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DEPARTMENT OF ELECTRONICS AND COMMUNICATION ENGINEERING

UNIT - III

CLASS : S7 ECE

SUBJECT CODE : OBT753

SUBJECT NAME : INTRODUCTION OF CELL BIOLOGY

REGULATION : 2017

UNIT 3

DIVISION

3.1 CELL CYCLE

3.1.1 DEFINITION

A series of events leading to the formation of new cell is known as **cell cycle**. The phenomenon changes leading to formation of new population take place in the cell cycle. The series of events include several phases.

3.1.2 DURATION OF CELL CYCLE

Different kinds of cells have varied duration for cell cycle phases. Eukaryotic cell divides every 24 hours. The cell cycle is divided into mitosis and interphase. In cell cycle 95% is spent for interphase whereas the mitosis and cytokinesis last only for an hour.

3.1.3 INTERPHASE

Longest part of the cell cycle, but it is of extremely variable length. At first glance the nucleus appears to be resting but this is not the case at all. The chromosomes previously visible as thread like structure, have dispersed. Now they are actively involved in protein synthesis, at least for most of the interphase.

3.1.4 G_1 PHASE

The first gap phase-2C amount of DNA in cells of G_1. The cells become metabolically active and grows by producing proteins, lipids, carbohydrates and cell organelles including mitochondria and endoplasmic reticulum.

Many checkpoints control the cell cycle.

The checkpoint called the **restriction point** at the end of G_1, determines a cells fate whether it will continue in the cell cycle and divide or enter a stage called **G_0** as a quiescent stage and probably as specified cell or die. Cells are arrested in G_1 due to:

- Nutrient deprivation
- Lack of growth factors or density dependent inhibition
- Undergo metabolic changes and enter into G_0 state

Biochemicals inside cells activates the cell division. The proteins called **kinases** and cyclins activate genes and their proteins to perform cell division.

Cyclins act as major checkpoint which operates in G_1 to determine whether or not a cell divides.

3.1.5 G_0 PHASE

Some cells exit G_1 and enters a quiescent stage called **G_0**, where the cells remain metabolically active without proliferation.

Cells can exist for long periods in G_0 phase. In G_0 cells cease growth with reduced rate of RNA and protein synthesis.

The G_0 phase is not permanent. Mature neuron and skeletal muscle cell remain permanently in G_0. Many cells in animals remains in G_0 unless called on to proliferate by appropriate growth factors or other extracellular signals. G_0 cells are not dormant.

3.1.6 S PHASE – SYNTHESIS PHASE – CELLS WITH INTERMEDIATE AMOUNTS OF DNA

Growth of the cell continues as replication of DNA occur, protein molecules called **histones** are synthesized and attach to the DNA. The centrioles duplicate in the cytoplasm. DNA content increases from 2C to 4C.

3.1.7 G_2 – THE SECOND GAP PHASE – 4C AMOUNT OF DNA IN CELLS OF G_2 AND MITOSIS

Cell growth continues by protein and cell organelle synthesis, mitochondria and chloroplasts divide. DNA content remains as 4C.

Tubulin is synthesized and microtubules are formed.

Microtubules organize to form spindle fibre. The spindle begins to form and nuclear division follows.

One of the proteins synthesized only in the G_2 period is known as **Maturation Promoting Factor (MPF)**.

It brings about condensation of interphase chromosomes into the mitotic form.

DNA damage checkpoints operates in G_1 , S and G_2 phases of the cell cycle.

3.2 MITOSIS

3.2.1 DEFINITION

Mitosis is the process of cell division in which one cell gives rise to two genetically identical daughter cells, resulting in cell duplication and reproduction.

- The number of chromosomes is preserved in both the daughter cells.
- Mitosis is a short period of chromosome condensation, segregation, and cytoplasmic division.

- The mitosis occurs in the somatic cells, and it is meant for the multiplication of cell numbers during embryogenesis and blastogenesis of plants and animals.
- As a process, mitosis is remarkably similar in all animals and plants.

3.2.2 CLOSED AND OPEN MITOSIS

In closed mitosis, the nuclear envelope remains intact and chromosomes migrate to opposite poles of a spindle within the nucleus. Eg: Many single celled eukaryotes including yeast and slime molds.

In open mitosis, the nuclear envelope breaks down and then reforms around the 2 sets of separated chromosome. Eg: Most plants and animals.

3.2.3 PURPOSE OF MITOSIS

The process of mitosis is significant in both cell division as well as cell reproduction. Some of the major significances/purposes are given below:

1. Continuous mitosis results in the increase in the number of cells enabling the organism to grow from a single cell to a complex living organism.
2. Different cells in the body like the cells on the skin and red blood cells are continuously replaced by mitosis.
3. About 5×10^9 cells are formed per day in humans via mitosis.
4. Mitosis is also involved in the repairmen and regeneration of body structures like in the starfish.
5. In multiple organisms, mitosis is the method of asexual reproduction.

3.2.4 STAGES

Mitosis is a part of the cell cycle and is preceded by the S phase of interphase and usually followed or accompanied by cytokinesis. Replication of chromosomes and synthesis of proteins required for spindle fiber formation are formed prior to the onset of mitosis.

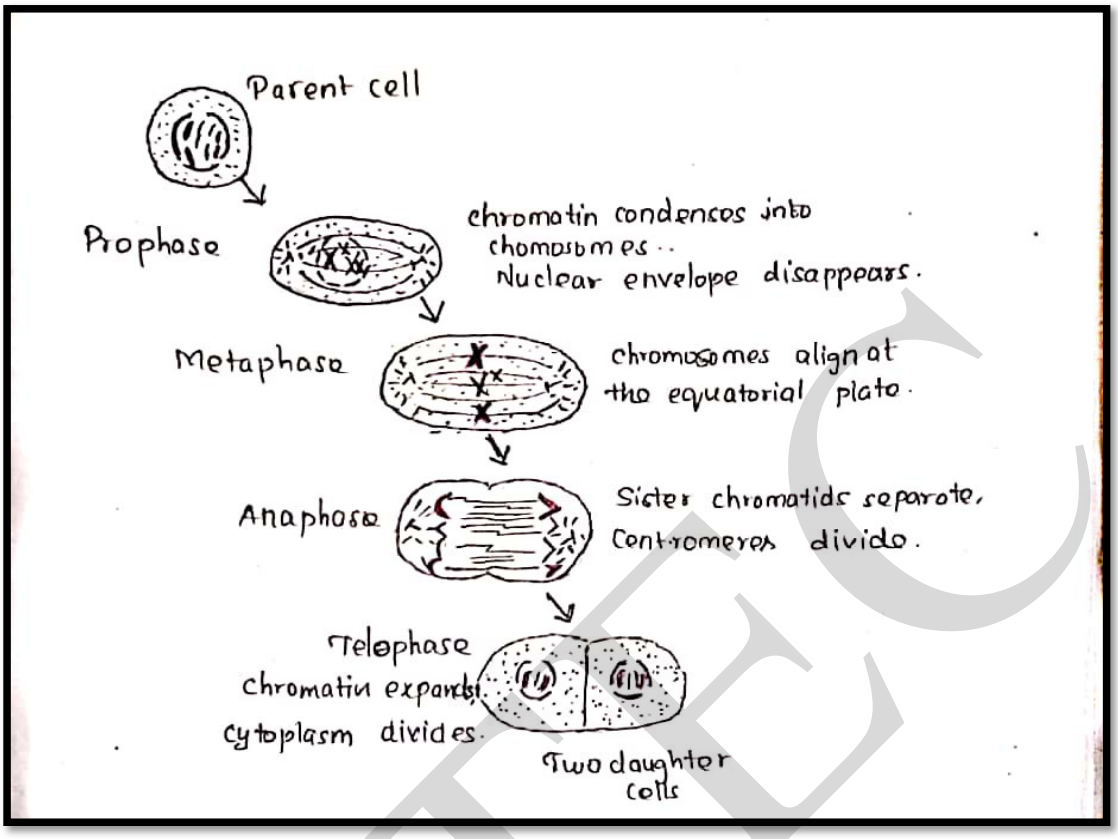

FIG 3.1: Structure of Mitosis

Mitosis is divided into the following phases based on the completion of one set of activities and the onset of the other.

1. Interphase

- Interphase is a part of the cell cycle where the cell copies its DNA as preparation for the M phase (mitotic phase).
- In interphase, metabolism of the cell increases, and it is often termed the most active phase of the cell cycle.
- A series of metabolic changes occur during this phase, all of which are divided into three subgroups.

G_1-phase or Pre-DNA synthesis phase

- It is the longest phase of the cell cycle and is followed by the M phase of the previous cell cycle.
- It is also termed as the “resting phase” as no DNA synthesis takes place during this phase.

- However, during G_1 phase, several cell organelles increase in size and cell rapidly synthesizes different types of RNA and proteins.
- Important events like the transcription of three types of RNAs, synthesis of regulatory proteins, enzymes required for DNA synthesis, and tubulin proteins along with other mitotic apparatus take place during this phase.

S-phase or DNA Synthesis phase

- S-phase involves the replication of nuclear DNA and the synthesis of histone proteins. The replication of cytoplasmic DNA can take place at any phase in the cell cycle.
- Thus, at the end of the S phase, each chromosome has two DNA molecules and a duplicate set of genes.
- This phase lasts for about 6-10 hours.

G_2-phase or Post DNA synthesis phase

- G_2 phase is termed the second gap phase or resting phase of the interphase.
- During this phase, the synthesis of RNA and proteins required for the cell continues.
- Cell division involves the enormous expenditure of energy, thus cell stores ATP in the G_2.
- By the end of this phase, the cell enters the division or M-phase of the cell cycle.

2. Prophase

- Prophase is the first stage of mitosis which is characterized by the appearance of thin-thread like condensing chromosomes.
- During prophase, the cell becomes spheroid while the cytoplasm becomes more refractile and viscous and pale.
- The chromosome in the prophase is composed of two coiled filaments, the chromatids, which are the result of the replication of DNA during the S phase.
- As prophase progresses, the chromatids become shorter and thicker, and two sister chromatids of each chromosome are held together by a special DNA-containing region, called the centromere**.**
- Similarly, the chromosomes approach the nuclear envelope, causing the central space of the nucleus to become empty.

- In the meantime, two pairs of centrioles surrounded by microtubules radiating in all directions migrate to opposite poles of the cell.
- Lastly, during prophase, the nucleolus gradually disintegrates, and this marks the end of prophase.
- However, in some primitive classes of plants and animals, the nuclear envelope does not dissolve during mitosis.

3. Prometaphase

- Prometaphase is initiated with the breakdown of the nuclear envelope, which enables the interaction of spindle fibers with the chromosomes.
- At this stage, the chromosomes are violently rotated and oscillated back and forth between the spindle poles because their centromeres are capturing the ends of microtubules and are being pulled by the captured microtubules.
- By the end of prometaphase, the sister chromatids are attached to the spindle fibers on the opposite ends and are held on the metaphase plate.

4. Metaphase

- During metaphase, the chromosomes are shortest and thickest.
- Their centromeres of the sister chromatids occupy the plane of the equator forming a metaphase plate, and the arms remain directed towards the poles.
- Two chromatids of a chromosome repulse each other with the microtubules remaining stationary and under tension.

5. Anaphase

- The anaphase begins abruptly with the synchronous splitting of each chromosome into its sister chromatids, called daughter chromosomes, separating for the centromere.
- The splitting of each centromere during prophase is caused by an increase in cytosolic Ca^{2+}.
- In anaphase, there is a movement of chromatids towards the pole due to the shortening of the microtubules.
- During their poleward migration, the centromeres remain forward so that the chromosomes characteristically appear U, V or J- shaped.
- Interzonal fibers expand and support the movement of chromosomes towards the pole.

- A total of 30 ATPs are required to carry chromosomes to the poles.

6. Telophase

- The end of the migration of the daughter chromosomes to the poles marks the beginning of the telophase
- During telophase, the events of prophase occur in reverse sequence.
- A nuclear envelope reassembles around each group of chromosomes to form two daughter nuclei.
- Events like the disappearance of mitotic apparatus, reduction in the viscosity of cytoplasm followed by synthesis of RNA take place during telophase.
- The chromosomes resume their long, slender, extended form and the nucleolus reappears at the end of telophase.

7. Cytokinesis

- Cytokinesis is the division of cytoplasm which is followed by mitosis, resulting in the formation of two separate daughter cells.
- Cytokinesis usually begins in anaphase and continues through telophase and into interphase.
- In animals, cytokinesis occurs through constriction and furrow formation.
- The first sign of cleavage in animal cells is constriction of the plasma membrane during anaphase.
- The constriction invariably occurs in the plane of the metaphase plate, at right angles to the long axis of the mitotic spindle apparatus.
- The constriction grows more in-depth from the outside to the inside, and ultimately a cell divides into two daughter cells.
- In plants, however, cytokinesis occurs by cell plate formation as constriction is not possible due to the presence of a rigid cell wall.
- Golgi apparatus arrange themselves on the equator to form phragmoplast, which later forms the cell plate in plants.

3.2.5 APPLICATIONS

Mitosis has been utilized for many lab-based techniques in molecular biology and biotechnology. Some of the common use of mitosis are:

1. **Cloning**

- Cloning is a technique employed in biotechnology to produce identical copies of cells or DNA fragments.
- In cloning, the number of organisms is increased by the process of mitosis, which is then used in a wide array of biological experiments like fingerprinting.

2. Tissue culture

- The growth of tissues or cells outside of the body of the organism in a liquid, semi-solid, or solid growth medium is called tissue culture.
- Tissue culture is based on the process of mitosis, where a cell undergoes division to form multiple tissues.
- Besides, tissue culture may lead to organ culture in various organisms.

3. Stem cell regeneration

- Stem cells are a group of cells that can be directed to form specialized cells in the body.
- Stem cells can undergo mitosis to regenerate and repair diseased or damaged tissues in people.

3.3 MEIOSIS

3.3.1 DEFINITION

- Meiosis is a type of cell division in sexually reproducing eukaryotes, resulting in four daughter cells (gametes), each of which has half the number of chromosomes as compared to the original diploid parent cell.
- The haploid cells become gametes, which by union with another haploid cell during fertilization defines sexual reproduction and formation of a new generation of diploid organisms.
- Meiosis occurs in the germ cells of sexually reproducing organisms.
- In both plants and animals, germ cells are localized in the gonads, but the time at which meiosis takes place varies among different organisms.

3.3.2 PURPOSE OF MEIOSIS

The process of meiosis is essential for all sexually reproducing organisms for the following reasons:

1. The meiosis maintains a constant number of chromosomes in sexually reproducing organisms through the formation of gametes.

2. By crossing over, the meiosis results in the exchange of the genes and, thus, causes the genetic variations among the species. These variations are the raw materials of the evolutionary process.

3.3.3 STAGES

- Meiosis is composed of two rounds of cell division, namely Meiosis I and Meiosis II.
- Each round of division contains a period of karyokinesis (nuclear division) and cytokinesis (cytoplasmic division).

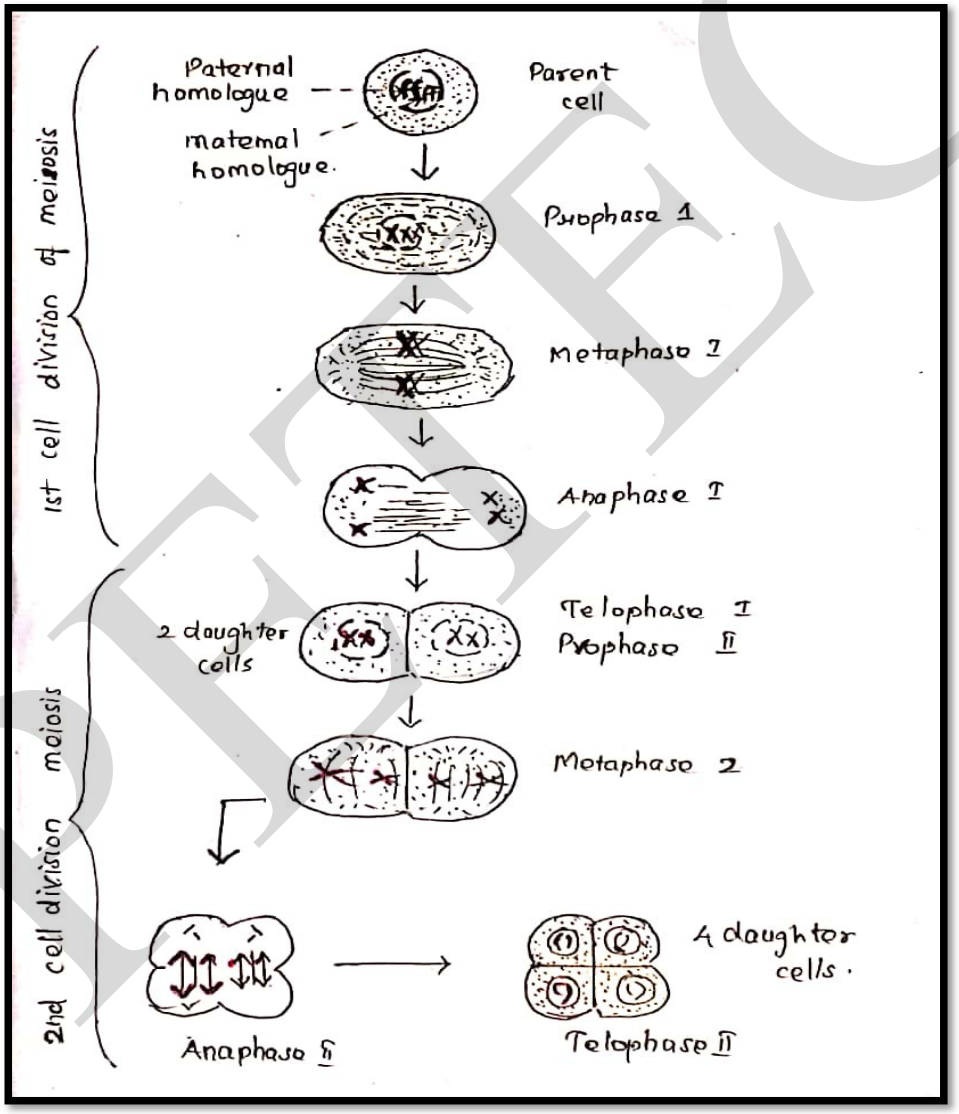

FIG 3.2: Structure of Meiosis

3.3.3.1 MEIOSIS I

- The first meiotic division consists of prolonged prophase in which the homologous chromosomes come in close contact with each other and exchange hereditary material between them.
- Similarly, in the first meiotic division, the reduction of chromosome number takes place and, thus, two haploid cells are resulted by this division.
- The first meiotic division is also known as the heterotypic division.

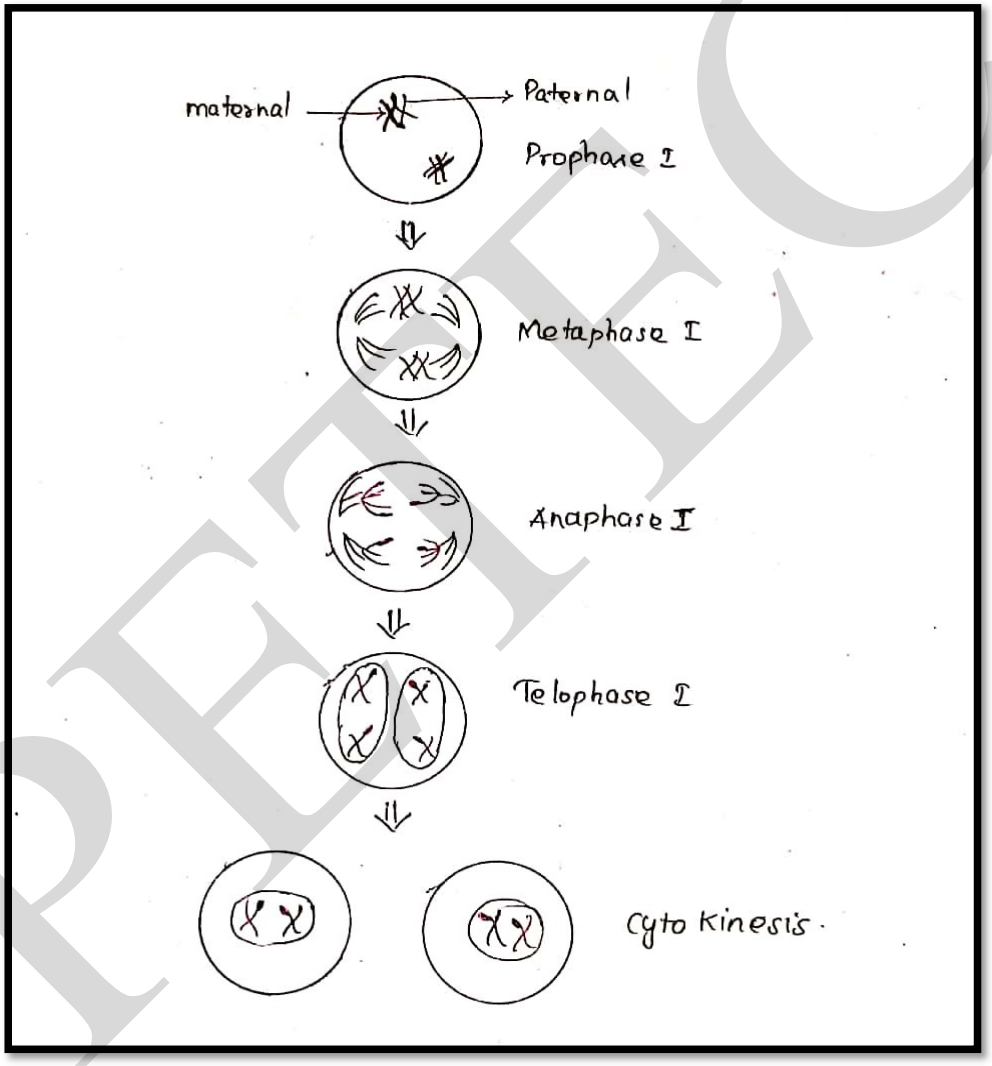

FIG 3.3: Structure of Meiosis I

- Meiosis I consists of the following steps:

Interphase

- Just like mitosis, meiosis also consists of a preparatory phase called interphase.

- The interphase is characterized by the following features :
- The nuclear envelope remains intact, and the chromosomes occur in the form of diffused, long, coiled, and indistinctly visible chromatin fibers.
- The DNA amount becomes double. Due to the accumulation of ribosomal RNA (rRNA) and ribosomal proteins in the nucleolus, the size of the nucleolus is significantly increased.
- In animal cells, a daughter pair of centrioles originates near the already existing centriole and, thus, an interphase cell has two pairs of centrioles.
- In the G_2 phase of interphase, there is a decisive change that directs the cell toward meiosis, instead of mitosis.
- At the beginning of the first meiotic division, the nucleus of the dividing cell starts to increase in size by absorbing the water from the cytoplasm, and the nuclear volume increases about three folds.

Prophase I

Prophase I is the longest stage of the meiotic division. It includes the following substages:

Leptotene

- In the leptotene stage, the chromosomes become even more uncoiled and resemble a long thread-like shape, and they develop bead-like structures called chromomeres.
- The chromosomes at this stage remain directed towards centrioles, so the chromosomes in the nucleus appear like a bouquet in the animal cell. Therefore, this stage is also called the Bouquet Stage.

Zygotene or Synaptotene

- The zygotene stage begins with the pairing of homologous chromosomes, which is called synapsis.
- The paired homologous chromosomes are connected by a protein-containing framework called a synaptonemal complex.
- The synaptonemal complex helps to stabilize the pairing of homologous chromosomes and to facilitate recombination or crossing over.
- The synapsis might begin at one or more points along the length of the homologous chromosomes.

- Synapsis might start from the ends of the chromosomes and continues towards their centromeres (proterminal synapsis), or it might start at the centromere and proceed towards the ends (procentric pairing).
- In some cases, the synapsis occurs at various points of the homologous chromosomes (random pairing).

Pachytene

- In this stage, the pair of chromosomes become twisted spirally around each other and cannot be distinguished separately.
- In the middle of the pachytene stage, each homologous chromosome splits lengthwise to form two chromatids, but they continue to be linked together by their common centromere.
- The chromosomes at this point are termed bivalent because it consists of two visible chromosomes, or as a tetrad because of the four visible chromatids.
- This stage is particularly crucial as a critical genetic phenomenon called " crossing over" takes place in this stage.
- The crossing over involves redistribution and mutual exchange of hereditary material between two homologous chromosomes.
- The enzyme endonuclease breaks the non-sister chromatids at the place of crossing over.
- After the breaking of chromatids, the interchange of chromatid segments takes place between the non-sister chromatids of the homologous chromosomes.
- Another enzyme, ligase, binds the broken chromatid segments with the non-sister chromatid.
- The process of mutual exchange of chromatin material between one non-sister chromatid of each homologous chromosome is known as the crossing over.

Diplotene

- The synaptonemal complex appears to be dissolved, leaving chromatids of the paired homologous chromosome physically joined at one or more localized points called
- In diplotene, chiasmata move towards the end of chromosomes in a zip like a manner.

Diakinesis

- In this stage, the bivalent chromosomes become more condensed and uniformly distributed in the nucleus.
- At this point, the nuclear envelope breaks down, and the nucleolus disappears.
- Further, the chiasmata reach the end of the chromosomes, and the chromatids remain attached until metaphase.

Metaphase I

- Metaphase I consists of spindle fiber attachment to chromosomes and chromosomal alignment at the equator.
- During metaphase I, the spindle fibers are attached with the centromeres of the homologous chromosomes, which are directed towards the opposite poles.

Anaphase I

- At anaphase I homologous chromosomes are separated from each other, and due to the shortening of chromosomal fibers or microtubules, each homologous chromosome with its two chromatids and undivided centromere move towards the opposite poles of the cell.
- Because during the chiasma formation, one of the chromatids has changed its counterpart, therefore, the two chromatids of a chromosome are not genetically identical.

Telophase I

- The onset of telophase I is defined by the movement of a haploid set of chromosomes at each pole.
- The nuclear envelope is formed around the chromosomes, and the chromosomes become uncoiled. The nucleolus reappears and, thus, two daughter nuclei are formed.

Cytokinesis I

- In animals, cytokinesis occurs by the constriction of the cell membrane while in plants, it occurs through the formation of the cell plate, resulting in the creation of two daughter cells.

3.3.3.2 MEIOSIS II

- In the second phase of the meiotic division, the haploid cell divides mitotically and results in four haploid cells. This division is also known as the homotypic division.

- This division does not include the exchange of the genetic material and the reduction of the chromosome number as in the first meiotic division.

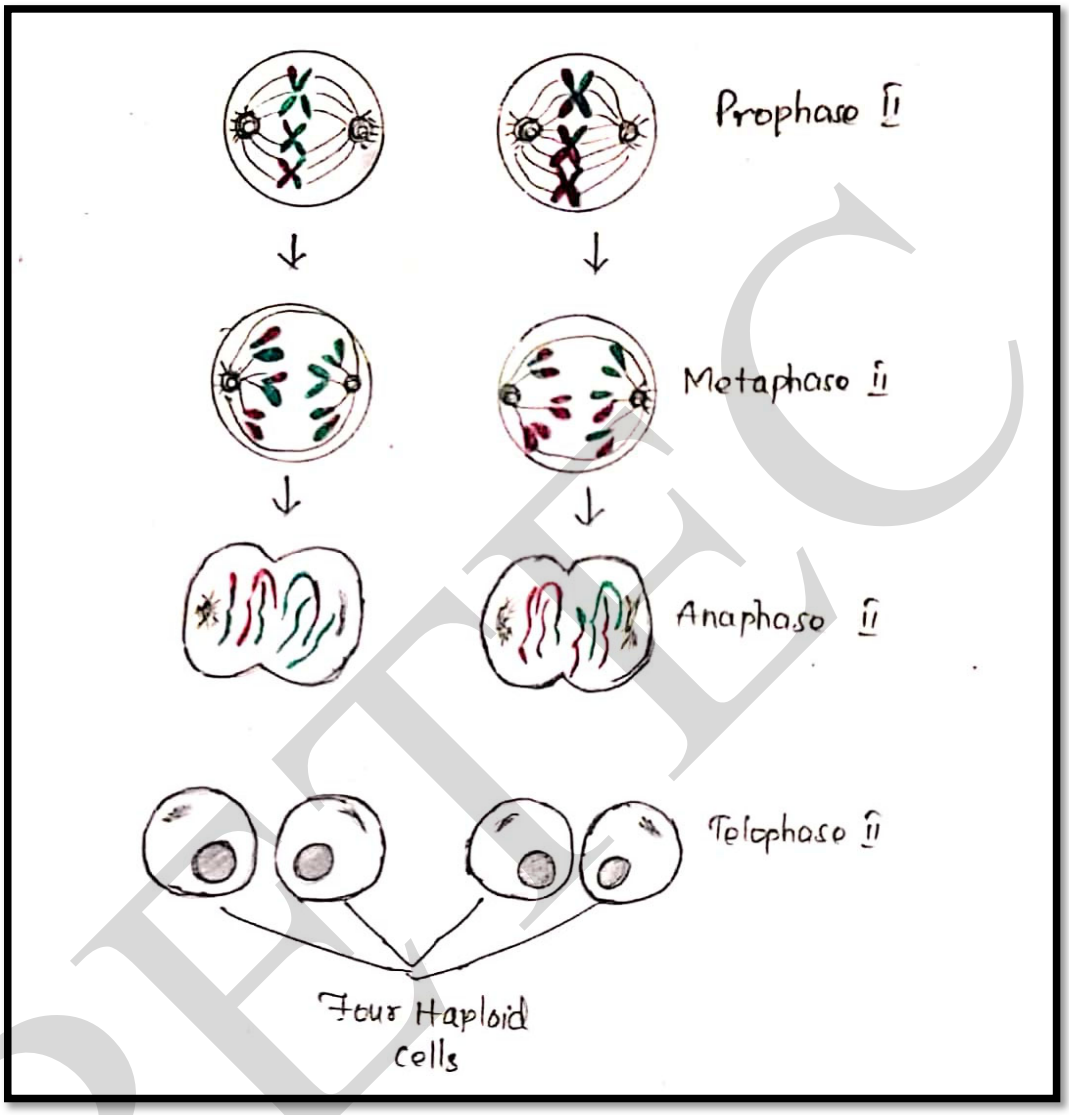

FIG 3.3: Structure of Meiosis II

- Meiosis II consists of the following steps:

Prophase II

- In prophase II, each centriole divides, resulting in two pairs of centrioles.
- The centrioles move towards the opposite poles and the nuclear membrane, and the nucleolus disappears.

Metaphase II

- During metaphase II, the chromosomes get arranged on the equator of the cell through the spindle fibers.
- The centromere divides and, thus, each chromosome produces two daughter chromosomes.
- The spindle apparatus is attached to the centromere of each chromosome.

Anaphase II

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

Telophase II

- The chromatids migrate to the opposite poles and now known as chromosomes.
- The endoplasmic reticulum forms the nuclear envelope around the chromosomes, and the nucleolus reappears due to the synthesis of ribosomal RNA.

Cytokinesis II

- The process of cytokinesis is identical to cytokinesis I resulting in the division of cytoplasm for each of the four daughter cells formed.

3.3.4 APPLICATIONS

Meiotic like mitosis is used for several lab-based technologies, some of which are given below:

Tissue culture

- Like mitosis, meiosis is also used in biotechnology to acquire a gametic condition in cells.
- Meiosis often accompanies mitosis to generate variation which aids in studies regarding evolutionary processes.

In-vitro gamete formation

- In various gamete failure-derived infertility issues, the embryonic stem cells are differentiated into germ-like cells through the meiotic division.
- These gametes are formed in-vitro via meiosis and are inserted into the individuals with such disorders.

3.4 APOPTOSIS

3.4.1 DEFINITION

- Apoptosis is a normal genetically programmed cell death where an aging cell at the end of its life cycle shrinks and its remaining fragments are phagocytosed without any inflammatory reaction**.**
- It consists of a series of biochemical changes that lead to changes in the cell's morphology or death.
- It results in the death of 50 to 70 billion cells per day in an average adult human being.
- It is also termed as 'cellular suicide' as cells undergo a highly regulated process for the programmed removal of cells from the body.

3.4.2 WHY DO CELLS UNDERGO APOPTOSIS?

- Most cells are provided with an in-built mechanism of apoptosis as a part of the cell cycle.
- This mechanism allows the body to get rid of unnecessary cells or infected cells.
- Apoptosis is considered a vital part of various processes including normal cell cycle, proper development and functioning of the immune system, embryonic development, and chemical-induced cell death.
- Apoptosis is a part of development as it is essential in the differentiation of a mass of tissue into various groups.
- Apoptosis occurs in cells that might have been infected with viruses or might even be cancerous. This process usually takes place when the cell detects defects in the **DNA** and is not able to repair it.
- Apoptosis is also an essential part of the immune system as it clears the pathogen-specific immune cells once the foreign particle is removed from the body.
- This also helps to remove the immune cells that might react against the body's cells and cause autoimmune diseases.
- Another reason for apoptosis is to maintain homeostasis in the body by removing old cells to make space for the new ones.

3.4.3 APOPTOSIS MECHANISMS

The process of apoptosis is highly complex and sophisticated, involving an energy-dependent series of molecular events. Three different pathways work on different mechanisms to achieve apoptosis. All three of these pathways converge at

the same terminal pathway, which results in the sequential degradation of cellular organelles.

3.4.3.1 Extrinsic or death receptor pathway

- The extrinsic pathway that initiates apoptosis involves transmembrane receptor-mediated interactions.
- These interactions take place between ligands and their corresponding death receptors that are all part of the tumor necrosis factor (TNF) family.
- All members of the TNF receptor family share a common cysteine-rich extracellular domain with about 80 amino acids called the "death domain".
- The death domain plays a vital role in transmitting the death signal from the cell surface to the intracellular signaling pathways.
- The events or interactions that take place in the extrinsic phase of apoptosis involve two models; FasL/FasR and TNF-α/TNFR1 models, both of which include the clustering and binding of receptors and their ligands.
- Upon ligand binding, cytoplasmic adapter proteins are activated, which causes the receptors to exhibit death domains.
- The binding of FasL to FasR results in the activation of the adapter protein FADD whereas the binding of TNF ligand (TNF α) to TNF receptor (TNFR1) results in the binding of the adapter protein TRADD with activation of FADD and RIP.
- These events cause the dimerization of the death effector domain, causing FADD to bind with procaspase-8.
- As a result of the binding, a death-inducing signaling complex (DISC) is formed, resulting in the auto-catalytic activation of procaspase-8.
- Once caspase-8 is activated, the terminal phase or execution phase of apoptosis is triggered.

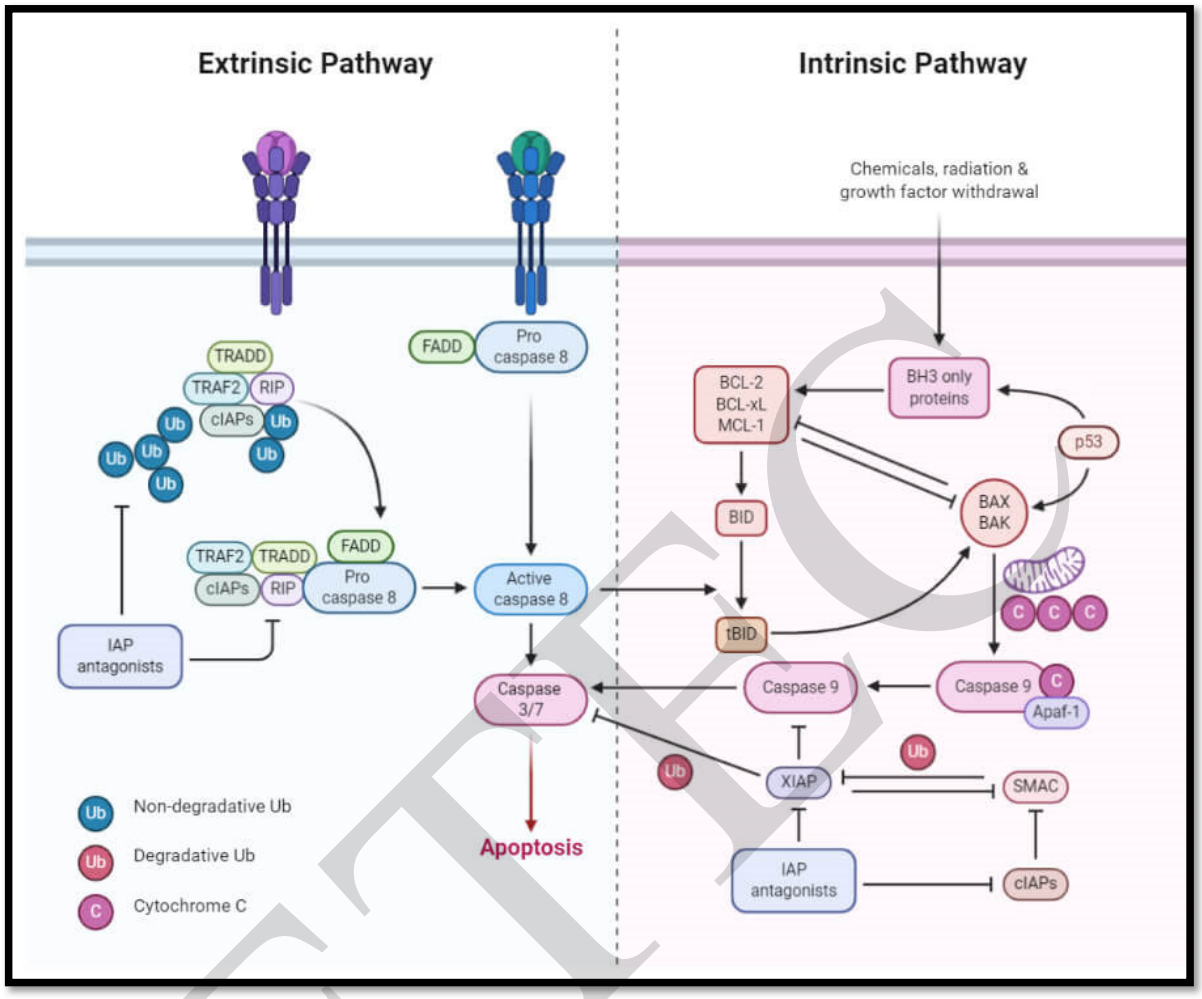

FIG 3.4: Structure of Extrinsic Vs Intrinsic Pathway

3.4.3.2 The intrinsic or mitochondrial pathway

- The intrinsic pathway that initiates apoptosis involves a series of non-receptor-mediated processes that produce intracellular signals and act directly on targets within the cell.
- This pathway involves mitochondrial-initiated events.
- The factors that initiate the intrinsic pathway produce intracellular signals that might act in either a positive or negative fashion.
- Negative signals include the absence of certain growth factors, cytokines, and hormones that can lead to failure of inhibition of death programs, thereby triggering apoptosis.
- In simple words, the withdrawal of factors causes loss of apoptotic suppression and subsequent activation of apoptosis.

- The factors that act positively include, radiation, toxins, hypoxia, hyperthermia, viral infections, free radicals, among others.

- All of these factors cause changes in the inner mitochondrial membrane that causes the opening of the mitochondrial permeability transition (MPT) pore and release of two main groups of pro-apoptotic proteins from the intermembrane space into the cytosol.

- The first group consists of cytochrome *c that* binds and activates Apoptotic protease-activating factor – 1(Apaf-1) as well as procaspase-9, forming a protein complex termed, apoptosome.

- The apoptosome cleaves the procaspase into the active form, caspase 9, which further cleaves and activates procaspase into the effector caspase 3.

- The first group also has other proteins like SMACs (second mitochondria-derived activator of caspases) and HtrA2/Omi that promote apoptosis by inhibiting the activity of IAPs (inhibitors of apoptosis proteins).

- The second group of pro-apoptotic proteins is released from the mitochondria during apoptosis, but this occurs as a part of the terminal phase after the cell has committed to die.

- These proteins translocate to the nucleus and cause DNA fragmentation and condensation of peripheral nuclear chromatin.

3.4.3.3 Perforin/granzyme pathway

- Perforin/granzyme pathway is a novel pathway employed by cytotoxic T lymphocytes that exert their cytotoxic effects on tumor cells and virus-infected cells.

- This involves secretion of the transmembrane pore-forming molecule, *perforin*, with a subsequent release of cytoplasmic granules through the pore and towards the target cell.

- The granules consist of two crucial serine proteases; granzyme A and granzyme B that activate different proteins in the pathway.

- Granzyme B cleaves proteins at aspartate residues and therefore activates procaspase-10 and can cleave factors like ICAD (Inhibitor of Caspase Activated DNAse.

- It has also been observed that granzyme B can utilize the mitochondrial pathway for amplification of the death signal by induction of cytochrome *c release*.

- But granzyme B can also directly activate caspase-3. In this pathway, there is a direct induction of the execution phase of apoptosis.

- Granzyme A also has an essential role in cytotoxic T cell-induced apoptosis and activates caspase-independent pathways.
- As granzyme A reaches the cell, it activates DNA nicking by DNAse enzyme that prevents cancer through the induction of tumor cell apoptosis.
- Granzyme A protease cleaves the SET complex that inhibits the production of the DNAse enzyme.
- The proteins that make up the SET complex together protect chromatin and DNA structure. Thus, the inactivation of the SET complex by granzyme A contributes to apoptosis by blocking the maintenance of DNA and chromatin structure integrity.

3.4.3.4 Execution pathway

- Both the extrinsic and intrinsic pathways end at the point of the execution phase, considered the terminal pathway of apoptosis.
- This phase of apoptosis is initiated by the activation of various caspases that activate cytoplasmic endonucleases and proteases.
- The cytoplasmic endonucleases degrade the nuclear material, whereas the proteases degrade the nuclear and cytoskeletal proteins.
- Caspase-3 is the most important protein of the executioner caspases and is activated by any of the initiator caspases (caspase-8, caspase-9, or caspase-10).
- Caspase-3 precisely activates the endonuclease Caspase-activated DNase (CAD). CAD then causes chromatin condensation by degrading chromosomal DNA within the nuclei.
- Caspase-3 also causes cytoskeletal reorganization and disintegration of the cell into apoptotic bodies.
- Gelsolin, an actin-binding protein, is considered as one of the critical substrates of activated caspase-3. Caspase-3 cleaves gelsolin and the cleaved fragments of gelsolin, in turn, cleave actin filaments, resulting in disruption of the cytoskeleton and formation of apoptotic bodies.
- The later stages of apoptosis cause the appearance of phosphatidylserine on the outer leaflet of apoptotic cells.
- This facilitates noninflammatory phagocytic recognition, allowing for their early uptake and disposal.
- As the process takes place without the release of cellular components, no inflammatory response is elicited.

3.4.4 INHIBITION OF APOPTOSIS

- Inhibition of apoptosis inhibits the cell death signaling pathways, which helps the tumor cells to escape apoptosis.
- Different groups of proteins act as negative regulators of apoptosis which are categorized as anti-apoptotic factors like IAPs and Bcl-2.
- IAP (Inhibitor of apoptosis) proteins represent a group of negative regulators of both caspases and cell death.
- IAP group in humans consists of 8 proteins, all of which have a characteristic BIR (Baculovirus IAP Repeat) domain that binds with the caspases and other proteins involved in apoptosis.
- Proteins like XIAP bind caspase-9 and caspase-3, thus inhibiting their activation and preventing apoptosis.
- Another factor, Bcl-2, governs mitochondrial membrane permeability and can be either pro-apoptotic or anti-apoptotic.
- The anti-apoptotic proteins include some proteins like Bcl-2, Bcl-x, and BAG that inhibit the release of cytochrome c and also modify the permeability of the mitochondrial membrane, thus inhibiting the intrinsic pathway of apoptosis.
- The ability of cells to escape apoptosis is the major cause of cancers like leukemia and multiple myeloma.
- Inhibition of apoptosis also induces loss of immune function by the immune system. The mutation of inhibition protein XIAP results in a rare genetically-mediated immunodeficiency.

3.4.5 REGULATION OF APOPTOSIS

- Several proteins and genes regulate apoptosis. Specific families of proteins are involved in the regulation of apoptosis in various steps.
- Among all the factors, IAPs and Bcl-2 are two of the most important proteins involved that decide whether the apoptosis is going to complete or inhibit.
- The extrinsic pathway of apoptosis is inhibited by a protein called c-FLIP which will bind to FADD and caspase-8, rendering them ineffective.
- Another mechanism of apoptosis regulation in the extrinsic pathway involves a protein called Toso, which blocks Fas-induced apoptosis in T cells by inhibition of caspase-8 activation.
- In the intrinsic pathway, members of the Bcl-2 family play an important role in

the regulation and control of the pathway.

- The Bcl-2 family of proteins controls mitochondrial membrane permeability, and the proteins can be either be pro-apoptotic or anti-apoptotic.
- The proteins of the Bcl-2 family regulate apoptosis by regulating the release of cytochrome *c* from the mitochondria via alteration of the mitochondrial membrane permeability.
- Proteins like Puma and Noxa are members of pro-apoptotic factors that facilitate the activation of apoptosis by preventing the action of anti-apoptotic factors.
- A group of proteins released from the mitochondria, Smac, promote apoptosis by inhibiting the action of IAPs in the mitochondrial pathway.

3.4.6 APOPTOSIS ASSAYS

- Because the process of apoptosis is regulated tightly at various points, it is possible to evaluate the activity of different proteins involved.
- It is essential to confirm the mechanism of cell death by two different assays as the process of apoptosis and necrosis might overlap.
- The first essay detects the initial events of apoptosis, whereas the second identifies the execution or terminal phase.
- Apoptosis assays have been divided into six different groups, which are:

3.4.6.1 Cytomorphological altercation

- The observation of hematoxylin and eosin-stained tissue sections with light microscopy allows the visualization of apoptotic cells.
- This method detects the cells in the later events of apoptosis, but the cells in the early stage of apoptosis are not recognized.
- Transmission electron microscopy (TEM) is the gold standard for the confirmation of apoptosis.
- In TEM, cells undergoing apoptosis reveals several structural characteristics. These characters include:
  - ✓ Electron-dense nucleus (marginalization of the nucleus in the early phase)
  - ✓ Nuclear fragmentation
  - ✓ Intact cell membrane even late in the cell disintegration phase
  - ✓ Disorganized cytoplasmic organelles

✓ Large clear vacuoles

Phosphatidylserine at the cell surface.

- With the progression of apoptosis, these cells will lose the cell-to-cell adhesions and will separate from neighboring cells.
- Eventually, the cell will fragment into apoptotic bodies with intact cell membranes and will contain cytoplasmic organelles with or without nuclear fragments.

3.4.6.2 DNA fragmentation

- The DNA laddering technique is another method of detection of apoptosis that visualizes the products of endonuclease cleavage during apoptosis.

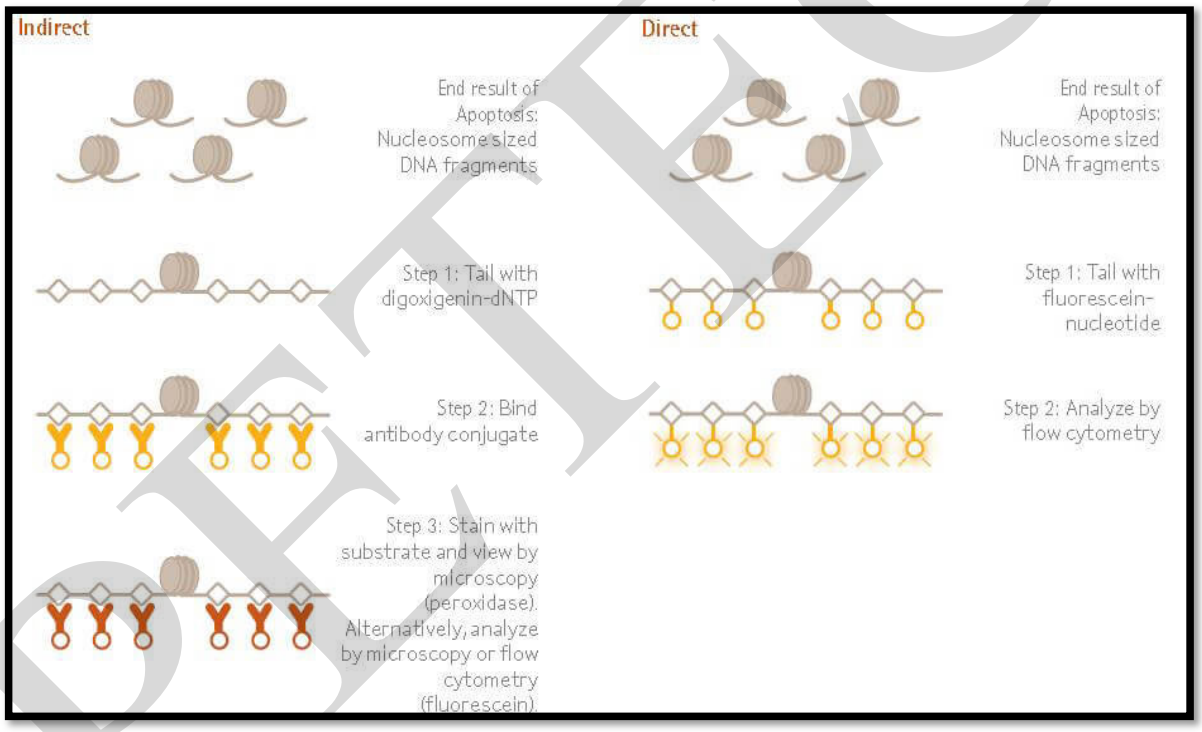

FIG 3.5: Process of DNA Fragmentation

- This involves the extraction of DNA from a lysed cell homogenate separation by agarose gel electrophoresis.
- The resulting bands of DNA form a DNA ladder that can be used to detect apoptosis in tissues where the number of apoptotic cells is high.
- However, DNA fragmentation only occurs during the later stages of apoptosis, and thus this cannot detect cells in the early stages.

- Another method is the TUNEL (Terminal dUTP Nick End-Labeling) method which also detects the endonuclease cleavage products by enzymatically labeling the ends of DNA strands.
- Terminal transferase is used to attach dUTP to the 3′-end of the DNA fragments.
- The dUTP is then labeled with a variety of probes to allow detection by light microscopy, fluorescence microscopy or flow cytometry.
- Although this technique is fast and can be conducted in a few hours, it might give false-positive results from necrotic cells.

3.4.6.3 Detection of caspases, cleaved substrate, regulators and inhibitors

- Various types of caspase activity assays are available that detect more than 13 known caspases involved in apoptosis.
- Some immunoassays can detect cleaved substrates such as PARP and known cell modifications such as phosphorylated histones.
- A variety of assays including western blot, immunoprecipitation, and immunohistochemistry can be used to detect caspase activation.
- Apoptosis PCR microarray is a comparatively new method that uses real-time PCR to indicate the expression of about 112 genes involved in apoptosis.
- The microarrays are designed to produce the expression profile of genes that encode essential receptors, ligands, intracellular regulators, and transcription factors involved in the regulation of programmed cell death.
- The genes involved in anti-apoptosis can also be assessed with this methodology.
- This technique can only provide an estimate of the number of apoptotic cells and thus has to be accompanied by other assays.

3.4.6.4 Membrane altercations

- The presence of phosphatidylserine residues on the outer plasma membrane of apoptotic cells can be detected via An-nexin V in tissues, embryos, or cultured cells.
- The apoptotic cells are first bound with FITC-labeled Annexin V and then visualized with fluorescent microscopy.
- This technique has a disadvantage as the membranes of necrotic cells might also be labeled. In order to avoid this, necrotic cells can be stained with membrane-impermeant nucleic acid dyes like propidium iodide and trypan blue to detect the loss of membrane integrity.

- In contrast, the membrane integrity of apoptotic cells can be detected by the absence of these dyes.
- The movement of phosphatidylserine to the outside of the cell membrane will also allow the transport of some dyes into the cell in a unidirectional manner.
- Thus, the cell might accumulate dye and shrinks in volume. As a result, the cell dye content becomes more concentrated and can also be visualized with light microscopy.

3.4.6.5 Detection of Apoptosis in Whole Mounts

- Dyes like acridine orange (AO), Nile blue sulfate (NBS), and neutral red (NR) can be used to visualize whole mounts of embryos or tissues.
- As these dyes are acidophilic, they tend to concentrate in areas of high lysosomal and phagocytotic activity.
- This technique should be associated with another assay as it cannot differentiate the apoptotic debris from the debris of microorganisms.
- These dyes, however, have certain disadvantages where acridine orange is mutagenic and toxic, and NBS and neutral red do not penetrate deep into the tissues and can be lost during preparation.
- Another dye, Lyso-Tracker Red, can be used with laser confocal microscopy to provide 3-dimensional imaging of apoptotic cells.

3.4.6.6 Mitochondrial assays

- Mitochondrial assays demonstrating cytochrome *c* release permit the detection of changes during the early stages of the intrinsic pathway.
- The Laser scanning confocal microscopy (LSCM) creates thin optical slices of living cells that are then used to monitor various mitochondrial events in intact single cells throughout a period of time.
- This technique measures parameters like mitochondrial permeability, depolarization of the inner mitochondrial membrane, mitochondrial redox status, Ca^{2+}fluxes, and reactive oxygen species.
- However, these changes also occur during necrosis and thus cannot be used as detection of apoptosis exclusively.
- Other mitochondrial dyes that measure the redox potential or metabolic activity of the mitochondria in cells are also available. To determine the mechanism of apoptosis, however, a caspase detection assay should accompany this technique.

- Cytochrome *c* release from the mitochondria of living or fixed cells can also be assayed using fluorescence and electron microscopy.

- Pro-apoptotic or anti-apoptotic regulator proteins like Bax, Bid, and Bcl-2 can also be detected using fluorescence and confocal microscopy. However, the fluorescent protein tags might alter the interaction between the proteins and thus should be accompanied by other assays for confirmation.

3.4.7 APOPTOSIS SIGNIFICANCE

- ❖ Apoptosis is essential during development where many cells undergo programmed cell death, thus contributing to the formation of various tissues and organs from a single mass of tissue. It can even be used as an important determinant of fetal abnormalities.

- ❖ The regular removal of old cells from the body enables the body to produce new cells, helping maintain the cell population in the body. The inability to do so might result in dramatic consequences depending on the type of cells.

- ❖ Apoptosis helps in the removal of redundant and damaged cells from the body. At the same time, cells that are infected with a virus, and the ones that cannot be repaired are removed via apoptosis.

- ❖ Apoptosis is often used by the body's immune system to check if the newly formed cells are self-destructive or not. If the immune cells are found to be destructive against the body's cell, these are removed, preventing the possibility of autoimmune diseases.

- ❖ Apoptosis plays a critical role in the immune system of the body. Different cytotoxic cells like T lymphocytes are produced in advance during infection by foreign material. Once the invader is cleared from the body, the existing pathogen-specific immune cells are removed from the body via apoptosis.

- ❖ Apoptosis has also been observed in regulating the development of thymocytes and the shaping of T cells and the coordination of various immune responses.

- ❖ Apoptosis is also involved in the removal of about 50% of neurons produced during early embryonic development and in the formation of reproductive parts.

- ❖ Excess apoptosis might result in neurodegenerative diseases, and deficient apoptosis occurs in conditions like cancer and autoimmune diseases.

TWO MARKS

UNIT 3

DIVISION

1. Define cell cycle.

A cell cycle is a series of events that takes place in a cell as it grows and divides. A cell spends most of its time in what is called interphase, and during this time it grows, replicates its chromosomes, and prepares for cell division.

2. Define interphase.

Before a cell can reproduce, it has to perform a variety of activities to get ready. The stage of the cell cycle when a cell is preparing itself to duplicate is called interphase. Since so many things are happening in the cell at this time, most of the cell's life is spent in this stage. While preparing to reproduce, the cell makes more cytoplasm (the gel-like substance found inside the cell membrane that bathes the organelles) and increases its supply of proteins. When it's ready, it goes through three sub-phases of interphase: G1, S, and G2.

3. Define Maturation Promoting Factor.

A protein complex responsible for triggering mitosis in somatic cells and for maturation of oocytes into egg cells. Consisting of cyclin B (see cyclin) bound to a cyclin-dependent kinase, it catalyses the phosphorylation of proteins that in turn bring about the events of mitosis, including condensation of chromosomes, formation of the mitotic spindle, and breakdown of the nuclear envelope. Levels of cyclins and MPF rise as the cell enters mitosis, reach a peak during mitosis, and then fall during anaphase.

4. Define Mitosis.

Mitosis, a process of cell duplication, or reproduction, during which one cell gives rise to two genetically identical daughter cells. Strictly applied, the term mitosis is used to describe the duplication and distribution of chromosomes, the structures that carry the genetic information.

5. Difference between open and closed mitosis.

In organisms undergoing an open mitosis, the NPC (Nuclear Pore Complex) is disassembled along with the nuclear envelope, and therefore nuclear transport does not occur during open mitoses. However, in organisms undergoing a closed mitosis, the NPC must still function as a conduit between the nucleus and the cytoplasm.

6. What is the purpose of mitosis?

The purpose of mitosis is to make more diploid cells. It works by copying each chromosome, and then separating the copies to different sides of the cell. That way, when the cell divides down the middle, each new cell gets its own copy of each chromosome.

7. What are the stages of mitosis?

- ❖ Prophase
- ❖ Metaphase
- ❖ Anaphase
- ❖ Telophase
- ❖ Cytokinesis typically overlaps with anaphase and/or telophase.

8. Define Meiosis.

Meiosis is a process where a single cell divides twice to produce four cells containing half the original amount of genetic information. These cells are our sex cells – sperm in males, eggs in females. During meiosis one cell divides twice to form four daughter cells.

9. What is the purpose of meiosis?

- ✓ The meiosis maintains a constant number of chromosomes in sexually reproducing organisms through the formation of gametes.
- ✓ By crossing over, the meiosis results in the exchange of the genes and, thus, causes the genetic variations among the species. These variations are the raw materials of the evolutionary process.

10. What are the stages in meiosis?

Since cell division occurs twice during meiosis, one starting cell can produce four gametes (eggs or sperm). In each round of division, cells go through four stages:

- ✓ Prophase
- ✓ Metaphase
- ✓ Anaphase
- ✓ Telophase

11. Define Zygotene.

The zygotene stage, also known as zygonema, from Greek words meaning "paired threads", occurs as the chromosomes approximately line up with each other into homologous chromosome pairs. In some organisms, this is called the bouquet stage because of the way the telomeres cluster at one end of the nucleus.

12. Difference between mitosis and meiosis.

Meiosis	**Mitosis**
Starts as diploid; ends as haploid	Starts as diploid; ends as diploid
Chromosome number is reduced	Chromosome number is conserved
Chromosome pairs undergo synapsis	No synapsis occurs
Used for sexual reproduction	Used for growth/healing/asexual reproduction
2 nuclear divisions	1 nuclear division
8 phases	5 phases
Daughter cell not identical to parent cell	Daughter cell identical to parent cell
Results in 4 daughter cells	Results in 2 daughter cells
Produces germ cells	Produces somatic cells
Occurs only in sexual organisms	Occurs in asexual and sexual organisms

13. Define Pachytene.

Pachytene, also referred to as pachynema. It is defined as the stage when a fully formed synaptonemal complex exists. During pachytene the homologous chromosomes thicken and become recombinant.

14. Define Leptotene.

The leptotene stage, also known as the leptonema. During the leptotene stage those duplicated chromosomes—each consisting of two sister chromatids—condense from diffuse chromatin into long, thin strands that are more visible within the nucleoplasm.

15. Define Diakinesis.

Diakinesis the last stage of prophase in meiosis, in which the nucleolus and nuclear envelope disappear, spindle fibers form, and the chromosomes shorten in preparation for metaphase.

16. Define cytokinesis.

Cytokinesis is the physical process of cell division, which divides the cytoplasm

of a parental cell into two daughter cells. Cytokinesis starts during the nuclear division phase called anaphase and continues through telophase in mitosis.

17. Define cell cycle regulation.

The progression of cells through the cell cycle is controlled by checkpoints at different stages. These detect if a cell contains damaged DNA and ensure those cells do not replicate. ... This cell cycle is also closely regulated by cyclins which control cell progression by activating cyclin-dependent kinase (CDK) enzymes.

18. What is purpose of cell cycle regulation?

Cell cycle regulation is crucial for proper cellular homeostasis. Communication between or within a cell is done through cell signaling and a change in the activity of the cell is sent as a signal that may trigger a cascade of reaction for the body to respond accordingly.

19. Define apoptosis and what is the purpose of apoptosis?

Apoptosis is a form of programmed cell death, or "cellular suicide" in which cells die due to injury. **Apoptosis** removes cells during development, eliminates potentially cancerous and virus-infected cells, and maintains balance in the body.

20. What are the steps in apoptosis?

- ✓ The decision to activate the pathway;
- ✓ The actually "suicide" of the cell;
- ✓ Engulfment of the cell remains by specialized immune cells called phagocytes;
- ✓ Degradation of engulfed cell.

21. Difference between Apoptosis and Necrosis.

Apoptosis	**Necrosis**
Apoptosis is a regular process of death of the cell that occurs in the body where cell itself takes part in the death	Necrosis is a cellular process of death occurring when the cells are highly exposed to extreme external conditions
It is a natural process and not caused by external factors	It is caused by external agents such as infection, trauma, toxins.
The organelles are still functional even after the death of the cell	The organelles are not functional after the death of cell

The cell membrane breaks into several apoptotic bodies	The cell membrane breaks and releases the cell contents
No symptoms are observed during the process of apoptosis	The symptoms like Inflammation, tissue death and decreased blood flow at the infected site are observed during the process
It is caused due to the self-generated signals within a cell.	It is caused by Bacterial and fungal infections, mycobacterial infections, denatured proteins, pancreatitis, or by the deposits of antibodies and antigens
This process does not require energy since the enzymes carry out the process.	This process requires energy to carry out the process