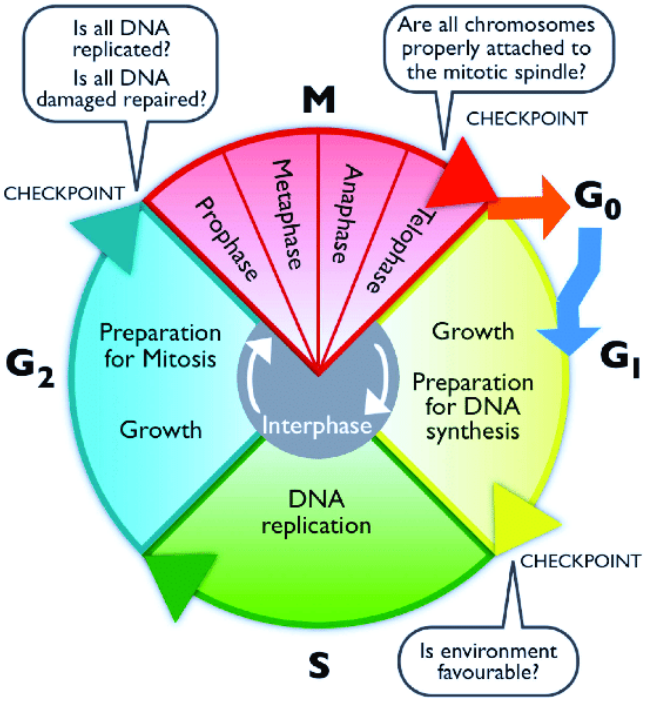
18MBO13C	PAPER – III CELL BIOLOGY

Unit – V

Cell cycle – four phases – biochemical and cellular activities. Cell division types - amitosis, endomitosis, polyteny, Mitosis and Meiosis. Kinetochore, Role of centromere and spindle fibers- Spindle apparatus – Cytokinesis. Apoptosis and its significance.

The *cell cycle* is a term that describes the changes that occur through the life cycle of a cell, and, like most cellular processes, it is influenced by environmental conditions. The cell cycle is also influenced by circadian rhythms, which ensure that the ultraviolet-sensitive phases of the cell cycle occur at night.

The cell cycle is divided into four phases known as G1, S, G2, and M. The nucleus is typically in interphase and the chromatin is decondensed. Interphase is composed of the G1-, S-, and G2-phases. DNA synthesis takes place during the S-phase, and S stands for synthesis. S-phase is preceded by G1-phase and followed by G2-phase, where G stands for gap, since G1 and G2 were named at times when it appeared that nothing was happening during these phases. The G2-phase is followed by mitosis (or meiosis), which is known as M-phase. The newly formed nuclei soon return to the G1-phase.



In some cells, notably the antipodal and synergid cells of the female gametophyte, the tapetal cells surrounding the male gametophyte, and the endosperm

cells surrounding the developing intervening M-phase. Endoreduplication results in cells with large polytenic chromosomes with DNA contents around 24,576 times greater than the DNA content of normal haploid chromosomes. The genes and proteins involved in regulating the phases and transitions of the cell cycle are particularly well known in yeast although new ones are being discovered in both yeast and other organisms.

The durations of the various phases of the cell cycle vary from species to species and from cell type to cell type. As an example, in *Vicia faba* meristematic cells, G1 takes about 2.5 hours, S-phase takes about 6 hours, G2 takes about 5 hours, and M takes about 0.5 hours. Most differentiated cells spend the majority of their life in G1, but are still capable of dividing as evidenced by the ability of plants and some animals to regenerate lost parts after wounding. The cell cycle can be best studied in cells, including tobacco BY-2 suspension cultured cells, in which the cell cycle can be readily synchronized. While in other cell lines, the mitotic index, which is the percentage of cells undergoing mitosis at the same time, was about 10 percent, in tobacco BY-2 cells, the mitotic index is greater than 70percent.

Progression through the cell cycle is central to cell proliferation and fundamental to the growth and development of all multicellular organisms, including higher plants. The periodic activation of complexes containing cyclins and cyclin-dependent kinases mediates the temporal regulation of the cell-cycle transitions.

What regulates the cell cycle? In 1970, it was found that a protein extract stimulated frog oocytes to divide when it was microinjected into the eggs. The protein in the active extract was called ***maturation promoting factor*.** Later it was found that a mutant of yeast, called *CDC* (for cell-division cycle), was unable to produce a protein necessary for cell division, and consequently, the mutant stops dead in its tracks during cell division. The CDC protein turned out to be the same as the protein that stimulates frog oocytes to divide. This protein is a protein kinase and occurs in all cell types l of the CDC protein kinase is enriched with the ability of the cell to divide. Intracellularly, the CDC protein kinase is enriched in the pre-prophase band. Surprisingly, the CDC protein kinase is always present in dividing cells, so how does it regulate the cell cycle? The CDC protein kinase is activated by another class of proteins called *cyclins*, and the concentration and type of cyclin change throughout the cell cycle. Each cyclin causes the CDC protein kinase to phosphorylate a different set of proteins necessary for a given phase. Currently, more and more regulatory proteins are being discovered that are involved in regulating the cell cycle. The synthesis, availability, degradation, and phosphorylation states of these proteins are important in initiating each phase of the cell cycle. The genes involved in switching a plant cell over from a mitotic

to a meiotic cell cycle, as well as the genes involved in all stages of meiosis including bivalent formation and recombination, are being discovered. Cells mature and may even senesce in time. Some of the developmental events that occur as cells age are regulated by extrinsic factors, whereas others are regulated by intrinsic factors. For example, cells may keep track of, in some way, the number of cell cycles, and then initiate differentiation after a particular number of divisions. One way to register the number of cell divisions involves dilution. An initial cell may have a given amount of a particular molecule. Each time the cell divides, the compound will be diluted until there is a sub threshold level, and the cell can no longer divide. This compound or compounds, at least in part, are known to occur at the ends of chromosomes called *telomeres*. After each division, the telomere gets shorter and shorter. When it is no longer there, the cells tops dividing. By contrast, cancer cells produce a protein called *telomerase*. Telomerase is an enzyme that rebuilds the telomeres wrote that "the somatic cells cease to multiply. From an egoist being, the cell becomes an altruist, in the sense that it proceeds to new division only when the needs of the whole demand it." While no one knows how cells tell time, we know that some gene on the human chromosome #1 causes cells to lose their immortality. It is known that when human chromosome #1 is transferred into an immortal line of Syrian hamster cells, the cells acquire a limited lifespan. On the other hand, oncogenes make products that cause the cell to be immortal. Likewise, Agrobacterium causes a cancer like condition in plants.

The senescence of organisms may be a manifestation of the senescence of cells.This idea comes from studies in mammals, including humans, where the maximum number of divisions in culture cells is directly proportional to the lifespan of the species and inversely proportional to the age of the donor. Moreover, cells derived from donors with heritable premature aging syndromes usually senesce after fewer divisions than cells derived from people the same age who do not have the syndrome in question. The causes of programmed cell death, known as apoptosis, are being vigorously.

Types of Cell divisions:

Organisms exhibit two types of cell divisions. This is based on the pattern of distribution of parental chromosomes to the daughter cells. They are Mitosis and Meiosis.

Amitosis,

In prokaryotic organisms like bacteria and blue green algae, where there is no organized chromosomes and the nucleus; the cell division is equational and it is called Amitosis, for the mitotic apparatus and such complicated chromosomal movements are absent, but their parental DNA is separated in equal numbers. However the genetic materials like in DNA

(ssDNA or dsDNA) or genetic RNA in viruses do undergo replication cum separation, such division and separation exist in many viruses. Viral DNA or RNA are replicated in host cells. In most of the viruses replicated genetic material, only one DNA or RNA is loaded into their newly formed capsids. Whatever may be the types, all cellular prokaryotic and eukaryotic cell divisions involve two important events viz, DNA replication, nuclear division called Karyokinesis and then cytoplasmic division called Cytokinesis.

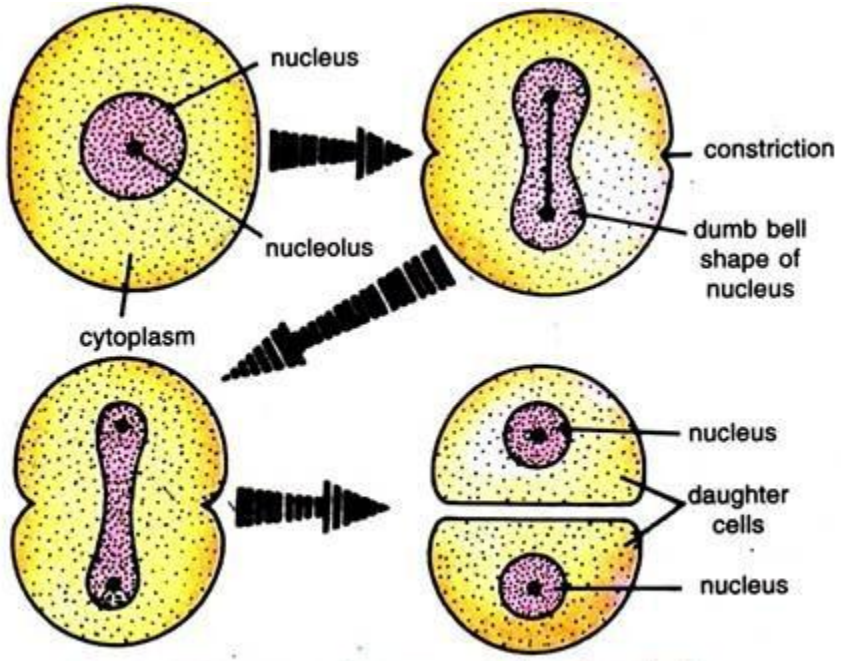


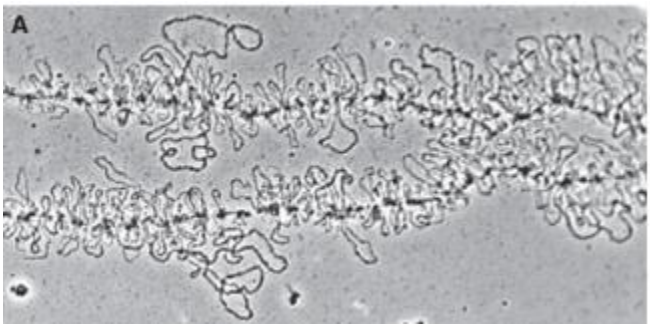
Fig. 5.1. Diagrammatic representation of amitosis.

Amitosis is also referred to as Binary fission or direct division. This type of division is employed by prokaryotes, not only for multiplication of cells but also for reproduction. In this context, a bacterial cell has been taken as an example of describe the process.

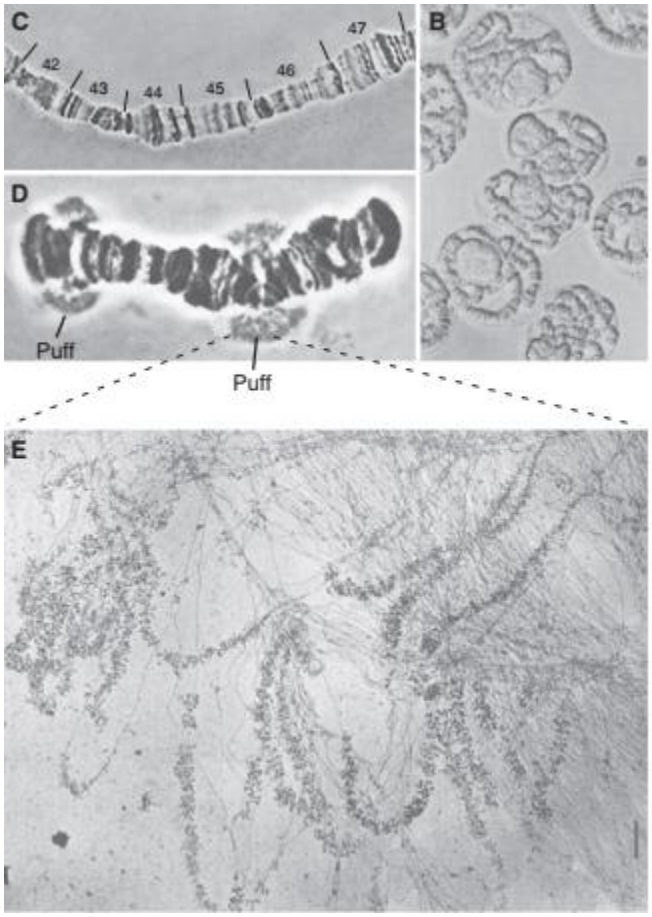
Endomitosis:-

Endomitosis is defined as the replication of chromosomes in the absence of cell or nuclear division, resulting in numerous copies within each cell. It occurs notably in the salivary glands of Drosophila and other flies. Cells in these tissues contain giant chromosomes (Polyteny), each consisting of over a thousand intimately associated, or synapsed, chromatids.

Polytene chromosomes were discovered by Balbiani (1881) in larval salivary glands, Malpighian tubules, intestine, hypoderm and muscles of *Chironomus plumosus*. Polytene chromosomes are specific interphase chromosomes consisting of thousands of deoxyribonucleic acid (DNA) strands. For this reason they are very large and display a characteristic band–interband morphology.



A, Phase contrast view of the left end of meiotic lamp brush chromosome 6 from the newt *Notophthalmus viridescens*.



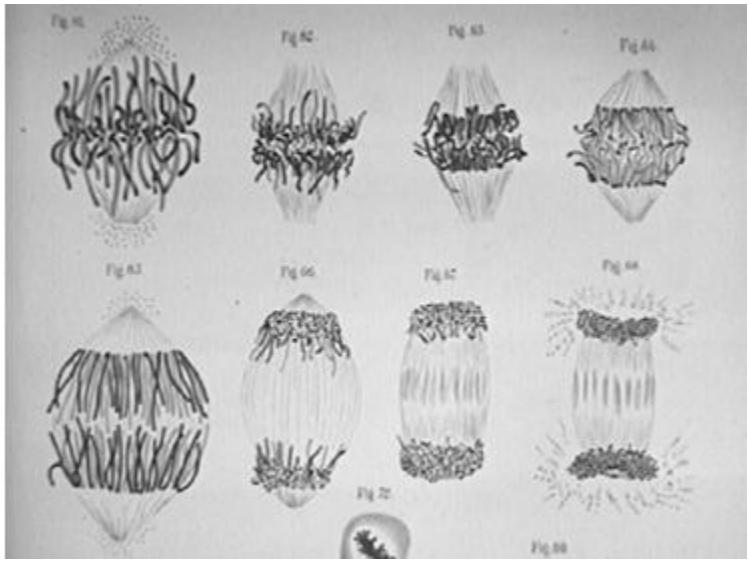
B–D, The Domain organization of polytene chromosomes. Once Drosophila larvae reach a certain developmental stage, most cells stop dividing, and larval growth proceeds via an increase in the size of individual cells. To keep the protein synthesis machinery of these huge cells supplied with messenger RNA, DNA replication is uncoupled from cell division so that ultimately, the cells contain many times the

normal complement of cellular DNA (ie, they are polyploid). In certain tissues, the numerous copies of the chromosomes are maintained in strict alignment with respect to one another, making giant polytene chromosomes, the best known of which occur in the salivary gland B, Giant polytene chromosomes are visible within isolated salivary gland nuclei. **C**, A portion of a high-resolution map of the *Drosophila* polytene chromosomes. *D,* Polytene chromosome showing puffs. The nset box shows an area analogous to that used in panel E.E, Electron micrograph of puff showing transcribing DNA loops. These loops are covered with a "fuzz" corresponding to growing RNA chains coated with proteins.

Polyteny arises in tissues, organs and at developmental stages when there is need for the rapid development of an organ at an unaltered high level of function. Organs containing cells with polytene chromosomes are, as a rule, involved in intense secretory functions accomplished during a short time against a background of rapid growth. Chromosome rearrangements and in situ hybridization on polytene chromosomes allow genes to be mapped to a resolution of a few tens of kilobases. Polytene chromosomes allow a specific narrow region to be dissected out with a micromanipulator and a library of DNA clones to be derived from the region.

Mitosis

Mitosis was first defined by Walther Flemming (1882) as a mode of indirect nuclear division that provides the daughter nuclei with identical chromosomes. Mitosis is part of the process where the cell changes from oneness to twoness. While mitosis involves the equal division of chromosomes between two daughter cells, there are structural and motile elements involved in bringing them together to the metaphase plate and then distributing them to the daughter cells.



The segregation of the genome occurs in all dividing cells; however, mitosis only occurs in eukaryotes. In prokaryotes, genomes are segregated by the growth of the plasma membrane between the attachment points of the two replicated DNA molecules. The process of mitosis is far more complex and it has gone through evolutionary change in each eukaryotic kingdom. In general, the segregation of the genome depends less and less on membranes and more and more on spindle fibers as one progresses up the evolutionary ladder

Mitosis is a continuous process and for convenience, however, it is divided into six phases: prophase, prometaphase, metaphase, anaphase, telophase, and cytokinesis. Karyokinesis, or the division of the nucleus, is typically, but not always, followed by cytokinesis, where the cytoplasm is divided into two. It should always be kept in mind that mitosis is a three-dimensional process.

1 Prophase

Prophase begins as the chromatin starts to condense from its metabolically active state into well-defined chromosomes that are more easily moved through the cytoplasm. Each chromosome is composed of two sister chromatids. Each sister chromatid contains one of the two strands of DNA that result from the semi-conservative DNA replication process that took place during the previous S-phase. Typically, each somatic cell has an even number of homologous chromosomes, one set from each parent.

During prophase, the mitotic spindle begins to form outside the nuclear envelope from microtubule organizing centers (MTOCs), which are sometimes called centrosomes or centrospheres. In the fungi, the centrosomes are known as spindle pole bodies. The centrosome of animal cells typically contains a centriole, and the centriole has been often, although erroneously, considered to be the organizer of the spindle during prophase for over 100 years.

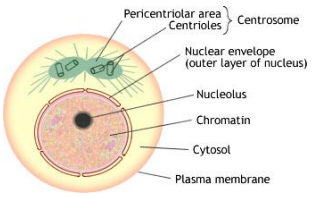
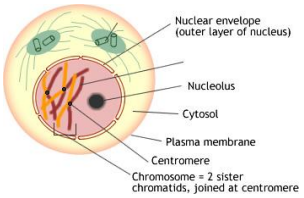
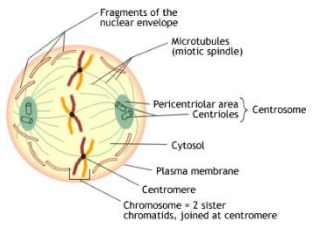
Evidence that the centriole is not required for spindle formation or mitosis comes from the observation that most plant cells do not have centrioles, although they still form mitotic spindles and undergo mitosis.

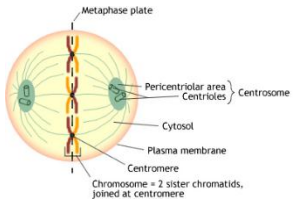
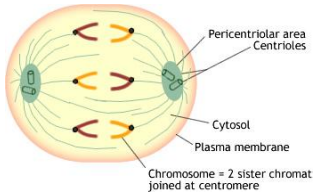
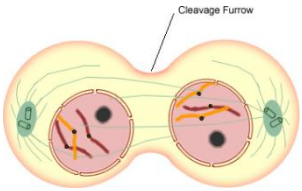
Thus, the MTOC, and not the centriole, contains the fundamental substances necessary to initiate the prophase spindle. The MTOC can have a diverse array of shapes, and in many plant cells, the MTOC may be dispersed along the nuclear envelope. The microtubules generated from this MTOC form an optically clear zone around the nucleus.. The division of the MTOC is another sign that a cell is going from a state of oneness to a state of twoness.

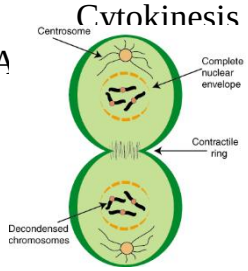
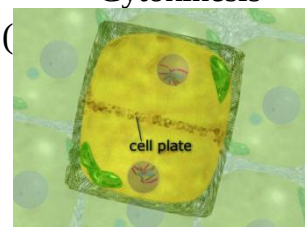
The mitotic apparatus can exist in a multitude of forms. It can appear spindle shaped, barrel shaped, or almost amorphous. Forms that are even more diverse can appear in hybrids.

Ivor Cornman (1944) suggested that each chromosome has an independent spindle, and Wada (1966) suggested that each more or less independent spindle joins together to form a bipolar mitotic apparatus of which the shape depends on the shape of the cell. Thus, the concept of independent spindles explains the lack of bipolar organization that exists in the mitotic apparatus of long, thin pollen tubes.

Stages of Mitosis

Stage of Mitosis / Graphic	*Important Notes*
Interphase  	• G_1 phase, S phase, and G_2 phase • Grows by producing protein and organelles • Chromatin duplicated during the "S" or synthesis phase • In the G_2 stage, duplicated chromosomes can not be seen yet, because they have not condensed • Nuclear envelope is still intact, cell contains two centrosomes with centriole pairs, and the nuclei is still present
Prophase  	• Chromosomes condense and can now be viewed through a light microscope • Nucleoli disappears • Duplicated chromosomes take the form of two sister chromatids, bound at their centromeres, and joined at their arms through cohesin proteins, or sister chromatid cohesion • Mitotic spindle forms from centrosomes, containing the centrosomes and microtubules extending from them; shorter microtubule arrays are called "asters" for their star-like appearance • Microtubules lengthen, propelling the centrosomes away from each other, to opposite sides of the cell
Prometaphase  	• Nuclear envelope fragments, allowing the extending microtubules to come into the nuclear area • Two kinetochores (specialized proteins) form at the centromeres on each sister chromatid; the chromosomes are also now even more condensed • Microtubules that connect to the kinetochores are

	"kinetochore microtubules" and can move the chromosomes about; other microtubules interact with microtubules from the centrosomes on the other side
Metaphase  	• Longest stage of mitosis at roughly 20 minutes • Centrosomes are now completely opposite one another • The metaphase plate is an imaginary plane equidistant from the two centrosomes, just like an equatorial line drawn across the cell; the chromosomes align themselves on this metaphase plate, with their centrosomes centered • All kinetochores of each sister chromatids are attached to kinetochore microtubules, coming from opposite poles
Anaphase  	• Shortest stage of mitosis lasting only a few minutes • Cohesin proteins binding chromatids are cleaved; the chromatids are separated, thus becoming chromosomes • Kinetochore microtubules shorten, pulling chromosomes to the centrosomes on opposite poles; they move centromeres first • The cell elongates as the nonkinetochore microtubules lengthen • Two ends of the cell now have complete sets of chromosomes
Telophase  	• Two nuclei form in the cell; the nucleoli reappear • Two nuclear envelopes form from the parent cell's nuclear envelope fragments and other parts of the endomembrane system • Chromosomes become less condensed • Mitosis is complete

Cytokinesis (A  	• Cytokinesis is the division of the cell's cytoplasm • Well underway by late telophase • In animal cells, the formation of a cleavage furrow pinches the cell in two • The cytoplasmic side of the furrow has a contractile ring of actin microfilaments that interacts with the myosin molecules –causing the ring to contract, and the cell to pinch inward until the parent cell is now two daughter cells
Cytokinesis ( 	• Cytokinesis is the division of the cell's cytoplasm • Vesicles from the Golgi apparatus move along microtubules to the center of the plant cell, where they coalesce • The vesicles form a cell wall and the cell wall materials they carry collect in the cell plant • The cell plate enlarges and elongates until it fuses with the plasma membrane, separating the parent cell into two daughter cells, each with their own plasma membrane

Meiosis

(Gk. meioum = to reduce, osis = state)

(1) **Definition :** It is a special type of division in which the chromosomes duplicate only once, but cell divides twice. So one parental cell produces 4 daughter cells; each having half the chromosome number and DNA amount than normal parental cell. So meiosis is also called reductional division.

(2) **Discovery :** It was first demonstrated by Van Benden (1883) but was described by Winiwarter (1900). Term "meiosis" was given by Farmer and Moore (1905).

(3) **Occurrence :** It is found in special types and at specific period. It is reported in diploid germ cells of sex organs (e.g. primary spermatocytes of testes to form male gametes called spermotozoa and primary oocytes to form female gametes called ova in animals) and in pollen mother cells (microsporocytes) of anther and megasporocyte of ovule of ovary of flowers in plant to form the haploid spores. The study of meiosis in plants can be done in young flower buds.

Process of Meiosis

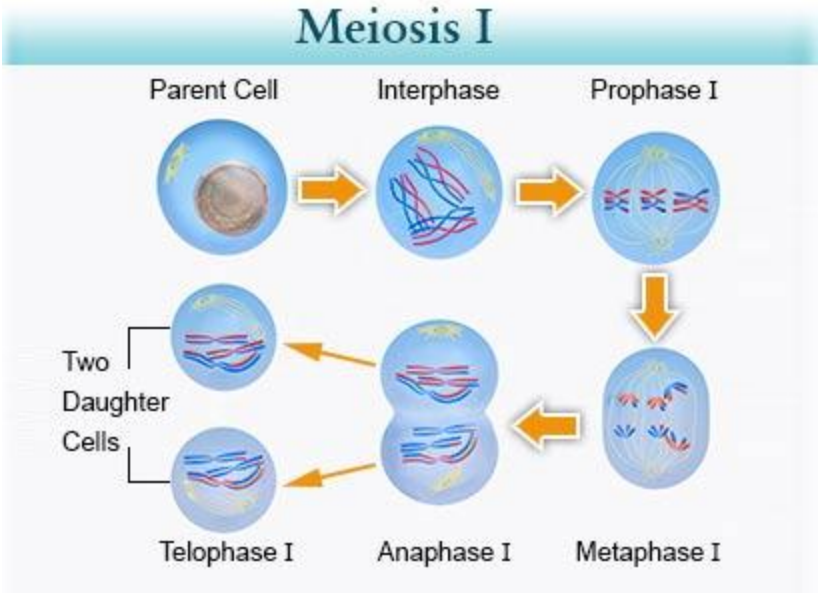
Meiosis is completed in two steps, meiosis I and meiosis II

Meiosis I

In which the actual chromosome number is reduced to half. Therefore, meiosis I is also known as reductional division or heterotypic division. It results in the formation of two haploid cells from one diploid cell. It is divided into two parts, karyokinesis I and cytokinesis I.

Karyokinesis I

It involves division of nucleus. It is divided into four phases i.e. prophase, metaphase, anaphase, telophase.



Prophase I

It is of longest phase of karyokinesis of meiosis. It is again divisible into five subphases i.e. leptotene, zygotene, pachytene, diplotene and diakinesis.

Leptotene/Leptonema

(a) Chromosomes are long thread like with chromomeres on it.

(b) Volume of nucleus increases.

(c) Chromatin network has half chromosomes from male and half from female parent.

(d) Chromosome with similar structure are known as homologous chromosomes.

(e) Leptonemal chromosomes have a definite polarization and forms loops whose ends are attached to the nuclear envelope at points near the centrioles, contained within an

aster. Such peculiar arrangement is termed as bouquet stage (in animals) and syndet knot (in plants).

(f) E.M. (electron microscope) reveals that chromosomes are composed of paired chromatids, a dense proteinaceous filament or axial core lies within the groove between the sister chromatids of each chromosome.

(g) Lampbrush chromosome found in oocyte of amphibians is seen in leptotene.

Zygotene / Zygonema

(a) Pairing or "synapsis" of homologous chromosomes takes place in this stage.

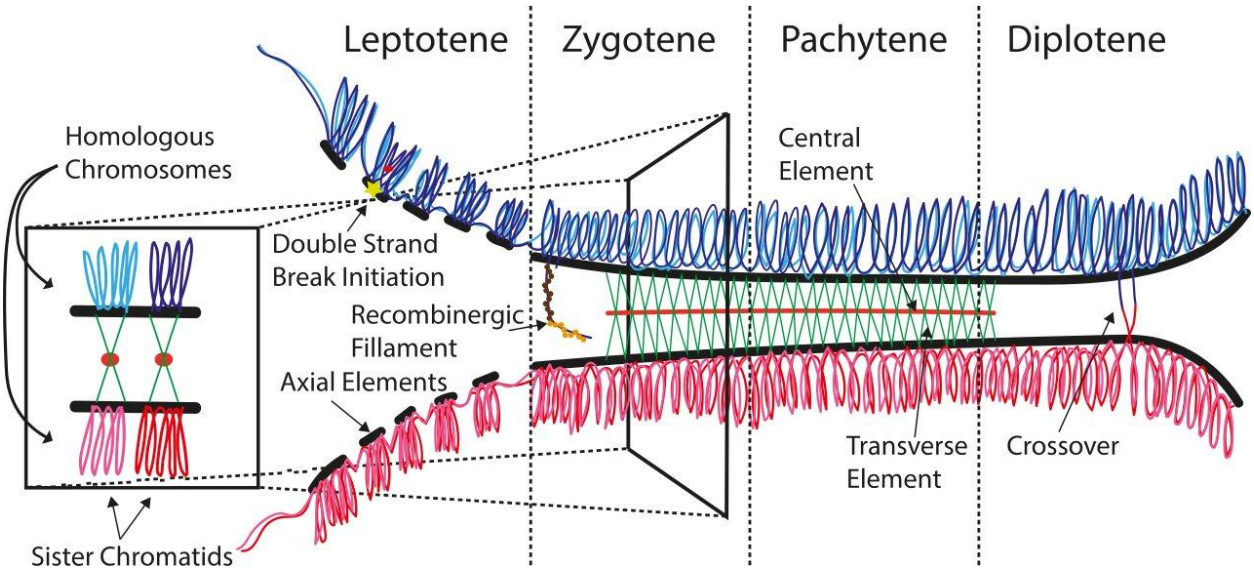
(b) Synapsis may be of following types.

- **Procentric :** Starting at the centromere.
- **Proterminal :** Starting at the end.
- **Localised random** : Starting at various points.

(c) Paired chromosomes are called bivalents, which by furthur molecular packing and spiralization becomes shorter and thicker.

(d) Pairing of homologous chromosomes in a zipper-fashion. Number of bivalents (paired homologous chromosomes) is half to total number of chromosomes in a diploid cell. Each bivalent is formed of one paternal and one maternal chromosome (i.e. one chromosome derived from each parent).

(e) Under EM, a filamentous ladder like nucleoproteinous complex, called synaptinemal. Complex between the homologous chromosomes which is discovered by "Moses" (1953).



Schematic of the synaptonemal complex at different stages of prophase I and the chromosomes arranged as a linear array of loops.

Pachytene/Pachynema

(a) In the tetrad, two similar chromatids of the same chromosome are called sister chromatids and those of two homologous chromosomes are termed non-sister chromatids.

(b) Crossing over i.e. exchange of segments between non-sister chromatids of homologous chromosome occurs at this stage.

It takes place by breakage and reunion of chromatis segments. Breakage called nicking, is assisted by an enzyme endonuclease and reunion termed annealing is added by an enzyme ligase. Breakage and reunion hypothesis proposed by Darlington (1937).

(c) Chromatids of pachytene chromosome are attached with centromere.

(d) A tetrad consists of two sets of homologous chromosomes each with two chromatids. Each tetrad has four kinetochore (two sister and two homologous).

(e) A number of electron dense bodies about 100 nm in diameter are seen at irregular intervals within the centre of the synaptonemal complex, known as recombination nodules.

(f) DNA polymerase is responsible for the repair synthesis.

Diplotene/Diplonema

(a) At this stage the paired chromosomes begin to separate (desynapsis).

(b) Cross is formed at the place of crossing over between non-sister chromatids.

(c) Homologous chromosomes move apart they remain attached to one another at specific points called chiasmata.

(d) At least one chiasma is formed in each bivalent.

(e) Chromosomes are attached only at the place of chiasmata.

(f) Chromatin bridges are formed in place of synaptonemal complex on chiasmata.

(g) This stage remains as such for long time.

Diakinesis

(a) Chiasmata moves towards the ends of chromosomes. This is called terminalization.

(b) Chromatids remain attached at the place of chiasma only.

(c) Nuclear membrane and nucleolus degenerates.

(d) Chromosome recondense and tetrad moves to the metaphase plate.

(e) Formation of spindle.

(f) Bivalents are irregularly and freely scattered in the nucleocytoplasmic matrix.

When the diakinesis of prophase-I is completed than cell enters into the metaphase-I.

Metaphase I

It involves;

(i) Chromosome come on the equator.

(ii) Due to repulsive force the chromosome segment get exchanged at the chiasmata.

(iii) Bivalents arrange themselves in two parallel equatorial or metaphase plates. Each equatorial plate has one genome.

(iv) Centromeres of homologous chromosomes lie equisdistant from equator and are directed towards the poles while arms generally lie horizontally on the equator.

(v) Each homologous chromosome has two kinetochores and both the kinetochores of a chromosome are joined to the chromosomal or tractile fibre of same side.

Anaphase-I

(i) It involves separartion of homologous chromosomes which start moving opposite poles so each tetrad is divided into two daughter dyads. So anaphase-I involves the reduction of chromosome number, this is called disjunction.

(ii) The shape of separating chromosomes may be rod or J or V-shape depending upon the position of centromere.

(iii) Segregation of mendalian factors or independent asortment of chromosomes take place. In which the paternal and maternal chromosomes of each homologous pair segregate during anaphase-I which introduces genetic variability.

Telophase-I

(i) Two daughter nuclei are formed but the chromosome number is half than the chromosome number of mother cell.

(ii) Nuclear membrane reappears.

(iii) After telophase I cytokinesis may or may not occur.

(iv) At the end of Meiosis I either two daughter cells will be formed or a cell may have two daughter nuclei.

(v) Meiosis I is also termed as reduction division.

(vi) After meiosis I, the cells in animals are reformed as secondary spermatocytes or secondary oocytes; with haploid number of chromosomes but diploid amount of DNA.

(vi) Chromosomes undergo decondensation by hydration and despiralization and change into long and thread like chromation fibres.

Interphase

Generally there is no interphase between meiosis-I and meiosis-II. A brief interphase called interkinesis, or intermeiotic interphase. There is no replication chromosomes, during this interphase.

Cytokinesis-I

It may or may not be present. When present, it occurs by cell-furrow formation in animal cells and cell plate formation in plant cells.

Significance of meiosis-I

(i) It separates the homologous chromosomes to reduce the chromosome number to the haploid state, a necessity for sexual reproduction.

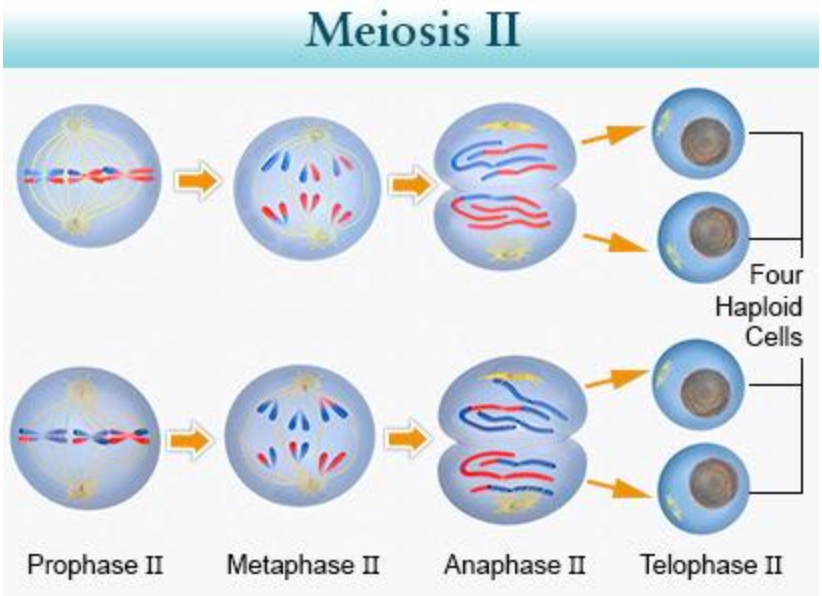
(ii) It introduces variation by forming new gene combinations through crossing over and randon assortment of paternal and maternal chromosomes.

(iii) It may at times cause chromosomal mutation by abnormal disjunction.

(iv) It induces the cells to produce gametes for sexual reproduction or spores for asexual reproduction.

Meiosis-II

It is also called equational or homotypical division because the number of chromosomes remains same as after meiosis-I. It is of shorter duration than even typical mitotic division. It is also divisible into two parts, Karyokinesis-II and Cytokinesis-II.



Karyokinesis-II

It involves the separation of two chromatids of each chromosome and their movement to separate cells. It is divided in four phases i.e., Prophase-II, Metaphase-II. Anaphase-II and Telophase-II.

Almost all the changes of Karyokinesis-II resembles to mitosis which involves.

(i) It starts just after end of telophase I.

(ii) Each daughter cell (nucleus) undergoes mitotic division.

(iii) It is exactly similar to mitosis.

(iv) At the end of process, cytokinesis takes place.

(v) Four daughter cells are formed after completion.

(vi) The sister kinetochores of one chromosome are separated.

(vii) The four daughter cells receive one chromatid each of the tetravalent.

(viii) Centromere divide at anaphase II.

(ix) Spindle fibres contract at prophase II.

Cytokinesis-II

It is always present and occurs by cell furrow formation in animal cell and cell plate formation in plant cell.

So by meiosis, a diploid parental cell divides twice forming four haploid gametes or sex cells, each having half the DNA amount than that of the parental cell and one-fourth of DNA present in the cell at the time of beginning of meiosis.

Significance of Meiosis

(i) Constancy of chromosome number in successive generation is brought by process.

(ii) Chromosome number becomes half during meiosis.

(iii) It helps in introducing variations and mutation.

(iv) It brings about gamete formation.

(v) It maintains the amount of genetic informative material.

(vi) Sexual reproduction includes one meiosis and fusion.

(vii) The four daughter cells will have different types of chromatids.

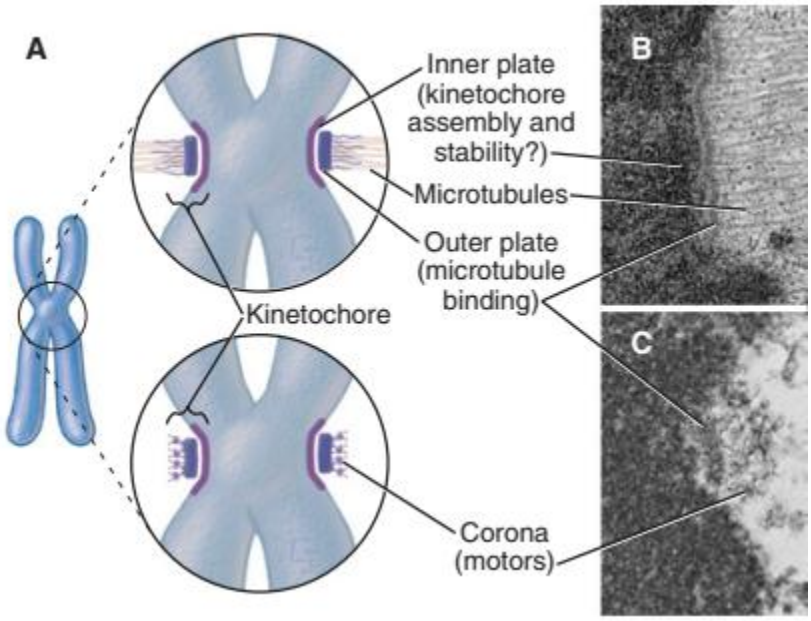
Why the necessity of Meiosis-II

The basic aim of meiosis is to reduce the number of chromosomes to half. The chromosomes that separate in the anaphase of meiosis-I are still double. Each consist of two chromatids and has 2n amount of DNA. Thus reduction of DNA content does not occur in meiosis-I. Truely haploid nuclei in terms of DNA contents as well as chromosome number are formed in meiosis-II. When the chromatids of each chromosome are separated into different nuclei. Thus meiosis-II is necessary.

Kinetochore, Role of centromere and spindle fibers:-
Spindle apparatus – Cytokinesis.

The Chromosome's Control Center: The Kinetochore

The centromere is the genetic locus that specifies the site where a kinetochore assembles on the chromosomal DNA molecule. The kinetochore is a button-like structure embedded in the surface of the centromeric chromatin of most eukaryotic mitotic chromosomes . When thin sections of centromeres are examined by electron microscopy, the kinetochore often appears to have several layers. The inner kinetochore is embedded in the surface of the centromere and is composed of a specialized form of chromatin. The outer kinetochore consists of an outer plate with a fibrous corona on its outer surface. It is constructed from protein complexes that link the chromatin to microtubules of the mitotic spindle.



KINETOCHORE STRUCTURE. A, Diagram of the major layers of the kinetochore. B, Thin-section electron micrograph of a kinetochore with attached microtubules. C, Thin-section micrograph of an unattached kinetochore.

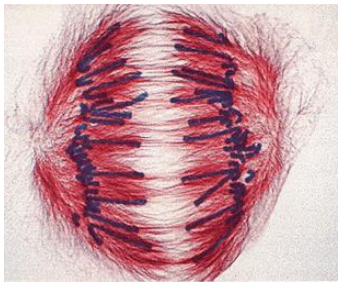
During interphase, the kinetochore persists as a condensed ball of heterochromatin that resembles other areas of condensed chromatin within the nucleus. The distinct multilayered kinetochore structure forms on the surface of the centromere during an early stage of mitosis called prophase, reaching its mature state following nuclear envelope

breakdown when the chromosome comes into contact with microtubules at the onset of mitotic prometaphase.

There are three types of centromeres known in eukaryotes (see Fig. 7.9). Point centromeres found in budding yeasts assemble kinetochores on defined DNA sequences and do not require epigenetic activation to function. They bind one microtubule. Regional centromeres, found in organisms ranging from fission yeast to humans, are based on preferred DNA sequences but require epigenetic activation to function. They bind two to 20 or more microtubules. In holocentromeres, as found in *Caenorhabditis elegans* and many plants and insects, the microtubules (roughly 20 in C. elegans) bind all along the poleward-facing surface of the mitotic chromosome. Given this diversity of centromeres, it is remarkable that the proteins responsible for kinetochore assembly and function are well conserved across evolution.

Spindle fibers:-Spindle apparatus (Mitotic Apparatus)

The spindle apparatus is a structure of the eukaryotic cytoskeleton involved in mitosis and meiosis, often referred to as the mitotic spindle during mitosis and the meiotic spindle during meiosis. Its function is to segregate chromosomes during cell division (either mitosis or meiosis) to the daughter cells. It consists of a bundle of microtubules joined at the ends but spread out in the middle, vaguely ellipsoid in shape. In the wide middle portion, known as the spindle midzone, antiparallel microtubules are bundled by kinesins. At the pointed ends, known as spindle poles, microtubules are nucleated by the centrosomes in the cells of most animals.



During spindle assembly in prometaphase, some of the spindle's microtubules attach to the kinetochores that assemble on the centromere portion of the chromosomes. The chromosomes are pulled into alignment along the spindle midzone to form the metaphase spindle. Once all the chromosomes are aligned with sister chromatids pointing to opposite ends of the spindle, the cell enters anaphase, in which the chromatids separate and move toward their respective poles. Since the center of the spindle specifies the plane along which the cell will divide during cytokinesis, this ensures that each daughter cell

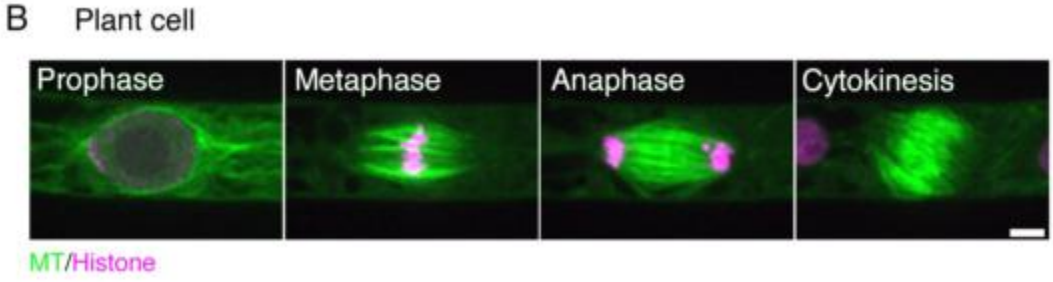
will receive one of each chromatid. The mitotic kinase aurora A is required for proper spindle assembly and separation.

Mechanism of Mitotic Spindle Assembly in Seed Plants

Researchers have elucidated the mechanism of acentrosomal spindle formation in land plants through microscopic observation and have revealed the processes common to, and different from, animal somatic cells.

The two main differences between animal and plant spindles are (1) the presence of centrosomes and well-developed astral MTs in animal spindles, and (2) the morphology of the anaphase spindle (the 'phragmoplast' in plants.

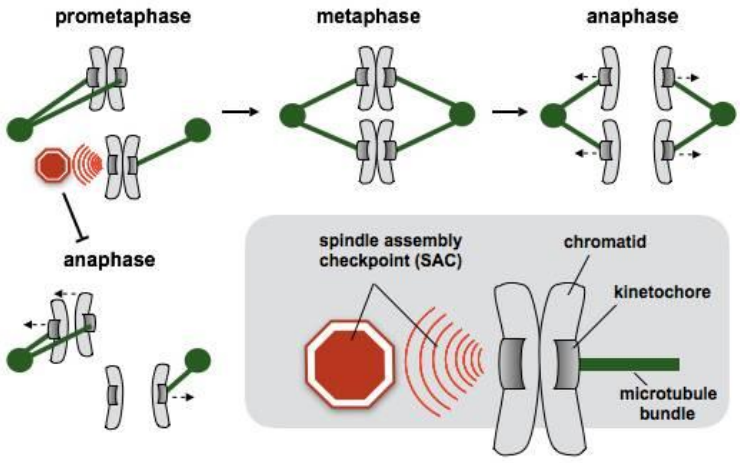
One of the best-characterised cell types with regard to mitotic spindle assembly is the endosperm of African blood lily *Haemanthus*. In the absence of centrosomes, abundant MTs (microtubules) are detected around the nuclear envelope during prophase. Immunofluorescence microscopy identified MT converging centres within the MT cloud, which was consistent with the idea that they are the major MT nucleation sites at this stage. MTs around the nucleus are gradually organized into a spindle-like structure, called the 'prophase spindle' (or 'prospindle'). The prophase spindle has either a bipolar fusiform or multipolar structure. After nuclear envelope breakdown (NEBD), MTs emanating from the converging centres associate with kinetochores to form kinetochore MTs. MTs are also likely nucleated near the chromosome/kinetochore independent of prophase spindles during the prometaphase as an MT depolymerisation/regrowth assay detected chromosome-proximal MT formation. Those MTs are then organised into an overall bipolar configuration.



Electron microscopy showed that the majority of the MTs are oriented in such a way that plus ends are pointed to the chromosome/kinetochore, similar to animal spindles. However, the metaphase spindle is barrel-shaped rather than fusiform,as the pole is not tightly focused at one point; multiple kinetochore and non-kinetochore MTs are converged or cross-linked locally and, thus, multiple mini-poles are observed. Immunostaining of MTs also identified 'fir tree' structures within the spindle, in which many MTs branched off from kinetochore MTs. With the start of anaphase, sister

chromatids are separated and then segregated to the pole by kinetochore MT depolymerisation, analogous to animal spindles. During telophase, the phragmoplast forms and is followed by centrifugal expansion towards the cell cortex.

During the mitotic phase of the cell cycle, the duplicated genome in the form of condensed chromosomes is partitioned to the daughter cells. From S-phase onward, the two copies of each chromosome (referred to as sister chromatids) are connected by molecular rings and each copy attempts to establish attachments to spindle microtubules that emanate from two spindle poles. If one sister has attached to one pole, the other needs to attach to the other pole. Since the two poles are on opposite sides of the cell, such a 'bipolar' attachment allows the cell to drag each sister to opposite sides as soon as the linkages are removed. The cell only initiates this phase of sister separation when each and every chromosome is attached in a bipolar fashion. After chromosome segregation, the cell creates a cleavage site in between the two segregating genomes, resulting in the generation of two distinct but genetically identical daughter cells.



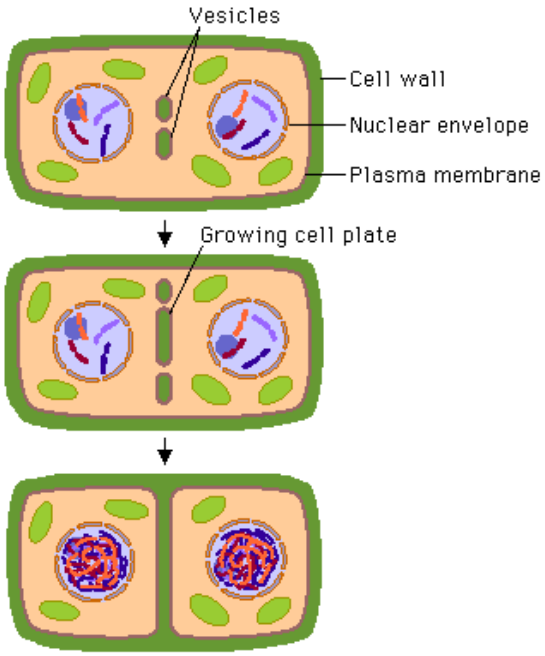
Scheme of chromosome segregation and the function of the Spindle Assembly Checkpoints (red octagon). The SAC (Spindle Assembly Checkpoints) prevents premature anaphase when one of the chromatids has not stably captured a microtubule.

The process to generate a bipolar attachment is known as biorientation. Biorientation is a highly dynamic and relatively fast process that is an interplay between the microtubule-based pulling apparatus (the mitotic spindle), the spindle attachment site on each sister (the kinetochore), and proteins that regulate the interaction between kinetochore and spindle (Figure 1). To prevent genetic imbalances after cell division, the cell has to make sure that all 46 duplicated chromosomes (in case of a diploid human cell) are bioriented and ready to be pulled in opposite directions. For this, it has evolved a

surveillance mechanism (the spindle assembly checkpoint) that monitors the attachments status of each individual kinetochore to spindle microtubules (Figure 1). The cell is not allowed to proceed with chromosome segregation if even a single kinetochore is not properly attached to the spindle (and thus the chromosome pair not properly bioriented).

Cytokinesis:-

Plant cells have walls, so cytokinesis cannot proceed with a cleavage furrow. Instead, during telophase a cell plate forms across the cell in the location of the old metaphase plate.



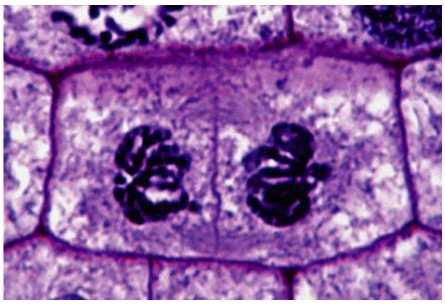
During telophase, membrane-enclosed vesicles derived from the Golgi apparatus migrate to the center of the cell where the metaphase plate used to be and fuse to form a cell plate.

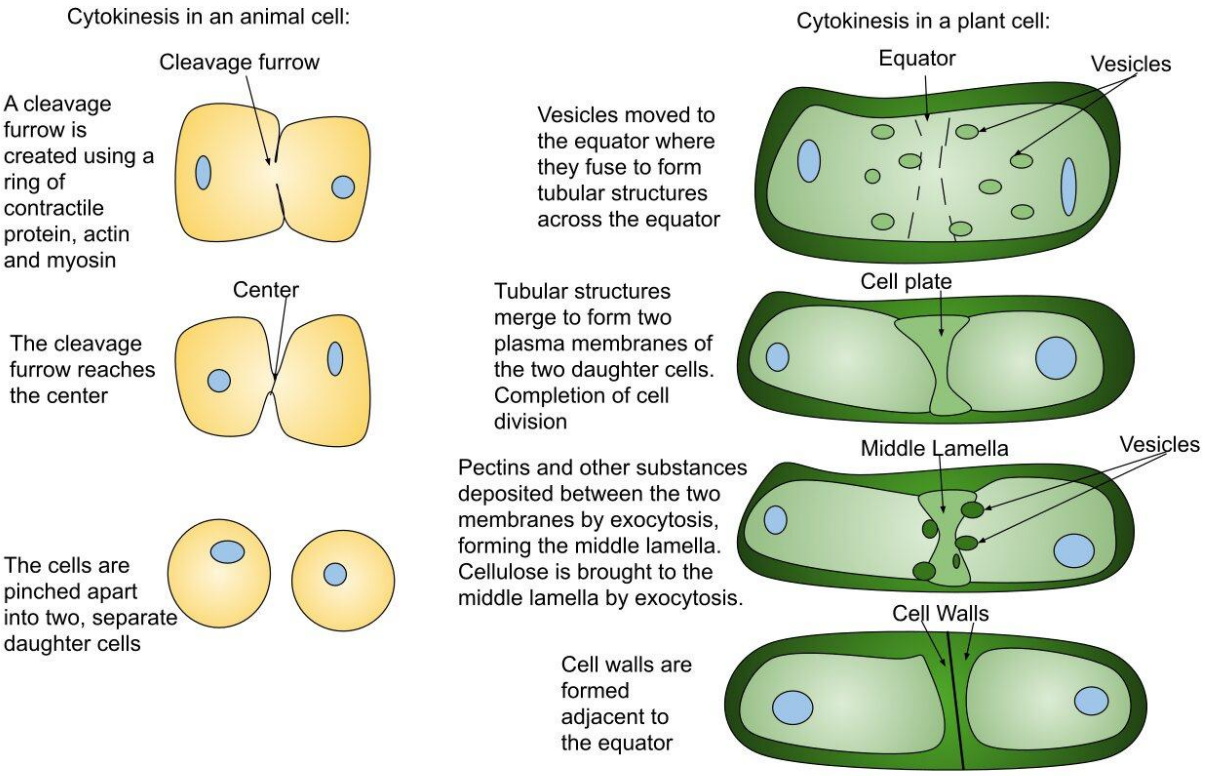
Eventually, the growing cell plate fuses with the existing plasma membrane, producing two daughter cells, each with its own plasma membrane.

A new cell wall forms between the two membranes of the cell plate.

Cytokinesis is the partitioning of the cytoplasm following nuclear division. This process presents a number of challenges for the plant cell: first, to avoid losing or bisecting the nucleus, this event needs to be carefully coordinated with respect to the nuclear cycle in space and in time. Second, a structure as complex as the plant cell wall needs to be laid down during the brief period of time between anaphase and telophase. The spatial and temporal regulation of cytokinesis requires a series of links between the nuclear cycle, the cell cortex, the Golgi apparatus, and the membrane trafficking

apparatus. A number of genes have recently been identified that could enable us to probe such links.





Cytokinesis in higher plants may be considered as a specialized form of secretion. At the end of anaphase, Golgi-derived secretory vesicles carrying cell wall materials are transported to the equator of a dividing cell. Fusion of these vesicles gives rise to a membrane-bound compartment, the cell plate. The cell plate expands from the middle out (centrifugally) until it reaches the “zone of attachment” or division site on the mother cell wall. Once this attachment has taken place, the cell plate undergoes a complex process of maturation during which callose is replaced by cellulose and pectin (Samuels et al., 1995, and references therein).

Apoptosis and its significance in plants.

Cell death can be driven by a genetically programmed signalling pathway known as programmed cell death (PCD). In plants, PCD occurs during development as well as in response to environmental and biotic stimuli. Apoptosis is an integral part of plant ontogenesis and two lines of critical evidence are available to support the claims: (1) the presence of apoptotic-like bodies, and (2) the importance of structural similarity suggesting functional conservation. It is controlled by cellular oxidative status, phytohormones, and DNA methylation. Genes controlling Programmed cell death (PCD) are conserved across wide evolutionary distances from *Caenorhabditis elegans* to humans.

Multicellular organisms possess intrinsic and extrinsic programs for cell suicide. Programmed cell death (PCD) is genetically controlled either by so-called death receptors or intrinsic signals that direct the cell to eliminate itself during development and in response to pathogens or environmental insults. It is thought that PCD arose during evolution in response to viruses to prevent virion replication, thereby sparing surrounding cells. Hallmarks of PCD have been observed in bacteria and yeast, presumably selected for by viruses or other evolutionary pressures. The presence of prokaryote-derived chloroplasts and mitochondria in plant cells, while yet to be shown as essential toplant PCD, are major sources of reactive oxygen species (ROS) and have been implicated in plant PCD regulation4–7, with the latter organelle playing a crucial role in animal PCD.

Significance in apoptosis plants

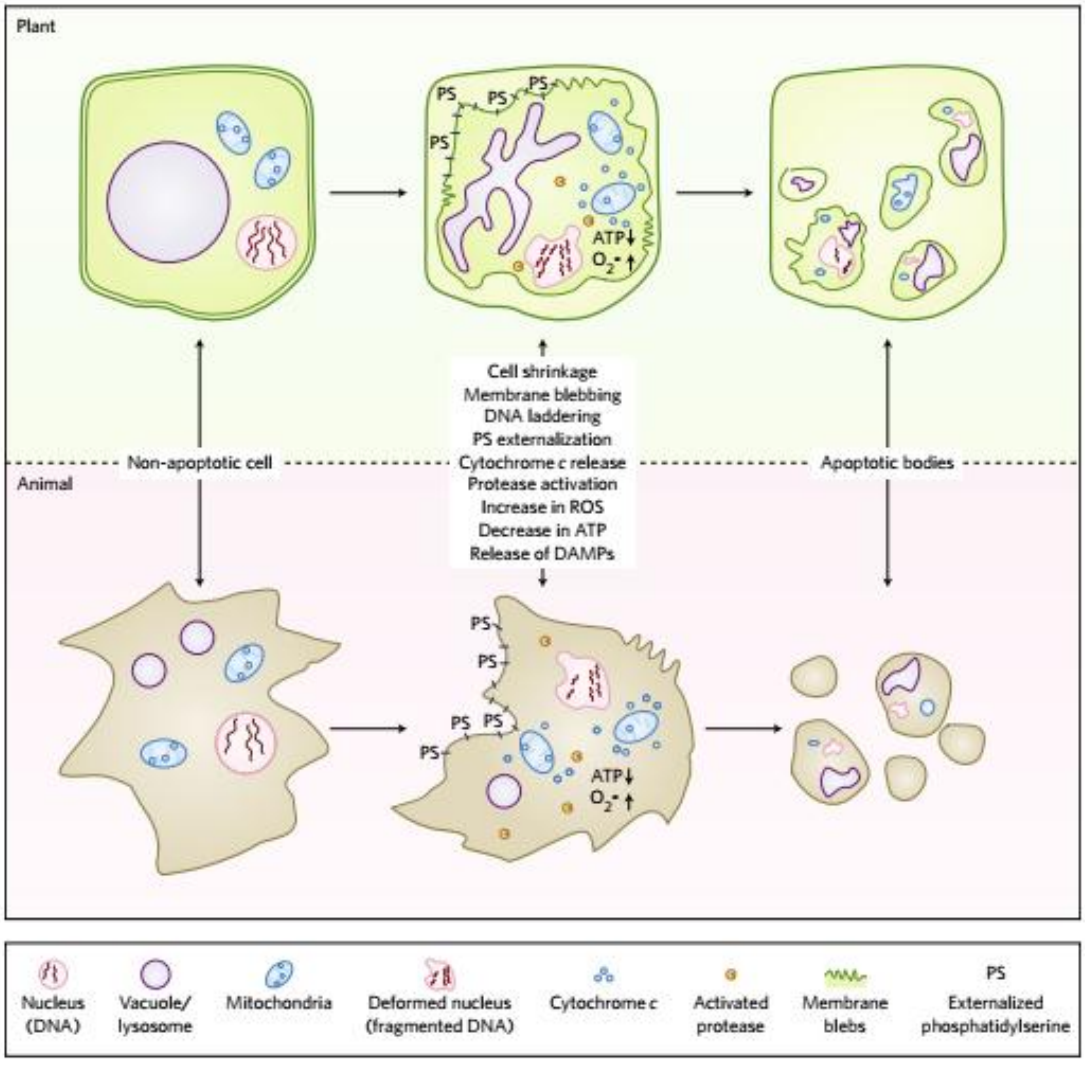
Like animals, plants use a suite of regulatory pathways to program the demise of cells. Plant PCD has been observed during development (for example, xylem formation, seed maturation, leaf senescence and several plant reproductive processes) and in plant–pathogen interactions (for example, *Sclerotinia sclerotiorum*, *Cochliobolus victoriae*, tobacco mosaic virus (TMV) and Botrytis cinerea), as well as during environmental stresses (for example, drought, heat, cold and hypersalinity).

In wheat plants apoptosis appears at early stages of development in coleoptile and initial leaf of 5- to 6-day-old seedlings.

Distinct ultrastructural features of apoptosis observed are (1). compaction and vacuolization of cytoplasm in the apoptotic cell, (2). specific fragmentation of cytoplasm and appearance in the vacuole of unique single-membrane vesicles containing active organelles, (3). cessation of nuclear DNA synthesis, (4). condensation and margination of

chromatin in the nucleus, (5). internucleosomal fragmentation of nuclear DNA, and (6). intensive synthesis of mitochondrial DNA in vacuolar vesicles.

Peroxides, abscisic acid, ethylene releaser ethrel, and DNA methylation inhibitor 5-azacytidine induce and stimulate apoptosis. Modulation of the reactive oxygen species (ROS) level in seedling by antioxidants and peroxides results in tissue-specific changes in the target candidate for the appearance and the intensity of apoptosis.



Comparison of apoptotic-like cell death in animal and plant cells. Plant cells (top) can display many of the hallmark features of animal apoptotic cell death (bottom). Similar to animals, subsets of apoptotic-like features are only observed in plants under certain conditions

Plants exhibit nearly all of the biochemical and morphological features of apoptosis. One difference between plant and animal PCD is the absence of phagocytosis

in plants. Evidence is emerging that the vacuole may be key to removal of unwanted plant cells, and may carry out functions that are analogous to animal phagocytosis.