) Variation in Absolute Chromosome Size:

Variation in absolute chromosome size refers to the differences in the physical length of chromosomes within a karyotype or between species. Chromosomes may vary significantly in size due to differences in the amount of DNA content, heterochromatin distribution, repetitive sequences, and structural rearrangements such as deletions, duplications, or amplifications. In many organisms, karyotypes show a bimodal pattern, with a few large chromosomes and several smaller ones (microchromosomes), while others display a gradual size series from largest to smallest. These size variations are important because they influence karyotype symmetry, genome organization, and chromosomal behavior during cell division. Larger chromosomes often carry more genes, while smaller ones may be enriched in heterochromatin. Comparing absolute chromosome sizes helps in species identification, evolutionary studies, and cytogenetic classification, making it a key parameter in karyological analysis.

a) The heterosporous pteridophytian taxa such as *Selaginella* and *Marsilea* tend to have smaller chromosomes than the homosporous ones.

b) Gymnosperms usually have largest mean and modal size of chromosomes.

Among angiosperms monocots generally have larger chromosomes than dicots. E.g.: *Paeonia* (Ranunculaceae) is a striking exception with large dicot chromosomes. In primitive leptosporangiate fern families like Osmundaceae and Hymenophyllaceae have relatively large chromosomes, those of Cyatheaceae and Polypodiaceae are of intermediate size while the smallest chromosomes are seen in Salviniaceae. The best example of phylogenetic increase in absolute chromosome size is in the family Gramineae. Avdulov considered phylogenetic increase in size has occurred as an adaptation to cool climate and he has suggested that it took place during the period of pleistocene glaciation/Quaternery glaciation/ice age (2.58 my or 11, 700 years).

iv) Variation in Number and Size of Satellites: Satellites are small chromosomal segments separated from the main chromosome body by a secondary constriction, usually associated with nucleolar organizer regions (NORs) that contain rRNA gene clusters. The number and size of satellites can vary widely among species and even among individuals within the same species. This variation arises from differences in the amount of ribosomal DNA, the degree of heterochromatin, and evolutionary changes such as duplications, deletions, or amplification of rDNA sequences. In some karyotypes, only a few chromosomes (often acrocentric ones) carry satellites, whereas in others multiple satellite-bearing chromosomes may be present. The size of satellites can also differ, ranging from minute knobs to large, conspicuous segments. These variations are important cytological markers used in karyotype characterization, taxonomic differentiation, and evolutionary studies, since satellite patterns often show species-specific or population-specific signatures. Satellites also influence chromosome behavior during meiosis and may be involved in genome stability and nucleolar function.

v) **Distribution of Euchromatin and heterochromatin and repetitive DNA:** Depending on the distribution of Euchromatin and heterochromatin, different types of interphase nuclei have been recognized. These being a) diffuse staining type b) Cap nucleus c) Multiple chromo centre nucleus and d) Prochromosome type. About 70% of DNA in plants is repetitive and is responsible for speciation to some extent. E.g.: Most of the conifers differ from each other in the amount of repetitive DNA. In gymnosperms, the chromosome number remains the same but the intra-chromosomal repetitive DNA changes are responsible for speciation.

a) **Diffuse staining type nuclei:** Diffuse staining type nuclei is a term often used in histology and pathology to describe the appearance of cell nuclei under a microscope. It refers to nuclei that exhibit a uniform or lightly dispersed chromatin pattern, making them appear diffusely stained rather than having distinct clumps of chromatin. Possible Implications of Diffuse Staining Nuclei includes the following types.

   1) **Normal Cellular Activity:** Some cells naturally have diffuse chromatin, indicating active transcription and a less condensed nuclear structure.
   2) **Cancerous or Abnormal Cells:** In pathology, diffuse nuclear staining can be a feature of certain malignancies, including some lymphomas, leukemias, and undifferentiated tumors.
   3) **Infectious or Inflammatory Conditions:** Certain viral infections (like CMV or EBV) can lead to nuclear changes with diffuse chromatin staining.
   4) **Apoptotic or Necrotic Changes:** Some dying cells may exhibit diffuse nuclear staining as chromatin decondenses before fragmentation.

b) **Cap Nucleus:** The term "cap nucleus" in relation to chromatin likely refers to a situation where chromatin is unevenly distributed within the nucleus, forming a cap-like appearance. Possible Explanations for "Cap Nucleus" in Relation to Chromatin includes the following types.

   1) **Plasma Cells:** Plasma cells, responsible for antibody production have condensed chromatin at one pole of the nucleus, forming a "chromatin cap."
   2) **Apoptosis:** During programmed cell death (apoptosis), chromatin undergoes margination, give the appearance of a chromatin cap or ring.
   3) **Viral Infections:** Cytomegalovirus (CMV) and other viral infections leading to chromatin displacement, forming a cap-like pattern.
   4) **Nucleolar Chromatin Capping in Certain Tumors:** Some cancers show abnormal chromatin organization, where chromatin clusters at one pole of the nucleus. E.g.: Hematologic malignancies (e.g., multiple myeloma) and certain carcinomas.

c) **Multiple Chromocenter Nucleus:** It refers to a nuclear structure where chromatin is organized into several chromocenters. Chromocenters are clusters of highly condensed heterochromatin within the nucleus. They contain repetitive DNA sequences, such as pericentromeric and telomeric regions. Eg: Drosophila salivary gland cells, plant cells, and some mammalian immune cells. The biological Significance of this karyotype includes **1. Gene Regulation:** Heterochromatin-rich regions suppress gene expression. **2. Chromosome Stability:** Helps in genome organization and maintaining nuclear architecture. **3. Developmental Changes:** Some cells transition from a single to multiple chromocenter arrangements during differentiation.

d) Prochromosome: A prochromosome refers to a highly condensed form of chromatin that appears in certain resting or interphase nuclei. These are highly condensed chromatin, unlike regular interphase chromatin, which is loosely packed, prochromosomes appear as short, thick chromatin bodies. Resemble mitotic chromosomes, but they are present in interphase. Eg: Insect cells, plant cells, and some mammalian cells (e.g., hepatocytes, embryonic cells), polyploid cells. These chromosomes involved in chromosome organization during non-dividing phases and may protect genetic material in long-lived cells.

6.4. EUCHROMATIN AND HETEROCHROMATIN

Euchromatin:

Euchromatin is the lightly packed form of chromatin that is rich in gene concentration (Figure 6.10). It is often under active transcription. Euchromatin comprises the most active portion of the genome within the nucleus, 92% of the human genome is euchromatic. The structure of Euchromatin is reminiscent of an unfolded set of beads represent Nucleosomes. Nucleosomes consist of eight proteins known as Histones, with approximately 147 base pairs of DNA wound around them. There are five main types of histones, H1, H2A, H2B, H3, and H4. These types are divided into two main classes. The core histones are H2A, H2B, H3, and H4. The linker histones are H1 and H5 (highest Lysine and Arginine ratio). In Euchromatin the wrapping is loose so that the raw DNA may be accessed. The basic structure of Euchromatin is an elongated, open 10 nm micro fibril, as noted by electron microscopy. Euchromatin participates in the active transcription of DNA to mRNA products. Individual chromosomes can be seen only during mitosis. During interphase, the general mass of chromatin is in the form of euchromatin. Euchromatin is less tightly packed than mitotic chromosomes. Regions of heterochromatin remain densely packed throughout interphase. Euchromatin contains m**ost** housekeeping genes, developmentally regulated genes, promoters, enhancers, and open regulatory regions and GC-rich DNA sequences. Since euchromatin is rich in functional genes, it plays a central role in maintaining normal cellular processes. Mutations or epigenetic changes in euchromatic regions often result in significant phenotypic effects, including developmental abnormalities and diseases.

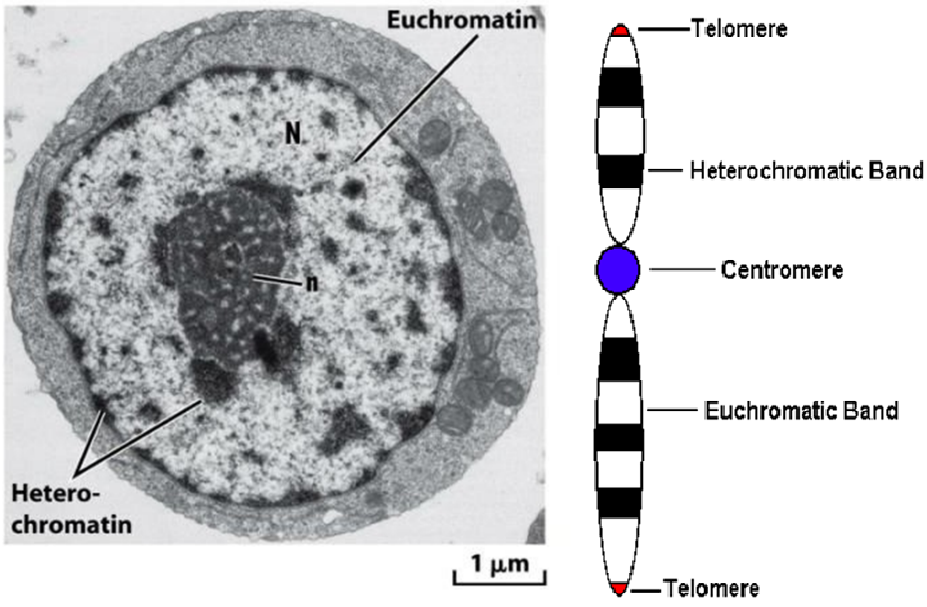

Figure-6.10: Euchromatin and Heterochromatin Of Chromosomes

Euchromatin is the primary hub of transcription, where active genes are continuously read and transcribed into RNA. Its open structure allows efficient binding of transcription machinery, making it essential for gene expression, cell differentiation, metabolic regulation and response to environmental signals. Because euchromatin contains regulatory elements, it also contributes to gene regulation networks, shaping cell identity and physiological functions. The dynamic remodeling of euchromatin enables quick shifts between active and inactive states, supporting adaptable gene expression.

Functions of Euchromatin:

1) **Active Gene Expression:** Euchromatin is the primary site of transcriptionally active DNA. Its loosely packed structure allows easy access for RNA polymerase and transcription factors, enabling continuous expression of housekeeping genes and regulated genes essential for cell function, metabolism, and growth.
2) **Regulation of Gene Activity:** Euchromatin contains promoters, enhancers, and regulatory elements that control gene activation and repression. Dynamic chromatin remodelling in euchromatin helps cells switch genes ON or OFF in response to developmental cues, environmental signals, or metabolic needs.
3) **Early DNA Replication:** Because euchromatin is less condensed, it undergoes early replication during S-phase. This ensures timely duplication of genes required for basic cellular processes, allowing smooth cell-cycle progression.
4) **Cellular Differentiation and Development:** Euchromatin plays a central role in cell fate determination, as differential activation of genes in euchromatic regions drives tissue-specific gene expression patterns during development and differentiation.
5) **Genome Flexibility and Rapid Adaptation:** Euchromatic regions allow easy chromatin remodelling, making them responsive to signalling pathways. This flexibility helps organisms quickly adapt to physiological and environmental changes by modifying gene expression.
6) **Facilitating Recombination and Genetic Diversity:** Euchromatin promotes meiotic and mitotic recombination because its open structure allows recombination machinery to access DNA. This contributes to genetic variation, evolution, and proper chromosome segregation.
7) **Maintenance of Nuclear Organization:** Euchromatin contributes to nuclear architecture by occupying the central regions of the nucleus. Its interaction with transcription factories and nuclear body components helps maintain functional compartmentalization of the nucleus.
8) **Prevention of Aberrant Gene Silencing:** Euchromatin prevents spreading of heterochromatin by maintaining an open, active state. This protects essential genes from accidental epigenetic repression and ensures stable gene expression.

Heterochromatin:

Heterochromatin is DNA which tends to be highly compacted and dark staining. The number of genes in heterochromatin is generally small relative to euchromatin. Heterochromatin lacks genes or they are inactive. Heterochromatin is nucleated at a specific sequence. Much heterochromatin is found in certain structural parts of the chromosomes:

centromeres and telomeres. The length of the inactive region varies from cell to cell. Inactivation of genes in this vicinity causes position effect variegation. Similar spreading effects occur at: telomeres, the silent cassettes in yeast mating type. HP1 is the key protein in forming mammalian heterochromatin. It acts by binding to methylated H3 histone. RAP1 initiates formation of heterochromatin in yeast by binding to specific target sequences in DNA. The targets of RAP1 include telomeric repeats and silencers at HML and HMR. RAP1 recruits SIR3/SIR4, which interact with the N-terminal tails of H3 and H4. In the dark-staining regions, the chromatin remains in the condensed state and is called heterochromatin. In 1928, Heitz defined heterochromatin as those regions of the chromosome that remain condensed during interphase and early prophase and form the so-called chromocentre. Heterochromatin is characterized by its especially high content of repetitive DNA sequences and contains very few, if any, structural genes (i.e., genes that encode proteins). It is late replicating (i.e., it is replicated when the bulk of DNA has already been replicated) and is not transcribed. It is thought that in heterochromatin the DNA is tightly packed in the 30 nm fibre.

In an interphase nucleus, usually there is some condensed chromatin around the nucleolus, called perinucleolar chromatin, and some inside the nucleolus, called intranucleolar chromatin. Both types of this heterochromatin appear to be connected and together, they are referred to as nucleolar chromatin. Dense clumps of deeply staining chromatin often occur in close contact with the inner membrane of the nuclear envelope (i.e., with the nuclear lamina) and is called condensed peripheral chromatin. Between the peripheral heterochromatin and the nucleolar heterochromatin are regions of lightly staining chromatin, called dispersed chromatin. In the condensed chromosomes, the heterochromatic regions can be visualized as regions that stain more strongly or weaklier than the euchromatic regions, showing the so-called positive or negative heteropyknosis of the chromosomes (Gr., hetero = different + pyknosis = staining).

Types of heterochromatin: Heterochromatin has been further classified into the following types.

a) Constitutive Heterochromatin: The DNA is permanently inactive and remains in the condensed state throughout the cell cycle. This is the most common type of heterochromatin occurs around the centromere, in the telomeres and in the C-bands of the chromosomes. In *Drosophila virilis*, constitutive heterochromatin exists around the centromeres and such pericentromeric heterochromatin occupies 40 per cent of the chromosomes. In many species, entire chromosomes become heterochromatic and are called B chromosome, satellite chromosomes or accessory chromosomes and contain very minor biological roles. Such chromosomes comprising wholly constitutive heterochromatin occur in corn, many phytoparasitic insects and salamanders. In the fly *Sciara*, large metacentric heterochromatic chromosomes are found in the gonadal cells, but are absent in somatic cells. Entire Y chromosome of male Drosophila is heterochromatic, even though containing six gene loci which are necessary for male fertility. Constitutive heterochromatin contains short repeated sequences of DNA, called satellite DNA. This DNA is called satellite DNA because upon ultracentrifugation, it separates from the main component of DNA. The exact significance of constitutive heterochromatin is still unexplained.

b) Facultative Heterochromatin: Heterochromatin is not permanently maintained in the condensed state, instead it undergoes periodic dispersal and during these times is transcriptionally active. Frequently, in facultative heterochromatin one chromosome of the pair becomes either totally or partially heterochromatic. The best known case is that of the X-chromosomes in the mammalian female, one of which is active and remains euchromatic, whereas the other is inactive and forms at interphase, the sex chromatin or Barr body (Named after its discoverer, Canadian cytologist Murray L. Barr). Barr body contains DNA which is not transcribed and is not found in males. Indeed, the number of Barr bodies is always one less than the number of X chromosomes (i.e., in humans, XXX female has two Barr bodies and XXXX female has three Barr bodies).

Functions of Heterochromatin:

1) **Maintenance of Chromosome Stability:** Heterochromatin ensures structural stability at **centromeres** and **telomeres**, preventing chromosome breakage, degradation, or fusion. It supports proper attachment of spindle fibres during cell division.
2) **Gene Silencing and Regulation:** By remaining condensed, heterochromatin prevents the expression of nearby genes. This is essential for X-chromosome inactivation, Genomic imprinting, Developmental gene repression, Controlling transposable elements
3) **Prevention of Recombination:** Heterochromatic regions suppress illegitimate recombination events, thereby maintaining genomic integrity particularly in repetitive DNA-rich regions.
4) **Nuclear Organization:** Heterochromatin forms structural domains that help organize the nucleus. It often localizes to the nuclear periphery, creating a spatial architecture that separates active and inactive chromatin compartments.
5) **Protection against Transposable Elements:** Repetitive DNA sequences in heterochromatin attract silencing complexes that keep transposons inactive, preventing genome instability.
6) **Cytogenetic Behaviour:** Heterochromatin is easily visible in chromosome banding techniques. C-banding specifically stains constitutive heterochromatin at centromeres. Heterochromatin appears dark in G-banding due to its AT-rich nature. In R-banding, heterochromatin appears light because gene-rich GC regions stain dark instead. Heterochromatin replicates late in S-phase due to its dense packing and low transcriptional activity.
7) **Evolutionary Significance:** Heterochromatin evolves rapidly due to its repetitive nature, making it useful in species differentiation, karyotype evolution, phylogenetic studies. The variation in heterochromatin amount and distribution contributes to chromosome number diversification and genome size variation among species.
8) **Heterochromatin and Disease:** Aberrations in heterochromatin structure or epigenetic marking can lead to diseases such as cancer (loss of heterochromatin leading to genomic instability), developmental disorders, premature aging syndromes, faulty X-inactivation disorders in females. Altered heterochromatin can disrupt nuclear organization and activate transposons, contributing to disease progression.

6.5. SUMMARY:

Chromosomal banding is a set of cytogenetic staining techniques that produce reproducible patterns of alternating dark and light bands along the chromosomes, allowing precise identification of individual chromosomes and their structural features. These banding patterns reflect underlying differences in DNA composition, chromatin organization, and replication timing. Techniques such as G-banding (Giemsa), Q-banding (Quinacrine), C-banding, R-banding, and NOR-banding highlight specific regions such as AT-rich areas, GC-rich gene-dense regions, constitutive heterochromatin, or nucleolar organizer regions. Chromosomal banding revolutionized cytogenetics by enabling accurate detection of chromosomal abnormalities like deletions, translocations, duplications, inversions, and aneuploidies. It also plays a crucial role in karyotyping, comparative genomics, evolutionary cytology, cancer diagnosis, and prenatal screening. Through these staining methods, cytogeneticists can construct detailed chromosomal maps and study genome architecture with high resolution.

A karyotype represents the complete chromosomal complement of an organism, arranged systematically into homologous pairs based on size, centromere position, and banding pattern. It provides a visual and structural overview of all chromosomes during metaphase, making it a fundamental tool in cytogenetics and diagnostic biology. Karyotyping allows detection of both numerical abnormalities—such as aneuploidy (trisomy 21, monosomy X), polyploidy, or mosaicism—and structural alterations including translocations, deletions, duplications, and inversions. Beyond medical applications, karyotypes offer insights into evolutionary biology, species differentiation, genome organization, and chromosomal evolution. In clinical settings, karyotype analysis is used in prenatal testing, infertility investigations, cancer cytogenetics, and identification of genetic syndromes. Differences in chromosome size, shape, satellite regions, telomere length, and heterochromatin distribution contribute to karyotypic diversity within and across species, making the karyotype an essential tool for understanding genetic stability and variation.

Euchromatin is the lightly stained, open, and transcriptionally active region of chromatin that contains the majority of the genome's functional genes. Its loose nucleosome packing and high accessibility allow transcription factors and RNA polymerase to bind easily, facilitating active gene expression. Euchromatin is enriched in GC-rich sequences and replicates early during the S-phase of the cell cycle. It plays a central role in regulating gene activity, genome plasticity, recombination, and DNA repair. Dynamic epigenetic modifications—such as histone acetylation, specific methylation patterns, and chromatin remodelling—maintain euchromatin in an extended conformation that is permissive for transcription. Because of its flexibility and transcriptional openness, euchromatin is essential for cellular differentiation, developmental regulation, and adaptation to environmental signals. Errors in euchromatin regulation can lead to abnormal gene expression, contributing to developmental disorders and diseases.

Heterochromatin is the densely packed, darkly stained region of chromatin that is generally transcriptionally inactive due to its compact structure. It contains repetitive, AT-rich DNA sequences and plays essential roles in maintaining chromosomal stability and structural integrity. Heterochromatin is classified into constitutive heterochromatin, which is permanently condensed (e.g., centromeres and telomeres), and facultative heterochromatin,

which becomes condensed in specific developmental or environmental conditions (e.g., the inactive X-chromosome forming the Barr body). It replicates late in S-phase and is enriched in epigenetic marks such as histone H3K9 methylation and DNA methylation. Functionally, heterochromatin suppresses transposable elements, facilitates proper chromosome segregation during cell division, protects chromosome ends, and contributes to gene silencing and epigenetic regulation. Abnormalities in heterochromatin formation or maintenance can lead to genome instability, chromosomal missegregation, and various human diseases including cancers and congenital disorders.

6.6. TECHNICAL TERMS:

AT-rich regions, GC-rich regions, Idiogram, Arm ratio (p/q), Satellite chromosomes, Active chromatin state, Condensed chromatin, Telomeric and centromeric regions, Barr body.

6.7. SELF-ASSESSMENT QUESTIONS:

1) How the chromosomal banding patterns useful in identifying chromosomal abnormalities?
2) Describe in detail the principles, techniques, and applications of various chromosomal banding methods (G, C, R, Q, and NOR banding).
3) Discuss the significance of banding patterns in comparative cytogenetics and evolution.
4) Explain the structure of a karyotype, methods of preparation, and its applications in cytogenetics.
5) Give an account on euchromatin and heterochromatin and their significance.

6.8. SUGGESTED READINGS:

1) C.B. Powar. 2010. Cell Biology. Himalaya Publishing House, Mumbai – 400004.
2) De Roberties E.D.P & De Roberties (Jr.) 2017. Cell and Molecular Biology (8th Edition).
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6) Lehninger Principles of Biochemistry – Nelson & Cox (for regulation concepts)
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8) P.S. Verma & V. K. Agarwal, 2021. Cell Biology, Genetics, Molecular Biology, Evolution and Ecology. S.Chand And Company Limited, New Delhi – 110044.

Dr. Kakumanu Babu

LESSON-7

SPECIAL TYPES OF CHROMOSOMES

7.0 OBJECTIVE:

- To provide the information to the students about the unique structural, functional, and developmental features of specialized chromosomes such as polytene, lamp-brush, B-chromosomes, and sex chromosomes in order to analyze their roles in gene expression, chromosome organization, evolution, and cytogenetic behavior.

STRUCTURE:

7.1 Introduction

7.2 Special Types of Chromosomes

7.2.1. Polytene Chromosomes

7.2.2. Lamp-Brush Chromosomes

7.2.3. B-Chromosomes

7.2.4. Sex Chromosomes

7.3 Summary

7.4 Technical Terms

7.5 Self-Assessment Questions

7.6 Suggested Readings

7.1. INTRODUCTION:

Special types of chromosomes are uniquely structured chromosomes that differ from typical mitotic chromosomes in size, morphology, function, and behavior, and they arise in specific developmental stages or tissues. These include polytene chromosomes, lamp-brush chromosomes, B-chromosomes and sex chromosomes. Polytene chromosomes are giant chromosomes formed by repeated rounds of DNA replication without cell division, a process known as endoduplication. They are most famously found in the salivary glands of *Drosophila* larvae. These chromosomes consist of many aligned chromatids, producing thick, banded structures easily visible under a light microscope. Polytene chromosomes display distinct dark and light bands corresponding to condensed and transcriptionally active regions, and they often exhibit “puffs” which represent sites of intense gene transcription. Because of their size and banding pattern, polytene chromosomes serve as powerful tools for gene mapping, studying chromatin organization, and analysing gene expression at high resolution. Lamp-brush chromosomes are exceptionally large meiotic chromosomes found in the growing oocytes of amphibians, birds, and some invertebrates. They derive their name from their characteristic “brush-like” appearance created by numerous lateral loops extending from each chromosome axis. These loops are regions of active transcription, producing high quantities of RNA needed for oocyte growth and early embryogenesis. Lamp-brush chromosomes are valuable for studying gene activity, transcriptional regulation, chromatin

structure, and RNA synthesis during meiosis. Their large size and extended configuration make them ideal cytological models for observing chromosome organization in a decondensed, functional state.

B-chromosomes, also known as supernumerary or accessory chromosomes, are extra, non-essential chromosomes found in addition to the standard A-chromosome complement of an organism. They vary widely in size, structure, and number between individuals within the same species and do not follow Mendelian inheritance. Often composed largely of heterochromatin, B-chromosomes typically lack essential genes but may carry repetitive DNA, transposable elements, or occasionally gene fragments. Although traditionally considered genetically inert, recent studies suggest they can influence phenotype, fertility, and adaptation in some plants and animals. Their unpredictable behaviour, transmission patterns, and evolutionary significance make B-chromosomes key subjects in cytogenetics and genome evolution. Sex chromosomes are a specialized pair of chromosomes responsible for determining the sex of an organism and controlling various aspects of sexual development and reproduction. They differ from autosomes in size, gene content, and structural features. In most animals, sex determination systems include XY (male heterogamety) or ZW (female heterogamety), with one chromosome typically gene-rich (X or Z) and the other highly degenerated and heterochromatic (Y or W). Sex chromosomes house genes crucial for sex differentiation, fertility, and inheritance of sex-linked traits. Their evolution from ordinary autosomes, involvement in dosage compensation, and role in genetic disorders make them fundamentally important in genetics, developmental biology, and evolutionary studies.

7.2. SPECIAL TYPES OF CHROMOSOMES:

Special types of chromosomes are structurally modified chromosomes that appear in certain organisms or specific tissues, exhibiting unique morphological and functional features not seen in typical somatic chromosomes. These chromosomes provide valuable insights into gene expression, chromatin organization, and developmental biology. Some cells at certain particular stages contain large nuclei with giant or large-sized chromosomes. The giant chromosomes are the polytene and lampbrush chromosomes. The major special chromosomes include 1. Polytene chromosomes 2. Lamp-brush chromosomes 3. B-chromosomes 4. Sex chromosomes.

1. Polytene chromosomes
2. Lamp-brush chromosomes
3. B. Chromosomes
4. Sex chromosomes

7.2.1. Polytene Chromosome:

Other name is salivary gland chromosomes. The polytene chromosome was proposed by Kollar due to the occurrence of many chromonemata (DNA) in them. Giant chromosomes were first time observed by E.G. Balbiani in the year 1881 in nuclei of certain secretory cells (salivary glands) of *Chironomas* larvae (Diptera). They were conclusively reported for the first time in insect cells (*Drosophila*) by Theophilus Painter (1933). In plants, polytene chromosomes have been observed in only a few species e.g., anther tapetum.

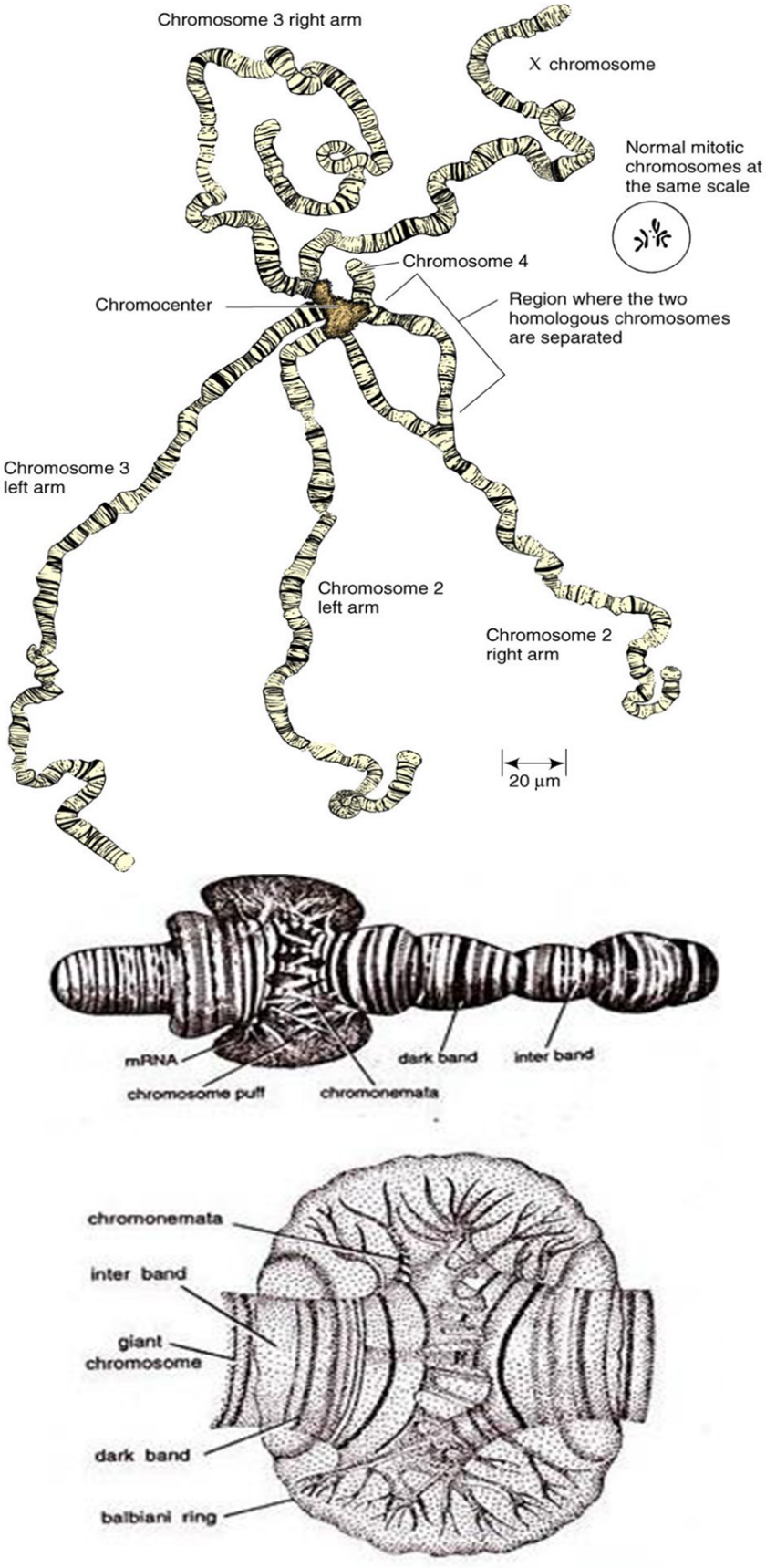

Figure-7.1: Polytene Chromosomes

Cells in the larval salivary gland of *Drosophila*, mosquito and *Chironema* contain chromosomes with high DNA content (Figure 7.1). E.g.: malphigian tubules, rectum, gut, foot pads, fat bodies, ovarian nurse cells. Polyteney of giant chromosomes happens by replication of the chromosomal DNA several times without nuclear division (endomitosis) and the resulting daughter chromatids do not separate but remain aligned side by side. During endomitosis the nuclear envelope does not rupture and no spindle formation takes place. The polytene chromosomes are visible during interphase and prophase of mitosis. A series of dark transverse bands alternates with clear zones of inter bands. Such individual bands can be correlated with particular genes. About 85% of the DNA in polytene chromosomes is in bands and rest 15% is in inter bands. Certain regions become "puffed out" due to active DNA transcription at particular stages of development. These chromosome puffs are also termed Balbiani rings. Puffs may appear and disappear depending on the production of specific proteins. The paternal and maternal chromosomes remain associated side by side and the phenomenon is termed somatic pairing.

Functions:

1) Polytene chromosomes increasing the volume of the cell's nuclei and causing cell expansion, It is also have a metabolic advantage as multiple copies of genes permits a high level of gene expression.
2) In Drosophila melanogaster, for example, the chromosomes of the larval salivary glands undergo many rounds of endoduplication to produce large quantities of adhesive mucoprotein before pupation.
3) Another example within the fly itself is the tandem duplication of various polytene bands located near the centromere of the X chromosome which results in the Bar phenotype of kidney-shaped eyes.
4) The interbands are involved in the interaction with the active chromatin proteins, nucleosome remodeling, and origin recognition complexes.
5) Their primary functions are: to act as binding sites for RNA pol II, to initiate replication and, to start nucleosome remodeling of short fragments of DNA.

7.2.2. Lamp-Brush Chromosome:

Lamp-brush chromosomes (figure 7.2) were first observed by Flemming in 1882 in Salamander oocytes and later described by Ruckert in shark in the year 1892. They appeared like brushes used for cleaning lamps, hence the name lamp-brush chromosome. Thorpe (1984) and Burns and Bottino (1989) preferred the term test tube brush chromosomes. They are transitory structures and can be observed during the diplotene stage of prophase I in meiosis in the oocytes of all animal species both vertebrates and invertebrates. They have been described in Sepia (Mollusca), Echinaster (Echinodermata) and in several species of insects, shark, amphibians, reptiles, birds and mammals (humans), spermatocytes and oocytes.

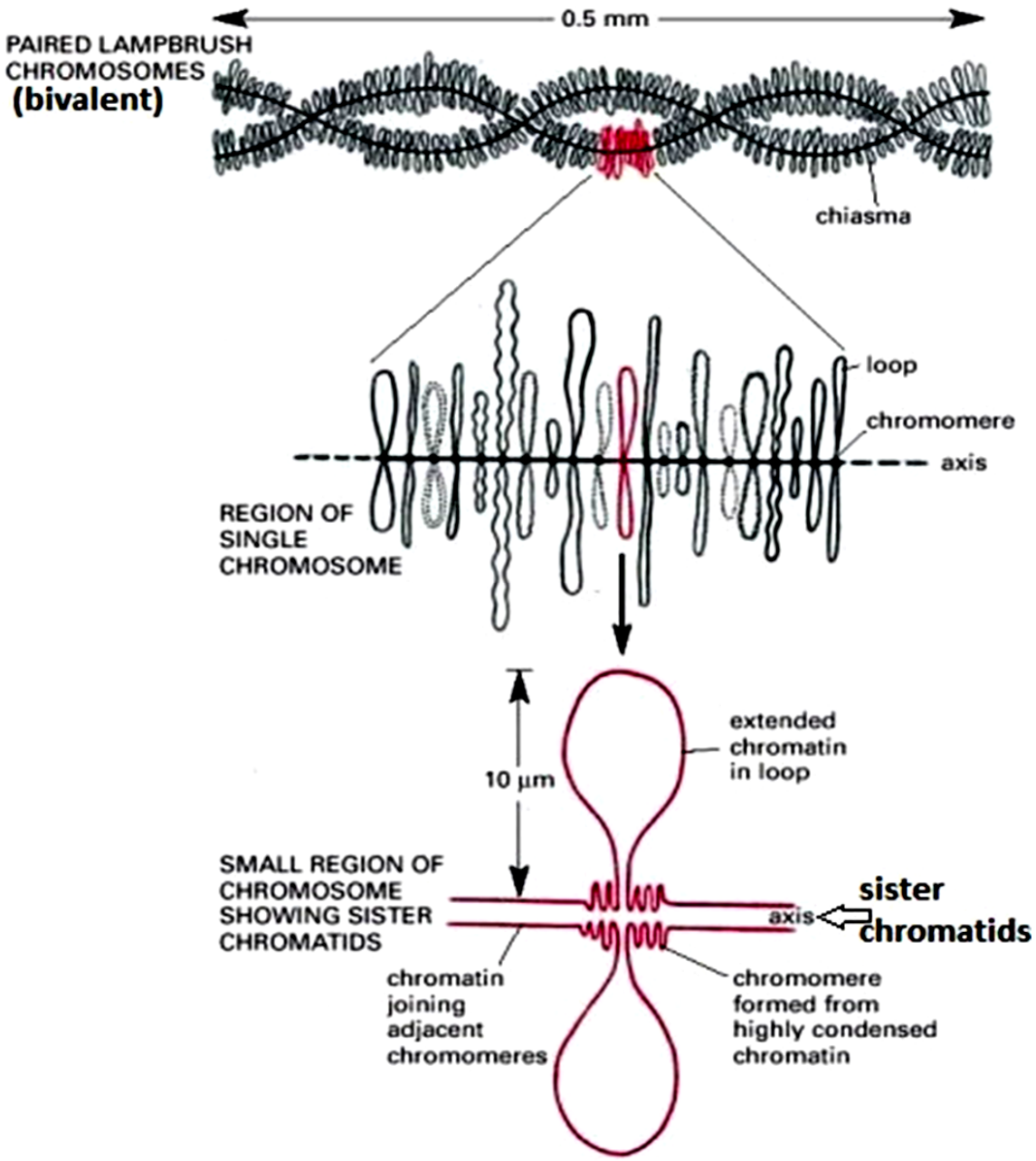

Figure-7.2: Lamp-Brush Chromosomes

The total length of the entire lamp-brush chromosome set is 5 to 6 µm and is organized into about 5,000 chromomeres. They are of exceptionally large sizes and present in bivalent form. They are formed due to the active synthesis of mRNA molecules for future use by the egg cells. They are organized into a series of chromomeres with large chromatin symmetrical loops extending laterally. Each loop appears at a constant position in the chromosome (10,000 loops per chromosome set or haploid set). Each loop has an axis made up of DNA unfolded from the chromosome and is transcriptionally highly active.

Functions:

1) Useful to study chromosome organization and genome function and gene expression during meiotic prophase.
2) Lampbrush chromosomes used for construction of detail cytological maps of individual chromosomes.

7.2.3. B-Chromosomes:

Many plant (maize, etc.) and animal (insects and small mammals) species, besides having autosomes (A-chromosomes) and sex-chromosomes possess a special category of chromosomes called B-chromosomes (Figure 7.3) without obvious genetic function. Other names for B-chromosomes are supernumerary chromosomes, accessory chromosomes, accessory fragments, etc. Usually have a normal structure, are somewhat smaller than the autosomes and can be predominantly, heterochromatic (many insects, maize, etc.) or predominantly euchromatic (rye). In maize, their number per cell can vary from 0 to 30 and they adversely affect, development and fertility only when occur, in large amount. In animals, the B-chromosomes disappear from the non-reproductive (somatic) tissue and are maintained only in the cell-lines that lead to the reproductive organs. B-chromosomes have negative consequences for the organism, as they have deleterious effect because of abnormal crossing over during the meiosis of animals and abnormal nucleus divisions of the gametoophyte plants. In animals, B-chromosomes occur more frequently in females. The origin of the B-chromosomes is uncertain. In some animals they may be derivatives of sex chromosomes. They generally do not show any pairing affinity with the' A-chromosomes. In humans, B chromosomes interact with standard chromosomes and play an important role in the evolution of genetic material.

Features of B-Chromosomes:

They are the primary cause of Turner syndrome, which affects development in females. B-chromosomes are found in all vertebrates, from fish to humans, and have been found to have significant roles in several pathways of the cell. Their number can vary among individuals of the same species. They do not follow normal Mendelian patterns of inheritance. They can undergo drive mechanisms to increase their transmission to the next generation. Many B chromosomes contain repetitive DNA and transposable elements. Some may carry functional genes that can affect the host.

Functions and Effects of B Chromosomes:

1) **Genetic Parasitism:** Many B chromosomes act as genetic parasites, increasing their own transmission without benefiting the host.
2) **Gene Expression Influence:** Some may regulate or suppress genes on A chromosomes.
3) **Adaptive Advantage:** In rare cases, B chromosomes can provide benefits, such as resistance to environmental stress or pathogens.

Figure-7.3: B-Chromosomes

7.2.4. Sex Chromosomes:

Sex chromosomes are a distinct pair of chromosomes responsible for determining the sex of an organism as well as regulating various aspects of sexual development, fertility, and inheritance of sex-linked traits. Unlike autosomes, which occur in identical pairs in both males and females, sex chromosomes differ between the sexes and can vary greatly in size, gene composition, and structural organization. They are believed to have evolved from a pair of homologous autosomes. One chromosome gradually acquired a key sex-determining gene, such as the SRY gene on the mammalian Y chromosome, and suppression of recombination between these proto-sex chromosomes eventually led to degeneration of the Y or W chromosome and expansion of functional genes on the X or Z chromosome.

Different sex-determining systems have evolved across species. In the widely known XY system of mammals and Drosophila, females possess XX chromosomes while males are XY, with the Y chromosome triggering male development. In contrast, birds and Lepidoptera follow a ZW system where males are homogametic (ZZ) and females are heterogametic (ZW). Other mechanisms include the XO system, where males possess a single X chromosome and females have two, and the haplodiploid system of bees and ants, where males are haploid and females are diploid. Sex chromosomes have unique structural features. The X chromosome is usually gene-rich, euchromatin-rich, and involved in vital cellular functions, whereas the Y chromosome is highly heterochromatic, contains relatively few genes, and houses male-determining and fertility-related loci. Recombination occurs only in the pseudoautosomal regions shared between X and Y, the remainder of the Y chromosome is non-recombining and subject to gradual gene loss.

Dosage compensation is essential for balancing gene expression between males and females. In mammals, one X chromosome in females undergoes epigenetic silencing to form a Barr body, whereas in Drosophila the single male X is hyper transcribed, and in *C. elegans* hermaphrodites both X chromosomes undergo partial down regulation. These mechanisms ensure that X-linked genes are expressed at comparable levels in both sexes. Cytologically, sex chromosomes show distinctive staining properties, with the Y chromosome usually appearing highly condensed and heterochromatic, while the inactive X chromosome in females forms a visible Barr body during interphase.

Functionally, sex chromosomes play crucial roles in sex determination, initiating and maintaining pathways for male or female differentiation. They also carry numerous sex-linked genes responsible for traits such as color blindness and hemophilia. The Y chromosome contains genes essential for spermatogenesis, including the AZF regions, making it important for male fertility. Sex chromosome abnormalities arise from nondisjunction during meiosis and lead to conditions such as Turner syndrome (45,X), Klinefelter syndrome (47,XXY), Triple X syndrome (47,XXX), and XYY syndrome (47,XYY), each associated with characteristic phenotypic effects.

Sex Chromosomes in Plants:

In plants, sex chromosomes also exist, as in *Silene latifolia,* though their evolution and structure can differ significantly from those in animals. Plant sex chromosomes often show independent origins and have diverse patterns of recombination and degeneration. Studying sex chromosomes provides insights into human genetic disorders, reproductive biology, epigenetic regulation such as X-inactivation, and evolutionary processes that shape genome organization. It also has practical applications in medical diagnostics, fertility studies, and understanding mechanisms of chromosome evolution and speciation.

XY System:

Similar to mammals, some plants have XY systems where males are XY and females are XX, with the Y chromosome carrying male-determining genes (e.g., *Silene latifolia*, *Asparagus*).

ZW System:

Some plants, like certain willows (Salix), show ZW systems (ZW males, ZZ females).

Heteromorphic vs. Homomorphic:

Chromosomes can be different sizes (heteromorphic, like X and Y) or identical (homomorphic), with differences arising from gene loss or duplication.

Genetic Degeneration:

Plant Y chromosomes often show signs of degeneration, losing genes over time, similar to animal Y chromosomes, though dosage compensation (balancing gene expression) isn't always seen.

7.3. SUMMARY:

Polytene chromosomes are giant, multi-stranded chromosomes formed through repeated rounds of DNA replication without cell division, a process called endoreduplication. Found mainly in the salivary glands of *Drosophila* larvae, they display distinct dark and light banding patterns and chromosomal "puffs" that indicate regions of active gene transcription. Their enormous size and detailed banding make them excellent tools for cytogenetic studies, gene mapping, and understanding chromatin organization and gene expression dynamics. Lampbrush chromosomes are large, extended meiotic chromosomes present in the growing oocytes of amphibians, birds, and some invertebrates. Characterized by numerous lateral loops that resemble the bristles of a lampbrush, these structures represent highly active transcriptional regions producing RNA required for oocyte development. Their unique morphology and accessibility make lampbrush chromosomes important models for studying transcriptional regulation, chromatin decondensation, and RNA synthesis during meiosis. B-chromosomes are extra, non-essential chromosomes found in addition to the normal chromosomal set of an organism. They vary in number, size, and genetic content among individuals and often consist largely of heterochromatin. Although not required for survival, B-chromosomes may influence phenotypic traits, fertility, or adaptation in some species. Their irregular inheritance and evolutionary persistence make them significant in understanding chromosomal behavior, genome evolution, and genetic diversity. Sex chromosomes are a specialized pair of chromosomes that determine the sex of an organism and regulate various aspects of sexual development and reproduction. They differ from autosomes in size, gene content, and heterochromatin distribution. Systems such as XY (mammals, Drosophila) and ZW (birds, butterflies) illustrate how one chromosome remains gene-rich while the other becomes reduced and heterochromatic over evolutionary time. Sex chromosomes play critical roles in sex determination, dosage compensation, inheritance of sex-linked traits, and the study of genetic disorders and chromosomal evolution.

7.4. TECHNICAL TERMS:

Chromosome Puffs (Balbiani Rings), Salivary Gland Chromosomes, Lateral Loops, Chromomere, Supernumerary Chromosomes, Sex Determination.

7.5. SELF-ASSESSMENT QUESTIONS:

1) Define polytene chromosomes and mention their significance in gene expression studies.
2) State the structural features of lamp-brush chromosomes.
3) How do sex chromosomes evolve? Briefly explain with an example.
4) Describe the structure, formation, and functional significance of polytene chromosomes. Discuss their role in developmental and molecular biology.
5) Explain in detail the organization of lamp-brush chromosomes. How do they serve as excellent models for studying transcriptional regulation?
6) What are B-chromosomes? Discuss their origin, inheritance patterns, effects on phenotype, and evolutionary significance.
7) Write an essay on sex chromosomes.
8) Compare and contrast polytene chromosomes, lamp-brush chromosomes, B-chromosomes, and sex chromosomes based on structure, function, and biological significance.

7.5. SUGGESTED READINGS:

1) C.B. Powar. 2010. Cell Biology. Himalaya Publishing House, Mumbai – 400004.
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Dr. Kakumanu Babu

LESSON-8

PHASES OF CELL CYCLE, CHECK POINTS AND CYCLIN DEPENDENT KINASES

8.0 OBJECTIVE:

- Students will understand the process and different phases of the cell cycle, need for check points and role of cyclin dependent kinases in cell division.

STRUCTURE:

8.1 Introduction

8.2 Phases of Cell Cycle

8.3 Check points of the Cell Cycle

8.4 Role of Cyclins

8.5 Cyclin Dependent Kinases

8.6 Summary

8.7 Technical Terms

8.8 Self-Assessment Questions

8.9 Suggested Readings

8.1. INTRODUCTION:

The cell cycle is a regulated series of events that a cell undergoes to grow, duplicate its genetic material, and divide into two daughter cells. It consists of two main stages: interphase and the mitotic phase. During interphase, the cell spends most of its time carrying out normal metabolic activities and preparing for division through three sub-phases: G_1, where the cell grows and synthesizes proteins, S phase, where DNA replication occurs and G_2, where the cell prepares the necessary components for mitosis. The mitotic phase includes mitosis, the division of the nucleus, and cytokinesis. The division of the cytoplasm, resulting in two genetically identical daughter cells. The progression of the cell cycle is tightly controlled by cyclins**,** cyclin-dependent kinases (CDKs), and checkpoint mechanisms that ensure accurate DNA replication and proper chromosome segregation. Proper regulation of the cell cycle is essential for growth, development, and tissue repair, while its disruption can lead to uncontrolled cell division and diseases such as cancer.

8.2. PHASES OF CELL CYCLE:

The cell cycle is defined as the full sequence of events that occur between the end of one nuclear division and beginning of the next. Howard and Pelc (1953) have divided cell cycle into G_1, S, G_2 and M phases (Figure 8.1). The G_1, S and G_2 phases together form the classical interphase.

G_1 Phase:

The G_1 phase is also known as the initial growth phase or the post-mitotic gap phase. It is the most extended phase of cell division. In this phase, various types of RNA (mRNA, tRNA, and rRNA) and proteins are synthesized. In plant cells, all cell organelles multiply, including the endoplasmic reticulum, mitochondria, the Golgi complex, ribosomes, and plasmids. The duration of G_1 Phase differs from cell to cell. It is shorter in frequently dividing cells. The duration of the G_1 phase varies greatly, ranging from 30 to 50 percent of the total time of the cell cycle. G_1 phase is completely absent in rapidly proliferating cells such as blastomeres of early embryos in frogs and mammals. G_1 phase cells have three possibilities. a) Continues the cycle and enters S phase. b) Stops the cell cycle and enters quiescent or G_0 phase. c) Terminates the cell cycle and initiates cell differentiation. The availability of mitogens and energy rich substances determines the aforementioned option. This point is known as a checkpoint. The following proteins are produced during the G_1 phase: (1) regulatory proteins that regulate different aspects of mitosis; (2) enzymes (like DNA polymerase) required for DNA synthesis; and (3) tubulin and other proteins of the mitotic apparatus.

S Phase:

DNA replication and histone protein synthesis occur during interphase's S phase, also known as the synthetic phase. To provide nucleosomes to the newly synthesized DNA, enormous numbers of new histones are required immediately at the start of the S phase. At the end of S phase, each chromosome contains two DNA molecules and a double set of genes. S phase accounts for around 35 to 45 percent of the cell cycle.

G_2 Phase:

During the G_2 phase of the cell cycle, RNA and protein synthesis continues, which is necessary for cell growth. The G_2 phase may take up 10 to 20% of the cell cycle's time. As the G_2 phase ends, the cell enters the M phase, where DNA synthesis stops and RNA and protein synthesis continue. All cell organelles multiply, and spindle formation occurs. The synthesis of tubulin protein is essential for spindle formation. The synthesis of protein is essential for plasma membrane development. A large number of ATP molecules are required to transfer chromosomes from the equator to the pole (30 ATP/chromosome). It lasts between 2 and 5 hours in most cells. Some proteins generated during this period trigger chromosomal condensation, which initiates mitosis.

G_0-Phase:

The G_0 phase, also known as the quiescent stage, is a period in which cells do not divide further (i.e., do not enter S-phase after G_1-phase) and undergo differentiation. This is due to a lack of mitogens and energy rich compounds, and the cells remain metabolically active, grow in size, and differentiate for a specific function after achieving a specific shape. However, some cells remain undifferentiated as reserve cells, and they may divide when necessary.

Interphase:

The nuclear envelope is still intact. Chromosomes have the form of dispersed, lengthy, coiled, and barely visible chromatin fibers. The amount of DNA doubles. In animal cells, a daughter pair of centrioles develops near an existing centriole, thus an interphase cell has two pairs of centrioles. Net membrane biosynthesis in animal cells increases shortly before cell division (mitosis). This excess membrane appears to be stored as blebs on the surfaces of cells that are preparing to divide.

M Phase or Mitotic Phase:

Somatic cells go through mitosis (Greek for "thread"). It is intended to increase the number of cells during plant and animal embryogenesis and blastogenesis. Mitosis initiates at the end of interphase (G_2 phase). It is a brief stage that includes chromosomal condensation, segregation, and cytoplasmic division. Mitosis is essential for replacing cells destroyed due to natural friction. The mitotic phase of the cell cycle is divided into four distinct phases: prophase, metaphase, anaphase, and telophase. During prophase, chromatin begins to condense and chromosomes become visible. Metaphase can be divided into two stages 1. Prometaphase and 2. Metaphase, during which the nuclear envelope begins to break down and the chromosomes begin to align at the cell's equatorial plane. In anaphase, sister chromatids divide and migrate to opposing poles, whereas in telophase, daughter chromosomes reach opposite poles and form two daughter nuclei. This is followed by cytokinesis, which is the last stage of mitosis. During cytokinesis, the cytoplasm is divided into two halves, and the cell divides into two daughter cells.

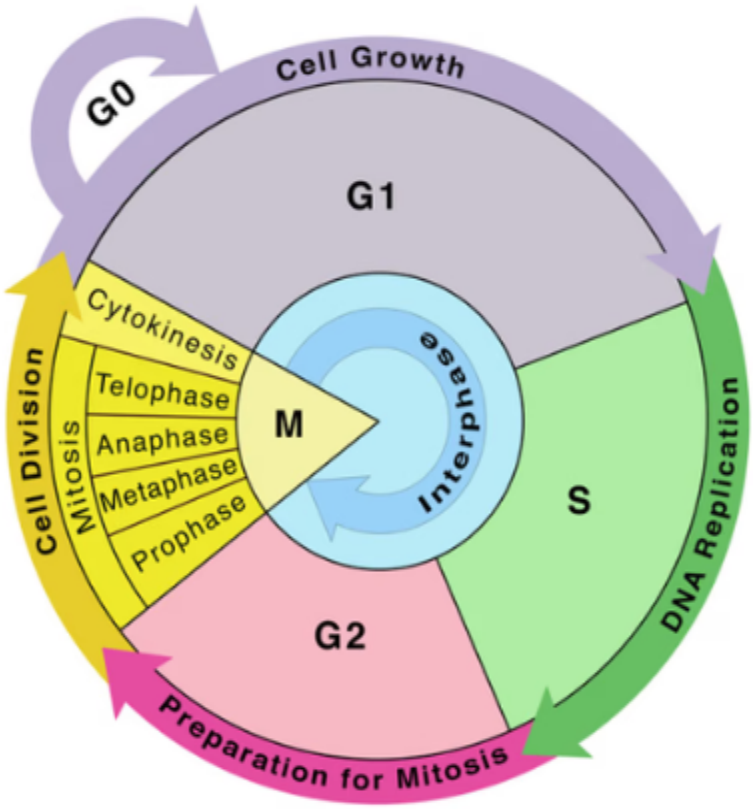

Figure-8.1: Phases of Cell Cycle

8.3. CHECKPOINTS OF THE CELL CYCLE:

Checkpoints in the cell cycle ensure that each phase of the cell division cycle is completed correctly. The checkpoints in the cell division cycle ensure that specific conditions can be fulfilled before the cell progresses from one phase to the next. The cell division cycle has three key checkpoints: G_1, G_2, and M (Figure 8.2).

G1 Check Point:

The G_1 checkpoint, sometimes referred to as the G_1 checkpoint, activates when the circumstances are ideal for cell division. At this checkpoint, the cell examines its size, nutrition, DNA damage, and all the preparations such as proteins, ATP, etc. that are necessary for the S phase. The final step is to determine whether the Cdk complex and S phase cyclins are activated to start DNA replication. The cell then moves on to the subsequent S phase. The cell is now dedicated to completing the full cell cycle after passing the G_1 phase. When an unfavorable circumstance arises, the cell either tries to fix itself or moves into the G_0 phase of the cell division cycle. The cell activates the G_2 checkpoint after passing the G_1 checkpoint.

G_2 Check Point:

This check often occurs following the S phase. The G_2 checkpoint's principal role is to monitor DNA quality and ensure appropriate DNA replication. After establishing that proper replication occurred in the S phase, the cells enter metaphase via spindle assembly. Apoptosis occurs when cells are unable to fix replication mistakes. This prevents the passing of damaged DNA to the daughter cells. The cell also checks for all preparations (e.g., all proteins, ATP, etc.) required in the M phase. Cells also check for tubulin production and whether M phase cyclins and the Cdk complex are active to commence mitosis. The cell then moves on to the next M phase.

M Check Point:

The third checkpoint, also known as the M checkpoint, occurs during the metaphase to anaphase transition. This checkpoint is also known as the spindle checkpoint because it ensures that all sister chromatids are appropriately linked to the spindle microtubules that separate them. The cell remains in mitosis until all sister chromatids are properly joined.

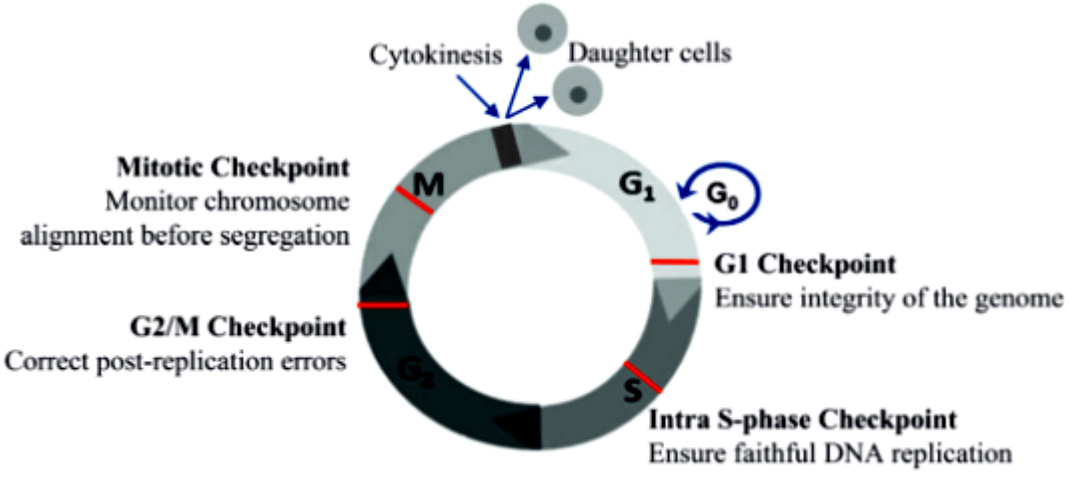

Figure-8.2: Check Points of Cell Cycle

8.4. ROLE OF CYCLINS:

There are controls on the cell cycle. The cell's preparations are examined by regulatory molecules. It involves identifying and fixing genetic damage and stopping unchecked cell division. The correct progression of a cell through the cell cycle is determined by two crucial types of regulatory molecules. These are 1. Cyclins 2. Kinases that are cyclin-dependent (Cdk). Leland H. Hartwell, Tim Hunt, and Sir Paul M. Nurse shared the 2001 Nobel Prize in Physiology or Medicine in recognition of their identification of important cell cycle regulators.

Cyclins:

Cyclins regulate CDK activity. Cyclins are classified into four types based on their presence and activity during the cell cycle. 1. D Cyclins 2. Cyclin E 3. S-phase cyclins 4. M-phase cyclins (Figures 8.3).

Cyclin D:

G_1 cyclins regulate the cell cycle and extracellular events. Their activity is regulated by signal transduction pathways that detect the presence of growth stimulants or cell proliferation inhibitory signals. The G_1 cyclin interacts with CDK4 and CDK6 to induce cell cycle entry.

Cyclin E:

During the late G_1 phase, G_1 cyclins begin to accumulate, achieving peak levels as cells progress into the S phase, followed by a subsequent decline during S phase. Cyclin E, which binds to CDK 2, is a key player in this process. The cyclin E-CDK 2 complex, in collaboration with the cyclin D-CDK4/6 complex, primarily functions to promote the G_1-S phase transition. This transition, known as START, marks the definitive point at which cells are permanently committed to division, thus eliminating the possibility of reverting to the G_1 phase.

S Phase Cyclins:

The synthesis of S phase cyclins occurs at the end of the G_1 phase, with their concentrations remaining elevated during the S phase and not diminishing until the early stages of mitosis. Two distinct types of S phase cyclins are responsible for triggering the S phase: cyclin E, which also aids in the cell cycle entry and is categorized as a G_1/S cyclin, and cyclin A. Both cyclins interact with CDK2 and are essential for the initiation of DNA synthesis.

Mitotic Cyclins:

The binding of mitotic cyclins, namely cyclin A and cyclin B, to CDK1 is essential for the transition into and the progression of mitosis. The synthesis of these cyclin-CDK complexes occurs during the S and G_2 phases, but their activation is delayed until the DNA synthesis process is complete. The various types of cyclins exhibit significant differences in their protein sequences; however, they all share a conserved region referred to as the cyclin box and display comparable three-dimensional structures.

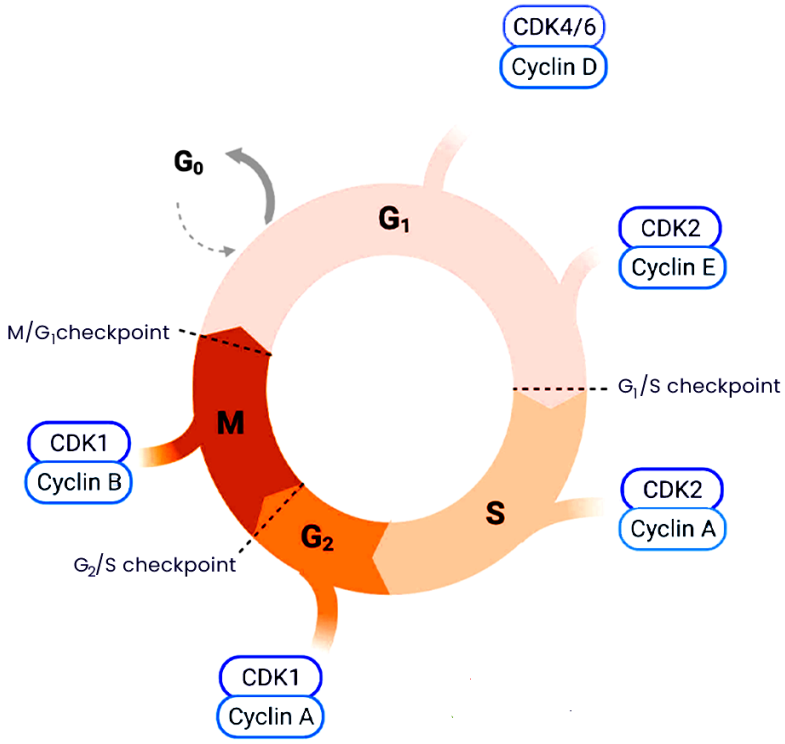

Figure-8.3: Cyclin Dependent Kinases (CDK) Complexes

Three Key Features of Cyclins:

1) Cyclins serve to bind and activate CDKs. The specific activity and substrate targeting of any CDK are mainly influenced by the specific cyclin that it is associated with.
2) The occurrence of cyclins is limited to the stages of the cell cycle that they stimulate, and they are not present in any other cell cycle stages.
3) Beyond their regulatory function at specific cell cycle stages, cyclins initiate a series of events that prepare the cell for the next phase. This action is vital for the continuous progression of the cell cycle.

8.5. CYCLIN DEPENDENT KINASES (CDKS CELL CYCLE):

Cyclin-dependent kinases (CDKs) are protein kinases characterized by needing a separate subunit a cyclin that provides domains essential for enzymatic activity. CDKs play important roles in cell cycle regulation (the control of cell division and modulate transcription in response to several extra- and intracellular cues). The evolutionary expansion of the CDK family in mammals led to the division of CDKs into three cell-cycle-related subfamilies i.e. 1. G_1 Cdk (Cdk 4) 2. S-phase Cdk (Cdk 2) 3. M-phase Cdk (Cdk 1).Their levels in the cell remain stable and remain inactive. They bind to the appropriate cyclin in order to be activated. Their function is to provide phosphate group to a number of proteins that control processes in the cell cycle. A CDK binds a regulatory protein (cyclin). Without cyclin, CDK has little kinase activity, only the cyclin-CDK complex is an active kinase but its activity can be typically further modulated by phosphorylation and other binding proteins, like p27. Various types of Cyclin and Cdks complexes are formed during cell cycle regulation and their functions are represented in Table 8.1

Table-8.1: Cyclin - CDKs Complex

Phase of cell cycle	Cyclin	Cdk	Cyclin-Cdk complex	Function
G1	Cyclin D	Cdk 4	G1 Cyclin-G1 Cdk	Inhibits Rb protein and signals the cell to prepare the chromosome for replication
S	Cyclin E and Cyclin A	Cdk 2	S phase cyclin – S phase Cdk	Activates DNA replication
G2	Cyclin B	Cdk 1	Mitotic cyclins – M phase Cdk	Activates mitosis

Mechanism of Cell Cycle Regulation by CDKs Activation

Multiple mechanisms ensure that CDKs are active in the right stage of the cell cycle. CDK activity is regulated by multiple mechanisms and the cells utilize multiple mechanisms to restrict cyclins to the appropriate cell cycle stage and to keep them at the right concentration.

1) Regulating of Cyclin Mechanisms
2) Action of CDK-activating kinase (CAK)
3) Inhibitory phosphorylations on CDK
4) Action of CDK Inhibitors

1. Regulation of Cyclin Mechanisms:

Cyclin-dependent kinases (CDKs) operate similarly to other protein kinases by targeting the covalent bonds within phosphate groups derived from ATP, which are then transferred to protein substrates. This action leads to the disintegration of the nuclear membrane. CDKs facilitate the progression of the cell from the G_1 phase to the S phase, as well as from the G2 phase to the M phase during the cell division cycle. The fluctuating levels of cyclins are crucial for enabling the cell to transition between these phases. This phase transition is made possible through the periodic phosphorylation of specific components involved in the cell cycle. The activation of cyclin-dependent kinases (CDKs) is not possible without their association with specific regulatory proteins known as cyclins. Consequently, the variations in CDK activity during the cell cycle are mainly driven by changes in the concentration of cyclins. During each stage of the cell cycle, distinct cyclins are synthesized, leading to the formation of various cyclin-CDK complexes specific to that stage (Figure 8.4). Each of these complexes plays a crucial role in initiating specific events associated with the cell cycle. Additionally, the levels of cyclins and the activity of CDKs are regulated by a variety of cellular mechanisms, forming a network that governs the overall regulation of the cell cycle.

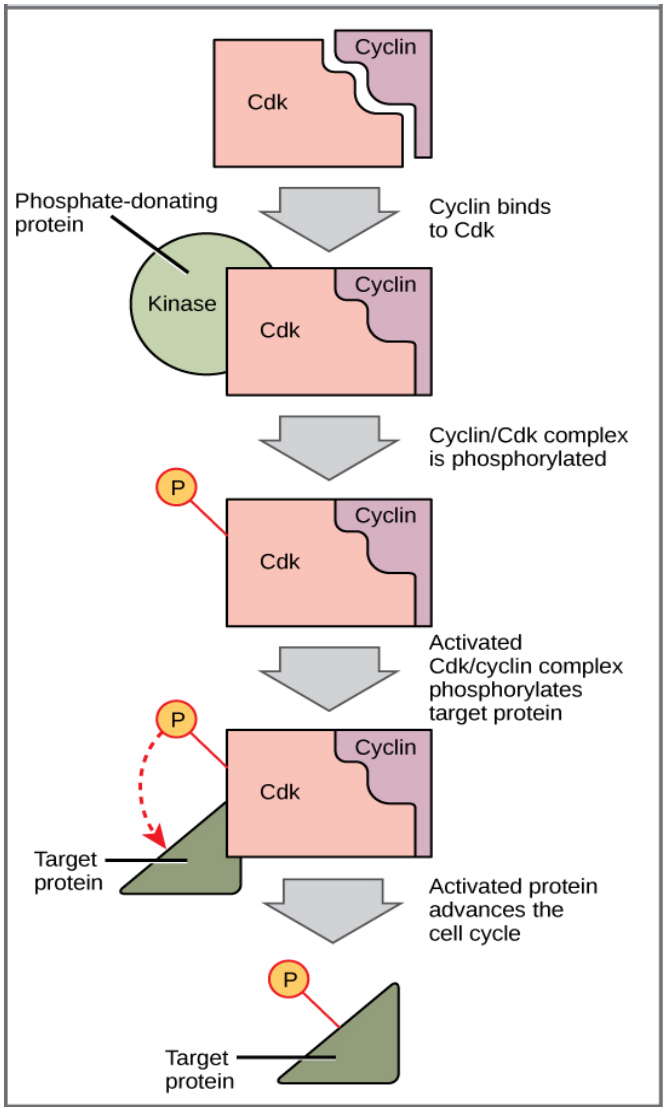

Figure-8.4: Regulation of Cyclin Dependant Kinases (CDK)

Series of steps involved in CDKs Regulation

1) Transcriptional control of cyclin genes.
2) Degradation of cyclins.
3) Transcriptional control of the cyclin subunits is one mechanism that ensures proper temporal expression of the cyclins.
4) Degradation of cyclins.

The most important regulatory control that restricts cyclins to the appropriate cell cycle stage is ubiquitin-mediated protein degradation. Cyclins are degraded through the action of two different ubiquitin-proteins:

a) SCF (Skp1, Cullin and f-box proteins)

b) APC/C (Anaphase-Promoting Complex or Cydosome)

The SCF complex regulates the transition from the G_1 phase to the S phase by facilitating the degradation of G_1 cyclins, specifically Cyclin D (Figure 8.5). Meanwhile, the APC/C is responsible for the degradation of S phase and mitotic cyclins, which aids in the exit from mitosis. The CDK activity is not controlled solely by cyclin levels. The presence of inhibitors, as well as activating and inhibitory phosphorylation events on the CDK subunit, is required to regulate cyclin-CDK activity.

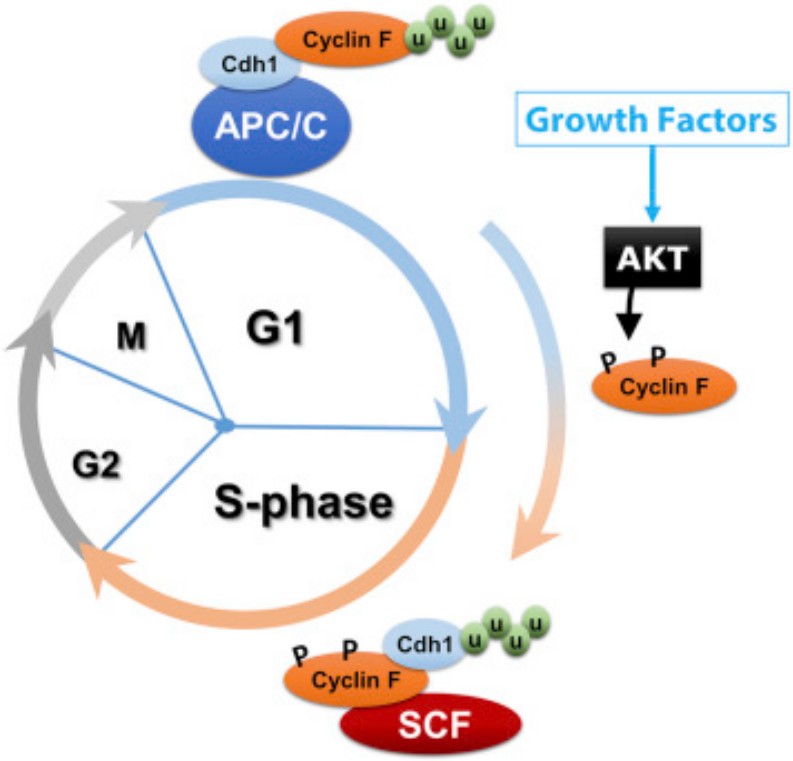

Figure-8.5: SCF Regulation of Cell Cycle

2. Action of CDK-Activating Kinase (CAK):

CDK activity requires threonine phosphorylation. The CDK-activating kinase (CAK) mediates this phosphorylation. The CAK activity remains consistent throughout the cell cycle and phosphorylates CDK as soon as a cyclin-CDK complex forms.

3. Inhibitory Phosphorylations on CDK:

CDK activity is controlled by inhibitory phosphorylation. This inhibitory phosphorylation is caused by a kinase known as "Weel".

4. Action of CDK Inhibitors:

CDK inhibitors regulate Cyclin-CDK activity. CDK inhibitors, or CKIs, are a protein family that binds directly to the cyclin-CDK complex, inhibiting its activity. These proteins are very crucial in regulating the G1-S phase transition (entrance into the cell cycle). The genes encoding these CKIs are frequently found mutated in human malignancies. CKIs that regulate S phase and mitotic CDKs are all required to prevent early activation of S and M

phase CDKs. Inhibitors of G1 CDKs are necessary for affecting a G1 arrest in response to proliferation inhibitory signals. Examples: INK4s, p53, p21.

CDK Inhibitors:

Cyclin-dependent kinase inhibitors (CKIs) are cyclins that are essential for controlling the cell cycle. They are proteins that work with cyclin-CDK complexes to limit kinase activity, typically during G1 or in reaction to environmental cues or damaged DNA. The two main CKI families found in animal cells are the CIP/KIP family and the INK4 family. The CDK monomers are bound by the strictly inhibitory INK4 family proteins. The crystal structures of CDK6-INK4 complexes reveal how INK4 binding distorts cyclin binding and kinase activity by twisting the CDK. Both the cyclin and the CDK of a complex are bound by the CIP/KIP family proteins, which have the ability to either activate or inhibit.

8.6. SUMMARY:

The cell cycle is the fundamental process through which cells reproduce, ensuring the accurate duplication and distribution of genetic material. It consists of a series of precisely coordinated and tightly regulated molecular events that control genome replication in the parent cell and the subsequent segregation of duplicated DNA and cytoplasm into two daughter cells. The speed at which cells progress through the cycle varies with developmental stage and cell type. Embryonic cells divide rapidly to support tissue and organ formation, whereas most adult somatic cells reside in a non-proliferative, metabolically active G_0 phase. The cell cycle is organized into distinct phases, with the majority of time spent in interphase, which comprises the G_1 (Gap 1), S (Synthesis), and G_2 (Gap 2) phases. During interphase, the cell grows, replicates its genome, and repairs any DNA damage. In contrast, only a small portion of the cycle is devoted to the M phase, where mitosis and cytokinesis occur, leading to the physical separation of the genome and cytoplasmic contents. Progression through the cell cycle is regulated by complexes formed between cyclins and cyclin-dependent kinases (CDKs). Cyclin D–CDK4/6 and cyclin E/A–CDK2 complexes function during the G_1 and G_1/S transition, whereas cyclin A/B–CDK1 complexes drive the G_2/M transition. Activation of CDKs requires cyclin binding; therefore, CDK activity is controlled by the synthesis, degradation, and subcellular localization of cyclins. Further regulation is mediated by CDK inhibitors (CDKIs) belonging to the INK4 and CIP/KIP families, which bind to CDKs and prevent their activation. Additionally, cyclins, CDKs, and CDKIs are modulated by phosphorylation and ubiquitination, ensuring precise control of cell-cycle progression.

8.7. TECHNICAL TERMS:

Interphase, G_1/S checkpoint (Restriction point), G_2/M checkpoint, Ubiquitin-mediated proteolysis, SCF complex, Cylclins, Cyclin dependent kinases.

8.8. SELF ASSESSMENT QUESTIONS:

1) Describe the phases of the cell cycle and explain the molecular events occurring in each phase.
2) Discuss the regulation of the cell cycle by cyclins and cyclin-dependent kinases (CDKs).

3) Explain the major cell cycle checkpoints and their significance in maintaining genomic integrity.
4) Describe the mechanisms of the G_1/S transition and its regulation by cyclin–CDK complexes.

8.9. SUGGESTED READINGS:

1) C.B. Powar. 2010. Cell Biology. Himalaya Publishing House, Mumbai – 400004.
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Prof. K. Mallikarjuna

LESSON-9

CELL DIVISION-MITOSIS AND MEIOSIS

9.0 OBJECTIVE:

- Students will understand the concept of cell division and realise the role of mitotic and meiotic divisions in the growth and development of organisms.

STRUCTURE:

9.1 Introduction

9.2 Mitosis

9.3 Meiosis

9.4 Summary

9.5 Technical Terms

9.6 Self-Assessment Questions

9.7 Suggested Readings

9.1. INTRODUCTION:

Cell division is fundamental to the continuity of life**.** In unicellular organisms, it serves as the primary means of reproduction, allowing a single cell to generate two daughter cells and thereby maintain its lineage. In multicellular organisms, repeated cycles of cell division are required to produce the vast number of cells necessary for growth, development, tissue maintenance, and regeneration. Because cell division ensures the transmission of genetic information from one generation of cells to the next, it must occur with exceptional accuracy. High-fidelity segregation of duplicated chromosomes, along with the equitable distribution of cytoplasmic components and the plasma membrane, is crucial. Errors in this process can result in cell death or, in multicellular organisms, mis-segregation of genetic or cytoplasmic material, which may contribute to developmental abnormalities and diseases such as cancer. Cells divide through two distinct mechanisms, mitosis and meiosis depending on their type and biological role. Mitosis results in two genetically identical daughter cells that retain the same chromosome number as the parent cell. This form of cell division supports growth, repair, and routine maintenance of tissues and therefore occurs in somatic or vegetative cells.

Meiosis, in contrast, produces four daughter cells, each containing half the number of chromosomes of the original cell. This reduction in chromosome number is vital for sexual reproduction and ensures genetic variation among offspring. Meiosis typically takes place in reproductive or germ cells. Meiosis is a specialized type of cell division, often called *reduction division*, because it reduces the chromosome number by half in the resulting cells. This process is essential for the formation of gametes in animals. The term *meiosis* (from the Greek *meioun*, meaning “to make smaller” or “to reduce”) was introduced in 1905 by J.B.

Farmer, reflecting the fundamental reduction in chromosome number that occurs during this division. Meiosis occurs in all sexually reproducing eukaryotes, including many single-celled organisms, and is indispensable for sexual reproduction. It produces haploid (1N) cells from a diploid (2N) nucleus. Although meiosis shares similarities with mitosis, it differs in that one round of chromosome replication is followed by two successive nuclear divisions, known as meiosis I and meiosis II, ultimately yielding four haploid cells. A hallmark of meiosis is the pairing of homologous chromosomes and the genetic recombination that contributes to genetic diversity. Historically, meiosis was first observed in 1876 by the German biologist Oscar Hertwig in sea urchin eggs, and later described at the chromosomal level by Belgian zoologist Edouard Van Beneden in *Ascaris* worm eggs in 1883. In 1911, American geneticist Thomas Hunt Morgan further documented its significance. In higher plants, the reproductive cycle includes a short multicellular haploid gametophyte stage and a dominant diploid sporophyte stage. The sporophyte undergoes meiosis within specialized tissues to produce haploid spores, which give rise to the male and female gametophytes.

9.2. MITOSIS:

In 1873, mitosis was first observed in the corneal cells of frogs, rabbits, and cats. A few years later, in 1875, the Polish histologist Wacław Mayzel provided the earliest formal description of the process. The term “mitosis” was introduced in 1882 by the German biologist Walther Flemming, who is credited with conducting the first detailed study of mitotic cell division. Flemming described mitosis in the late 19th century as occurring in two major phases. The first, known as the progressive phase, is characterized by chromosome condensation and their alignment along the equatorial plane of the cell. This is followed by the regressive phase, during which sister chromatids separate and migrate toward opposite poles. Modern biology now recognizes mitosis as a more elaborate process consisting of five distinct stages, interphase, prophase, metaphase, anaphase, and telophase (Figure 9.1).

Prophase:

The appearance of thin-thread-like condensing chromosomes marks the first phase of mitosis, known as prophase (Gr., pro = before; phasis = appearance). It has two stages namely Early Prophase and Late prophase. In early prophase **t**he cell becomes more spherical, refractile, and viscous. Chromosomes condense and become visible when stained. The chromosomes are made up of two identical chromatids known as sister chromatids, each carrying one DNA molecule, which are linked at the centromere. The two centrosomes that are replicated in the G2 phase right before prophase migrate to opposite poles of the nucleus. During prophase, proteins of kinetochores begin to deposit. Furthermore, during early prophase, the chromosomes are equally dispersed within the nuclear cavity. In late prophase spindle fibers (protein microtubules) begin to emerge from the centrosomes (consists of two centrioles in animal cells). As prophase continues, the chromosomes approach the nuclear envelope, leaving the center area of the nucleus vacant. Finally, during prophase, the

nucleolus slowly disintegrates. The degeneration and removal of the nuclear envelope indicate the end of prophase.

Prometaphase and Metaphase:

The disintegration of the nuclear membrane indicates the beginning of prometaphase and allows the mitotic spindle to engage with the chromosomes. At the metaphase plate, the spindle seems to be attempting to align and confine the chromosomes. By acting as a "cap," the kinetochores help to prevent the plus end from depolymerizing. Chromosomes are held on the metaphase plate by balanced bipolar forces, whereas sister chromatids are joined to opposite poles by their kinetochores. During metaphase (Gr., meta=after; phasis = appearance), the chromosomes are the shortest and thickest. Centrosomes reach opposite poles. Spindle fibres (protein microtubules) continue to extend from centrosomes. Chromosomes align along the equator of the spindle (also known as the metaphase plate), equidistant from the two centrosome poles. Spindle fibres (protein microtubules) reach the chromosomes and bind to the centromeres. Each sister chromatid is connected to a spindle fibre that originates from opposite poles.

Anaphase:

Anaphase (Gr., ana=up; phasis=appearance) begins abruptly with the simultaneous splitting of each chromosome into sister chromatids known as daughter chromosomes, each with a single kinetochore. Ca^{2+}containing membrane vesicles assemble at the spindle poles and release calcium ions, triggering anaphase. Later, pole ward movement of chromatids occurs due to shortening of the kinetochore microtubules. As the migration towards the poles occurs, the centromeres and kinetochores are positioned at the forefront, which causes the chromosomes to exhibit typical U, V, or J shapes. This is followed by the separation of poles themselves accompanied by the elongation of the polar microtubules. The astral microtubules also help in anaphase B by their attractive interaction with cell cortex.

Telophase:

The telophase begins with the termination of the daughter chromosomes' polar movement. Mitosis is completed by the rearrangement of two new nuclei and their entry into the G1 phase of interphase. During this phase, prophase events unfold in reverse order. A nuclear envelope reassembles around each set of chromosomes, resulting in two daughter nuclei. The mitotic apparatus, with the exception of the centrioles, disappears. The high viscosity of the cytoplasm reduces. As the coils relax, the chromosomes return to their long, slender, and stretched state. RNA production resumes, causing the nucleolus to reappear.

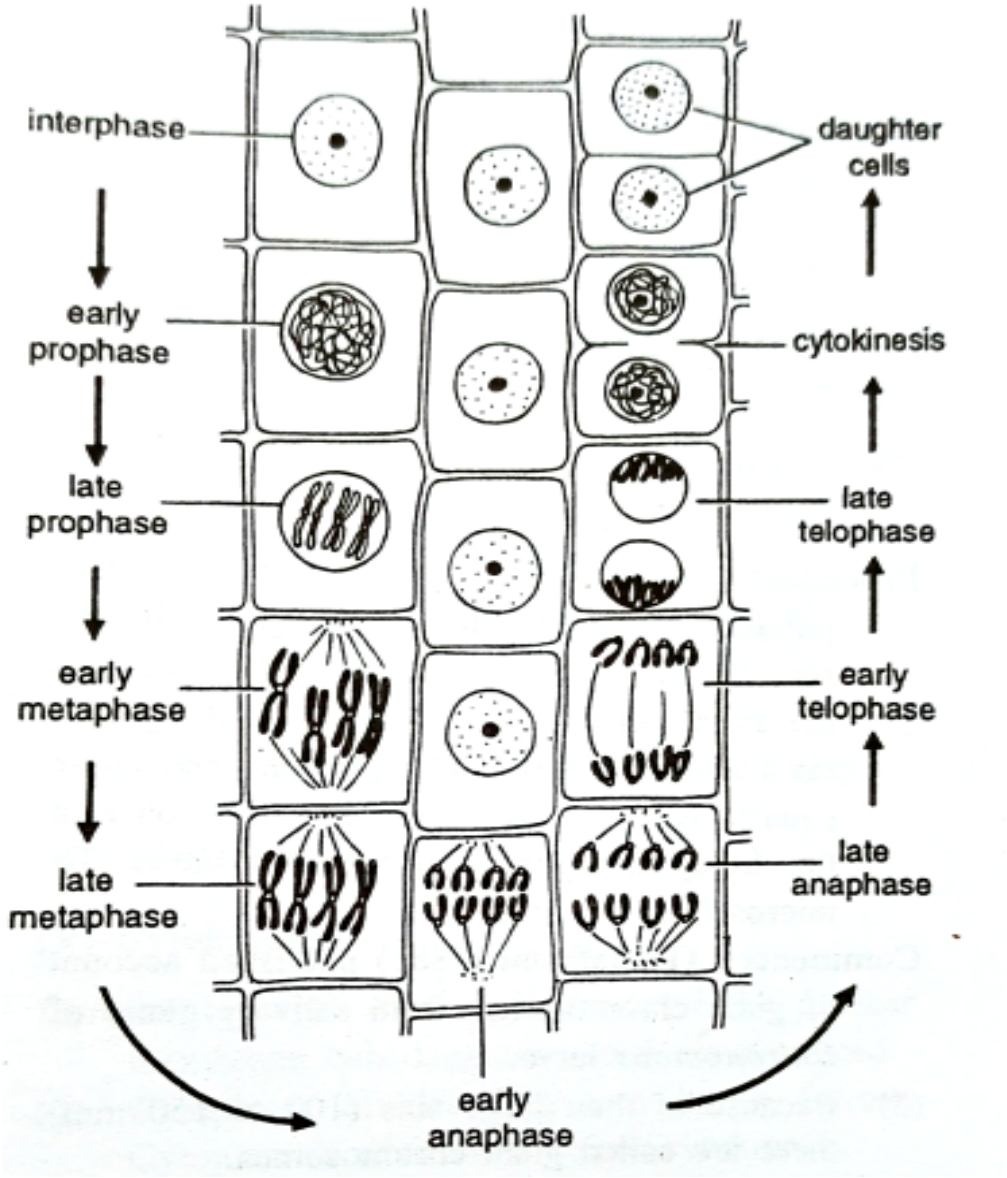

Figure-9.1: Stages of Mitosis

Cytokinesis:

Up to telophase nuclear division/karyokinesis occurs, followed by the division of cytoplasm known as cytokinesis. Cytokinesis is of two types.

1) Cell Furrow method – occurs in animals
2) Cell plate method – occurs in plants

Both DNA synthesis and mitosis are coupled to cytoplasmic division, or cytokinesis—the constriction of cytoplasm into two separate cells. During cytokinesis, the cytoplasm divides by a process, called cleavage. The mitotic spindle plays an important role in determining where and when cleavage occurs. Cytokinesis usually begins in anaphase and continues through telophase and into interphase. Karyokinesis is followed by division of cytoplasm (cytokinesis) thus forming two daughter cells.

1. Cell Furrow Method:

In animal cells, first sign of cleavage is puckering and furrowing of the plasma and separates daughter cells (Figure 9.2). The furrowing invariably occurs in the plane of the metaphase plate, at right angles to the long axis of the mitotic spindle. Cleavage is accomplished by the contraction of a ring composed mainly of actin filaments. This bundle of filaments, called contractile ring, is bound to the cytoplasmic face of the plasma membrane by unidentified attachment proteins. The Cell Furrow method includes 1. The contractile ring assembles in early anaphase. 2. This force is generated due to muscle-like sliding of actin and myosin filaments in the contractile ring. 3. The actin-myosin interaction pulls the plasma membrane down into a furrow. 4. When cleavage ends, the contractile ring is finally dispensed. Cytokinesis greatly increases the total cell-surface area as two cells form from one. Therefore, the two daughter cells resulting from cytokinesis require more plasma membrane than in the plant cell. Lastly, prior to cytokinesis, in M phase large membrane-bounded organelles such as Golgi apparatus and the endoplasmic reticulum break up into smaller fragments and vesicles.

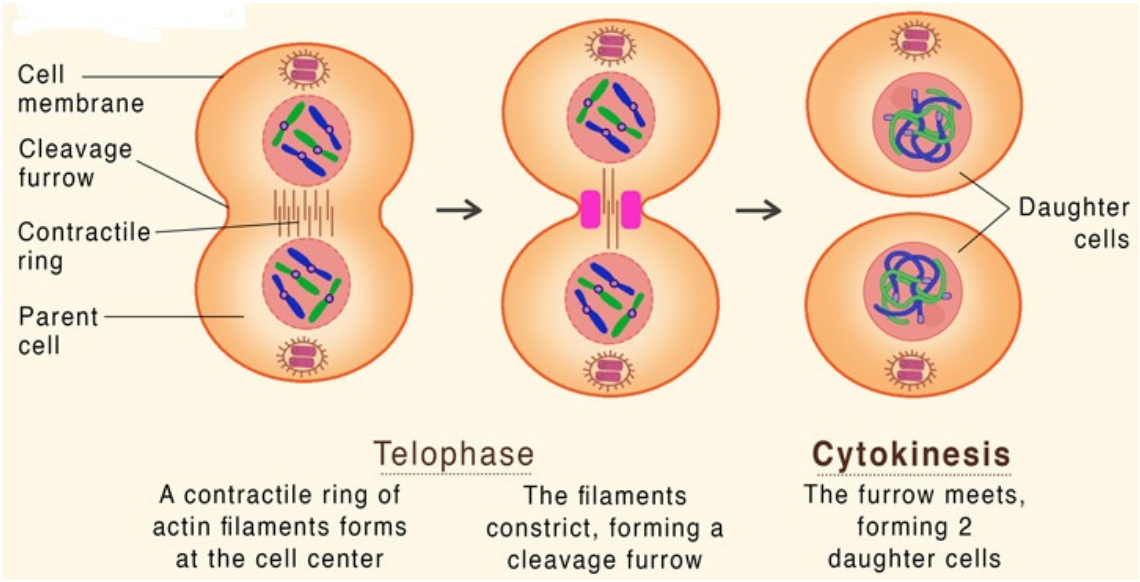

Figure-9.2: Cell Furrow Method

2. Cell Plate Method:

Due to the presence of a cell wall, cytokinesis in plant cells is significantly different from that in animal cells, Rather than forming a contractile ring, plant cells construct a cell plate in the middle of the cell (Figure 9.3). The stages of cell plate formation include (1) Creation of the phragmoplast, an array of microtubules that guides and supports the formation of the cell plate. (2) Trafficking of vesicles to the division plane and their fusion to generate a tubular-vesicular network. (3) Continued fusion of membrane tubules and their transformation into membrane sheets upon the deposition of callose, followed by deposition of cellulose and other cell wall components. (4) Recycling of excess membrane and other material from the cell plate. (5) Fusion with the parental cell wall.

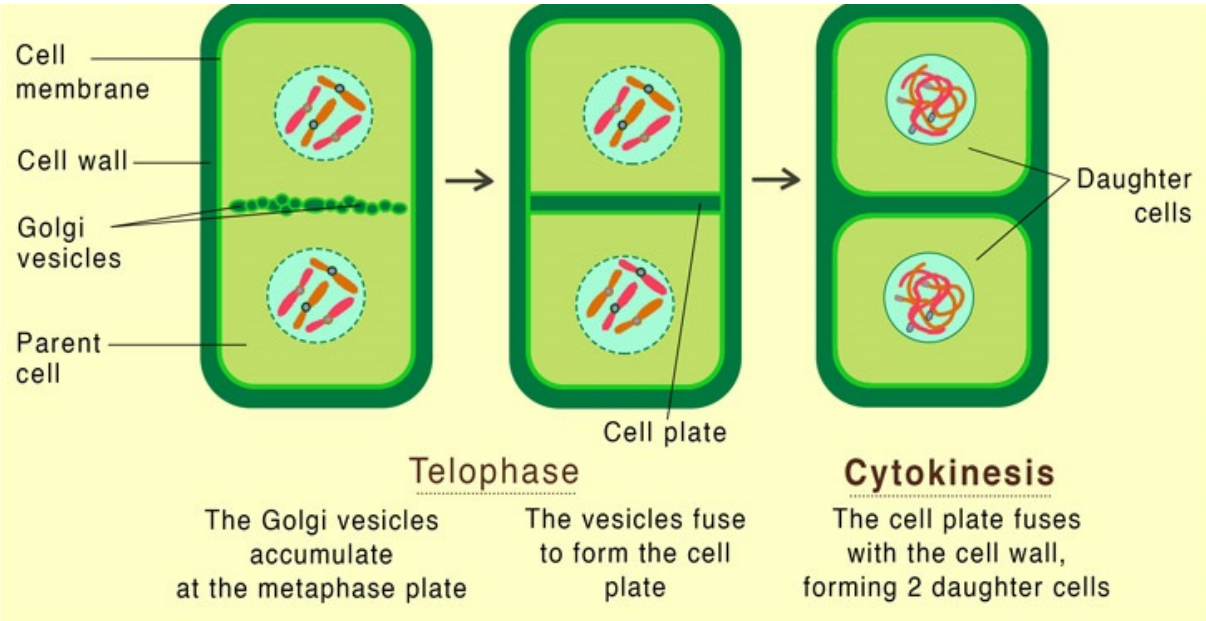

Figure-9.3: Cell Plate Method

Significance of Mitosis:

1. Growth and Development:

Through recurrent mitosis, a single cell zygote develops into a full-grown child (6×1022 cells). Plants can grow throughout their lives thanks to mitotic division in the apical and lateral meristems. Increases in tissue mass are caused by an increase in cell number, which is known as hyperplastic. Hence, mitosis is crucial for the growth and development of a multicellular organ.

2. Maintenance of Cell Size:

An over-grown somatic cell is stimulated to divide, allowing mitosis to maintain a suitable surface volume ratio. It also has a high nucleo-cytoplasmic ratio, which is restored to an efficient level during division. These ratios are critical for the proper functioning of cell dispersion across all chromosomes. This promotes correct coordination among daughter cells.

3. Healing and Regeneration:

Mitosis produces new cells to heal wounds, and some organisms may regenerate missing parts of their bodies as well as the entire organism through mitosis.

4. Repairing:

The process of replacing old or worn-out cells is known as repair. In the human body, approximately 5 ×109 cells are lost from the skin, alimentary canal lining, blood cells, and other areas. These cells are replaced by new ones created during mitosis.

5. Evidence of Basic Relationship:

The majority of organisms have a similar mitotic mechanism, indicating fundamental similarities and relationships.

9.3. MEIOSIS:

In sexually reproducing species, meiosis takes place in the germ cells. Germ cells can be found in the gonads of both plants and mammals. Because meiosis occurs at different times in different organisms, it can be categorized as follows:

1) **Terminal Meiosis:** It is also called gametic meiosis and commonly occurs in animals and few lower plants. In terminal meiosis, the meiotic division occurs immediately before the formation of gametes or gametogenesis.
2) **Intermediary or Sporic Meiosis:** It is the characteristic division of flowering plants. This meiosis takes place at some intermediate time between fertilization and the formation of gametes. It is also involved in the production of microspores (in anthers) and megaspores (in ovary or pistil) or in microsporogenesis and megasporogenesis.
3) **Initial or Zygotic Meiosis:** It occurs in some algae, fungi, and diatoms. Meiotic division occurs immediately after fertilization.

Process of Meiosis:

The cells that undergo meiosis are known as meiocytes. The gonad meiocytes are known as gonocytes, and they might be spermatocytes in males or oocytes in females. The plant sporangium's meiocytes are known as sporocytes. Meiosis appears to be two mitotic divisions with no gap for DNA replication. The first meiotic division comprises a long prophase in which homologous chromosomes become closely linked and exchange genetic material. Furthermore, the first meiotic division causes a drop in chromosomal number, resulting in two haploid cells. In the first meiotic division, also known as the heterotypic division, the haploid cell divides mitotically, resulting in four haploid cells. In the second meiotic division, also known as the homotypic division, the chromosomes are not paired, the genetic material is not exchanged, and the chromosome number is not reduced. Both meiotic divisions are continuous and include the typical stages of meiosis. The prophase of first meiotic division is very significant phase because the most cytogenetical events such as synapsis, crossing over, etc., occur during this phase. The prophase 1 is the longest meiotic phase, therefore, for the sake of convenience, it has been divided into six sub stages, viz., 1. Proleptonema (proleptotene), 2. Leptonema (leptotene), 3. Zygonema (zygotene), 4. pachynema (pachytene), 5. Diplonema (Diplotene) and 6. Diakinesis (Figure 9.4).

Heterotypic Division or First Meiotic Division:

Meiosis occurs following an interphase that is similar to an intermitotic interphase. During the premeiotic interphase, DNA duplication occurred at the S phase. In the G2 phase of interphase, there appears to be a significant shift that drives the cell toward meiosis rather than mitosis. At the start of the first meiotic division, the nucleus of the meiocyte begins to grow by absorbing water from the cytoplasm, and the nuclear volume increases by nearly threefold. Following these alterations, the cell enters the first stage of first meiotic division, known as prophase.

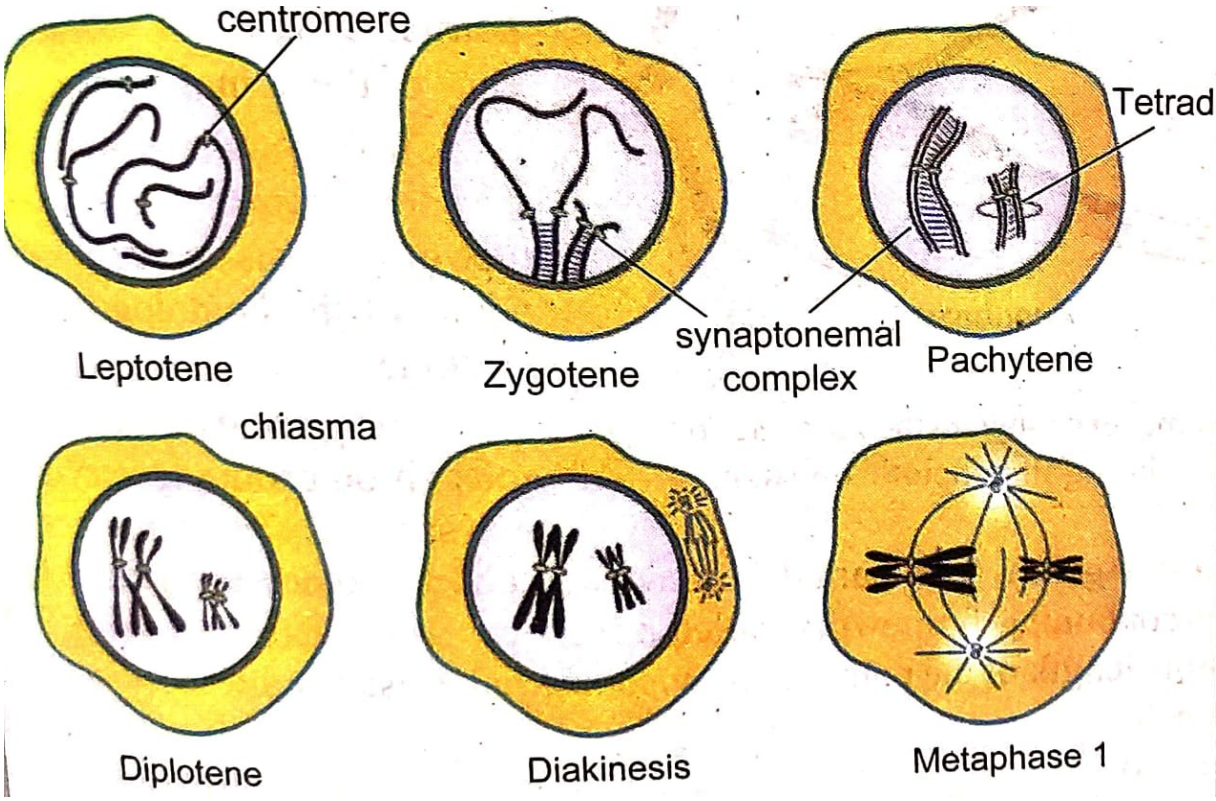

Figure-9.4: Various Stages of Prophase 1 of Meiosis I

Prophase I:

During prophase-I, DNA is exchanged between homologous chromosomes in a process known as homologous recombination, which frequently leads to chromosomal crossover. The paired and replicated chromosomes are known as bivalents or tetrads, and the process of pairing the homologous chromosomes is known as synapsis. Non-sister chromatids may cross-over at a point known as Chiasmata.

Proleptotene or Prolepto-nema:

The proleptotene stage is closely related to the early mitotic prophase. At this stage, the chromosomes are extremely thin, long, uncoiled, longitudinally single, slender thread-like structures.

Leptotene/Leptonema:

During the leptotene stage, the chromosomes become less coiled and take on a long thread-like structure. At this step, the chromosomes adopt a certain orientation within the nucleus. The chromosomal ends converge toward one side of the nucleus, which contains the centrosome (the bouquet stage). The centriole replicates, and each daughter centriole migrates to the opposite pole of the cell. When each centriole reaches the poles, it duplicates, and each cell pole now has two centrioles from a single diplosome. The two sister chromatids remain so firmly connected that they are indistinguishable from one another.

Zygotene:

During the zygotene stage, homologous chromosomes from the mother (via ova) and father (via sperm) are attracted to each other and pair, a process known as synapsis. The synapsis begins at one or more points along the length of the homologous chromosomes.

Three types of synapsis have been recognized.

i) **Proterminal Synapsis:** In proterminal type of synapsis the pairing in homologous chromosomes starts from the end and continues towards their centromeres.

ii) **Procentric Synapsis:** In procentric synapsis the homologous chromosomes start pairing from their centromeres and the pairing progresses towards the ends of the homologues.

iii) **Intermediate or Random Synapsis:** The intermediate type of synapsis occurs at various points of the homologous chromosomes.

The paired homologous chromosomes are joined by a roughly 0.2-µm thick, protein-containing framework called a synaptonemal complex (SC) (Figure 9.5), which extends along the entire length of the paired chromosomes and is typically anchored at either end to the nuclear envelope. The SC serves to stabilize the pairing of homologous chromosomes and to facilitate the cytogenetical activity, known as recombination or crossing over (occurring during pachynema). The organisms in which crossing over does not occur do not have the SC. e.g., male fruit fly -*Drosophila melanogaster*.

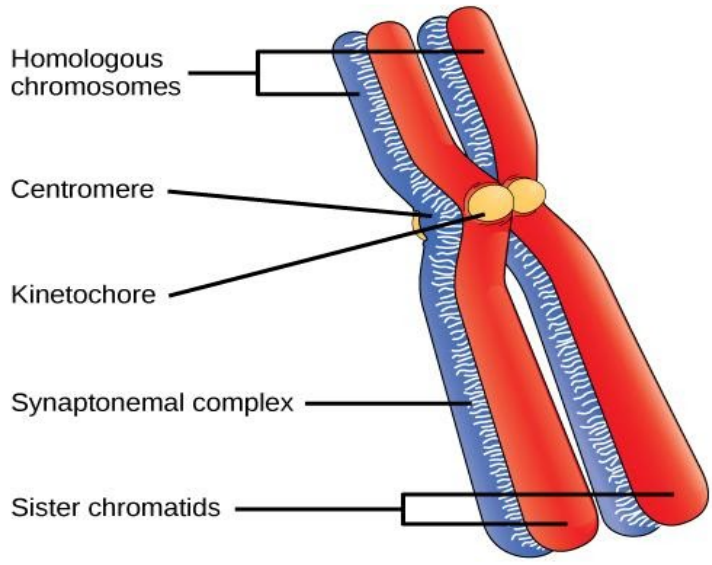

Figure-9.5: Synaptonemal Complex

Pachytene or Pachynema:

During the pachynema stage, an important genetic phenomenon known as "crossing over" occurs, which involves the rearranging, redistribution, and mutual exchange of hereditary material from two parents between two homologous chromosomes. At this point, each synaptonemal pair is commonly referred to as bivalent or dyads because it consists of two visible chromosomes, or as a quadrivalent or tetrad because it consists of four visible

chromatids. Recent opinions suggest that one chromatid of each homologous chromosome of a bivalent may divide transversely with the help of an enzyme called endonuclease. Following the chromatid division, chromatid segments are exchanged between the non-sister chromatids of the homologous chromosomes, and the presence of an enzyme called ligase unites the broken chromatid segments with the chromatids. This process of exchanging chromatin material between one non-sister chromatid of each homologous chromosome is called crossing over, and it is accompanied by the formation of chiasmata. During the pachytene and zygotene stages, small quantities of DNA are synthesized. This amount of DNA is used to repair broken DNA molecules in chromatids during chiasmata formation and crossing over. Up to this point, the nucleolus stays prominent and is connected with the chromosome's nucleolar organizer region.

Diplotene or Diplonema:

In diplonema, homologous chromosome unpairing or desynapsis begins, and the first chiasmata appear. At this stage, the chromatids of each tetrad are usually visible, but the synaptonemal complex appears to be dissolved, leaving participating chromatids of the paired homologous chromosome physically joined at one or more discrete points known as chiasmata (singular, chiasma). Crossing over occurs at these locations. During this stage, the chromatids frequently unfold, allowing for RNA synthesis and cellular development.

Diakinesis:

During diakinesis, the bivalent chromosomes become more evenly distributed in the nucleus, the nucleolus detaches from the nucleolar organizer and eventually disappears, the nuclear envelope degrades, and the chiasma moves from the centromere to the end of the chromosomes. This movement of the chiasmata is known as terminalization, and the chromatids remain connected by the termina.

Prometaphase:

During prometaphase, the nuclear membrane disintegrates and microtubules form a spindle between the two centrioles, which serve as the cell's two opposed poles. The chromosomes coil tightly in a spiral pattern and are positioned on the spindle's equator.

Metaphase I:

Chromosome alignment at the equator and spindle fibre attachment to chromosomes comprises metaphase I. The centromeres of each tetrad's homologous chromosomes are joined to the spindle's microtubules during metaphase I. Every chromosome has a centromere that faces the opposing poles. The homologous chromosomes become more repelled of one another and are prepared to split apart.

Anaphase I:

At anaphase I, homologues are separated and, as chromosomal fibres or microtubules shorten, each homologous chromosome, with its two chromatids and undivided centromere, moves towards the opposite poles of the cell. This is where the actual reduction and disjunction occur. It should be noted that homologous chromosomes that move to opposite

poles are either paternal or maternal in origin. During the formation of a chiasma between two chromatids of a chromosome, one of the chromatids changes its counterpart; thus, the two chromatids of a chromosome are genetically distinct.

Telophase I:

The onset of telophase I is marked by the arrival of a haploid set of chromosomes at each pole, during which time the nuclei are reassembled, the endoplasmic reticulum forms the nuclear envelope around the chromosomes, the chromosomes uncoil, the nucleolus reappears, and two daughter chromosomes are formed. Following karyokinesis, cytokinesis takes place, resulting in the formation of two haploid cells, both of which go through a brief resting phase of interphase, during which no DNA replication takes place, leaving the chromosomes at the second prophase identically double-stranded.

Cytokinesis:

Often, cytokinesis occurs after Meiosis II rather than after Meiosis I. If the meiosis I cell goes through cytokinesis, the cell membrane constricts, and two daughter cells are created. These progeny cells have a chromosome made up of one of their original chromatids and another made up of segments from their own and a chromatid from their homologue.

Homotypic or Second Meiotic Division:

The mitotic division that splits each haploid meiotic cell into two haploid cells is actually the homotypic or second meiotic division (Figure 9.6). The only difference is that the centromeres duplicate while the DNA does not. Meiosis II, also known as the mitotic or equational phase, consists of the following four stages.

Prophase II:

Each centriole splits in half during the second prophase, creating two pairs of centrioles. Every centriole pair moves to the pole on the other side. The microtubules form a spindle-like arrangement. Both the nucleolus and the nuclear membrane vanish. The two-chromatid chromosomes became thicker and shorter.

Metaphase II:

During metaphase II, the chromosomes get positioned on the equator of the spindle. The centromere separates into two and, thus, each chromosome creates two monads or daughter chromosomes. The microtubules of the spindle are associated with the centromere of the chromosomes.

Anaphase II:

The daughter chromosomes move towards the opposite poles due to the shortening of chromosomal microtubules and stretching of inter zonal microtubules of the spindle.

Telophase II:

Reconstitution of nuclei takes place. The chromosomes begin to uncoil and become thin. Nucleolus and nuclear membrane reappears.

Cytokinesis:

Cytokinesis of the daughter cells causes the formation of two cells, in other words from the two daughter cells of the first meiotic division, four cells are produced each with haploid set of chromosomes.

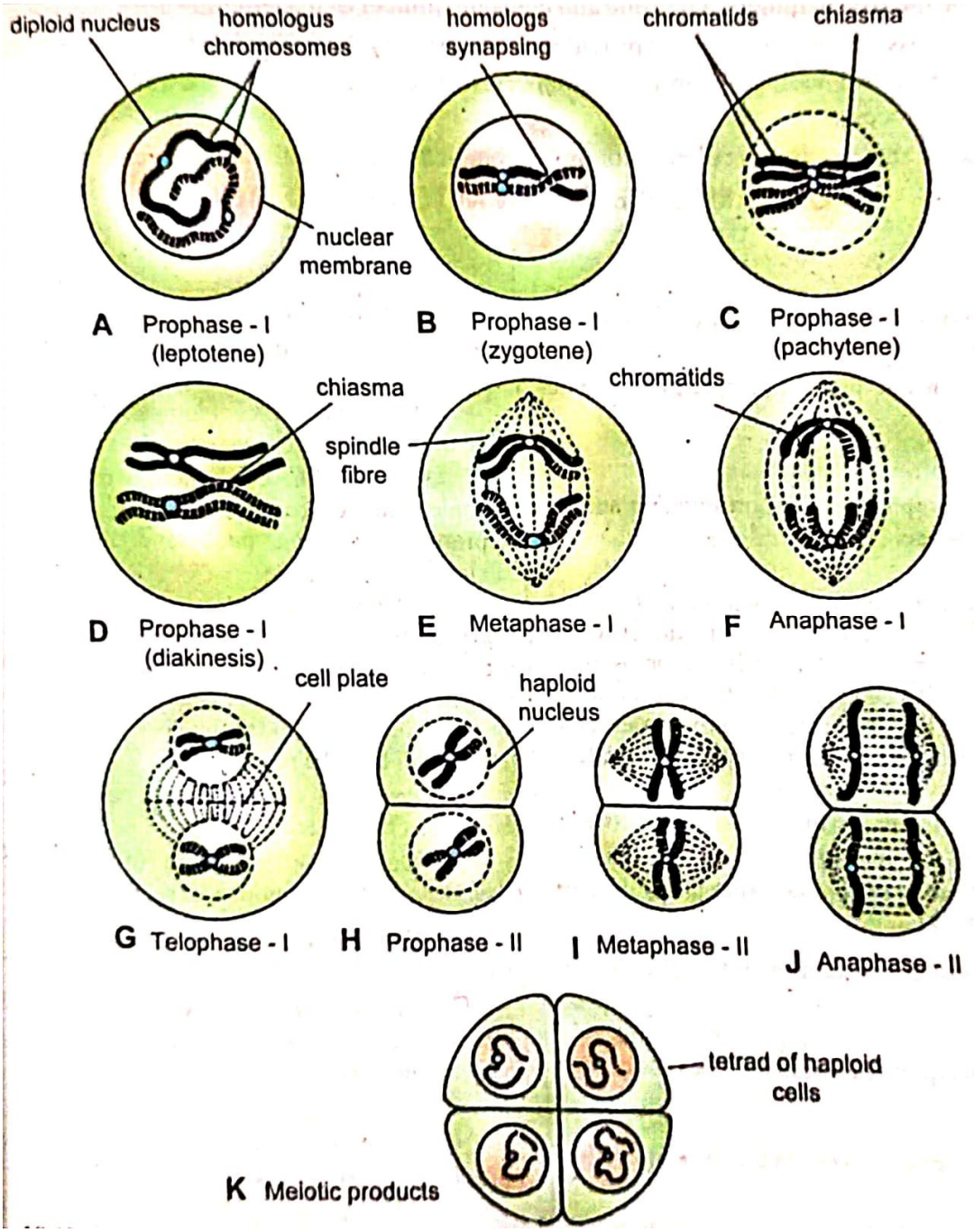

Figure-9.6: Various Stages of Heterotypic and Homotypic Meisois

Significance of Meiosis:

1) Meiosis is responsible for the formation of sex cells or gametes that are responsible for sexual reproduction.
2) It activates the genetic information for the development of sex cells and deactivates the sporophytic information.
3) It maintains the constant number of chromosomes by halving the same. This is important because the chromosome number doubles after fertilization.
4) In this process independent assortment of maternal and paternal chromosomes takes place. Thus, the chromosomes and the traits controlled by them are reshuffled.
5) The genetic mutation occurs due to irregularities in cell division by meiosis.
6) The mutations that are beneficial are carried on by natural selection.
7) Crossing over produces a new combination of traits and variations.

9.5. SUMMARY:

Mitosis is commonly occurring in five sequential stages prophase, metaphase, anaphase, telophase, and cytokinesis. During interphase, the nucleus is enclosed by the nuclear envelope, DNA replication occurs in the S phase, and sister chromatids are produced and held together at the centromere, located near the chromosome's center. Centrosomes, positioned at opposite poles of the cell, play a crucial role in organizing chromosome movements to ensure that each daughter cell receives a complete set of genetic material. These centrosomes assemble the mitotic spindle, a network of microtubules that facilitates the separation of sister chromatids. In prophase, chromatin condenses into visible chromosomes, each consisting of two identical sister chromatids joined at the centromere. The mitotic spindle begins to form, and the centrosomes start migrating toward opposite poles as microtubules elongate between them.

During prometaphase, the nuclear envelope disintegrates, allowing spindle microtubules to enter the nuclear region and attach to chromosomes. Microtubules bind at kinetochores, specialized protein complexes located at the centromere. Some spindle microtubules do not attach to kinetochores; instead, they interact with microtubules from the opposite pole, helping to stabilize the spindle. By metaphase, the centrosomes are fully positioned at opposite poles, and all chromosomes align along the metaphase plate, an imaginary plane equidistant between the two spindle poles. Each chromosome is attached to spindle microtubules via its kinetochores. Anaphase, the shortest stage of mitosis, begins when sister chromatids separate, becoming individual chromosomes that move toward opposite poles. By the end of anaphase, each side of the cell contains an identical set of chromosomes. In telophase, two new daughter nuclei form as the nuclear envelope reassembles around each set of chromosomes. The chromosomes begin to de-condense, and spindle microtubules depolymerize. At this point of mitosis the division of the nucleus is

complete. Finally, cytokinesis divides the cytoplasm, resulting in two genetically identical daughter cells.

Meiosis is the process of cell division in reproductive cells. This two-phase procedure splits the chromosomes of a diploid germ cell, resulting in four haploids. During prophase I, the nuclear envelope begins to break down and nuclear chromatin begins to condense into individual chromosomes composed of two sister chromatids. During metaphase I, pairs of homologous chromosomes (known as tetrads) migrate along their microtubule attachments, lining up along the metaphase plate. Anaphase I is the following stage, in which the homologous chromosome attachments break down and kinetochores pull the homologous chromosomes to opposing poles. Telophase and cytokinesis are the final stages of meiosis I, when the cells separate into two daughter cells. Prophase II is the first stage of meiosis II, in which the nuclear envelope degrades and the spindles regenerate. In metaphase II, the chromosomes align along the metaphase plate. Sister chromatids (individual chromosomes when separated) travel to opposing poles of the meiotic spindle during anaphase II. The chromosomes reach the poles in the final stage of meiosis II, the spindle disintegrates, and the nuclear envelopes reconstitute. Cytokinesis divides the original diploid cell into four haploid daughter cells.

9.6. TECHNICAL TERMS:

Chromatids, Centromeres, Chromatin, Sister Chromatids, Centromere, Kinetochore, Centrosome, Mitotic spindle, Microtubules, Spindle poles, Spindle Fibers. Chaismata, Crossing Over, Gametes, Homologous chromosomes, Synapsis, Chiasmata, Kinetochore, Centromere, Tetrad / Bivalent, Meiotic spindle, Independent assortment, Segregation, Nondisjunction, Gametogenesis

9.7. SELF ASSESSMENT QUESTIONS:

1) Differentiate between prophase and prometaphase.
2) Distinguish between karyokinesis and cytokinesis.
3) Give an account on mitotic cell division in eukaryotes.
4) Describe in detail the stages of meiosis I, highlighting the events of synapsis, crossing over, and chromosome segregation.
5) Explain the molecular mechanisms of recombination and its significance in generating genetic diversity.
6) Compare and contrast meiosis I and meiosis II with respect to chromosome behaviour and outcomes.
7) Explain the significance of mitosis and meiosis.

9.8. SUGGESTED READINGS:

1) C.B. Powar. 2010. Cell Biology. Himalaya Publishing House, Mumbai-400004.
2) De Roberties E.D.P & De Roberties (Jr.) 2017. Cell and Molecular Biology (8^{th} Edition).
3) Wolters Kluwer (India Pvt. Ltd.), New Delhi.
4) Alberts, B. et. al. (Molecular Biology of the Cell) – Garland Science
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7) Lehninger Principles of Biochemistry – Nelson & Cox (for regulation concepts)
8) Watson et al., Molecular Biology of the Gene – Pearson
9) P.S. Verma & V.K. Agarwal, 2021. Cell Biology, Genetics, Molecular Biology, Evolution and Ecology. S. Chand and Company Limited, New Delhi-110044.

Prof. K. Mallikarjuna

LESSON-10

APOPTOSIS

10.0 OBJECTIVE:

- Students will understand how the programmed cell death occurs and also the differences between an apoptosis and necrosis modes of cell death.

STRUCTURE:

10.1 Introduction

10.2 Mechanisms (Pathways) of Apoptosis

10.3 Intrinsic Pathway (Mitochondrial Pathway)

10.4 Extrinsic Pathway (Death Receptor Pathway)

10.5 Significance of Apoptosis

10.6 Summary

10.7 Technical Terms

10.8 Self-Assessment Questions

10.9 Suggested Readings

10.1. INTRODUCTION:

The term apoptosis is derived from the Greek word meaning dropping or falling off. In 1842, German scientist Carl Vogt was first to describe the principle of apoptosis. In 1885, Walther Flemming gave a more precise description of the process of programmed cell death. The term "Apoptosis" was first introduced by John Kerr, Andrew Wyllie, and Alastair Currie. In 2002, Nobel Prize in Medicine was awarded to Sydney Brenner, H. Robert Horvitz and John E. Sulston for their work in identifying the genes that control apoptosis. The term apoptosis can be defined as a natural biological process of programmed cell death in which the cells destroy themselves for maintaining the smooth functioning of the body."

There are two forms of cell death.

1) Programmed death of cells called Apoptosis.
2) An uncontrolled death of cells called Necrosis.

Both apoptosis and necrosis occur under different circumstances and involve different steps.

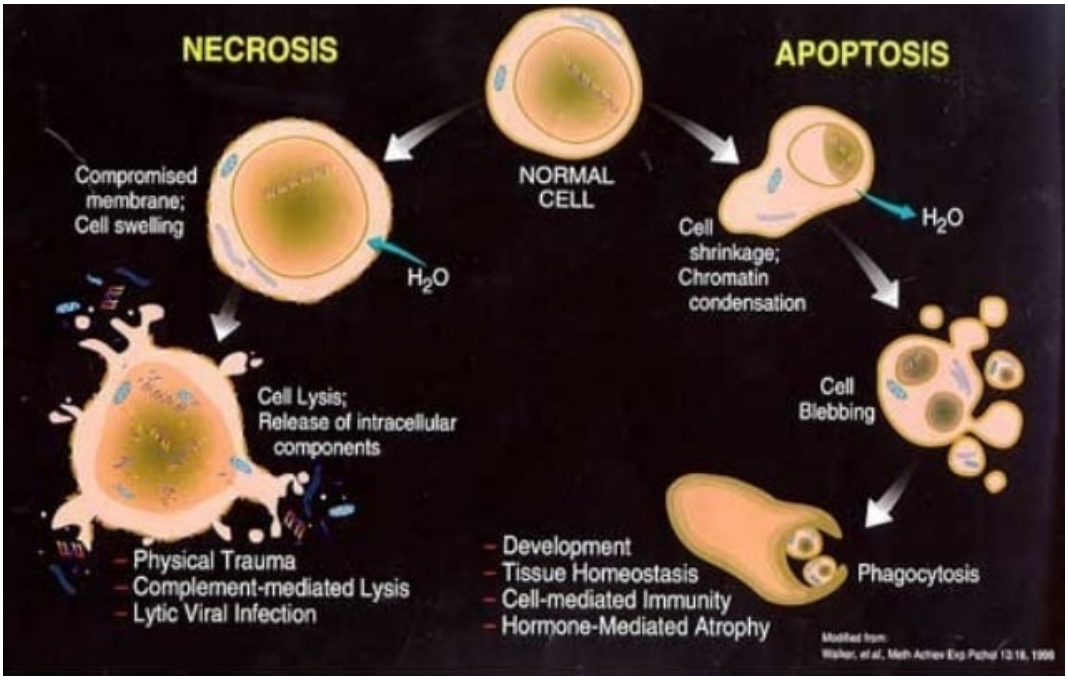

Fig. 10.1

Apoptosis is a biological process which occurs in all multicellular organisms including plants and animals. It removes the cells from the organisms that should no longer be a part of the organism. This process plays a major role in developing and maintaining a healthy immune system. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, DNA fragmentation, and mRNA decay.

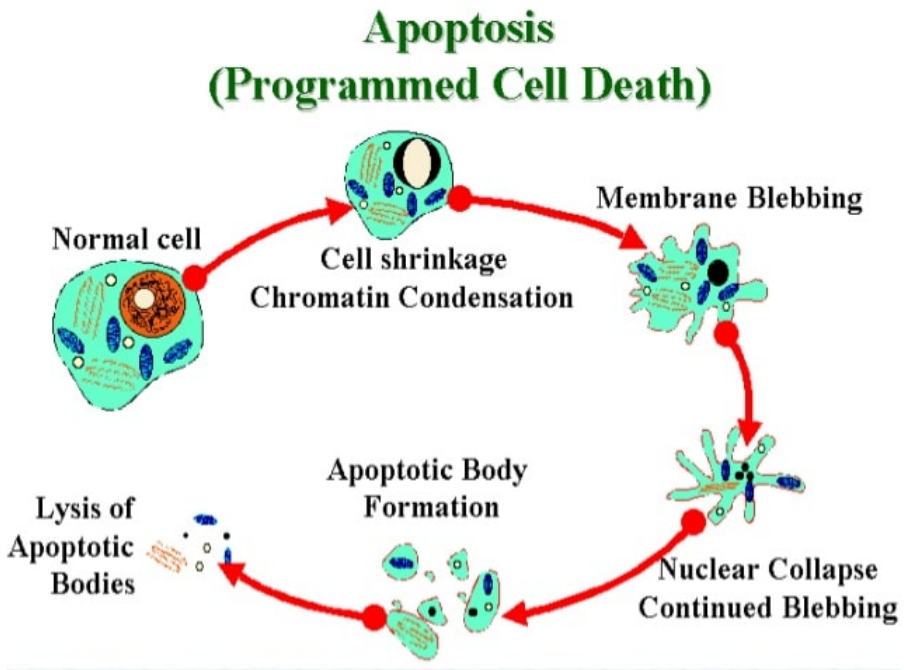

Fig. 10.2

During this biological process, infected cells, pre-cancerous cells and other cancer cells are eliminated successfully and maintain the balance of cells. Therefore, it is an essential process that is responsible for the normal development of cells, cell cycle maturation and maintaining the regular functions and activities of cells. In contrast to necrosis that results from acute cellular injury, apoptosis is a highly regulated and controlled process that confers advantages during an organism's life cycle.

10.2. MECHANISMS (PATHWAYS):

Apoptosis can be initiated through one of two pathways.

1) Intrinsic pathway: The cell kills itself because it senses cell stress. Weak external signals may also activate the intrinsic pathway of apoptosis.
2) Extrinsic pathway: The cell kills itself because of signals from other cells.

Both pathways induce cell death by activating caspases**.** These are proteases that degrade proteins. These two pathways activate initiator caspases, which then activate executioner caspases, which then kill the cell by degrading proteins indiscriminately.

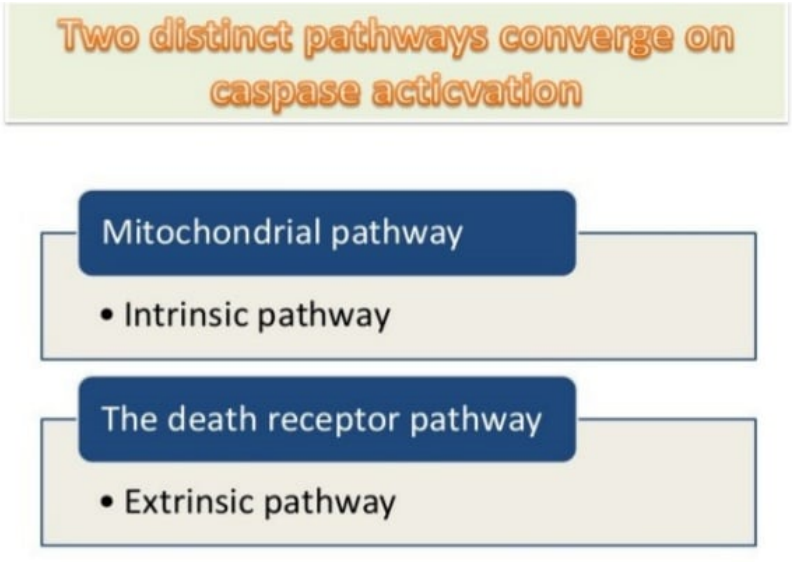

Fig. 10.3

A cell initiates intracellular apoptotic signaling in response to a stress, which may bring about cell suicide. The binding of nuclear receptors by glucocorticoids, heat, radiation, nutrient deprivation, viral infection, hypoxia, increased intracellular concentration of free fatty acids and increased intracellular calcium concentration, for example, by damage to the membrane, can all trigger the release of intracellular apoptotic signals by a damaged cell. A number of cellular components, such as poly ADP ribose polymerase, may also help regulate apoptosis. Single cell fluctuations have been observed in experimental studies of stress induced apoptosis.

Before the actual process of cell death is precipitated by enzymes, apoptotic signals must cause regulatory proteins to initiate the apoptosis pathway. This step allows those signals to cause cell death, or the process to be stopped, should the cell no longer need to die.

Several proteins are involved, but two main methods of regulation have been identified: the targeting of mitochondria functionality, or directly transducing the signal via adaptor proteins to the apoptotic mechanisms. An extrinsic pathway for initiation identified in several toxin studies is an increase in calcium concentration within a cell caused by drug activity, which also can cause apoptosis via calcium binding protease calpain.

In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in a wide variety of diseases. Excessive apoptosis causes atrophy (decrease in the size of the body), whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer. Some factors like Fas (First apoptosis signal) receptors and caspases promote apoptosis, while some members of the Bcl-2 (B- cell lymphoma 2) family of proteins inhibit apoptosis. The initiation of apoptosis is tightly regulated by activation mechanisms, because once apoptosis has begun, it inevitably leads to the death of the cell.

A cell initiates intracellular apoptotic signaling in response to a stress, which may bring about cell suicide. The binding of nuclear receptors by glucocorticoids, heat, radiation, viral infection, hypoxia, increased intracellular concentration of free fatty acids etc. For example, if the membrane is damaged, it can trigger the release of intracellular apoptotic signals by a damaged cell.

The two best-understood activation mechanisms are the intrinsic pathway and the extrinsic pathway.

10.3. INTRINSIC PATHWAY (MITOCHONDRIAL PATHWAY):

It is also called "**Mitochondrial pathway**". It is activated by intracellular signals generated when cells are stressed and depends on the release of proteins from the intermembrane space of mitochondria. Mitochondria are essential to multicellular life. Without them, a cell ceases to respire aerobically and quickly dies. This fact forms the basis for some apoptotic pathways. Apoptotic proteins that target mitochondria affect them in different ways. They may cause mitochondrial swelling through the formation of membrane pores, or they may increase the permeability of the mitochondrial membrane and cause apoptotic effectors to leak out. They are very closely related to intrinsic pathway, and tumors arise more frequently through intrinsic pathway than the extrinsic pathway because of sensitivity. There is also a growing body of evidence indicating that nitric oxide is able to induce apoptosis by helping to dissipate the membrane potential of mitochondria and therefore make it more permeable.

During apoptosis, cytochrome *c* (an essential component of the electron transport chain) is released from mitochondria through the actions of the proteins Bax (BCL2-associated X protein) and Bak (Bcl-2 homologues antagonist/killer). Once cytochrome *c* is released it binds with Apoptotic protease activating factor-1 (Apaf-1) and ATP, which then bind to pro-caspase-9 to create a protein complex known as an apoptosome. The apoptosome cleaves the pro-caspase to its active form of caspase-9, which in turn cleaves and activates pro-caspase into the effector caspase-3.

Mitochondria also release proteins known as SMACs (second mitochondria-derived activator of caspases) into the cells' cytosol following the increase in permeability of the mitochondria membranes. SMAC binds to proteins that inhibit apoptosis (IAPs) thereby deactivating them, and preventing the IAPs from arresting the process and therefore allowing apoptosis to proceed. IAP also normally suppresses the activity of a group of cysteine proteases called caspases, which carry out the degradation of the cell. Therefore, the actual degradation enzymes can be seen to be indirectly regulated by mitochondrial permeability.

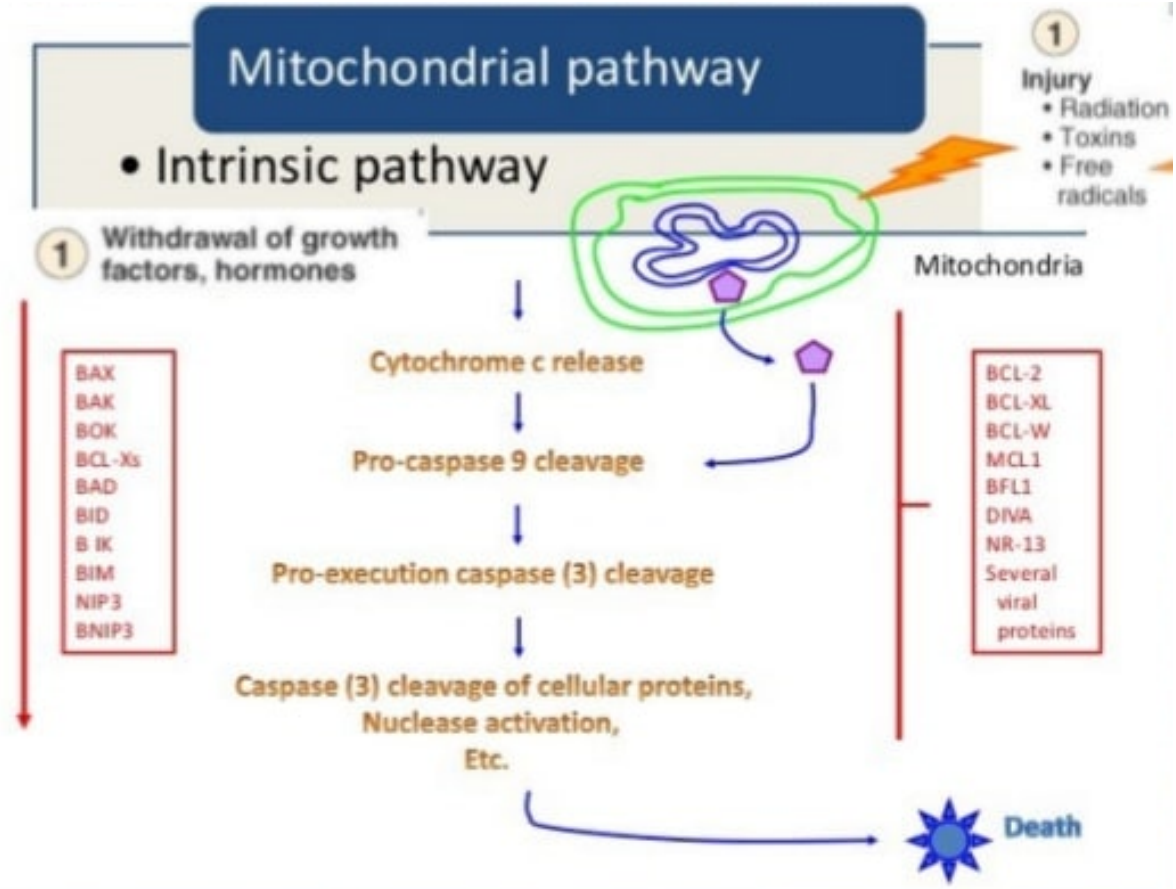

Fig. 10.4

10.4. EXTRINSIC PATHWAY (DEATH RECEPTOR PATHWAY):

It is activated by extracellular molecules binding to cell-surface death receptors, which leads to the formation of the 'death-inducing signaling complex' (DISC).

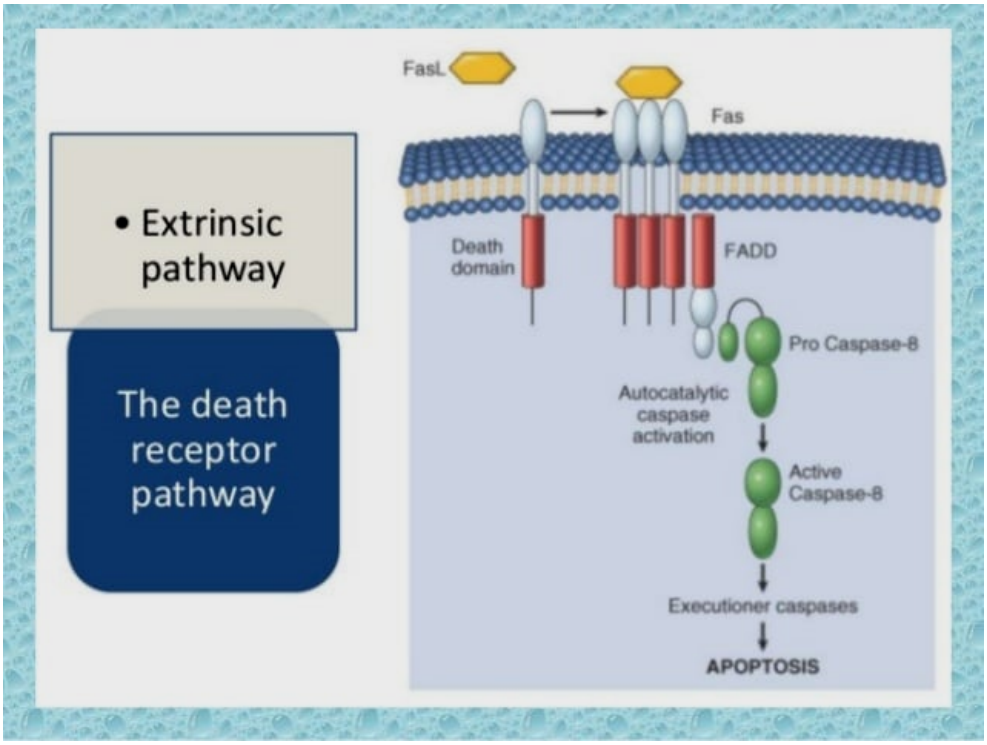

Fig. 10.5

Two theories of the direct initiation of apoptotic mechanisms in mammals have been suggested:

1) The TNF (tumor Necrosis factor) - induced model
2) The Fas-Fas (First apoptosis signal) ligand-mediated model

Both involving receptors of the TNF receptor (TNFR) family coupled to extrinsic signals.

TNF Pathway:

TNF-alpha is a cytokine produced mainly by activated macrophages, and is the major extrinsic mediator of apoptosis. Most cells in the human body have two receptors for TNF-alpha: TNFR1 and TNFR2. The binding of TNF-alpha to TNFR1 has been shown to initiate the pathway that leads to caspase activation via the intermediate membrane proteins TNF receptor-associated death domain (TRADD) and Fas-associated death domain protein (FADD). cIAP1/2 can inhibit TNF-α signaling by binding to TRAF2. FLIP inhibits the activation of caspase-8. However, signaling through TNFR1 might also induce apopotosis in a caspase-dependent manner. The link between TNF-alpha and apoptosis shows why an abnormal production of TNF-alpha plays a fundamental role in several human diseases, especially in autoimmune diseases. The TNF-alpha receptor superfamily also includes death receptors (DRs), such as DR4 and DR5**.** These receptors bind to the protein TRAIL (TNF-related apoptosis inducing ligand) and mediate apoptosis. Apoptosis is known to be one of the primary mechanisms of targeted cancer therapy. Luminescent iridium complex-peptide hybrids (IPHs) have recently been designed, which mimic TRAIL and bind to death receptors on cancer cells, thereby inducing their apoptosis.

FAS Pathway:

The fas receptor (First apoptosis signal), also known as Apo-1 or CD95, is a transmembrane protein of the TNF family which binds the Fas ligand (FasL). The interaction between Fas and FasL results in the formation of the *death-inducing signaling complex* (DISC), which contains the FADD, caspase-8 and caspase-10. In some types of cells (type I), processed caspase-8 directly activates other members of the caspase family, and triggers the execution of apoptosis of the cell. In other types of cells (type II), the Fas-DISC starts a feedback loop that spiral into increasing release of proapoptotic factors from mitochondria and the amplified activation of caspase-8.

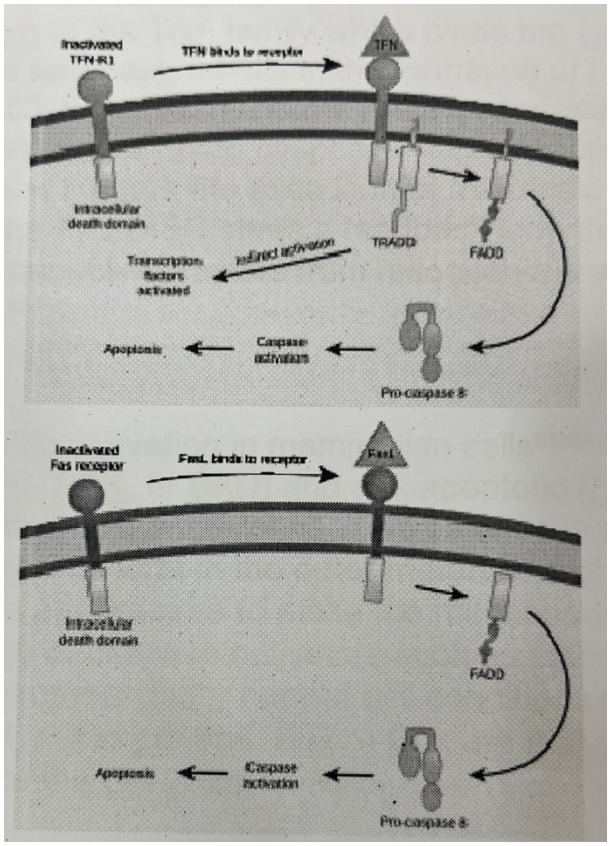

Fig. 10.6: TNF and Fas Signaling in Apoptosis

Common Components:

Following *TNF-R1* and *Fas* activation in mammalian cells[a balance between proapoptotic (BAX, BID, BAK, or BAD) and anti-apoptotic (Bcl-Xl and Bcl-2) members of the Bcl-2 family are established. This balance is the proportion of proapoptotic homodimers that form in the outer-membrane of the mitochondrion. The proapoptotic homodimers are required to make the mitochondrial membrane permeable for the release of caspase activators such as cytochrome c and SMAC. Control of proapoptotic proteins under normal cell conditions of nonapoptotic cells is incompletely understood, but in general, Bax or Bak are activated by the activation of BH3-only proteins, part of the Bcl-2 family.

Caspases:

Caspases play the central role in the transduction of ER apoptotic signals. Caspases are proteins that are highly conserved, cysteine-dependent aspartate-specific proteases.

There are two types of caspases:

1) **Initiator Caspases** e.g. caspase 2, 8, 9, 10, 11, 12

   The activation of initiator caspases requires binding to specific oligomeric activator protein.

2) **Effector caspases** e.g. caspase 3, 6, 7. Effector caspases are then activated by these active initiator caspases through proteolytic cleavage. The active effector caspases then proteolytically degrade a host of intracellular proteins to carry out the cell death program.

Caspase-Independent Apoptotic Pathway:

There also exists a caspase-independent apoptotic pathway that is mediated by AIF (apoptosis-inducing factor).

Apoptosis Model in Amphibians

The frog *Xenopuslaevis* serves as an ideal model system for the study of the mechanisms of apoptosis. In fact, iodine and thyroxine also stimulate the spectacular apoptosis of the cells of the larval gills, tail and fins in amphibian's metamorphosis, and stimulate the evolution of their nervous system transforming the aquatic, vegetarian tadpole into the terrestrial, carnivorous frog.

Negative Regulators of Apoptosis:

Negative regulation of apoptosis inhibits cell death signaling pathways, helping tumors to evade cell death and developing drug resistance. The ratio between anti-apoptotic (Bcl-2) and pro-apoptotic (Bax) proteins determines whether a cell lives or dies. Many families of proteins act as negative regulators categorized into either anti-apoptotic factors, such as IAPs and Bcl-2 proteins or prosurvival factors like cFLIP, BNIP3, FADD, Akt, and NF-kB.

Proteolytic Caspase Cascade: Killing the Cell:

Many pathways and signals lead to apoptosis, but these converge on a single mechanism that actually causes the death of the cell. After a cell receives stimulus, it undergoes organized degradation of cellular organelles by activated proteolytic caspases. In addition to the destruction of cellular organelles, mRNA is rapidly and globally degraded by a mechanism that is not yet fully characterized. mRNA decay is triggered very early in apoptosis.

A cell undergoing apoptosis shows a series of characteristic morphological changes. Early alterations include:

1) Cell shrinkage and rounding occur because of the retraction of lamellipodia and the breakdown of the proteinaceous cytoskeleton by caspases.
2) The cytoplasm appears dense, and the organelles appear tightly packed.
3) Chromatin undergoes condensation into compact patches against the nuclear envelope in a process known as pykniosis, a hallmark of apoptosis.
4) The nuclear envelope becomes discontinuous and the DNA inside it is fragmented in a process referred to as karyorrhexis. The nucleus breaks into several discrete chromatin bodies or nucleosomal units due to the degradation of DNA.

Apoptosis progresses quickly and its products are quickly removed, making it difficult to detect or visualize on classical histology sections. During karyorrhexis, endonuclease activation leaves short DNA fragments, regularly spaced in size. These give a charactestic "laddered" appearance on agar gel after electrophoresis. Tests for DNA laddering differentiate apoptosis from ischemic or toxic cell death.

Before the apoptotic cell is disposed of, there is a process of disassembly. There are three recognized steps in apoptotic cell disassembly.

1) Membrane blebbing: The cell membrane shows irregular buds known as blebs. Initially these are small surface blebs. Later these can grow into larger so-called dynamic membrane blebs. An important regulator of apoptotic cell membrane blebbing is ROCK1 (rho associated coiled-coil-containing protein kinase 1).
2) Formation of membrane protrusions: Some cell types, under specific conditions, may develop different types of long, thin extensions of the cell membrane called membrane protrusions. Three types have been described: microtubule spikes, apoptopodia, and beaded apoptopodia. Pannexin 1 is an important component of membrane channels involved in the formation of apoptopodia and beaded apoptopodia.
3) Fragmentation: The cell breaks apart into multiple vesicles called apoptotic bodies, which undergo phagocytosis. The plasma membrane protrusions may help bring apoptotic bodies closer to phagocytosis.

Removal of Dead Cells:

The removal of dead cells by neighboring phagocytic cells has been termed efferocytosis. Dying cells undergo the final stages of apoptosis display phagocytic molecules, such as phosphatidylserine, on their cell surface. Phosphatidylserine is normally found on the inner leaflet surface of the plasma membrane, but is redistributed during apoptosis to the extracellular surface by a protein known as scramblase. These molecules mark the cell for phagocytosis by cells possessing the appropriate receptors, such as macrophages. The removal of dying cells by phagocytes occurs in an orderly manner without eliciting an inflammatory response. During apoptosis, cellular RNA and DNA are separated from each other and sorted to different apoptotic bodies; separation of RNA is initiated as nucleolar segregation.

Pathway Knock-Outs:

Many knock-outs have been made in the apoptosis pathways to test the function of each of the proteins. Several caspases, in addition to APAF1 and FADD, have been mutated to determine the new phenotype. In order to create a tumor necrosis factor (TNF) knock-out, an exon containing the nucleotides 3704-5364 was removed from the gene. This exon encodes a portion of the mature TNF domain, as well as the leader sequence, which is a highly conserved region necessary for proper intracellular processing. TNF-/- mice develop normally and have no gross structural or morphological abnormalities. However, upon immunization with SRBC (sheep red blood cells), these mice demonstrated a deficiency in the maturation of an antibody response; they were able to generate normal levels of IGM, but could not develop specific IgG levels. Apaf-1 is the protein that turns on caspase 9 by cleavage to begin the caspase cascade that leads to apoptosis. Since a -/- mutation in the APAF-1 gene is embryonic lethal, a gene trap strategy was used in order to generate an APAF-1 -/- mouse. This assay is used to disrupt gene function by creating an intragenic gene fusion. When an APAF-1 gene trap is introduced into cells, many morphological changes occur, such as spina bifida, the persistence of interdigital webs, and open brain. In addition, after embryonic day 12.5, the brain of the embryos showed several structural changes. APAF-1 cells are protected from apoptosis stimuli such as irradiation. A BAX-1 knock-out mouse exhibits normal forebrain formation and a decreased programmed cell death in some neuronal populations and in the spinal cord, leading to an increase in motor neurons.

The caspase proteins are integral parts of the apoptosis pathway, so it follows that knock-outs made have varying damaging results. A caspase 9 knock-out leads to a severe brain malformation. A caspase 8 knock-out leads to cardiac failure and thus embryonic lethality. However, with the use of cre-lox technology, a caspase 8 knock-out has been created that exhibits an increase in peripheral T cells, an impaired T cell response, and a defect in neural tube closure. These mice were found to be resistant to apoptosis mediated by CD95, TNFR, etc., but not resistant to apoptosis caused by UV radiation, chemotherapeutic drugs, and other stimuli. Finally, a caspase 3 knock-out was characterized by ectopic cell masses in the brain and abnormal apoptotic features such as membrane blebbing or nuclear fragmentation. A remarkable feature of these KO mice is that they have a very restricted phenotype; Casp3, 9, APAF-1 KO mice have deformations of neural tissue and FADD and Casp 8 KO showed defective heart development, however, in both types of KO other organs developed normally and some cell types were still sensitive to apoptotic stimuli suggesting that unknown proapoptotic pathways exist.

10.5. SIGNIFICANCE OF APOPTOSIS:

1) Maintenance of normal tissue during development.
2) It helps to maintain homeostasis in the multicellular organisms. So, it maintains constant
3) balance between cell division and cell death.
4) It helps in the disposal of cells which are damaged, unwanted and dangerous. The dangerous T-lymphocytes are eliminated by apoptosis.
5) Avoids autoimmunity by eliminating self-reactive cell.

6) Elimination of virus infected cells.
7) Defence against infections and harmful chemicals.
8) Prevent tumor formation.

Role of Apoptosis:

Apoptosis plays an important role in the body of an organism which include-

1) The separation of the fingers during the development of the fetus is due to apoptosis.
2) It results in the closure of the neural tube in the dorsal part.
3) Programmed cell death results in the removal of vestigial remnants such as pronephros.
4) During the determination of sex of the fetus, the Wolffian ducts are removed by cell death.
5) In the urachus, apoptosis allows the removal of redundant tissues between the bladder and umbilicus.

Relationship between Apoptosis and Cancer:

Cancer is the uncontrolled division of cells that leads to the development of tumor. If the apoptotic signaling works properly, these unwanted cells can be removed from the body. The main reason for cancer is that they have the ability to prevent apoptosis and therefore multiply uncontrollably.

10.6. SUMMARY:

Apoptosis is a form of programmed cell death that occurs in multicellular organisms and in some eukaryotic, single-celled microorganisms such as yeast. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, DNA fragmentation, and mRNA decay. The average adult human loses 50 to 70 billion cells each day due to apoptosis. For the average human child between 8 and 14 years old, each day the approximate loss is 20 to 30 billion cells. In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis is a highly regulated and controlled process that confers advantages during an organism's life cycle. For example, the separation of fingers and toes in a developing human embryo occurs because cells between the digits undergo apoptosis. Unlike necrosis, apoptosis produces cell fragments called apoptotic bodies that phagocytes are able to engulf and remove before the contents of the cell can spill out onto surrounding cells and cause damage to them.

Apoptosis can be initiated through one of two pathways. In the intrinsic pathway, the cell kills itself because it senses cell stress, while in the extrinsic pathway the cell kills itself because of signals from other cells. Weak external signals may also activate the intrinsic pathway of apoptosis. Both the pathways induce cell death by activating caspases, which are proteases, or enzymes that degrade proteins. The two pathways both activate initiator caspases, which then activate executioner caspases, which then kill the cell by degrading

proteins indiscriminately. However, excessive apoptosis causes atrophy, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer. Some factors like Fas receptors and caspases promote apoptosis, while some members of the Bcl-2 family of proteins inhibit apoptosis.

10.7. TECHNICAL TERMS:

Apoptosis, Mitochondrial pathway, Intrinsic pathway, Extrinsic pathway, Death receptor pathway, Caspases.

10.8. SELF ASSESSMENT QUESTIONS:

1) Give an account on Mitochondrial pathway of apoptosis.
2) Write an essay on Extrinsic pathway of apoptosis.
3) Describe the different caspases, pro-apoptotic and anti-apoptotic proteins and their role in signal cascade activity during apoptosis.

10.9. SUGGESTED READINGS:

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Prof. V. Umamaheswara Rao

LESSON-11

ONCOGENE AND TUMOR SUPPRESSOR GENES

11.0 OBJECTIVE:

- Students will be aware of the oncogenes and suppressor genes and their role in causing different cancers.

STRUCTURE:

11.1 Introduction

11.2 Oncogenes

11.3 Tumor Suppressor Genes

11.4 Summary

11.5 Technical Terms

11.6 Self-Assessment Questions

11.7 Suggested Readings

11.1. INTRODUCTION:

The term 'Oncogene' was coined by George Todaro and Robert Heubnerin in 1969. The first confirmed oncogene was discovered in 1970 and was termed as src/SRC (pronounced 'sarc' as it is short form for sarcoma). SRC was first discovered as an oncogene in chicken retrovirus. There are two main classes of genes associate with cancers are Oncogenes and Tumor suppressor genes. Mutations in these genes cause cancers. Oncogenes act as dominantly accelerators. They encode proteins that promote the loss of growth control and the conversion of a cell to a malignant state. Tumor suppressor genes act as cell's brakes and they encode proteins that restrain cell growth and prevent cells from becoming malignant. Gain-in-mutation in proto-oncogenes and loss-in-mutation in tumor suppressor genes results in cancers. Basically, oncogenes are the mutant forms of normal cellular genes called as proto-oncogenes or cellular oncogenes. So, proto-oncogenes or cellular oncogenes are the cellular homologs of viral oncogenes.

11.2. ONCOGENES:

Encode proteins that promote loss of growth control and the conversion of a cell to malignant state. Derived from proto-oncogenes which are the genes that encode proteins having functions (cell growth control, signal transduction, progression through cell cycle etc.) in the normal cells. Approximately, 100 different oncogenes have been identified, most of which included as part of the genomes of RNA tumor viruses. Though the viral versions of each of these genes (v-onc genes) are derived from cellular versions (c-onc genes) present in mammalian genome, only a dozen of them have been shown to play a role in human carcinogenesis. In 1976, Dominique Stehelin, J. Michael and Harold E. Varmus of the

University of California, USA, demonstrated that oncogenes were activated proto-oncogenes that found in many organisms including humans.

Proto-Oncogene:

Most oncogenes began as proto-oncogenes. It is a normal gene that could become an oncogene due to mutations or increased expression.

Functions of Proto-Oncogenes:

1) They help to regulate cell growth and differentiation
2) They involve in signal transduction
3) They involve in execution of mitogenic signals

A proto-oncogene becomes a tumor-inducing agent, an oncogene. Examples of proto-oncogenes include RAS, WNT, MYC, ERK, and TRK.

Activation of Proto-Gene:

The proto-oncogene can become an oncogene by a relatively small modification of its original function. There are three basic methods of activation:

1) A mutation within a proto-oncogene, or within a regulatory region (for example the promoter region), can cause a change in the protein structure, causing – i) an increase in protein (enzyme) activity, ii) a loss of regulation.
2) An increase in the amount of a certain protein (protein concentration), caused by – i) an increase of protein expression (through misregulation), ii) an increase of protein (mRNA) stability, prolonging its existence and thus its activity in the cell, iii) gene duplication (one type of chromosome abnormality), resulting in an increased amount of protein in the cell.
3) A chromosomal translocation (another type of chromosome abnormality) occurs in 2 different types – i) translocation events which relocate a proto-oncogene to a new chromosomal site that leads to higher expression, ii) translocation events that lead to a fusion between a proto-oncogene and a 2^{nd} gene (this creates a fusion with increased cancerous/oncogenic activity).
    a) The expression of a constitutively active hybrid protein. This type of mutation in a diving stem cell in the bone marrow leads to adult leukemia.
    b) Philadelphia chromosome is an example of this type of translocation. This chromosome was discovered in 1960 by Peter Nowell and David Hungerford, and it is a fusion of parts of DNA from chromosome 22 and chromosome 9. The broken end of chromosome 22 contains the 'BCR' gene, which fuses with a fragment of chromosome 9 that contains the 'ABL_1'gene. When these two chromosome fragments fuse the genes also fuse creating a new gene – 'BCR-

ABL'. This fused gene encodes for a protein that displays high protein tyrosine kinase activity (this activity is due to the 'ABL_1' half of the protein). The unregulated expression of this protein activates other proteins that are involved in cell cycle and cell division which can cause a cell to grow and divide uncontrollably (the cell becomes cancerous). As a result, the Philadelphia chromosome is associated with Chronic Myelogenous Leukemia as well as other forms of leukemia.

The expression can be regulated by microRNAs (miRNAs), small RNAs 21-25 nucleotides in length that control gene expression by down regulating them. Mutations in such microRNAs (known as oncomirs) can lead to activation of oncogenes. Antisense messenger RNAs could theoretically be used to block the effects of oncogenes

Oncogene	Function / Activation	Cancer
AKT2	Encodes a protein – serine/threonine kinase	Ovarian cancer
ALK	Encodes a receptor tyrosin kinase	Lymphomas
ALK/NPM	Translocation creates fusion protein with nucleophosmin	Large cell lymphomas
RUNX1/AML1	Encodes a transcription factor	Acute myeloid leukemia

Oncogenes that Encode Growth Factors/Their Receptors:

The first connection between oncogenes and growth factors was made in 1983 when it was discovered that the cancer-causing Simian sarcoma virus contained an oncogene (sis) derived from cellular gene for platelet derived growth factor (PDGF), a protein present in human blood. The cultured cells infected with this virus become cancerous as they produce large amounts of PDGF that cause uncontrolled proliferation of cells. Gliomas, brain tumors occur due to over expression of PDGF. Avian erythroblastosis virus carry oncogene 'erb B' that directs the formation of EGF receptor that is missing part of the extracellular domain of the protein that binds to growth factor. The altered receptor for growth factors lead to malignancy.

Oncogenes That Code Cytoplasmic Protein Kinases:

A number of cytoplasmic protein kinases, including both serine-threonine kinases and tyrosine kinases are included among the list of oncogenes. Raf - a serine-threonine protein kinase leads the MAP kinase cascade, the primary growth control signaling pathway. Mutation in raf gene makes proto-oncogene to oncogene that contribute to loss of growth control. The first oncogene to be discovered is src, a protein kinase that phosphorylates serine residues. Transformation of a cell by a src-containing tumor virus is accompanied by the phosphorylation of a wide variety of proteins.

Oncogenes That Encode Nuclear Transcription Factors:

A number of oncogenes encode for nuclear transcription factors. The progression of cells through the cell cycle requires timely activation of large number of genes whose products contribute in various ways to cell growth and division. Any alteration in these proteins that control expression of genes seriously disturbs the cells normal growth. Best studied oncogene produced nuclear factor is myc. This Myc is one of the oncogenes found to be altered in human cancers, resulted from chromosome inversions or translocations. As a result, excess of Myc protein is produced due to increased level of this gene expression. E.g.: Burkitt's lymphoma, most common cancer among African people results from translocation of myc gene to a position adjacent to an Ab gene.

Oncogenes That Encode Products That Effects Apoptosis:

Apoptosis is one of the body's key mechanisms to rid itself of tumor cells at an early stage in their progression towards malignancy. Any alteration in this leads to tumor formation. bcl_2 is an oncogene closely linked to apoptosis encodes a membrane bound protein that inhibits apoptosis. The role of bcl2 in apoptosis is clearly revealed in phenotypes of knockout mice that lack bcl2. Like that myc, bcl2 gene product become oncogenic in increased levels than normal level, when translocate to an abnormal site on chromosome. Certain human lymphoid cancers, follicular B cell lymphomas are correlated with translocation of bcl2 gene next to a gene that codes for heavy chain of Ab molecules. Increased expression of bcl2 gene represses the apoptosis in lymphoid tissues allowing abnormal cells to proliferate to form lymphoid tumors.

11.3. TUMOR SUPPRESSOR GENES:

Function, Abnormalities, and Role in Cancer

Tumor suppressor genes are the genes that code for proteins that regulate the growth of cells and play an important role in preventing the development of cancer cells. These genes can work in different ways to either tell cells to stop dividing, repairing damaged DNA in cells that could lead to cancer or get rid of cells in which the damaged DNA cannot be repaired. When tumor suppressor genes are altered or inactivated due to a mutation (either one that is present at birth or one that occurs later in life), they make proteins that are less effective at controlling cell growth and / or repair. The resultant unchecked growth of damaged or abnormal cells leads to uncontrolled growth and the development of a cancerous tumor. Tumor suppressor genes are also known as anti-oncogenes or loss of function genes.

There are three main types of tumor suppressor genes:

- One type tells the cells to slow down and stop dividing
- Another type is responsible for fixing damages in DNA that can happen when cells divide (DNA repair genes or mutator genes)
- A third type is responsible for telling cells when to die, a process called apoptosis or programmed cell death

Oncogenes vs Tumor Suppressor Genes:

There are two primary types of genes involved in the development of cancer, oncogenes and tumor suppressor genes. The term oncogenes mean literally 'cancer genes' and these genes result in the uncontrolled growth of cells. (Proto-oncogenes are the genes that

helps cells grow, and when mutated so they function poorly are then referred to as oncogenes).

Tumor suppressor genes are easier to describe by using an analogy. Analogy to driving: Tumor suppressor genes are the brakes. With all the news about immunotherapy, and hearing bits and pieces about "on and off switches" with cancer, it may help to, very simplistically, think of cells as a car. In each cell, there is an accelerator and brakes. In normal cars, both are working fine. Multiple processes make sure they stay in balance so the car moves along steadily, but do not crash. Cancer begins with a series of mutations in genes. Genes function as a blueprint for making proteins with different functions. Some mutations are no big deal-we refer them as passenger mutations. The problem mutations are those that involve the driver. The driver can decide to go too fast or slow. One may hear about these as "driver mutations" not because they drive a car, but because they drive the growth of cancer cells. Cancer can be related to problems with either the accelerator or the brakes, but often, damage to both oncogenes and tumor suppressor genes occurs before a cancer develops. In other words, the accelerator has to be stuck to the floor and the brakes have to malfunction. The fact that cancer often requires a number of different mutations is one of the reasons why cancer is more common in old people. In this car analogy, i) oncogenes are the genes that control the accelerator and ii) tumor suppressor genes control the brakes.

Inheritance and Oncogenes vs Tumor Suppressor Genes

There are several important differences between oncogenes and tumor suppressor genes in cancer. In general, oncogenes are dominant. In our bodies, we have two sets of each of our chromosomes and two sets of genes: one from each of our parents. With dominant genes, only one of the two copies need to be mutated or abnormal for a negative effect to occur. An analogy is brown eyes. If people inherit one copy of the brown eyed gene and one copy of the blue-eyed gene, their eye color will always be brown. In the car analogy, it takes only one copy of a mutated gene controlling the accelerator for the car to run out of control (only one of the two proto-oncogenes need to be mutated to become an oncogene).

Tumor suppressor genes, in contrast, tend to be recessive. That is, just like you need two genes for blue eyes to have a blue-eyed baby, two suppressor genes must both be damaged in order to contribute to cancer. It is important to note that the relation between oncogenes and tumor suppressor genes is much more complex that this and the two are often intertwined. For example, a mutation in a suppressor gene may result in proteins that are unable to repair mutations in an oncogene.

Tumor Suppressor Genes and the "Two-Hit-Hypothesis":

Understanding the recessive nature of tumor suppressor genes can be helpful in knowing genetic predispositions and hereditary cancer. An example of tumor suppressor genes is the BRCA1/BRCA2 genes, otherwise known as the "breast cancer genes". People who have a mutation in one of these genes have an increased risk of developing breast cancer (among other cancers). But not everyone with the gene develops breast cancer. The first copy of these genes is mutated at birth, but it's not until another mutation occurs after birth (an acquired mutation or somatic mutation) that abnormal repair proteins are made that increase the risk of cancer. It is also important to note that there are several genes associated with the development of breast cancer (not just BRCA genes), for which genetic testing is available, and many of these are thought to be tumor suppressor genes.

This recessive nature is what is referred to in the "Two-hit hypothesis" of cancer. The first copy (in the above example, the inherited copy of the defective gene) is the first hit, and a later mutation in the other copy of the gene later in the life is the second hit. Of note is that having "2 hits" alone is not enough to lead to cancer. Damage to DNA cells (from the environment or due to normal metabolic processes in cells) must then occur, and together the two mutated copies of the tumor suppressor gene are unable to code for effective proteins to repair the damage.

Models of Tumor Suppression (Fig. 11.1)

1. Two-Hit Hypothesis (Knudson Hypothesis)

It was formulated by Knudson in 1971 and led indirectly to the identification of tumor suppression genes. It is the hypothesis that most tumor suppressor genes require both alleles to be inactivated, either through mutations or through epigenetic silencing to cause a phenotypic change. Knudson suggested that 'two hits' to DNA were necessary to cause the cancer. Unlike oncogenes, tumor suppressor genes generally follow the two-hit hypothesis, which states both alleles that code for a particular protein must be affected before an effect is manifested. If only one allele for the gene is damaged, the other can still produce enough of the correction protein to retain the appropriate function. In other words, mutant tumor suppressor alleles are usually recessive, whereas mutant oncogene alleles are typically dominant.

2. Mechanism of Acquired Uniparental Disomy (UPD)

Disomy refers to an abnormal chromosome represented twice in a single cell. It is a mechanism in cancer by which adventitious mutations are amplified leading to a growth advantage of these cells. Uniparental disomy probably occurs as a result of a mitotic error in somatic cells. Recently, a "Continuum model for tumor suppression" was published in "Nature" by Alice H. Berger, Alfred Knudson and Pier Paolo Pandolfi in 2011. Even partial inactivation of tumor suppressors can critically contribute to tumorigenesis.

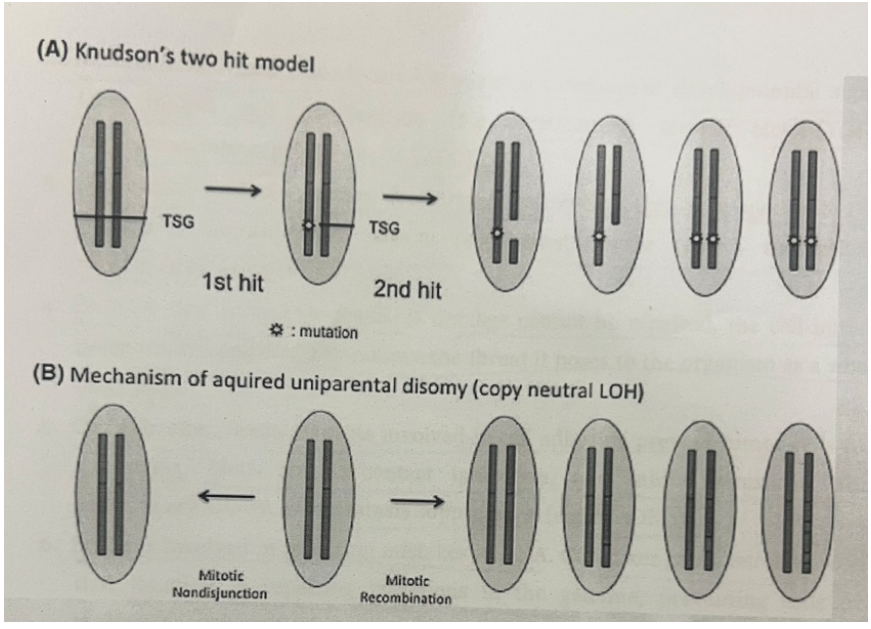

Fig-11.1: Models of Tumor Suppression

Tumor Suppressor Genes and Hereditary Cancer:

The inherited cancer syndromes account to 5% or less of cancers, but the percent of cancers that can be attributed to these genes is likely much higher. Genetic screening is now available for several of these syndromes, but in many cases, a genetic predisposition cannot be found with testing. In this case, it is very helpful for people to work with a genetic counselor who may be able to understand more about risk based on family history.

Two Basic Roles of Tumor Suppressor Genes: Gatekeepers and Caretakers:

The tumor suppressor genes may function as the "brakes" of the car in three primary ways but inhibiting cell growth, fixing broken DNA, or causing a cell to die. This type of tumor suppressor genes can be thought of as "gatekeeper" genes. Some tumor suppressor genes function in more of a caretaker role. These genes code for proteins that oversee and regulate many of the functions of other genes (proteins coded for by the genes) to maintain the stability of DNA.

There have now been many different tumor suppressor genes identified and it is likely that many more will be identified in the future. Tumor suppressor genes were first identified among children with retinoblastoma. In retinoblastoma, in contrast to many tumor suppressor genes, the tumor gene that is inherited is dominant and therefore allows cancers to develop in young children. If one parent carries the mutated gene, then 50 percent of their children will inherit the gene and be at risk for retinoblastoma. The tumor suppressor genes viz., Rb, APC and p53 function as gatekeepers. In contrast, BRCA1/BRCA2 genes function more as caretakers, and regulate the activity of other proteins involved in cell growth and repair.

Some Examples of Tumor Suppressor Genes Associated with Cancer Include-

Gene	Original Function	Two Hit?	Associated Carcinomas
Rb	DNA replication, cell division and death	Yes	Retinoblastoma
p53	Apoptosis	No	Half of all known malignancies
VHL	Cell division, death, and differentiation	Yes	Kidney cancer
APC	DNA damage, cell division, migration, adhesion, death	Yes	Colorectal cancer
BRCA2	Cell division and death, and repair of double-stranded DNA breaks	Yes	Breast /Ovarian cancer
NF1	Cell differentiation, division, development, RAS signal transduction	No	Nerve tumors, Neuroblastoma
PTCH	Hedgehog signaling	No	Medulloblastoma, Basal cell carcinoma

- RB gene: the suppressor gene responsible for retinoblastoma.

- p53 gene: the p53 gene encodes for protein p53 which regulates gene repair in cells. Mutations in this gene are implicated in around 50 percent of cancers. Inherited mutations in the p53 gene are much less common than acquired mutations, and result in the hereditary condition known as Li Fraumeni Syndrome. The p53 gene codes for proteins that tell cells to die if they are damaged beyond repair, a process referred to as apoptosis.

- BRCA1/BRCA2 genes: these genes are responsible for around 5 percent to 10 percent of breast cancers, but both BRCA1gene mutations and BRCA2 gene mutations are associated with an increased risk of other cancers as well (BRCA2 is also linked to an increased lung cancer risk in women).

- APC gene: these genes are associated with an increased risk of colon cancer in people with familial adenomatous polyposis (FAP). This inherited disorder is characterized by cancer of large intestine (colon) and rectum. People with classic FAP may develop multiple non-cancerous (benign) growths (polyps) in colon as early as their teenage years.

- PTEN gene: the PTEN gene is one of the non-BRCA genes that can increase the risk of a woman developing breast cancer (up to an 85% lifetime risk). It is associated with both PTEN hamartoma tumor syndrome and Cowden syndrome. The gene codes for proteins that aid in cell growth but also help cells stick together. When the gene is mutated, there is a greater risk that cancer cells will “break off” or metastasize.

- SWI/SNF complex: the SWI/SNF (Switch/Sucrose Non-Fermentable) complex is a crucial ATP-dependent family of chromatin remodelers in eukaryotes, acting as a molecular machine that uses energy from ATP to slide, evict, or restructure nucleosomes (DNA wrapped around histones) to control DNA accessibility for gene expression, replication, and repair. Essential for the development and genome stability, its subunits are frequently mutated in nearly 20-25% of human cancers, highlighting its role as a major tumor suppressor, making it a significant target in cancer research and therapy. Mutations in the individual complexes can lead to misfolding, which compromises the ability of the complex to work together as a whole. SWI/SNF has the ability to move nucleosomes, which condenses DNA, allowing for transcription or block transcription from occurring for certain genes. Mutation of this ability could cause genes to be turned on or off at the wrong times.

At the current time, over 1200 human tumor suppressor genes have been identified. The University of Texas has a tumor suppressor gene database that lists many of these genes.

Tumor Suppressor Genes and Cancer Treatments:

Understanding tumor suppressor genes may also help explain a bit why therapies, such as chemotherapy, don’t completely cure cancer. Some cancer treatments work to stimulate cells to commit suicide. Since some tumor suppressor genes are involved in the process of apoptosis (cell death) the cancer cells may not go through the process of apoptosis

as other cells might. Learning about the function of tumor suppressor genes and oncogenes involved in the formation of cancer, as well as the characteristics of cancer cells and how cancer cells differ from normal cells, can help researchers look at new ways to both identify people at risk of cancer and to treat cancers that occur. And not only changes in genes, but modifying the way of gene expressions without actual changes in the genome (known as epigenetics) plays a role in cancer. It is possible that changes in the environment of our tissues may affect the "expression" of tumor suppressor proteins made by these genes. For example, one study looked at the role medicinal herbs may play in the activation of tumor suppressor molecules, and several other studies have been looking at the role of dietary patterns in tumor suppressor activation.

Functions:

The proteins encoded by most tumor suppressor genes inhibit cell proliferation or survival. Inactivation of tumor suppressor genes therefore leads to tumor development by eliminating negative regulatory proteins. In most cases, tumor suppressor proteins inhibit the same cell regulatory pathways that are stimulated by the products of oncogenes. While tumor suppressor genes have the same main function, they have various mechanisms of action, that their transcribed products perform, which include the following –

- Intracellular proteins that control gene expression of a specific stage of the cell cycle. If these genes are not expressed, the cell cycle does not continue, effectively inhibiting cell division (e.g., pBR and p16).

- Receptors or signal transducers for secreted hormones or developmental signals that inhibit cell proliferation (e.g., transforming growth factor (TGF)-β and adenomatous polyposis coli (APC).

- Checkpoint control proteins that trigger cell cycle arrest in response to DNA damage or chromosomal defects (e.g., breast cancer type 1 susceptibility protein (BRCA1), p16 and p14.

- Proteins that induce apoptosis - If damage cannot be repaired, the cell initiates programmed cell death to remove the threat it poses to the organism as a whole (e.g., p53).

- Cell adhesion - Some proteins involved in cell adhesion prevents tumor cells from dispersing, block loss of contact inhibition, and inhibit metastasis. These proteins are known as metastasis suppressors (e.g., CADM1).

- Proteins involved in repairing mistakes in DNA – Caretaker genes encode proteins that function in repairing mutations in the genome, preventing cells from replicating with mutations.

- Certain genes can also act as tumor suppressor and oncogenes. Dubbed proto-oncogenes with tumor suppressor function, these genes act as 'double agents' that both positively and negatively regulate transcription (e.g., NOTCH receptors, TP_{53} and FAS).

11.4. SUMMARY:

An oncogene is a gene that has the potential to cause cancer. In tumor cells, these genes are often mutated, or expressed at high levels. Most normal cells will undergo programmed form of rapid cell death (apoptosis) when critical functions are altered and malfunctioning. A gene is involved in normal cell growth. Oncogenes may cause the growth of cancer cells. Mutations in genes that become oncogenes can be inherited, or caused by being exposed to substances in the environment that cause cancer. Oncogenes play an important role in the regulation or synthesis of proteins linked to tumorigenic cell growth. The resultant protein encoded by an oncogene is termed oncoprotein. Some oncoproteins are accepted and used as tumor markers.

Two main classes of genes associate with cancers – Oncogenes and Tumor suppressor genes. Mutations in these genes cause cancers. Oncogenes act dominantly as accelerators which encode proteins that promote the loss of growth control and result in conversion of cell to malignant state. Tumor suppressor genes act as cell's brakes that encode proteins which restrain cell growth and prevent cells from becoming malignant. Oncogenes are the mutant forms of normal cellular genes or proto-oncogenes that function in cell growth control, signal transduction, and progression through cell cycle. Proto-oncogenes or cellular genes are homologous to viral oncogenes. Approximately 100 different oncogenes identified and most of them included as part of genomes of RNA tumor viruses. Viral versions of these genes (V-onc genes) derived from cellular versions (C-onc genes) present in mammalian genome, only a dozen of them have been shown to play a role in human carcinogenesis.

11.5. TECHNICAL TERMS:

Oncogenes, Tumor suppressor genes, Gain-in-mutation, Loss-in-mutation, Proto-oncogenes, Tumor cells, Rb gene, p53 gene, BRCA1, BRCA2, Two-hit hypothesis.

11.6. SELF ASSESSMENT QUESTIONS:

1) Explain in detail about the Oncogenes.
2) Write an account on Tumor Suppressor Genes.
3) Describe the Two-hit mutation model of cancer.

11.7. SUGGESTED READINGS:

1) De Robertis, E.D.P. and De Robertis, E.M.F. 2010. Cell and Molecular Biology, 8th Edition, Wolters Kluwer, USA.
2) Jean Brachet and Mirsky, Alfred E. (Eds): he Cell, Academic Press, Inc. New York, USA.
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4) Wolfe, Stephen L. 1993, Molecular and Cellular Biology, Wordsworth Publishing Company, California, USA.

5) Roy, S.C. and Kalyan Kumar De., 1977. Cell Biology, New Central Book Agency, Calcutta.

6) P.S. Verma & V. K. Agarwal, 2021. Cell Biology, Genetics, Molecular Biology, Evolution and Ecology. S. Chand And Company Limited, New Delhi-110044.

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Prof. V. Umamaheswara Rao

LESSON-12

GENOMES OF MITOCHONDRIA AND CHLOROPLAST, ENDOSYMBIOTIC THEORY

12.0 OBJECTIVE:

- Students will acquaint with the nature of genomes of mitochondria and chloroplast as well as endosymbiotic theory

STRUCTURE:

12.1 Introduction

12.2 Genome of Mitochondria

12.3 Genome of Chloroplast

12.4 Endosymbiotic Theory

12.5 Summary

12.6 Technical Terms

12.7 Self-Assessment Questions

12.8 Suggested Readings

12.1. INTRODUCTION:

A genome is the complete set of genetic information in an organism / all genetic material of an organism. If consists of DNA (or RNA in some viruses). It includes the genes (the coding regions) and the non-coding DNA as well as mitochondrial DNA and chloroplast DNA. The study of genome is called genomics. Mitochondria are found in all eukaryotic cells. They are the energy transducing organelles. They convert the fuels into ATP through the process of oxidative phosphorylation. Mitochondria contain their own genome in their matrix. Mitochondrial DNA (mtDNA / mDNA) is the double stranded small circular chromosome found inside mitochondria. Mitochondrial DNA was discovered in 1960s by Margit M. K. Nass and Sylvan Nass by electron microscopy as DNase-sensitive threads inside mitochondria, and by Ellen Haslbrunner, Hans Tuppy and Gittfried Schatz by biochemical assays on highly purified mitochondrial fractions. Human mitochondrial DNA was the first significant part of the human genome to be sequenced. This sequencing revealed that the human mtDNA includes 16,569 base pairs and encodes 13 proteins. Nuclear and mitochondrial DNA are thought to be of separate evolutionary origin, with the mtDNA being derived from the circular genomes of bacteria engulfed by the early ancestors of today's eukaryotic cells. This theory id called the endosymbiotic theory.

12.2. GENOME OF MITOCHONDRIA (MITOGENOME):

In organisms, there are six main mitochondrial genome types. They are classified by their structure (i.e. circular versus linear), size, presence of introns or plasmid like structures, and whether the genetic material is a singular molecule or collection of homogeneous or heterogeneous molecules. In many unicellular organisms (e.g. the green alga *Chlamydomonas reinhardtii*), and in rare cases also in multicellular organisms (e.g. in some species of *Cnidaria*), the mtDNA is found as linearly organized DNA. Most of these linear mtDNAs possess telomerase-independent telomeres (i.e., the ends of the linear DNA) with different modes of replication. There are three different mitochondrial genome types found in plants and fungi. The first type is a circular genome that has introns (type 2) and may range from 19 to 1000 kbp in length. The second genome type is a circular genome (about 20-1000 kbp) that also has a plasmid-like structure (type 3). The final genome type that can be found in plants and fungi is a linear genome made up of homogeneous DNA molecules (type 5).

Great variation in mtDNA gene content and size exists among fungi and plants. Some plant species have enormous mitochondrial genomes, with *Silene conica* mtDNA containing as many as 11,300,000 base pairs. Surprisingly, even those huge mtDNAs contain the same number and kings of genes as related plants with much smaller mtDNAs. The genome of the mitochondrion of the cucumber (*Cucumis sativus*) consists of three circular chromosomes, which are entirely or largely autonomous with regard to their replication.

Protists contain the most diverse mitochondrial genomes, with five different types found in this kingdom. Type 2, type 3 and type 5 mentioned in the plant and fungal genomes also exist in some protists. One of these unique types is a heterogeneous collection of circular DNA molecules (type 4) while the other is a heterogeneous collection of linear molecules (type 6). The smallest mitochondrial genome sequenced to date is the 5,967 bp mtDNA of the parasite *Plasmodium falciparum*.

Replication:

Mitochondrial DNA is replicated by the DNA polymerase gamma complex. This complex is composed of a 140 kDa catalytic DNA polymerase encoded by the POLG gene (DNA polymerase Gamma Catalytic Subunit) and two 55 kDa accessory subunits encoded by the POLG2 gene. The replisome machinery is formed by DNA polymerase, TWINKLE and mitochondrial SSB (single stranded binding) proteins. TWINKLE is a helicase, which unwinds short stretches of dsDNA in 5’ to 3’ direction. All these peptides are encoded in the nuclear genome.

Structure of Genome in Human:

There are 37 genes on their respective H and L strands (Fig. 12.1). The two strands of the human mitochondrial DNA are distinguished as the heavy strand (outer circle) and the light strand (inner circle).

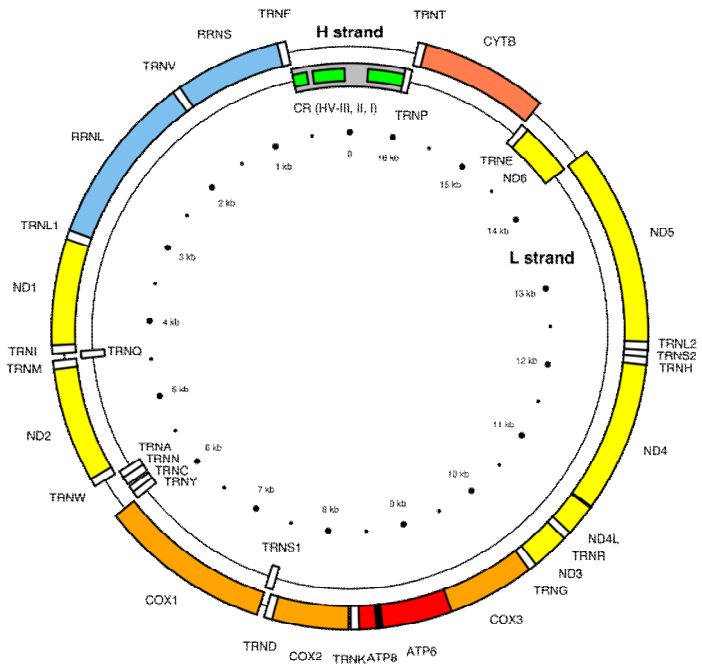


Figure-12.1: Mitochondrial Genome of Humans

1) The heavy strand (H strand / O_H) containing the majority of the genes is rich in guanine and encodes 12 subunits of the oxidative phosphorylation system, two ribosomal RNAs (12S and 16S), and 14 tRNAs.
2) The light strand (L strand / O_L) encodes one subunit, and 8 tRNAs.

So, altogether mtDNA encodes for two rRNAs, 22 tRNAs, and subunits of d13 proteins, all of which are involved in the oxidative phosphorylation process. Between most (but not all) protein coding regions, tRNAs are present. During transcription, the tRNAs acquire their characteristic L-shape that gets recognized and cleaved by specific enzymes. With the mitochondrial RNA processing, individual mRNA, rRNA, and tRNA sequences are released from the primary transcript. Folded tRNAs therefore act as secondary structure punctuations.

Organization of Genome of Plants:

The mitochondrial genome of higher plants is the largest and complex among the eukaryotes. However, the plant mitochondrial genome does not contain many genes. The mitochondrial size in *Brassica campestris* is 218 kb and contains direct repeat of 2000 kb, whereas in *Zea mays*, there are five direct repeat and inverted repeat sequences present in 570 kb DNA. The size of ribosomal RNA (26S and 18S) in plant mitochondria is larger than other mitochondria and there is a unique 5S rRNA in higher plants. The closely linked 18S and 5S genes in maize are separated from the gene for 25S rRNA. The rRNA sequence in plant mitochondria has several homologies with bacterial and chloroplast rRNA genes. Several evidences have shown that DNA sequences can move from one organelle to another organelle. In maize, 12 kb chloroplast sequences have been shown to be inserted into mitochondrial DNA.

The plant mitochondrial genes that encode proteins for cytochrome C oxidase and the apoprotein of cytochrome b have been sequenced. It is evidenced that mitochondria do not use the universal genetic code and uses various other alternatives, for example, in maize mitochondria CGG codes for tryptophan. However, this codon represents for arginine in universal code. Plant mitochondrial genes seem to lack UGA termination codon. In maize mitochondria, a gene for the apoprotein of cytochrome b is 1164 base pairs long and codes for protein of 42.9 kDa and its amino acid sequence exhibits almost fifty per cent homology with other corresponding proteins in yeast. The presence of introns in maize genes was not evidenced. Analysis of maize genome suggests that genes present in the mitochondria determine cytoplasmic male sterility, and its sterility problem can be reversed back by nuclear restorer (Rf) genes. It is difficult to identify the sequences responsible for cytoplasmic male sterility (cms) due to the larger size of the mitochondrial DNA.

Regulation of Transcription:

The promoters for the initiation of the transcription of the heavy and light strands are located in the main non-coding region of the mtDNA called the displacement loop (D-loop). There is evidence that the transcription of the mitochondrial rRNAs is regulated by the heavy-strand promoter 1 (HSP1), and the transcription of the polycistronic transcripts coding for the protein subunits are regulated by HSP2.

Inheritance of mtDNA:

In most multicellular organisms, mtDNA is inherited from the mother (maternally inherited). Whatever the mechanism, this single parent (uniparental inheritance) pattern of mtDNA inheritance is found in most animals, most plants and also in fungi. In exceptional cases, human babies sometimes inherit mtDNA from both their fathers and their mothers resulting in mtDNA heteroplasmy. Male mitochondrial DNA inheritance has been discovered in Plymouth Rock chickens. Evidence supports rare instances of male mitochondrial inheritance in some mammals as well.

Applications of Mitochondrial Genome:

1) In anthropological genetics, it is useful to trace geographic distribution of genetic variation.
2) It is widely applied in forensic science.
3) It is a powerful implement to identify human remains.

12.3. GENOME OF CHLOROPLAST (PLASTOME):

The chloroplasts are found in plant cells and eukaryotic algae. The main role of chloroplasts is to perform photosynthesis, where photosynthetic pigment chlorophyll captures the energy from sunlight and converts it and stores it in the energy storage molecules ATP and NADPH. Chloroplasts play a crucial role in sustaining life on earth.

The genetic material of the chloroplast is called as Chloroplast DNA (cpDNA). It is also called as plastid DNA. The chloroplast genome is also known as "Plastome". A chloroplast genome contains double stranded circular DNA containing genes necessary for functioning of the chloroplasts and maintaining their structure. It is located in the stroma of chloroplast. Chloroplast genomes are highly conserved among plant species. There is more than one copy of genome in each chloroplast. The exact number varies during development, but mesophyll cells in young leaves contain about 100 copies of genome. The presence of DNA chloroplasts was first suggested during the early 1950s. In 1963, Masahiro R. Ishida and Ruth Sager, were the first to extract the chloroplast DNA. They were able to isolate chloroplasts from the alga, *Chlamydomonas*, and found an enriched satellite DNA. The first complete chloroplast genome sequences were published in 1986, in *Nitotiana tabacum* (tobacco) by Sugiura and colleagues and *Marchantia polymorpha* (liverwort) by Ozeki et al. Later, more DNA molecules were obtained from the chloroplasts of higher plant species. At present, complete chloroplast genome sequences from more than 114 different plants have been studied / published. E.g. *Arabidopsis thaliana, Nitotiana tabacum, Coffea arabica, Eucalyptus globulus, Gossypium hirsutum, Helianthus annuus, Lycopersicon esculentum* etc.

Molecular Structure:

Chloroplast DNAs are circular, and are typically 1,20,000-1,70,000 base pairs long. They have a mass of about 80-130 million daltons. Most chloroplasts have their entire chloroplast genome combined into a single large ring. But the genome is broken up into about 40 small plasmids, each 2,000 – 10,000 base pairs long in dinophytes. Each minicircle contains one to three genes. Many chloroplast DNAs contain two inverted repeats, which separate a ling single copy (LSC) section from a short single copy (SSC) section. The inverted repeats vary in length, ranging from 4,000 to 25,000 base pairs long each. The inverted repeat regions usually contain three ribosomal RNA and two tRNA genes. Chloroplast DNA has long been thought to have a circular structure, but some evidence suggests that chloroplast DNA more commonly takes a linear shape. Over 95% of the chloroplast DNA in corn chloroplasts has been observed to be in branched linear form rather than individual circles.

Each chloroplast contains around 100 copies of its DNA in young leaves, reduced to 15-20 copies in older leaves. They are usually packed into nucleoids which can contain several identical chloroplast DNA rings. Many nucleoids can be found in each chloroplast. Though chloroplast DNA is not associated with true histones, a histone-like chloroplast protein (HC) coded by the chloroplast DNA that tightly packs each chloroplast DNA ring into a nucleoid has been found in red algae. In primitive red algae, the chloroplast DNA nucleoids are clustered in the center of a chloroplast, while in green plants and green algae, the nucleoids are dispersed throughout the stroma. In *Arabidopsis thaliana*, the DNA is circular and composed of 1,54,478 bp with a pair of inverted repeats (26,264 bp), which are separated by small and large single copy regions. A total of 87 potential protein coding genes including 8 genes duplicated in the inverted repeat regions. Four ribosomal RNA genes and 37 tRNA genes representing 20 amino acids were assigned to the genome (Figure 12.2).

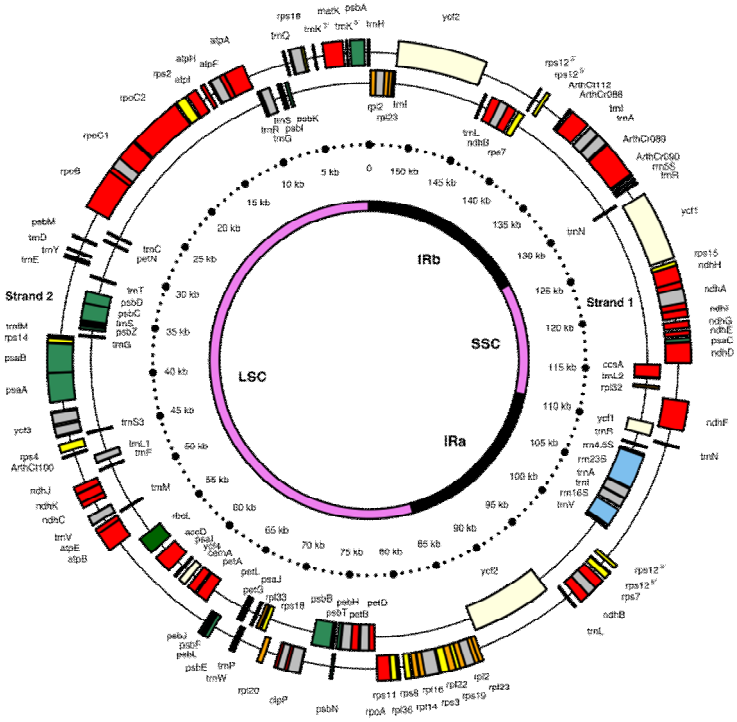


Figure-12.2: Chloroplast Genome of *Arabidopsis Thaliana*

DNA Replication:

Two main models have been proposed regarding the mechanism for chloroplast DNA (cpDNA) replication – *Leading model of cpDNA replication* and *Alternative model of replication*.

Leading model of cpDNA Replication:

The results of the microscopy experiments led to the idea that chloroplast DNA replicates using a double displacement loop (D-loop). As the D-loop moves through the circular DNA, it adopts a theta intermediary form, also known as a Cairns replication intermediate, and completes replication with a rolling circle mechanism. Replication starts at specific points of origin. Multiple replication forks open up, allowing replication machinery to replicate the DNA. As replication continues, the forks grow and eventually converge. The new cpDNA structures separate, creating daughter cpDNA chromosomes. In cpDNA, there are several A—G deamination gradients. DNA becomes susceptible to deamination events when it is single stranded. When replication forks form, the strand not being copied is single stranded, and thus at risk for A – G deamination. This mechanism is still the leading theory today.

Alternative Model of Replication:

One of the main competing models for cpDNA asserts that most cpDNA is linear and participates in homologous recombination and replication structures are similar to bacteriophage T4. It has been established that some plants have linear cpDNA, such as maize.

When the original experiments on cpDNA were performed, scientists did notice linear structures; however, they attributed these linear forms to broken circles. If the branched and complex structures seen in cpDNA experiments are real.

Gene Content and Protein Synthesis:

The chloroplast genome most commonly includes around 100 genes. The genes in chloroplast DNA are organized into operons. Introns are common in chloroplast DNA molecules. Among the land plants, the contents of the chloroplast genome are fairly similar. They code for four ribosomal RNAs, 30-31 tRNAs, 21ribosomal proteins, and 4 RNA polymerase subunits, involved in protein synthesis. For photosynthesis, the chloroplast DNA includes genes for 28 thylakoid proteins and the large Rubisco subunit. In addition, its genes encode eleven subunits of a protein complex which mediates redox reactions to recycle electrons.

Chloroplast Genome Reduction and Gene Transfer:

Over time, many parts of the chloroplast genome were transferred to the nuclear genome of the host, a process called 'endosymbiotic gene transfer'. As a result, the chloroplast genome is heavily reduced compared to that of free-living cyanobacteria. Chloroplasts may contain 60-100 genes whereas cyanobacteria often have more than 1500.

Proteins Encoded by the Chloroplast

Of the approximately three thousand proteins found in chloroplasts, some 95% of them are encoded by nuclear genes. Many of the chloroplast's protein complexes consist of subunits from both the chloroplast genome and the host's nuclear genome. As a result, protein synthesis must be coordinated between the chloroplast and the nucleus.

Protein Synthesis:

Protein synthesis within chloroplasts relies on an RNA polymerase coded by the chloroplast's own genome, which is related to RNA polymerases found in bacteria. Chloroplasts also contain a mysterious second RNA polymerase that is encoded by the plant's nuclear genome. The two RNA polymerases may recognize and bind to different kinds of promoters within the chloroplast genome.

Chloroplast DNA Inheritance:

In angiosperms, the chloroplast genome is maternally inherited in the majority of the species. In many species, however, at least occasional paternal transmission results in either paternal or biparental inheritance in some individuals.

Importance of Chloroplast Genome

1) Chloroplast genomes of land plants and algae contain generally between 100 and 150 genes. These genes are involved in plastid gene expression and photosynthesis and in various other tasks.
2) Some of the chloroplast genes appear to be essential for growth and survival.
3) Chloroplast genome sequences are most valuable for understanding plant evolution and phylogeny.

12.4. ENDOSYMBIOTIC THEORY (Symbiogenesis):

The Endosymbiotic Theory states that "some of the cell organelles like mitochondria and chloroplast in eukaryotic cells were once prokaryotic cells that were ingested by a large prokaryotic microbe". This theory explains the origin of eukaryotic cells. **Endosymbiotic theory, Symbiogenesis**, or **Serial endosymbiotic theory** is the leading evolutionary theory of the origin of eukaryotic cells from prokaryotic organisms. The theory explains that mitochondria, plastids such as chloroplasts, and possibly other organelles of eukaryotic cells are descended from formerly free-living prokaryotes (more closely related to bacteria) taken one inside the other in endosymbiosis. The idea that chloroplasts were originally independent organisms that merged into a symbiotic relationship with other one-celled organisms.

Mitochondria appear to be phylogenetically related to Rickettsiales proteobacteria, and chloroplasts to nitrogen-fixing filamentous cyanobacteria. The evidence supporting symbiogenesis are --

1) New mitochondria and plastids are formed only through binary fission, and that cells cannot create new ones.
2) The transport proteins called porins are found in the outer membranes of mitochondria, chloroplasts, and bacterial cell membranes;
3) Cardiolipin (an enzyme of the ETS) is found only in the inner mitochondrial membrane and bacterial cell membranes.
4) Some mitochondria and plastids contain single circular DNA molecules similar to the chromosomes of bacteria.

History:

Andreas Schimper (1883) had observed that the division of chloroplasts in green plants closely resembled that of free-living cyanobacteria and who proposed that green plants had arisen from a symbiotic union of two organisms. In 1918, the French scientist 'Paul Jules Portier' published Les Symbiotes, in which he claimed that the mitochondria originated from a symbiosis process. 'Ivan Wallin' advocated the idea of an endosymbiotic origin of mitochondria in the 1920s. The Russian botanist 'Konstantin Mereschkowski' first outlined the theory of symbiogenesis in 1905. The Russian botanist 'Boris Kozo-Polyansky' became the first to explain the theory in terms of Darwinian evolution. In 1924, in his book A New Principle of Biology- Essay on the Theory of Symbiogenesis, he wrote, that the theory of symbiogenesis is a theory of selection relying on the phenomenon of symbiosis. 'Lynn Margulis' advanced the theory with microbiological evidence in a 1967.

The fundamental theory of symbiogenesis as the origin of mitochondria and chloroplasts is now widely accepted.

From Endosymbionts to Organelles:

Modern endosymbiotic theory explains that simple life forms merged, forming cell organelles, like mitochondria. According to Keeling and Archibald**,** biologists usually distinguish organelles from endosymbionts by their reduced genome sizes. As an endosymbiont evolves into an organelle, most of its genes are transferred to the host cell genome. The host cell and organelle need to develop a transport mechanism that enables the return of the protein products needed by the organelle but now manufactured by the cell. Cyanobacteria and α-proteobacteria are the most closely related free-living organisms to plastids and mitochondria respectively. Both cyanobacteria and α-proteobacteria maintain a large genome encoding thousands of proteins. Plastids and mitochondria exhibit a dramatic reduction in genome size when compared with their bacterial relatives.

Nowack and her associates performed gene sequencing on the chromatophore (1.02 Mb) and found that only 867 proteins were encoded by these photosynthetic cells. Comparisons with their closest free living cyanobacteria of the genus *Synechococcus* (having a genome size 3 Mb, with 3300 genes) revealed that chromatophores underwent drastic genome shrinkage. Chromatophores contained genes that were accountable for photosynthesis but were deficient in genes that could carry out other biosynthetic functions; this observation suggests that these endosymbiotic cells are highly dependent on their hosts for their survival and growth mechanisms. Thus, these chromatophores were found to be non-functional for organelle-specific purposes when compared with mitochondria and plastids. This distinction could have promoted the early evolution of photosynthetic organelles. The loss of genetic autonomy, that is, the loss of many genes from endosymbionts, occurred very early in evolutionary time. Taking into account the entire original endosymbiont genome, there are three main possible fates for genes over evolutionary time.

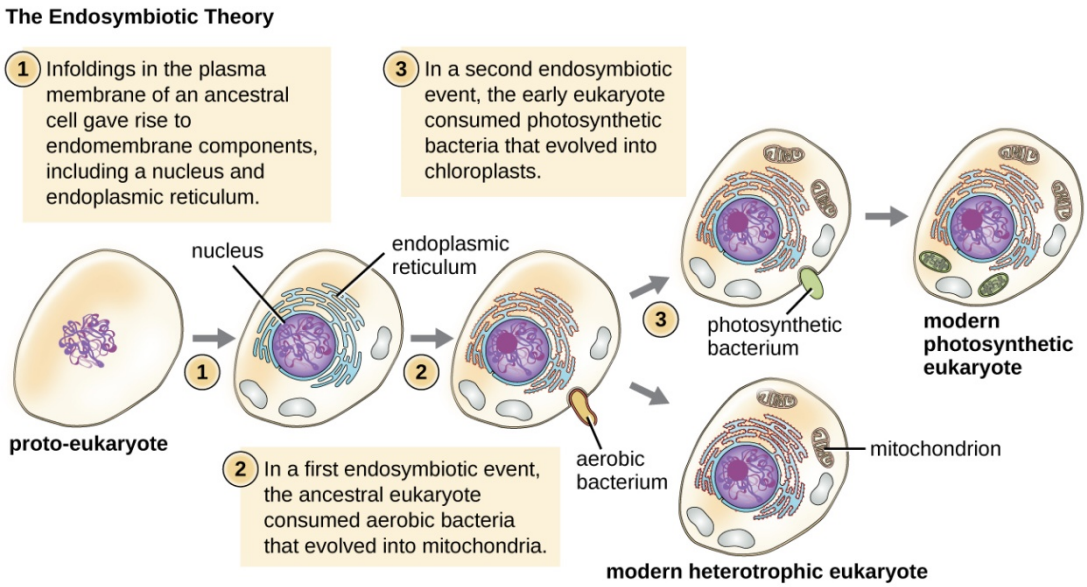
1) The first fate involves the loss of functionally redundant genes, in which genes that are already represented in the nucleus are eventually lost.
2) The second fate involves the transfer of genes to the nucleus. As organelle genomes have been greatly reduced over evolutionary time, nuclear genes have expanded and become more complex. As a result, many plastid and mitochondrial processes are driven by nuclear encoded gene products. In addition, many nuclear genes originating from endosymbionts have acquired novel functions unrelated to their organelles.

Several hypotheses have been proposed to explain the mechanism of gene transfer.

1) **The Complementary DNA (cDNA) hypothesis** involves the use of messenger RNA (mRNAs) to transport genes from organelles to the nucleus where they are converted to cDNA and incorporated into the genome. The cDNA hypothesis is based on studies of the genomes of flowering plants.
2) **The bulk flow hypothesis** is stating that escaped DNA, rather than mRNA, is the mechanism of gene transfer. According to this hypothesis, disturbances to organelles, including autophagy (normal cell destruction), gametogenesis (the formation of gametes), and cell stress, release DNA which is imported into the nucleus and incorporated into the nuclear DNA using non-homologous end joining (repair of double stranded breaks).

Endosymbiosis of Protomitochondra:

Endosymbiotic theory for the origin of mitochondria suggests that the proto-eukaryote engulfed a protomitochondria, and this endosymbiont became an organelle.



Steps in Endosymbiiotic Theory

- **Step 1**

  Prokaryotic cell membrane folded into cytoplasm
- **Step 2**

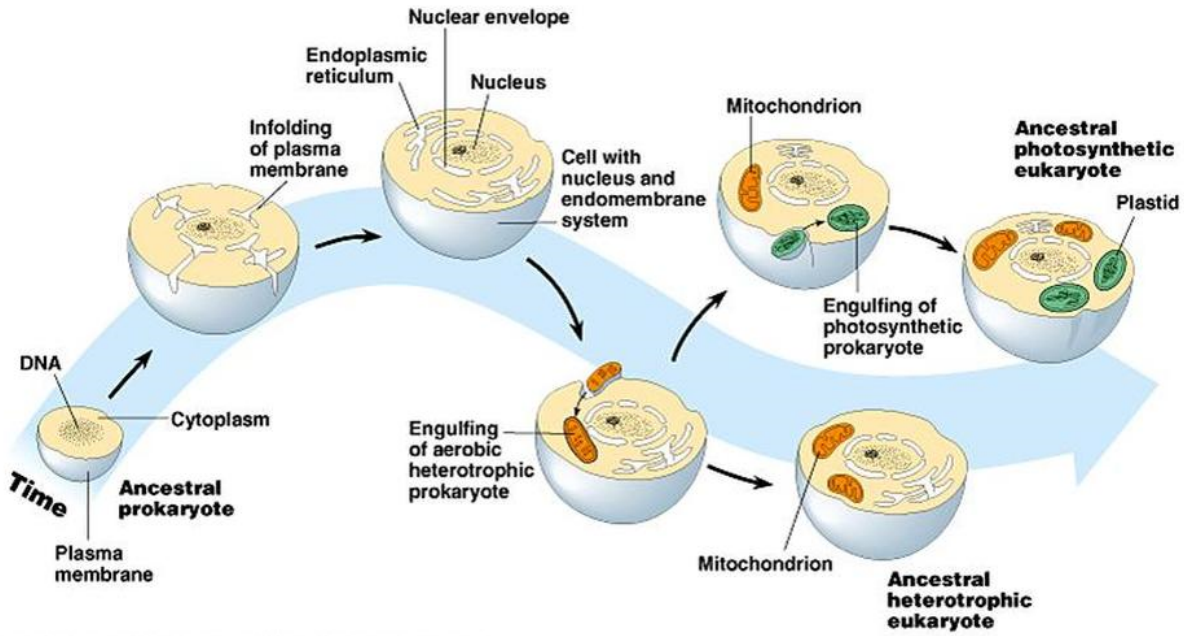
  Nuclear membrane, endoplasmic reticulum, and golgi body are now independent of external membrane
- **Step 3**

  Ancestoral eukaryote engulfed, but did not kill prokaryote
- **Step 4**

  The prokaryote survived inside the eukaryote and each evolved a dependence of each other
- **Step 5**

  In the ancestors of algae and land plants, photosynthetic prokaryotes were engulfed, but not killed
- **Step 6**

  The cells evolved dependence on each other. Multiple, independent symbioses led to different algal groups



Mitochondria:

The presence of deoxyribonucleic acid (DNA) in mitochondria and proteins, derived from mtDNA, suggest that this organelle may have been a prokaryote prior to its integration into the proto-eukaryote. Mitochondria are regarded as organelles rather than endosymbionts because mitochondria and the host cells share some parts of their genome, undergo mitosis simultaneously, and provide each other means to produce energy. The Endomembrane system and nuclear membrane were hypothesized to have derived from the protomitochondria.

Nuclear Membrane:

The presence of a nucleus is one major difference between eukaryotes and prokaryotes. Some conserved nuclear proteins between eukaryotes and prokaryotes suggest that these two types had a common ancestor. Another theory behind nucleation is that early nuclear membrane proteins caused the cell membrane to fold inwardly and form a sphere with pores like the nuclear envelope. This theory suggests that the vesicles leaving the protomitochondria may have formed the nuclear envelope.

Endomembrane System:

Modern eukaryotic cells use the endomembrane system to transport products and wastes in, within, and out of cells. The membrane of nuclear envelope and endomembrane vesicles are composed of similar membrane proteins. These vesicles also share similar membrane proteins with the organelle they originated from or are traveling towards. Prokaryotes do not have a complex internal membrane network like the modern eukaryotes, but the prokaryotes could produce extracellular vesicles from their outer membrane. After the early prokaryote was consumed by a proto-eukaryote, the prokaryote would have continued to produce vesicles that accumulated within the cell. Interaction of internal components of vesicles may have led to formation of the endoplasmic reticulum and contributed to the formation of Golgi apparatus.

Organellar Genomes:

Plastomes and Mitogenomes:

The human mitochondrial genome has retained genes encoding 2 rRNAs, 22 tRNAs, and 13 redox proteins. The third and final possible fate of endosymbiont genes is that they remain in the organelles. Plastids and mitochondria, although they have lost much of their genomes, retain genes encoding rRNAs, tRNAs, proteins involved in redox reactions, and proteins required for transcription, translation, and replication. There are many hypotheses to explain why organelles retain a small portion of their genome. The hydrophobicity hypothesis states that highly hydrophobic (water hating) proteins are not easily transported through the cytosol and therefore these proteins must be encoded in their respective organelles.

The Code Disparity Hypothesis:

This hypothesis states that the limit on transfer is due to differing genetic codes and RNA editing between the organelle and the nucleus. The redox control hypothesis states that genes encoding redox reaction proteins are retained in order to effectively couple the need for repair and the synthesis of these proteins. The final hypothesis states that the assembly of membrane proteins, particularly those involved in redox reactions requires coordinated synthesis and assembly of subunits.

Non-Photosynthetic Plastid Genomes

The majority of the genes in the mitochondria and plastids are related to the expression (transcription, translation and replication) of genes encoding proteins involved in either photosynthesis (in plastids) or cellular respiration (in mitochondria). Non-photosynthetic plastids tend to retain a small genome. There are two main hypotheses to explain this occurrence:

The essential tRNA hypothesis notes that there have been no documented functional plastid-to-nucleus gene transfers of genes encoding RNA products (tRNAs and rRNAs). As a result, plastids must make their own functional RNAs or import nuclear counterparts. The genes encoding tRNA-Glu and tRNA-fmet, however, appear to be indispensable. The plastid is responsible for haem biosynthesis, which requires plastid encoded tRNA-Glu (from the gene trnE) as a precursor molecule. Like other genes encoding RNAs, trnE cannot be transferred to the nucleus. In addition, it is unlikely trnE could be replaced by a cytosolic tRNA-Glu as trnE is highly conserved; single base changes in trnE have resulted in the loss of haem synthesis. The gene for tRNA-formylmethionine (tRNA-fmet) is also encoded in the plastid genome and is required for translation initiation in both plastids and mitochondria. A plastid is required to continue expressing the gene for tRNA-fmet so long as the mitochondrion is translating proteins. The limited window hypothesis offers a more general explanation for the retention of genes in non-photosynthetic plastids.[48] According to the bulk flow hypothesis, genes are transferred to the nucleus following the disturbance of organelles. Disturbance was common in the early stages of endosymbiosis, however, once the host cell gained control of organelle division, eukaryotes could evolve to have only one plastid per cell. Having only one plastid severely limits gene transfer as the lysis of the single plastid would likely result in cell death. Consistent with this hypothesis, organisms with multiple plastids show an 80-fold increase in plastid-to-nucleus gene transfer compared with organisms with single plastids.

Evidences for the Endosymbiontic Theory:

There are many lines of evidence that mitochondria and plastids including chloroplasts arose from bacteria.

1) New mitochondria and plastids are formed only through binary fission, the form of cell division used by bacteria and archaea.
2) If a cell's mitochondria or chloroplasts are removed, the cell does not have the means to create new ones.
3) Transport proteins called porins are found in the outer membranes of mitochondria and chloroplasts and are also found in bacterial cell membranes.
4) A membrane lipid cardiolipin is exclusively found in the inner mitochondrial membrane and bacterial cell membranes.
5) Some mitochondria and some plastids contain single circular DNA molecules that are similar to the DNA of bacteria both in size and structure.
6) Genome comparisons suggest a close relationship between mitochondria and Rickettsial bacteria.
7) Genome comparisons suggest a close relationship between plastids and cyanobacteria.
8) Many genes in the genomes of mitochondria and chloroplasts have been lost or transferred to the nucleus of the host cell. Consequently, the chromosomes of many eukaryotes contain genes that originated from the genomes of mitochondria and plastids.[60]
9) Mitochondrial and plastid ribosomes are more similar to those of bacteria (70S) than those of eukaryotes.
10) Proteins created by mitochondria and chloroplasts use N-formylmethionine as the initiating amino acid, as do proteins created by bacteria but not proteins created by eukaryotic nuclear genes or archaea.
11) Comparison of chloroplasts and cyanobacteria showing their similarities. Both chloroplasts and cyanobacteria have a double membrane, DNA, ribosomes, and thylakoids.

Secondary Symbiosis:

Primary endosymbiosis involves the engulfment of a cell by another free living organism. Secondary endosymbiosis occurs when the product of primary endosymbiosis is itself engulfed and retained by another free-living eukaryote. Secondary endosymbiosis has occurred several times and has given rise to extremely diverse groups of algae and other eukaryotes. One possible secondary endosymbiosis in process has been observed by Okamoto and Inouye (2005).

12.5. SUMMARY:

There are three different mitochondrial genome types found in plants and fungi. The first type is a circular genome that has introns (type 2) and may range from 19 to 1000 kbp in

length. The second genome type is a circular genome (about 20-1000 kbp) that also has a plasmid-like structure (type 3). The final genome type that can be found in plants and fungi is a linear genome made up of homogeneous DNA molecules (type 5). Protists contain the most diverse mitochondrial genomes, with five different types found in this kingdom. Type 2, type 3 and type 5 mentioned in the plant and fungal genomes also exist in some protists. One of these unique types is a heterogeneous collection of circular DNA molecules (type 4) while the other is a heterogeneous collection of linear molecules (type 6). The smallest mitochondrial genome sequenced to date is the 5,967 bp mtDNA of the parasite *Plasmodium falciparum*.

Each chloroplast contains around 100 copies of its DNA in young leaves, reduced to 15-20 copies in older leaves. They are usually packed into nucleoids which can contain several identical chloroplast DNA rings. Many nucleoids can be found in each chloroplast. Though chloroplast DNA is not associated with true histones, a histone-like chloroplast protein (HC) coded by the chloroplast DNA that tightly packs each chloroplast DNA ring into a nucleoid has been found in red algae. In primitive red algae, the chloroplast DNA nucleoids are clustered in the center of a chloroplast, while in green plants and green algae, the nucleoids are dispersed throughout the stroma. In *Arabidopsis thaliana*, the DNA is circular and composed of 1,54,478 bp with a pair of inverted repeats (26,264 bp), which are separated by small and large single copy regions. A total of 87 potential protein coding genes including 8 genes duplicated in the inverted repeat regions. Four ribosomal RNA genes and 37 tRNA genes representing 20 amino acids were assigned to the genome.

The Endosymbiotic Theory states that “some of the cell organelles like mitochondria and chloroplast in eukaryotic cells were once prokaryotic cells that were ingested by a large prokaryotic microbe”. This theory explains the origin of eukaryotic cells. **Endosymbiotic theory, Symbiogenesis**, or **Serial endosymbiotic theory** is the leading evolutionary theory of the origin of eukaryotic cells from prokaryotic organisms. The theory explains that mitochondria, plastids such as chloroplasts, and possibly other organelles of eukaryotic cells are descended from formerly free-living prokaryotes (more closely related to bacteria) taken one inside the other in endosymbiosis. The idea that chloroplasts were originally independent organisms that merged into a symbiotic relationship with other one-celled organisms.

12.6. TECHNICAL TERMS:

Mitogenome, Mitochondria, mtDNA, Plastome, Chloroplast, cpDNA, Endosymbiotic theory, Symbiogenesis, Serial endosymbiotic theory.

12.7. SELF ASSESSMENT QUESTIONS:

1) Give an account on mitochondrial genome and its application.
2) Write an essay on chloroplast genome and its importance.
3) Explain about the Endosymbiotic theory.

12.8. SUGGESTED READINGS:

1) De Robertis, E.D.P. and De Robertis, E.M.F. 2010. Cell and Molecular Biology, 8th Edition, Wolters Kluwer, USA.

2) Jean Brachet and Mirsky, Alfred E. (Eds): he Cell, Academic Press, Inc. New York, USA.

3) Stebbins, G.L., Chromosomal Evolution in Higher Plants, Edward Arnold Publications, London.

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5) Roy, S.C. and Kalyan Kumar De., 1977. Cell Biology, New Central Book Agency, Calcutta.

6) P.S. Verma & V. K. Agarwal, 2021. Cell Biology, Genetics, Molecular Biology, Evolution and Ecology. S. Chand And Company Limited, New Delhi-110044.

7) Alberts, B. et. al. (Molecular Biology of the Cell) – Garland Science

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Prof. V. Umamaheswara Rao

LESSON-13

STRUCTURAL ABERRATIONS OF CHROMOSOMES

13.0 OBJECTIVE:

- Students will understand what chromosomal aberrations is. The types of structural aberrations of chromosomes. Students also able to identify and significance of structural aberrations in growth and development of plant and animals along with their role in evolutionary development.

STRUCTURE:

13.1 Introduction
13.2 Structural Changes in Chromosomes
13.3 Types of Structural Changes in Chromosome
13.3.1. Deletion (or Deficiency)
13.3.2. Duplication
13.3.3. Inversion
13.3.4. Translocation
13.3.5. Variation in Chromosome Morphology
13.4 Summary
13.5 Technical Terms
13.6 Self-Assessment Questions
13.7 Suggested Readings

13.1. INTRODUCTION:

Chromosomal abnormalities, also referred to as chromosomal aberrations or anomalies, represent significant deviations from the typical chromosomal complement within an organism. These alterations can arise from errors during fundamental cellular processes, particularly meiosis and mitosis, which are critical for cell division and genetic transmission. The identification and characterization of these aberrations are typically achieved by comparing an individual's karyotype. These abnormalities are broadly classified into two principal categories 1. Numerical abnormalities - involves changes in the total count of chromosomes. 2. Structural abnormalities – change in physical architecture of one or more individual chromosomes. The integrity of chromosome structure is very important for normal cellular functions and the overall stability of the genome. In human beings, they are a major underlying cause of birth defects, intellectual disabilities, and an elevated risk for infertility or recurrent pregnancy loss. Furthermore, these aberrations are intimately linked to the development and progression of various cancers, often contributing to drug resistance. At the cellular level, chromosomal aberrations can induce changes in metabolic pathways, alter secretory phenotypes, and promote cellular heterogenization, which may lead to premature cellular senescence.

13.2. STRUCTURAL CHANGES IN CHROMOSOMES:

To understand the abnormalities of chromosome structure we should consider two important features of chromosome behavior. (1) During prophase I of meiosis, homologous regions of chromosomes show a great affinity for pairing and they often go through considerable contortions in order to pair. This property results in many curious structures observed in cells containing one normal chromosome set plus an aberrant set. (2) Structural changes usually involve chromosome breakage. The broken chromosome ends are highly "reactive" or "sticky", showing strong tendency to join with broken ends.

13.3. TYPES OF STRUCTURAL CHANGES IN CHROMOSOME:

Structural changes in chromosome may be of the following types 1. Deficiency or deletion: which involves loss of a broken part of a chromosome. 2. Duplication: involves addition of a part of chromosome. 3. Inversion in which broken segment reattached to original chromosome in reverse order. 4. Translocation in which the broken segment becomes attached to a nonhomologous chromosome resulting in new linkage relations. Structural abnormalities can occur in both homologous chromosomes of a pair or in only one of them. When both homologous chromosomes are involved, these are called structural homozygotes *e.g., deletion homozygote, duplication homozygote, etc.* When only one homologous chromosome is involved, it is called structural heterozygote.

13.3.1. Deletion (or Deficiency):

The simplest result of breakage is the loss of a part of a chromosome. Portions of chromosomes without a centromere (acentric fragments) lag in anaphase movement and are lost from reorganizing nuclei or digested by nucleases. Such loss of a portion of a chromosome (some genes) is called deletion. The chromosomes with deletions can never revert to a normal condition. If gametes arise from the cells having a deleted chromosome, this deletion is transmitted to the next generation. Further, a deletion can be 1. Terminal 2. Intercalary (interstitial).

i) **Terminal deletion:** In terminal deletion a terminal section of a chromosome is absent and it is resulted by only one break (Figure 13.1).

ii) **Intercalary Deletion:** In the intercalary deletion, an intermediate section or portion of chromosome is lost and it is caused by two breaks, one on either end of the deleted region. The chromosome is broken into three pieces; the middle one of which is lost and the remaining two pieces get joined again (Figure 13.1).

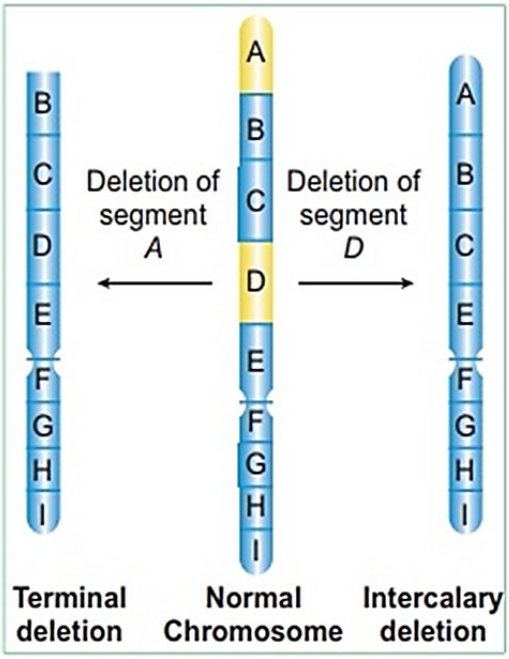

Figure-13.1: Terminal and Intercalary Deletion of Chromosomes

Deletion in Prokaryotes:

In *E. coli* deletion is up to 1 % of the chromosome. In λ phage 20% of genome is missing due to deletions.

Deletion in Human Beings:

Each deletion result in distinct set of syndromes (abnormalities). 1. Deletion in 22 chromosome results in "Philadelphia 22", which is associated with 'chronic myelogenous leukaemia'. 2. Deficiency in chromosome 5 results in 'cri-du-chat' (cry of cat syndrome). Having unique facial features and severe mental, physical retardness. 3. Wolf-Hirschhorn Syndrome results from a deletion of the most terminal portion of the short arm of chromosome 4. Affected individuals typically exhibit severe growth deficiency, profound intellectual disability, seizures, microcephaly, and a distinctive craniofacial appearance often described as a "Greek warrior helmet" due to a prominent forehead, hypertelorism, and a wide nasal bridge. 4. Jacobsen Syndrome caused by a deletion of genetic material from the end of the long arm (q arm) of chromosome 11. Clinical manifestations include developmental delays (affecting speech and motor skills), cognitive impairment, learning difficulties, and behavioral problems such as attention-deficit/hyperactivity disorder (ADHD). Distinctive facial features include small, low-set ears, widely set eyes, a broad nasal bridge, and downturned corners of the mouth. A significant proportion (over 90%) of affected individuals also have Paris-Trousseau syndrome, a lifelong bleeding disorder caused by platelet dysfunction. Heart defects and skeletal abnormalities are also frequently observed.

Genetic Significance of Deletion:

Organisms with homozygous deletion do not survive, it is lethal. Even individuals heterozygous for deletion (deletion in one of the homologous chromosomes) may not survive.

Smaller deletion in heterozygous condition can be tolerated by the organisms. Deletions are important cytological tools for mapping genes. Deletions play an important role in species formation and creating variability through chromosomal mutations. Pseudodominance results in deletion.

13.3.2. Duplication:

The **presence of a part of a chromosome in excess** of the normal complement is known as **duplication.** Extra segments in a chromosome may arise in a variety of ways such as follows:

i) Tandem Duplication: Duplicated region is situated just by the side of the normal corresponding section of the chromosome and the sequences of genes are the same in normal and duplicated region. For example, if the sequence of genes in a chromosome is ABCD.EFGH (The full stop depicts the centromere) and if the chromosomal segment containing the genes BCD is duplicated, the sequence of genes in tandem duplication will be ABCDBCD.EF GH (Figure 13.2). E.g.: Hemoglobins specialized for different life stages, varying numbers of milk-digesting Lactases among human populations, and an evolving arsenal of venom proteins in rattlesnakes.

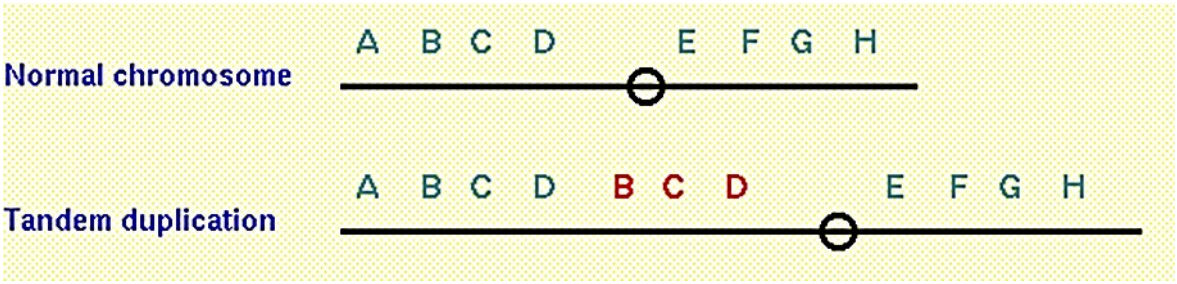

Figure-13.2: Tandem Duplication of Chromosomes

ii) Reverse Tandem Duplication: Here, the sequence of genes in the duplicated region of a chromosome is just the reverse of a normal sequence. The sequence of genes due to reverse tandem duplication will be reversed. E.g.: BCD is duplicated; the sequence of genes in tandem duplication will be ABCDDCB.EF GH (Figure 13.3).

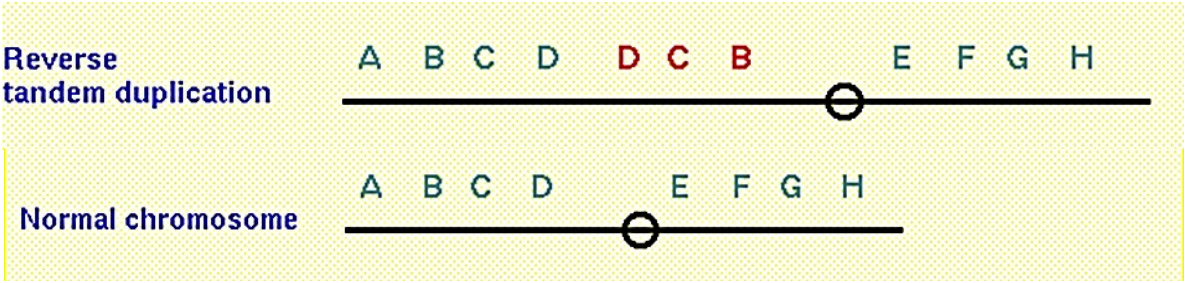

Figure-13.3: Reverse Tandem Duplication of Chromosomes

iii) **Displaced Duplication:** In this case the duplicated region is not situated adjacent to the normal section. The segment is repeated somewhere away from its original location but on the same arm (homobrachial displacement Figure 13.4) or on the other arm (heterobrachial displacement Figure 13.5). E.g.: *Drosophila* bar eye

Figure-13.4: Homobranchial duplication

Figure-13.5: Heterobranchial Duplication

iv) **Transposed Duplication:** The duplicated portion of chromosome becomes attached to a non-homologous chromosome. For example, if ABCD.EFGH and LMNO.PQRS represent the gene sequences of two nonhomologous chromosomes, a transposed duplication will result into chromosomes with gene sequence.

ABCD.EFGH **LMNO.PQBCDRS**

Such a transposed duplication may be either **interstitial (e.g., LMN BC OPQ. RST) or terminal (i.e., LMN OPQ. RST BC).**

v) **Non-homologous/Extra-Chromosomal Duplication:** In the presence of centromere the duplicated part of a chromosome act as independent chromosome (Figure 13.6). The retrogene formation and the subsequent duplication led to new enzymatic activities, which determined the evolution of a novel phenolic pathway in Brassicaceae. Eg: P-450 family in Brassicaceae.

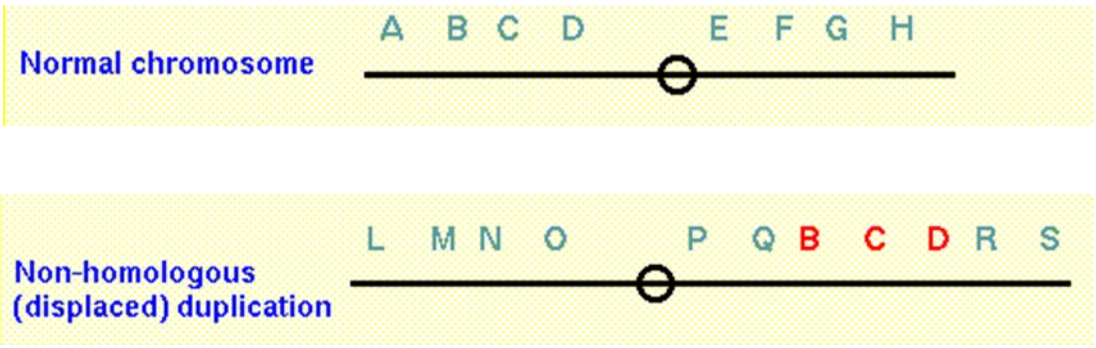

Figure-13.6: Non-Homologous Duplication of Chromosomes

Genetical Effects of Duplication:

1. Bar eye in *Drosophila* - (a) a reverse repeat in chromosome 4 causes eyeless dominant (Ey) (b) a tandem duplication in chromosome 3 causes confluens (Co) resulting in thickened veins, and (c) another duplication causes hairy wing (Hw). 2. In humans, unequal crossing over between homologous chromosomes results in deletions, tends to occur Lepore and Kenya variants of adult haemoglobin (HbA), both causing anaemia (i.e., one type of thalassemia*).* 3. **Charcot-Marie-Tooth Disease Type 1A (CMT1A) caused due to** tandem DNA duplication of a 1.4-Mb to 1.5-Mb genomic fragment on chromosome 17. Clinically, CMT1A is characterized by progressive muscle weakness and atrophy, particularly affecting the feet, lower legs, hands, and forearms, often resulting in repeated ankle sprains and sensory changes (paresthesia). Symptoms typically manifest during childhood. The disease is inherited in an autosomal dominant pattern.

13.3.3. Inversion:

Inversion involves a rotation of a part of a chromosome or a set of genes by 180°on its own axis. It essentially involves occurrence of breakage and reunion. The net result of inversion is neither a gain nor a loss in the genetic material but simply a rearrangement of the gene sequence. An inversion can occur in the following way: suppose that the normal order of segments within a chromosome is 1-2-3-4.5-6, 7; breaks occur in regions 2-3 broken piece is reinserted in reverse order. This results in an inverted chromosome having segments 1-3-2-4.5-6-7 (Figure 13.7).

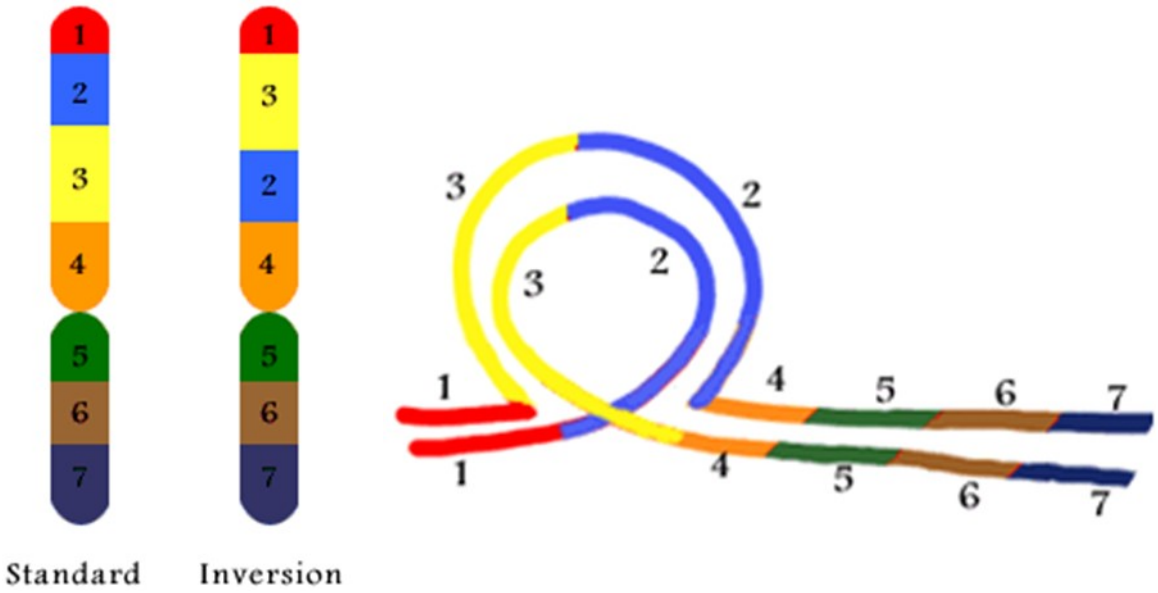

Figure-13.7 Inversion of Chromosomes

The location of the centromere relative to inverted segment determines the genetic behaviour of the chromosomes. If the centromere is not included in the inversion it is called paracentric inversion (Figure 13.8) and when inversion includes the centromere it is called pericentric inversion (Figure 13.9).

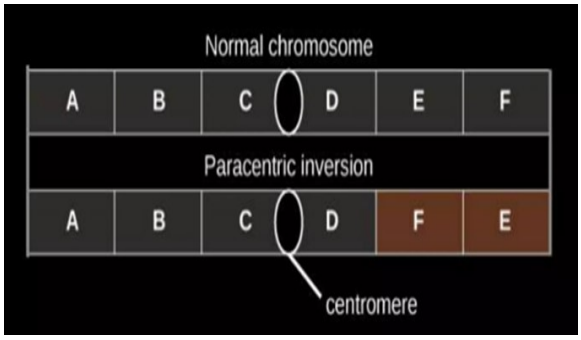

Figure-13.8: Paracentric Inversion

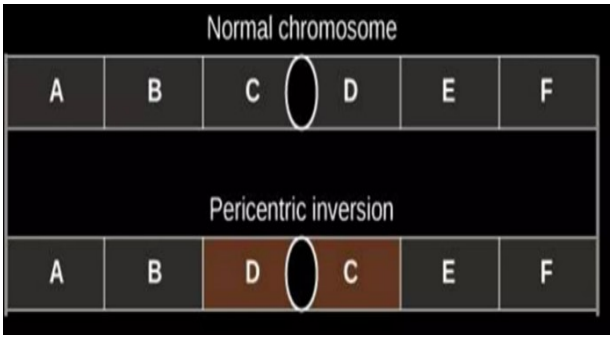

Figure-13.9: Pericentric Inversion

Advantages of Inversions:

Fertility of inversion homozygotes and sterility of inversion heterozygotes lead to establishment of two group (or varieties) which are mutually fertile but do not breed well with the rest of the species. Both varieties evolve in different directions and later become reproductively isolated species. Eg: *Drosophila*

Genetic Effects of Inversion:

The carriers of inversions are phenotypically in normal state, however the increased risk of infertility, recurrent miscarriage, or the birth of offspring with congenital anomalies and learning difficulties may occur.

13.3.4. Translocation:

The shifting or transfer of a part of a chromosome or a set of genes to a non-homologous one, is called translocation. There is no addition or loss of genes during translocations, only a rearrangement (*i.e., change* in the sequence and position of a gene). Translocations may be of following three types: 1. Simple translocation 2. Shift translocation 3. Reciprocal translocation.

1) **Simple Translocations:** They involve a single break in a chromosome. The broken piece gets attached to one end of a nonhomologous chromosome (Figure 13.10A).
2) **Shift Translocations:** In this type of translocation, the broken segment of one chromosome gets inserted interstitially in a nonhomologous chromosome (Figure 13.10B).
3) **Reciprocal Translocations:** In this case, a segment from one chromosome is exchanged with a segment from another nonhomologous one, so that in reality two translocation chromosomes are simultaneously achieved (Figure 13.10C).

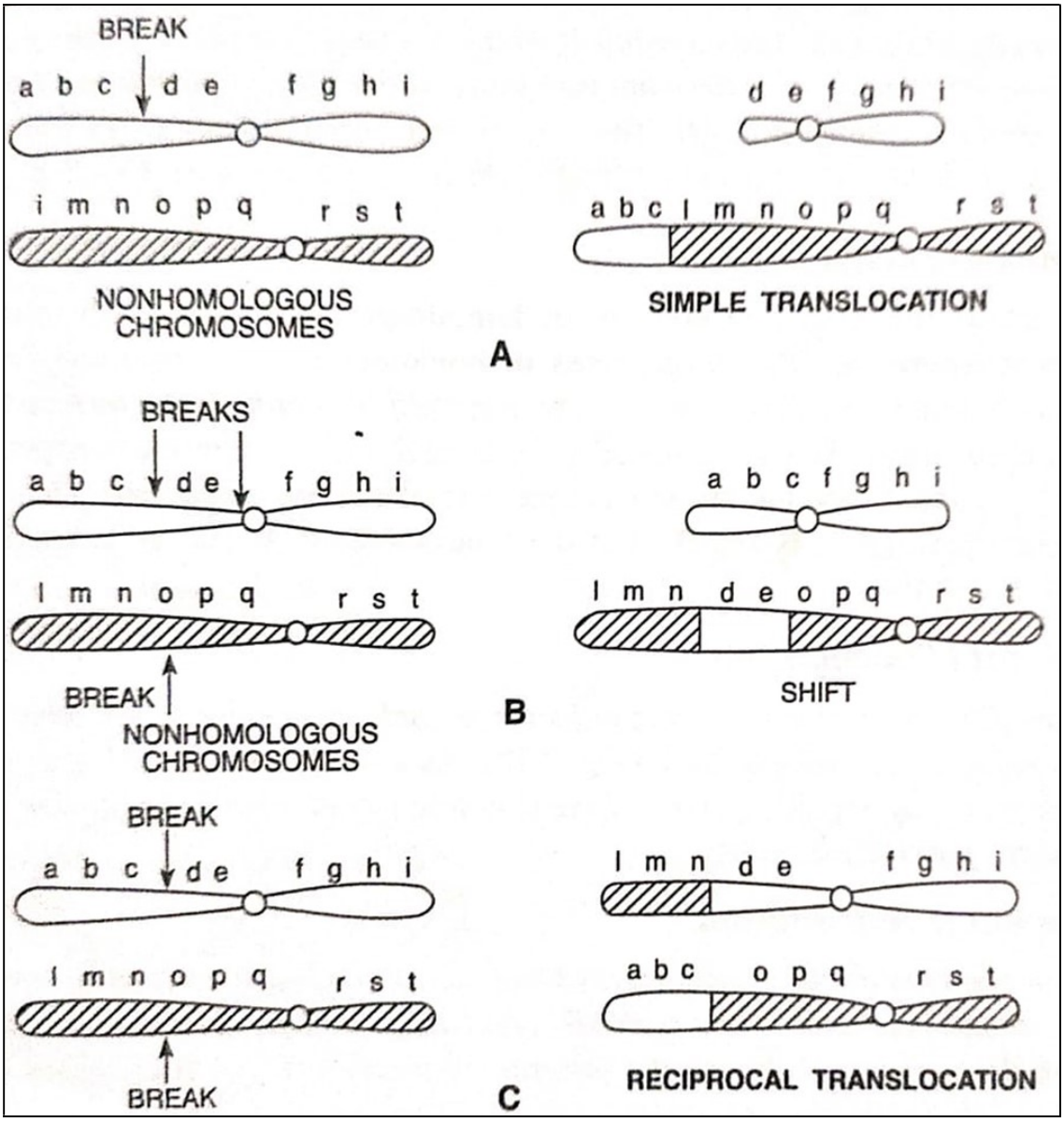

Figure-13.10: Types of Chromosomal Translocations

The exchange of chromosome parts between nonhomologous chromosomes creates new linkage relationships (Figure 13.11). Such translocations also drastically change the size of a chromosome as well as the position of its centromere. These are of two types

1) **Homozygous translocation: Both homologous chromosomes carry the same translocation**. This means that a segment of one chromosome has been exchanged with a segment of another non-homologous chromosome, and **the same exchange has occurred in both copies of the chromosome pair**.
2) **Heterozygous translocation:** A **heterozygous translocation** occurs when **only one of the two homologous chromosomes in a pair** carries a translocation, while the other remains in its normal configuration. This type of chromosomal rearrangement often has significant implications for **meiosis, fertility, and inheritance**.

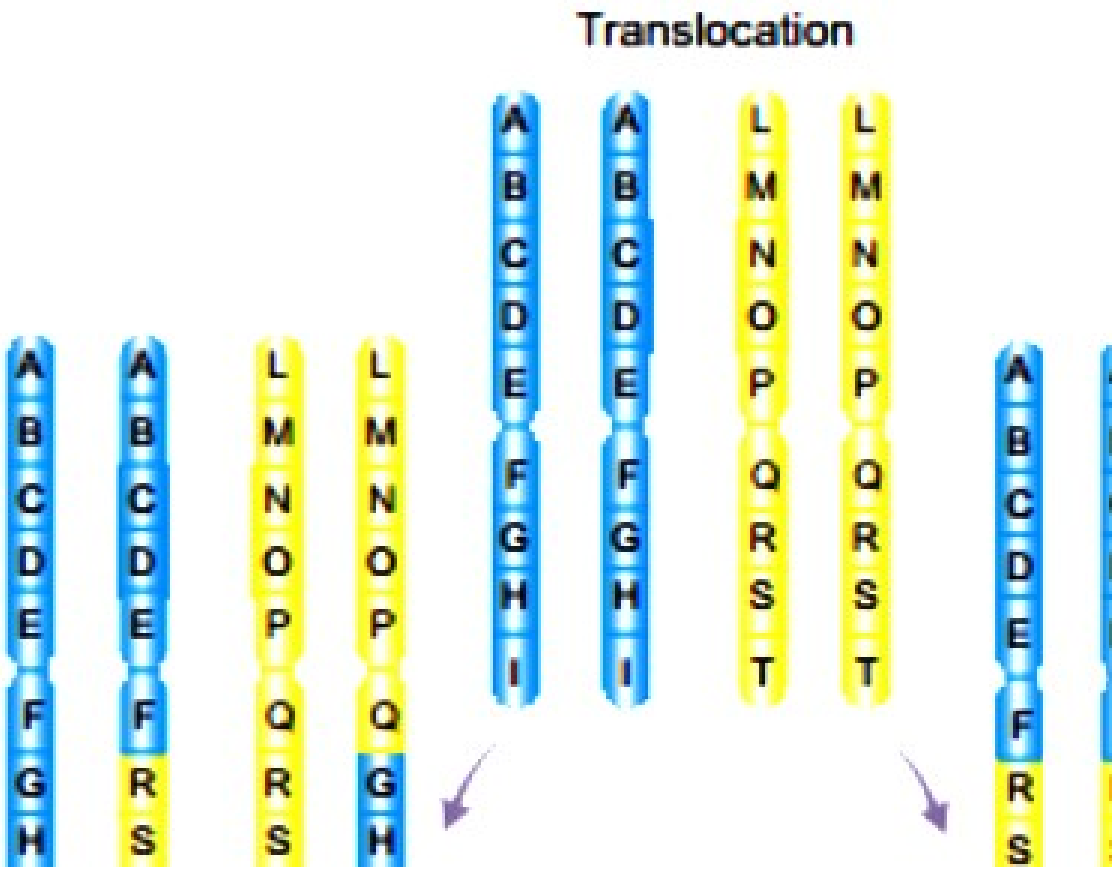

Figure-13.11: Homo and Heterozygous Translocation

The first case of translocation was studied in the evening primrose (*Oenothera)* which was originally described as a mutation by de Vries. In Drosophila, humans and other animals, translocations have also been induced by X-rays. Patients with chronic myelocytic (myologenous) leukemia (a kind of cancer) display an interesting chromosomal abnormality. In the bone marrow and in cells derived from it, is present a short chromosome, called the Philadelphia (Ph1) chromosome.

13.3.5 Variation in Chromosome Morphology:

Various changes in chromosome structure often produce variation in chromosome morphology such as isochromosomes, ring chromosomes and Robertsonian translocation.

1) **Isochromosomes:** An isochromosome is a chromosome in which both arms are identical. It is thought to arise when a centromere divides in the wrong plane, yielding two daughter chromosomes, each of which carries the information of one arm only but present twice (Figure 13.12). For example, telocentric X chromosome of Drosophila may be changed into an “attached-X” which is formed due to mis-division of the centromere. It can lead to genetic conditions like Turner syndrome and Down syndrome.

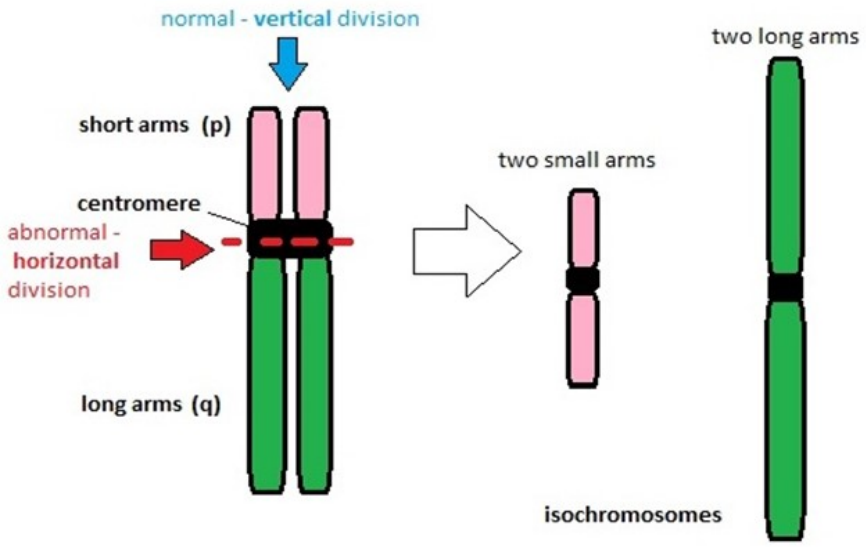

Figure-13.12: Isochromosomes

2) **Ring Chromosomes:** Chromosomes are not always rod-shaped. Occasionally ring chromosomes are encountered in higher organisms. Sometimes breaks occur at each end of the chromosome and broken ends are joined to form a ring chromosome (Figure 13.13). The most common feature of this condition is recurrent seizures (epilepsy) in childhood. The seizures may occur during the day or at night during sleep.

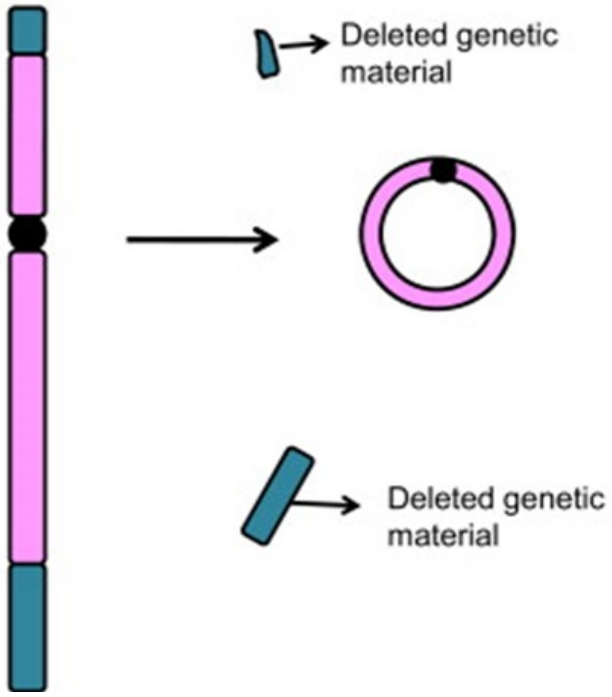

Figure-13.13: Ring Chromosome

3) **Robertsonian Translocation:** Sometimes whole arm fusions occur in the non-homologous chromosomes. It is called Robertsonian translocation. The break in one chromosome is near the front of the centromere and the break in the other chromosomes is immediately behind its centromere (Figure 13.14). The resultant smaller chromosome consists of largely inert heterochromatic material near the centromere. It normally contains no essential genes and tends to become lost.

Robertsonian translocation results in a reduction of the chromosome number. The Robertsonian translocation is found to have a role in the evolution of human beings. Humans have 46 chromosomes whereas the great apes (Chimpanzees, Gorillas and Orangutans) have 48 chromosomes. It is suspected that structural rearrangements of chromosomes may lead to reproductive isolation and the formation of new species. Robertsonian translocations cause chromosomal deletions or addition and result in syndromes of multiple malformations, including trisomy 13 (Patau syndrome) and trisomy 21 (Down syndrome).

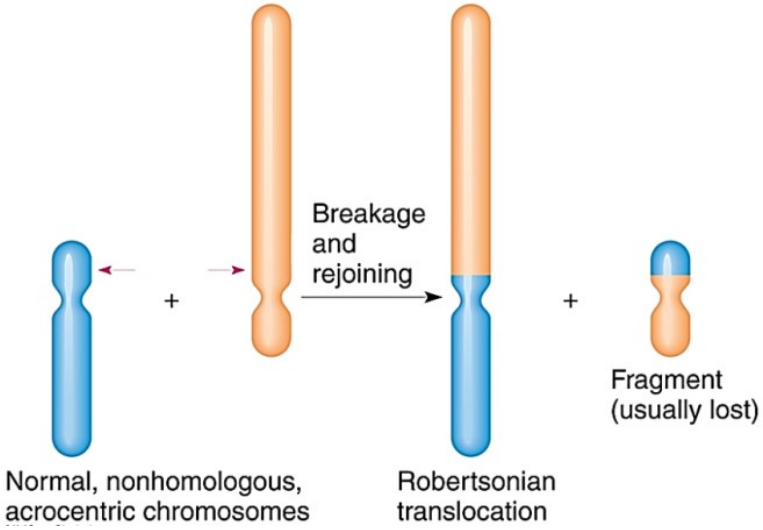

Figure-13.14: Robertsonian Translocation of Chromosomes

The overview of the chromosomal structural aberrations was represented in the following table 13.1.

Table-13.1: Overview of Structural Chromosomal Aberrations

Aberration Type	Primary Characteristic	Description	Net Genetic Material Change	Common Mechanism	Clinical Example
Deletion	Loss of segment	A portion of a chromosome is missing. Can be terminal (at end) or interstitial (within arm).	Loss	Chromosome breakage, unequal crossing over	Cri-du-chat Syndrome (5p-)
Duplication	Gain of segment	An extra copy of a chromosomal segment is present. Can be tandem, reverse tandem, or displaced.	Gain	Unequal crossing over, replication errors	Charcot-Marie-Tooth Type 1A (PMP22 dup)
Inversion	Segment reversal	A chromosomal segment is flipped 180 degrees and reinserted. Can be paracentric (no centromere) or pericentric (includes centromere).	No net change (balanced)	Chromosome breakage, ectopic recombination	(Often leads to reproductive issues in carriers)
Reciprocal Translocation	Exchange between non-homologous chromosomes	Segments from two different non-homologous chromosomes are exchanged.	No net change (balanced in carrier)	Chromosome breakage, non-homologous end-joining	Philadelphia Chromosome (CML)
Robertsonian Translocation	Fusion of acrocentric chromosomes	Fusion of long arms of two acrocentric chromosomes (13, 14, 15, 21, 22) with loss of short arms.	No net change (balanced in carrier, but 45 chromosomes)	Centromeric fusion after breaks in acrocentrics	Familial Down Syndrome
Ring Chromosome	Circularization	Chromosome ends break and fuse to form a ring.	Often loss of distal material	Two breaks and fusion, telomere dysfunction	(Variable clinical presentations)
Isochromosome	Mirror-image arms	An abnormal chromosome with two identical arms (e.g., two p arms or two q arms).	Partial monosomy and partial trisomy	Transverse centromere division	Turner Syndrome (i(Xq))

13.4. SUMMARY:

Structural chromosomal aberrations refer to alterations in the physical structure of chromosomes, leading to changes in the arrangement or dosage of genetic material. These abnormalities typically arise from errors during cell division (meiosis or mitosis), DNA replication, or DNA repair, often involving breakage and incorrect rejoining of chromosome segments. Common types include deletions (loss of a chromosome segment), duplications (presence of an extra copy of a segment), inversions (a segment being reversed within the chromosome), and translocations (exchange of segments between non-homologous chromosomes). While some structural aberrations can be balanced (no net gain or loss of genetic material, often with no immediate clinical effect on the carrier but potential reproductive issues), others are unbalanced (resulting in missing or extra genetic material), frequently leading to developmental delays, intellectual disabilities, birth defects, or increased risk of certain diseases like cancer.

13.5. TECHNICAL TERMS:

Aberration, Karyotype, Idiogram, Breakpoint, Robertsonian Translocation, Deletion, Inversion.

13.6. SELF-ASSESSMENT QUESTIONS:

1) Discuss the fundamental difference between balanced and unbalanced structural
2) chromosomal aberrations.
3) Describe the two main types of inversions, including how they are distinguished
4) cytogenetically. Explain the molecular events that lead to their formation.
5) Discuss the clinical significance of translocations, including their role in genetic disorders,
6) recurrent miscarriages, and cancer.
7) How would you cytogenetically distinguish between a pericentric and a Paracentric
8) Inversion.

13.7. SUGGESTED READINGS:

1) Thompson & Thompson Genetics in Medicine 8th Edition by Robert L. Nussbaum, Roderick R. McInnes, and Huntington F. Willard. ELSEVIER1600 John F. Kennedy Blvd. Ste 1800 Philadelphia, PA 19103-2899.
2) Medical Genetics, 6th Edition by Jorde, Carey, Bamshad, and White. ELSEVIER1600 John F. Kennedy Blvd. Ste 1800 Philadelphia, PA 19103-2899.
3) Human Molecular Genetics 5th Edition by Tom Strachan and Andrew Read. Garland Science Publishers, UK.
4) Gardner and Sutherland's Chromosome Abnormalities and Genetic Counselling by R. J. McKinlay Gardner and David J. Amor. Oxford University Press, U.K.

Prof. A. Amruthavalli

LESSON-14

NUMERICAL ABERRATIONS OF CHROMOSOMES

14.0 OBJECTIVE:

- Students will understand the types of numerical aberrations of chromosomes. Students also able know the significance of numerical aberrations in growth and development of plant and animals along with their role in evolutionary development.

STRUCTURE:

14.1 Introduction

14.2 Overview of Chromosomal Aberrations

14.3 Classification of Numerical Chromosomal Aberrations

14.3.1. Euploidy

14.3.2. Aneuploidy

14.4 Summary

14.5 Technical Terms

14.6 Self-Assessment Questions

14.7 Suggested Readings

14.1. INTRODUCTION:

Chromosomes are intricate, thread-like structures found within the nucleus of eukaryotic cells, serving as the organized repositories of an organism's genetic information. They are composed of tightly coiled deoxyribonucleic acid (DNA) intricately bound to various proteins, primarily histones. In humans, the typical somatic cell maintains a diploid (2n) complement of 46 chromosomes, arranged into 23 homologous pairs. This includes 22 pairs of autosomes and one pair of sex chromosomes, which determine biological sex (XX for females and XY for males). Gametes contain haploid (n) set of chromosomes. Sometimes irregularities occur in nuclear division or “accidents” may befall interphase chromosomes so that cells or entire organisms with aberrant genomes may be formed. Such chromosomal aberrations may include whole genomes and entire single chromosomes. Changes in number of whole chromosomes are called heteroploidy.

Heteroploidy may involve entire sets of chromosomes (euploidy), or loss or addition of single whole chromosomes (aneuploidy). Each may produce phenotypic changes, modifications of phenotypic ratio, or alteration of linkage groups. These aberrations are intimately linked to a wide array of human diseases and are a hallmark of spontaneous abortions, congenital birth defects, and nearly all human tumors. Disruptions to this numerical stability can lead to wide-ranging and severe consequences, from the earliest stages of conception through to the later stages of life, highlighting its critical role as a

cornerstone of biological function. The chromosomal constitution of an individual can be visually represented through a karyotype. Karyotyping serves as a foundational diagnostic tool in cytogenetics, enabling the detection of both numerical and large structural chromosomal abnormalities.

14.2. OVERVIEW OF CHROMOSOMAL ABERRATIONS:

Broadly, chromosomal aberrations are categorized into two principal types: numerical and structural. Numerical aberrations, also known as heteroploidy, involve changes in the total count of chromosomes. This can manifest as variations in entire sets of chromosomes, a condition termed euploidy, or more commonly, as the gain or loss of individual chromosomes, known as aneuploidy. The precise and equal distribution of genetic material into daughter cells during cell division is a fundamental biological imperative for all eukaryotic organisms. Any failure in this regulated process can lead to genomic alterations in the resulting daughter cells.

14.3. CLASSIFICATION OF NUMERICAL CHROMOSOMAL ABERRATIONS:

Numerical chromosomal aberrations can be broadly categorized into two main groups 1. Euploidy and 2. Aneuploidy, based on whether the change involves entire sets of chromosomes or individual chromosomes (Figure 14.1).

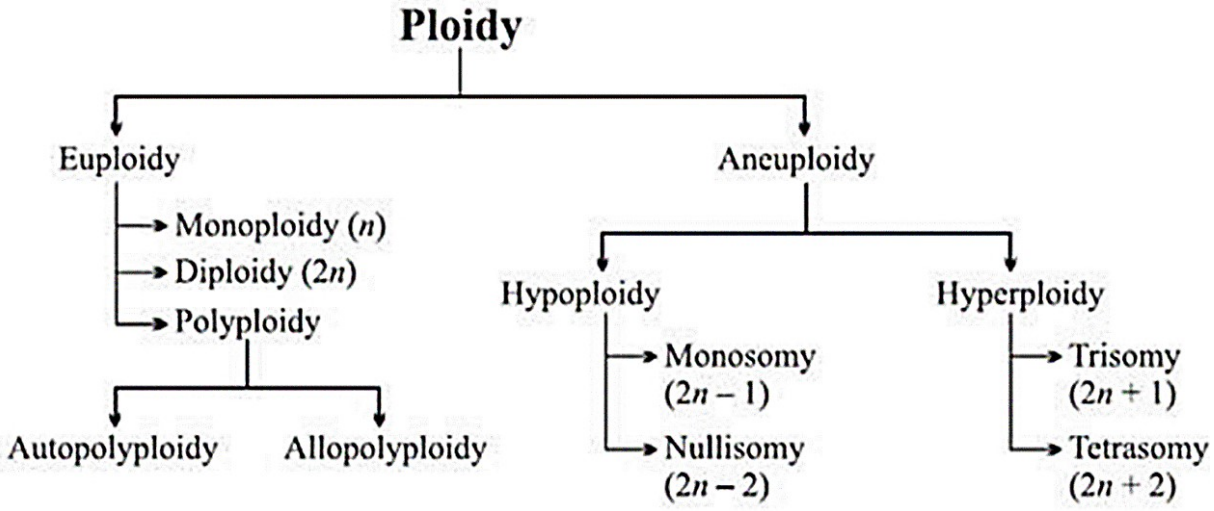

Figure-14.1: Types of Numerical Aberrations

14.3.1. Euploidy:

The term euploidy (Gr., *eu = even or true; ploid = unit)* describes a condition in which a cell or an organism possesses a chromosome number that is an exact multiple of the haploid number (n). Euploidy is of three types i. Monoplidy ii. Diploidy iii. Polyploidy based on the number of chromosomal sets.

i. Monoploidy or Haploidy (n):

Monoploidy refers to the presence of a single complete set of chromosomes, typically found in normal gametes (sperm and egg cells). In humans, a haploid set consists of 23 chromosomes. Monoploidy is common in plants and rare in animals. e.g., 7 in barley and 10 in corn.

a) **Origin and Production of Monoploids:** Monoploids in some cases are found normally and are produced due to parthenogenesis, as in male (drone) hymenopteran insects such as bees, wasps and ants. In these insects, queen and workers are diploid females (Figure 14.2).

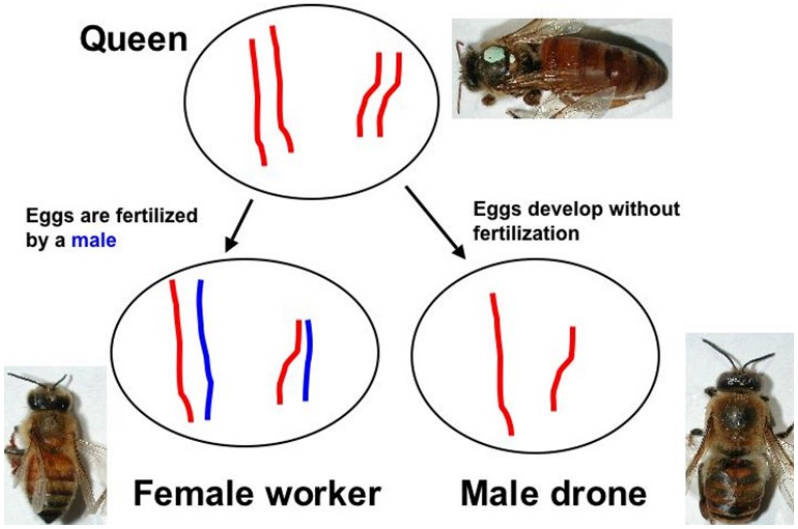

Figure-14.2: Origin of Monoploidy in Bees

In angiosperms (flowering plants) monoploids may also originate spontaneously due to parthenogenetic development of egg. Eg: tomatoes and cotton. Rarely monoploid plants may originate from the pollen tube, synergids and antipodals of the embryo sac and are called androgenic monoploids or androgenic haploids (Figure 14.3).

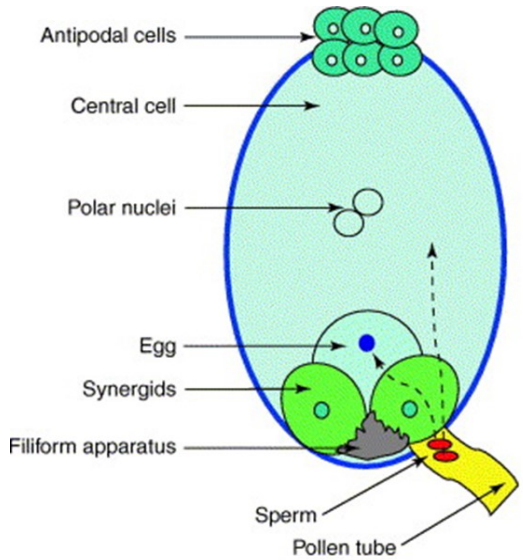

Figure-14.3: Centres of Monoploidy Origin in Embryosac

Monoploids can be produced by artificial means by the following methods: (1) X-ray treatments (2) delayed pollination (3) cold treatment (4) colchicine treatment (5) distant (interspecific or intergeneric) hybridization (6) anther or pollen culture. Among these techniques, the most important ones are distant hybridization and another culture.

Distant Hybridization:

Interspecific crosses in genera of Solanaceae (e.g., *Solanum* and *Nicotiana*) have been employed for the production of both parthenogenetic and androgenic monoploids. E.g.: Potato Kasha and Kao (1970) have used this technique for producing monoploids in large number in barley. When diploid barley, *Hordeum vulgare,* is pollinated using a diploid wild relative called *Hordeum bulbosum,* fertilization occurs, but during the ensuing somatic cell divisions, the chromosomes of *H. bulbosum* are preferentially eliminated from the zygote, resulting in a haploid embryo (caused by a genetic incompatiability between the chromosomes of the different species) (Figure 14.4). The resulting haploids can be doubled with colchicine treatment. This technique has resulted in the rapid production of new varieties of barley and applied to other plant species also.

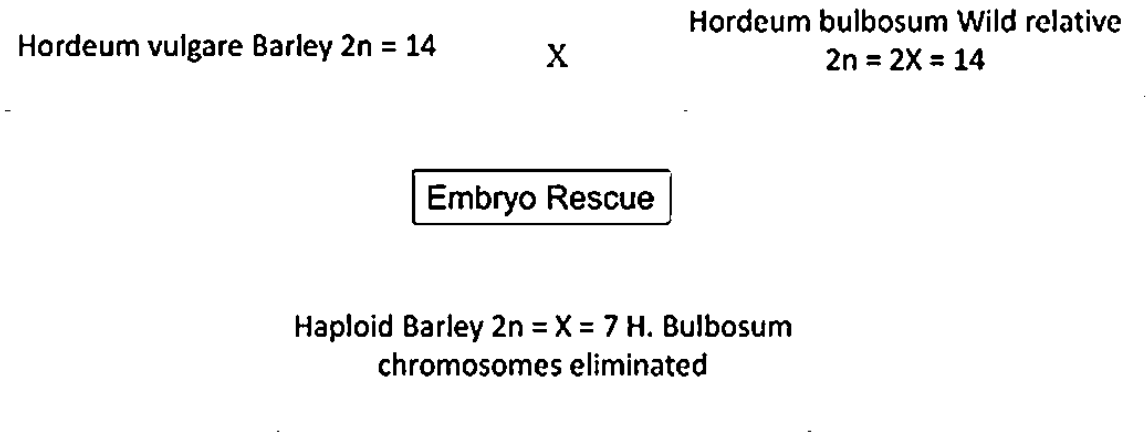

Figure-14.4: Distant Hybridization in Barley

Anther or Pollen Culture:

The production of monoploids in tobacco plant by anther and pollen culture was demonstrated by Prof. S.C. Maheshwari. Pollen grain, may be induced by cold treatment to grow instead into an embryoid, a small dividing mass of cells. The embryoid may be grown on agar to form a monoploid plantlet, which can then be potted in soil to mature (Figure 14.5). E.g.: soya bean, rice, wheat, mustard, and tobacco. Presently, this technique is regarded as a very potential source of monoploid production.

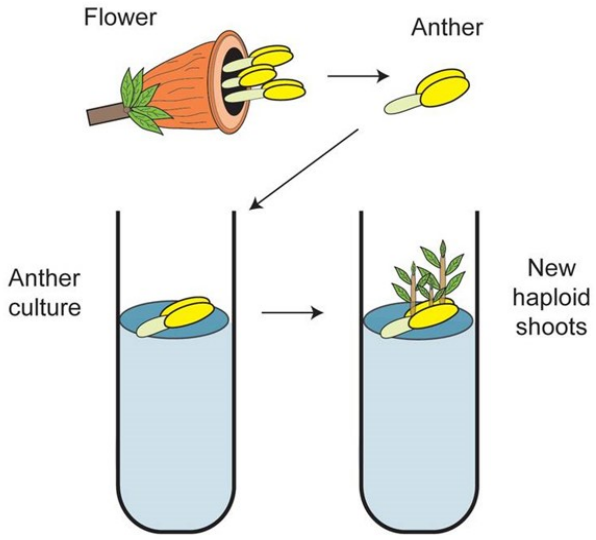

Figure-14.5: Anther Culture Procedure

Morphology of Monoploids:

Monoploid plants have reduced size of all vegetative and floral parts. The size of seed and stomata as well as diameter of pollen was found smaller in monoploids than in the diploids. Even the size of nucleus (or the nuclear volume) of a monoploid often was found to be just half than the nucleus of the diploid cell.

Cytology of Monoploids:

In monoploids, each chromosome is represented only once due to which there is no zygotene pairing and all the chromosomes appear as univalents on the metaphase plate at the time of meiosis. During anaphase each chromosome moves independently of the other and goes to either of the two poles. According to law of probability the chance that a particular chromosome will go to a particular pole as half and the chance that all the chromosomes of a monoploid set will go to the same pole is ½ X ½ X ½....n times, where n = number of chromosomes in the monoploid set. So, the frequency of gametes with the haploid set or 'n' number of chromosomes will be (½) n. This indicates that higher the number of chromosomes in a haploid set, lesser will be the frequency of all of them being included in the same gamete.

Gametes containing less than the haploid number of chromosomes are normally not viable. Therefore, monoploid organisms are highly sterile. For instance, a monoploid in maize (2n = 20) will have 10 chromosomes and the number of chromosomes in a gamete can range from 0–10. Consequently, considerable sterility is found in such monoploid maize plants. In contrast, in monoploid male honey bees during spermatogenesis the meiosis is bypassed by mitosis. As a result, their sperms are haploid and viable.

Uses of Monoploids:

1. In a monoploid, since there is only one copy of each chromosome and only one allele of each gene, each gene is expressed whether it is dominant or recessive 2. This

facilitates genetic experiments and this is the reason why microorganisms have been able helpful in genetic studies 3. Success has been achieved in developing monoploid strains of *Nicotiana, Datura* and *Triticum*. 4. We can develop pure breeding strains which are resistant to the insects.

ii. Diploidy:

It represents the normal chromosomal state for somatic cells in sexually reproducing organisms, containing two complete sets of chromosomes (2n). The standard human diploid number is 46 chromosomes (2n=46).

iii. Polyploidy:

Any organism with more than two genomes (2x) is called a polyploid. Ploidy levels higher than tetraploid are not commonly encountered in natural populations, however most of the crops and ornamental flowers are polyploids. e.g., wheat (Hexaploid 6x), strawberries (octaploid, 8x). Generally, polyploidy is common in plants (more common in monocots) but rare in animals.

Types of Polyploidy: There are following three different kinds of polyploids.

a) Autopolyploids
b) Allopolyploids and
c) Segmental or autoallopolyploids

Autopolyploids:

The autopolyploids are those polyploids, which consist of same basic set of chromosomes multiplied. For example, if a diploid species has two similar sets of chromosomes or genomes (AA), an autotriploid will have three similar genomes (AAA), and an autotetraploid, will have four such genomes (AAAA).

Origin and Production of Autopolyploids:

The autopolyploids may occur in nature or may be produced artificially. In nature, their autopolyploidy nature is deduced by their meiotic behaviour. E.g.: 'doob' grass (*Cynodon dactylon).*

Polyploids may arise naturally by following means. (i) As a result of interference with cytokinesis, once chromosome replication has occurred. (ii) It may occur either in somatic tissue which gives rise to tetraploid branches or during meiosis which produces unreduced gametes. (iii) Polyploidy may occur due to chilling. E.g.: Seedless varieties of watermelons, sugar beet, tomato, grapes, marigolds, snap dragons, apples and banana.

Induced Autopolyploidy:

The autopolyploidy have been induced in many plant and animal cells by artificial means such as chemical (e.g., chloral hydrate, colchicine, sulphanil amide, mercury chloride,

hexachlorcyclohexane, etc.), radioactive substances (e.g., radium and X-ray) and temperature shocks. These inducers usually disturb the mitotic or meiotic spindle and cause non-segregation of already duplicated chromosomes, during cell divisions.

Colchicine:

Colchicine is an alkaloid obtained from the corms of plants *Colchicum autmunale* and *C. luteum*. Its aqueous solution is found to prevent the formation and organization of spindle fibres, so the metaphase chromosomes of the affected cells called C-metaphase or colchicine metaphase do not move to a metaphase plate and remain scattered in the cytoplasm. Even the process of cytokinesis is prevented by colchicine and with duplications of chromosomes the number goes on increasing. As colchicine interferes with spindle formation, its effects are limited to dividing and meristematic cells.

Effects of Autopolyploidy:

Autopolyploidy results in gigantism of plant cells, *i.e., leaves, flowers and fruits of an* autopolyploid are larger in size than a diploid plant.

1) With the increase in cell size, the water content increases which leads to a decrease in osmotic pressure. This results into loss of resistance against frost, etc.
2) Due to slower rate of cell division, the plant's growth rate decreases. This leads to a decrease in auxin supply and a decrease in respiration.
3) Due to slow growth rate, the time of blooming of an autopolyploid is delayed.
4) At higher ploidy level, such as autooctoploids, the adverse effects become highly pronounced and lead to the death of the plants.
5) Polyploid varieties with an even number of genomes (E.g.: tetraploids) are often fully fertile, whereas those with an odd number (E.g.: triploids) are highly sterile.

Uses of Induced Polyploidy:

1) Seedless fruits can be produced by using triploids. Eg: seedless watermelons
2) These triploids are obtained from seeds raised by a cross of tetraploid and diploid plants.
3) The tetraploids have been produced from the diploids by colchicine treatment.
4) By adopting these methods a variety of
5) Triploids such as sugar beet, tomato and grapes.
6) Tetraploids such as rye, barley, corn, apple, grapes, marigolds, snapdragons, lily, phlox, etc., have been obtained.
7) Among the forage crops, tetraploid barseem is a very popular crop in Northern India.

b) Allopolyploids:

When the polyploidy results due the doubling of chromosome number in a F1 hybrid which is derived from two distinctly different species, then, it is called allopolyploidy and the resultant species is called an allopolyploid. Let **A** represent a set of chromosomes (genome)

in species ***X***, and let **B** represent another genome in a species ***Y***. The F1 hybrids of these species then would have one **A** genome and another **B** genome. The doubling of chromosomes in the F1 hybrids will give rise to allotetraploids with two **A** and two **B** genomes. *Raphano brassica* is a classic example of allopolyploidy or amphipolyploidy (Figure 14.6). In 1927, a Russian geneticist, G.D. Karpechenko performed a cross between radish (*Raphanus sativum*, 2n = 18) and cabbage (*Brassica oleracea*, 2n = 18) and in F1 got sterile (diploid) hybrids. Among these sterile F1 hybrids, he found certain fertile plants which were found to contain 36 chromosomes. These fertile tetraploids were called *Raphano brassica*.

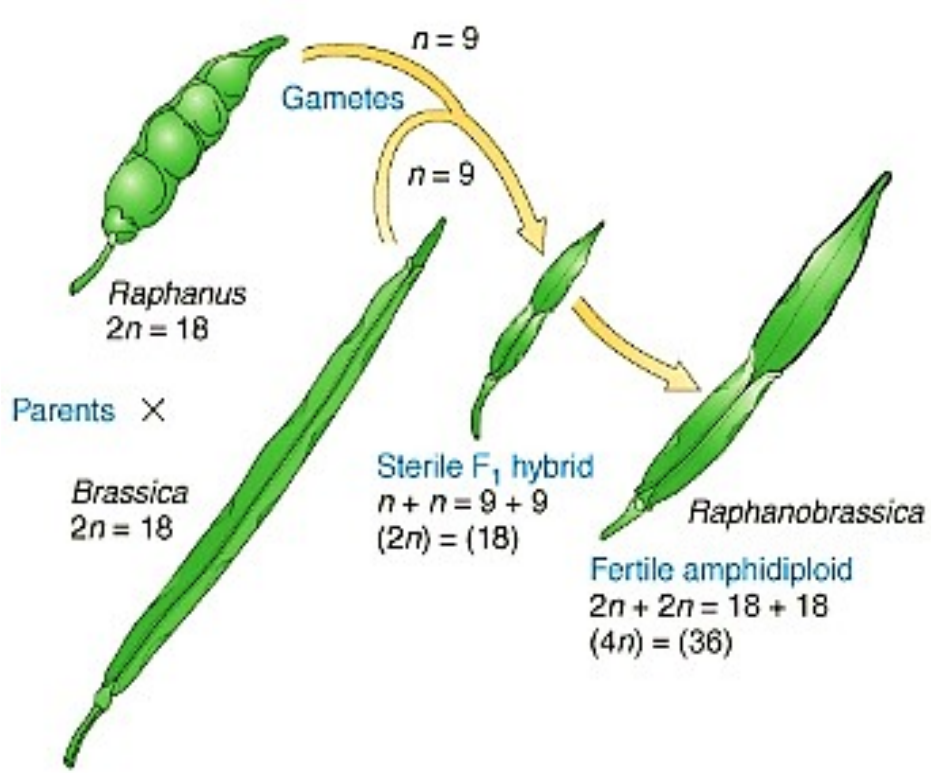

Figure-14.6: Formation of *Raphano Brassica* due to Allopolyploidy

Synthesized Allopolyploids:

To find out the origin of naturally occurring allopolyploids some cytogeneticists produced certain allopolyploids in laboratory by employing artificial means. Common hexaploid wheat and tetraploid cotton furnish two such examples.

i) ***Triticum spelta*:** It is a hexaploid wheat which was artificially synthesized in 1946 by E.S. McFadden and E. R. Sears and also by H. Kihara. They crossed an emmer wheat, *Triticum dicoccoides*, (tetraploid: 2n = 28) with goat grass, *Aegilops squarrosa* (diploid; 2n = 14) and doubled the chromosome number in the F1 hybrid. This artificially synthesized hexaploid wheat was found to be similar to the primitive wheat *T. spelta* (Figure 14.7). When the synthesized hexaploid wheat was crossed with naturally occurring *T. spelta*, the F1 hybrid was completely fertile. This suggested that hexaploid wheat must have originated in the past due to natural hybridization between tetraploid wheat and goat grass followed by subsequent chromosome doubling.

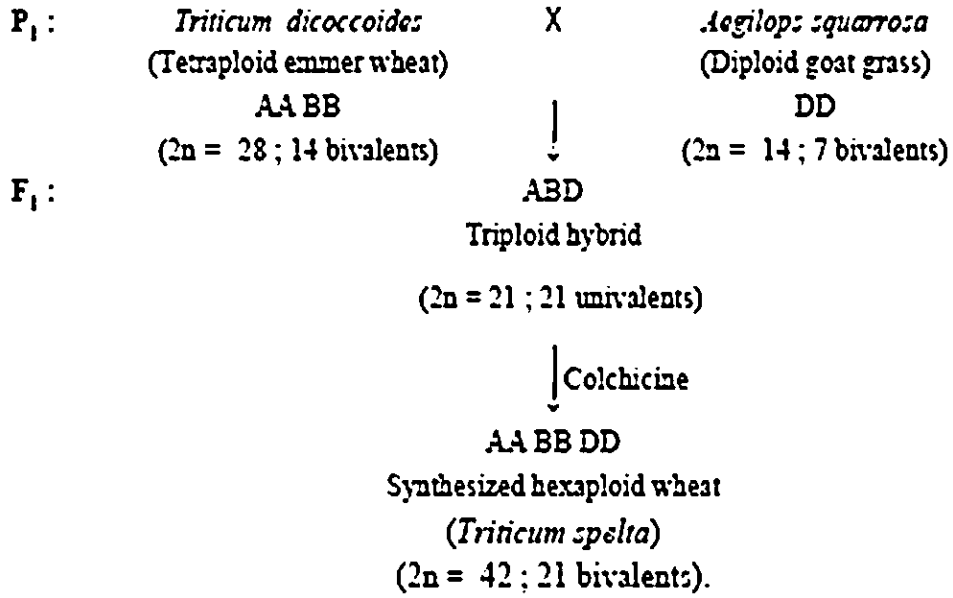

Figure-14.7: Artificial Synthesis of Hexaploid Wheat

ii) ***Gossypium hirsutum*:** The New world cotton plant, is another interesting example of allopolyploidy. Old world cotton, *Gossypium herbaceum*, has 13 pairs of chromosomes, while, American or "upland cotton" also contains 13 pairs of chromosomes. J.O. Beasley crossed the old world and American cottons and doubled the chromosome number in the F1 hybrids. The allopolyploids, thus, produced resembled the cultivated New world cotton and when crossed with it gave fertile F1 hybrids. Tetraploid *Gossypium hirsutum* originated from two diploid species, namely *G. herbaceum* (2n = 26) and *G. raimondii* (2n = 26) (Figure 14.8).

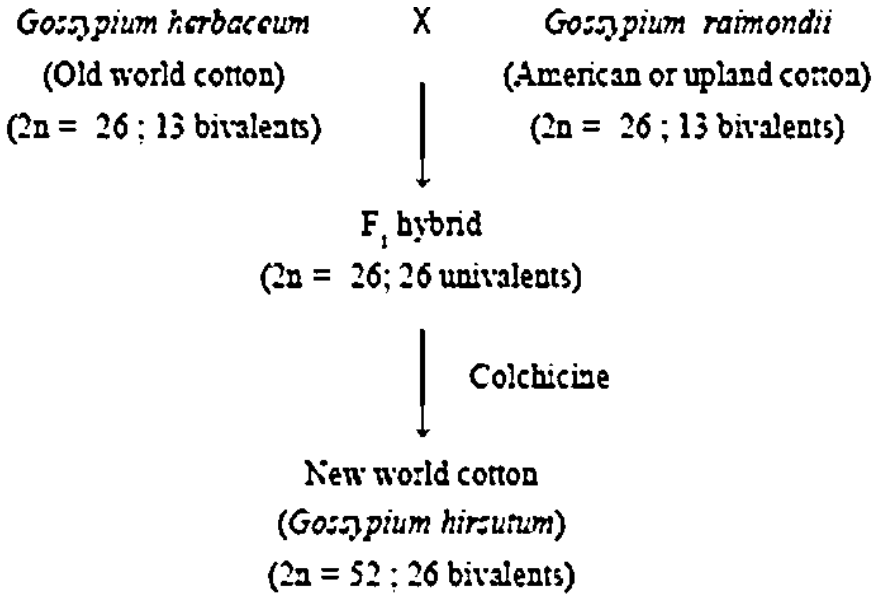

Figure-14.8: Artificial Synthesis of Hexaploid Wheat

c) Segmental or autoallopolyploids:

In these polyploids chromosomes belonging to different genomes do pair together to some extent. This indicates that segments of chromosomes and not the whole chromosomes are homologous. Therefore, such allopolyploids are called segmental allopolyploids. The segmental allopolyploids are intermediate between autopolyploids and allopolyploids and can be identified by their peculiar meiotic behaviour. It is generally believed that most naturally occurring polyploids are segmental allopolyploids. E.g.: Bread wheat.

Autoallopolyploidy between *Triticum aestivum* (common wheat) and *Secale cereale* (rye) results in the formation of a synthetic crop called **Triticale**, which combines the genomic characteristics of both parents. *Triticum aestivum* is a hexaploid species (2n = 6x = 42) with the genome composition **AABBDD**, while *Secale cereale* is a diploid (2n = 2x = 14) with the genome **RR**. When these two species are hybridized, the initial F_1 hybrid is sterile due to irregular chromosome pairing. However, through artificial chromosome doubling (often using colchicine), a fertile octoploid hybrid is produced with the genome constitution **AABBDDRR** (2n = 8x = 56) (Figure 14.9). This new plant, triticale, is considered an **autoallopolyploid** because it contains multiple sets of chromosomes from the same species i.e. the AABBDD genomes from wheat (auto component) and RR genome from rye (allo component).

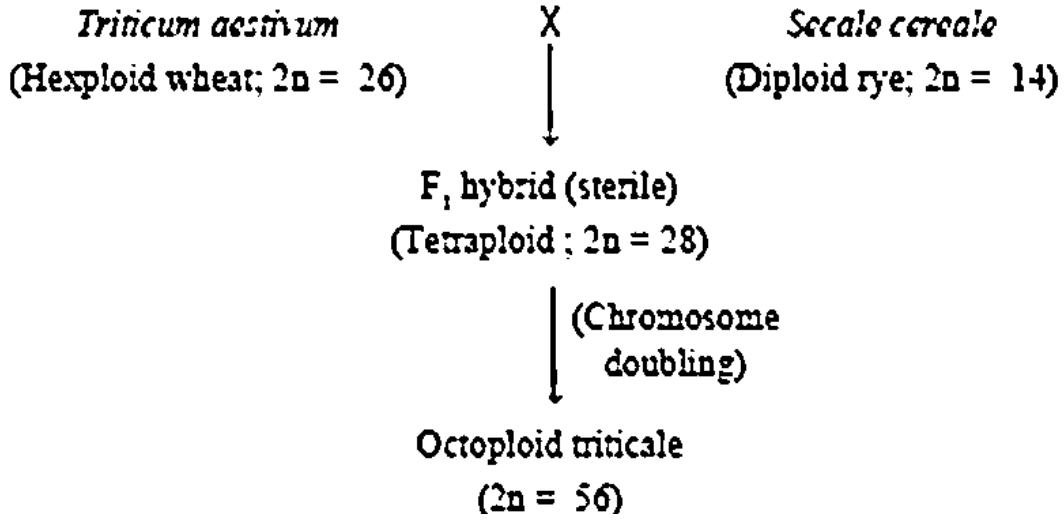

Figure-14.9: Segmental or Autoallopolyploidy in *Triticale*

Polyploidy in Animals:

Polyploidy is rare in animals but occur in flatworms, leeches and brine shrimp. In mice, also, 40 per cent liver cells are tetraploids, and about 5 per cent are octoploids. Polyploidy in humans have been found in liver cells and cancer cells. In them polyploidy is whether complete or as a mosaic, it leads to gross abnormalities and death.

Phenotypic Effects of Polyploidy:

i) **Morphological Effect of Polyploidy:** The polyploidy is invariably related with gigantism. The polyploid plants have been found to contain large-sized pollen grains, cells, leaves, stomata, xylem, etc. The polyploid plants are more vigorous than diploids.

ii) **Physiological Effect of Polyploidy:** The ascorbic acid content has been reported to be higher in tetraploid cabbages and tomatoes than in corresponding diploids. Likewise corn meal of a tetraploid maize seed contains 40 per cent more vitamin A than cornmeal from a diploid plant.

iii) **Effect on Fertility of Polyploidy:** It reduces the fertility of polyploid plants in variable degrees.

iv) **Evolution Through Polyploidy:** Interspecific hybridization combined with polyploidy. Offers a mechanism whereby new species may arise suddenly in natural populations.

14.3.2 Aneuploidy:

Changes that involve parts of a chromosome set results in individuals, called aneuploids (Gr.*aneu* = uneven; ploid = unit). Aneuploidy refers to a chromosomal constitution where the number of chromosomes in a cell or organism is not an exact multiple of the haploid number. This condition typically results from the gain (hyperploidy) or loss (hypoploidy) of one or a few individual chromosomes, rather than entire sets. All of these aneuploids are probably produced by nondisjunction during mitosis or meiosis. Aneuploidy is the most prevalent form of chromosomal abnormality detected in humans. It is a significant contributor to pathological states, serving as a hallmark of spontaneous abortions, birth defects, and is observed in virtually all human tumors. Aneuploidy is of following types i. Monosomy ii. Nullisomy iii. Trisomy iv. Double trisomy v. Tetrasomy (Figure 14.10).

1) **Monosomy:** Diploid organisms which are missing one chromosome of a single pair are monosomic with the genomic formula 2n –1. A monosomic individual forms gametes of two types, (n) and (n –1). The n –1 gametes do not survive in plants, but, in animals that may cause genetic imbalance, which is manifested by high mortality or reduced fertility of resulted organism. Monosomy in diploids is not tolerated, since it creates imbalance due to loss of one complete chromosome. The number of possible monosomics in an organism will be equal to the haploid chromosome number. For example, in common wheat, since 21 pairs of chromosomes are present, 21 possible monosomics are known. These 21 monosomics in wheat were produced by E.R. Sears in variety called “chinese spring” and being used for genetic studies all over the world. E.g.: cotton, tobacco, maize and tomato

   Double monosomics (2n–1–1) or triple monosomics: Double monosomic means that the chromosome number is 2n–2 like that in a nullisomic, but the missing chromosomes are nonhomologous. The 2n–1–1–1 could also be produced in polyploids such as wheat.

2) **Nullisomy:** An organism which has lost a chromosome pair is a nullisomic. The nullisomic organism has the genomic formula (2n–2). A nullisomic diploid often does not survive. Nullisomic polyploid (*e.g., hexaploid wheat, 6x–2)* may survive but exhibit reduced vigour and fertility.

3) **Trisomy:** Trisomics are those diploid organisms which have an extra chromosome (2n + 1). The number of possible trisomics will be equal to the haploid chromosome number. For example,haploid chromosome number of barley is 7, consequently in it seven trisomics are possible. Further, when the extra chromosome is identical to its homologs, such a trisomic is called primary trisomic. There are also secondary and tertiary trisomics. While the secondary trisomic means that the extra chromosome should be an isochromosome (i.e., both chromosome arms genetically similar), a tertiary trisomic would mean that the extra chromosome should be the product of translocation. Trisomics were obtained for the first time in jimson weed (*Datura stramonium)* by A.F. Blakeslee and J. Belling (1924). Most of the trisomics were identified by the size, shape and other morphological features of the fruit of jimson weed.

   Trisomy in humans: A. Down's syndrome (DS) or Trisomy-21 B. Edward's syndrome or Trisomy-18 C. Patau syndrome or Trisomy-13.

   Cytology of trisomics: The trisomics have an extra chromosome which is homologous to one chromosome of the diploid complement. Therefore, it forms trivalent which may take a variety of shapes in primary and secondary trisomics. In a tertiary trisomic a characteristic pentavalent is observed.

4) **Double Trisomy:** In a diploid organism when two different chromosomes are represented in triplicate, the double trisomic is resulted. The double trisomic causes great genetic imbalance and has the genomic formula 2n+1+1.

5) **Tetrasomy:** The diploid organisms having two extra chromosomes are known as tetrasomic. They have the genomic formula 2n+2. All the 21 possible tetrasomics are available in wheat.

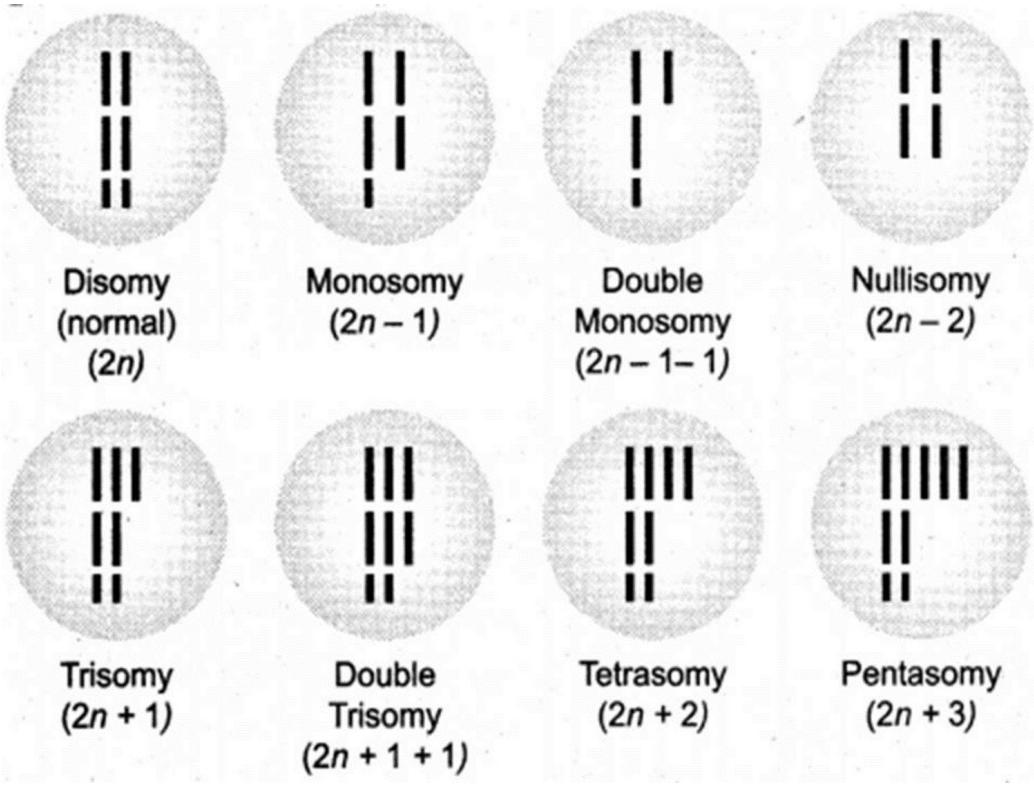

Figure- 14.10: Types of Aneuploidy

The various "-somies" and their associated notations including information on general viability in humans was given in Table 14.1.

Table-14.1: Somies and Their Effect on Human Beings

Type of Aneuploidy	Chromosomal Notation	Definition	General Viability in Humans
Hypoploidy			
Monosomy	2n-1	Loss of a single chromosome from a diploid set	Autosomal monosomies are almost universally lethal; sex chromosome monosomy (e.g., Turner Syndrome) is viable but with significant clinical features.
Nullisomy	2n-2	Loss of both homologous chromosomes of a pair	Incompatible with human life.
Hyperploidy			
Trisomy	2n+1	Gain of a single extra chromosome	Most common viable aneuploidy, but often leads to severe developmental disorders or miscarriage.
Tetrasomy	2n+2	Gain of two extra chromosomes to a pair	Generally rarer and more severe than trisomy for autosomes; more common for sex chromosomes.

14.4. SUMMARY:

Numerical chromosomal aberrations refer to changes in the number of chromosomes in a cell, resulting in an abnormal chromosome count. These aberrations are broadly classified into two types 1. **Aneuploidy** and 2. E**uploidy**. **Aneuploidy** involves the gain or loss of one or more individual chromosomes, but not entire sets such as **monosomy (2n−1)** or **trisomy (2n+1)**. It usually arises due to non-disjunction during meiosis and can lead to genetic disorders. E.g.: Down syndrome (trisomy 21). **Euploidy**, on the other hand, involves variations in the entire set of chromosomes. It includes **monoploidy (1n)** and **polyploidy** such as **triploidy (3n)** or **tetraploidy (4n)**. Polyploidy is more common in plants and plays a significant role in evolution and speciation, often improving vigour and adaptability. Numerical aberrations can severely affect cell function, development, and fertility, depending on the organism and the type of aberration.

14.5. TECHNICAL TERMS:

Ploidy, Haploid, Non-disjunction, Somatic mosaicism, Endopolyploidy

14.6. SELF ASSESSMENT QUESTIONS:

1) Define aneuploidy with an example.
2) Differentiate between monosomy and nullisomy.
3) What is non-disjunction and how does it lead to aneuploidy?
4) Mention two differences between autopolyploidy and allopolyploidy.
5) Explain the types of aneuploidy with suitable examples.
6) Define polyploidy. Discuss its types and significance in plants.
7) What are numerical chromosomal aberrations? Describe their classification in detail.

14.7. SUGGESTED READINGS:

1) Thompson & Thompson Genetics in Medicine 8th Edition by Robert L. Nussbaum,
2) Roderick R. McInnes, and Huntington F. Willard. ELSEVIER1600 John F. Kennedy
3) Blvd. Ste 1800 Philadelphia, PA 19103-2899.
4) Medical Genetics, 6th Edition by Jorde, Carey, Bamshad, and White. ELSEVIER1600 John
5) F. Kennedy Blvd. Ste 1800 Philadelphia, PA 19103-2899.
6) Human Molecular Genetics 5th Edition by Tom Strachan and Andrew Read. Garland
7) Science Publishers, UK.
8) Gardner and Sutherland's Chromosome Abnormalities and Genetic Counselling by R. J.
9) McKinlay Gardner and David J. Amor. Oxford University Press, U.K.

Prof. A. Amruthavalli