Indira Gandhi National
Open University
School of Sciences

BBCCT - 103

CELL BIOLOGY

Block

4

CELL CYLE AND ITS REGULATION

BLOCK 4 : CELL CYCLE AND ITS REGULATION

As you know the cell theory states that a cell arises from the preexisting cells via cell division. It is one of the fundamental properties of cells. Therefore, this block will explain you how cells divide and regulate the cell cycle. You will also learn the cell death and cell renewal in the last unit.

There are three units in this block.

Unit 11 Cell Cycle describes the type of cell division and subdivision of cell cycle (Interphase). The biochemical and morphological changes during the cell division (Mitosis and Meiosis) are discussed in detail.

Unit 12 deals with the regulation of Cell division. It explains cell cycle checkpoints and regulatory molecules: CDK (Cyclin Dependent Kinases) and cyclin.

Unit 13 is the Cell Death and Cell Renewal in which you will learn programmed cell death (apoptosis and necrosis) and cell renewal and their biological importance. The principle and application of Fluorescence Activated Cell Sorting (FACS), a technique of cell biology is also discussed in this unit.

Expected Learning Outcomes

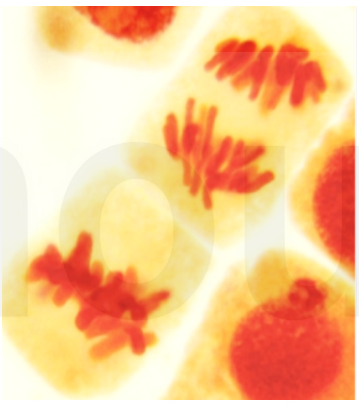
After studying this block, you should be able to:

- define the cell division and its types;
- describe the cell cycle and subdivision of interphase;
- explain the basic phases of Mitosis and Meiosis;
- point out the key difference between mitosis and meiosis;
- describe the cell cycle check-points and restriction point;
- highlight the regulatory molecules (CDK & Cyclin) of the cell cycle;
- explain how CDK and Cyclin regulate the cell cycle;
- indicate the relevance between cell death and cell renewal;
- explain the apoptosis and necrosis; and
- describe the principle and application of FACS.

UNIT 11

CELL CYCLE

Structure



11.1 INTRODUCTION

The last block of the cell biology course is entitled, 'Cell cycle and its regulation'. This unit deals with the variations in cell division cycle in prokaryotes and eukaryotes. Cell division is a result of a series of tightly regulated biochemical events that together constitute the cell cycle. In spite of the variations in cell division cycle the basic theme remains the same, that of duplication and segregation of the genetic material.

The major emphasis will be on binary fission in prokaryotes and mitosis and meiosis in eukaryotes. In prokaryotes, cell division results in an increase in the number of organisms whereas in multicellular organisms it is the process by which an organism is formed and their cell numbers are maintained through life.

The purpose of meiosis in specialized cells will be discussed. The distinguishing features of the cell cycle stages, dynamics of the cytoskeleton will be elaborated. The concept of checkpoints to monitor progress through critical stages of the cell cycle is the subject matter of unit 12. The unit ends with a comparison of mitosis and meiosis.

Expected Learning Outcomes

After studying this unit, you should be able to:

- explain why does a cell divide;
- indicate the types of cell division in prokaryotes and eukaryotes;
- describe the subdivision of interphase and their role;
- highlight the biochemical and morphological hallmarks of mitotic phase;
- describe the identifying features of meiosis-I and II;
- understand the consequences of meiosis and;
- point out the key difference between mitosis and meiosis.

11.2 CELL DIVISION

One of the fundamental properties of a cell is its ability to divide. A complete round of cell cycle includes an elaborate preparation followed by division perse to produce daughter cells genetically identical to the mother cell, with the exception of meiosis that results in non identical products. In order to achieve accuracy in the overall process the cell cycle in eukaryotes is elaborately controlled. It decides both on the need to divide and also monitors stringently the cell division process.

Why does a cell divide at all?

If we consider a cell to be a cube, then surface area of the cell can be given by the formula:

Surface area = length × width × number of sides…….. (i)

The volume of the cell, which is the total space occupied by the cytoplasm and other cellular contents would be,

Volume = length × width × height …… (ii)

Thus both surface area and volume of a cell increases with increasing cell size but the surface to volume ratio decreases because the increase in volume is more than surface area. A larger cell will have insufficient surface area for nutrient uptake and waste disposal. These factors limit the growth of cells up to a threshold level and then it may divide, distributing its contents to daughter cells.

11.3 TYPES OF CELL DIVISION

All living organisms have developed one or more ways to divide. The complexity of the process has no doubt increased as we evolved from simple to more complex organisms. The simplest version of cell reproduction is **binary fission** in prokaryotes. In eukaryotes, cell division can be classified into two major types: mitosis and meiosis.

Mitosis or equational division is the common type of division by which somatic or vegetative cells of the body divide to produce two daughter cells with identical number of chromosomes from a mother cell. Mitosis can be either open or closed. In the former chromosome segregation occurs after breakdown of the nuclear envelope and in the latter the nuclear envelope remains intact throughout the cell division cycle.

In some protists and yeast the nuclear envelope remains intact and spindle microtubules may be formed inside the nucleus or microtubules can pass through tunnels in the nuclear membrane. In higher eukaryotes mitosis is of the open type.

Meiosis is a specialized form of cell division that occurs in sexually reproducing organisms. It produces haploid gametes and involves two division cycles. During meiosis genetically diverse gametes are generated that gives these organisms an edge over asexually reproducing organisms in terms of survival and evolution. The haploid cells restore their chromosome number following fertilization.

11.3.1 Cell Division in Prokaryotes

In most prokaryotes their genome is a single circular DNA molecule that is extensively packaged to form a nucleoid. It lacks a nuclear membrane to demarcate its boundary. In these unicellular organisms growth is accompanied by an increase in cell number. They divide by **binary fission** (direct cell division) to segregate the duplicated genetic material and other cytoplasmic contents to the daughter cells.

It is a relatively simple, faster and primitive process than the complex mitotic cycle familiar to you. The basic molecular machinery for active partitioning of newly replicated DNA in bacteria is known and the process can be broadly divided into five steps (Fig.11.1). This process begins with the replication of DNA that is initiated from specific sites called the origin of replication. DNA replication is semi conservative and proceeds in both directions until the two forks reach the terminus.

The replicating chromosomes are tethered to different position on the cell membrane. As the cell grows it elongates and the replicated DNA is partitioned. This is followed by the formation of a ring composed of repeating units of **FtsZ protein** at midpoint. The FtsZ protein is a conserved protein that shows some similarity to the tubulin protein in eukaryotes.

The ring directs the inward growth of new membrane (septum) and facilitates septation. Many other proteins are also recruited and the septum continues to grow inwards. The completion of septation results in two genetically identical daughter cells.

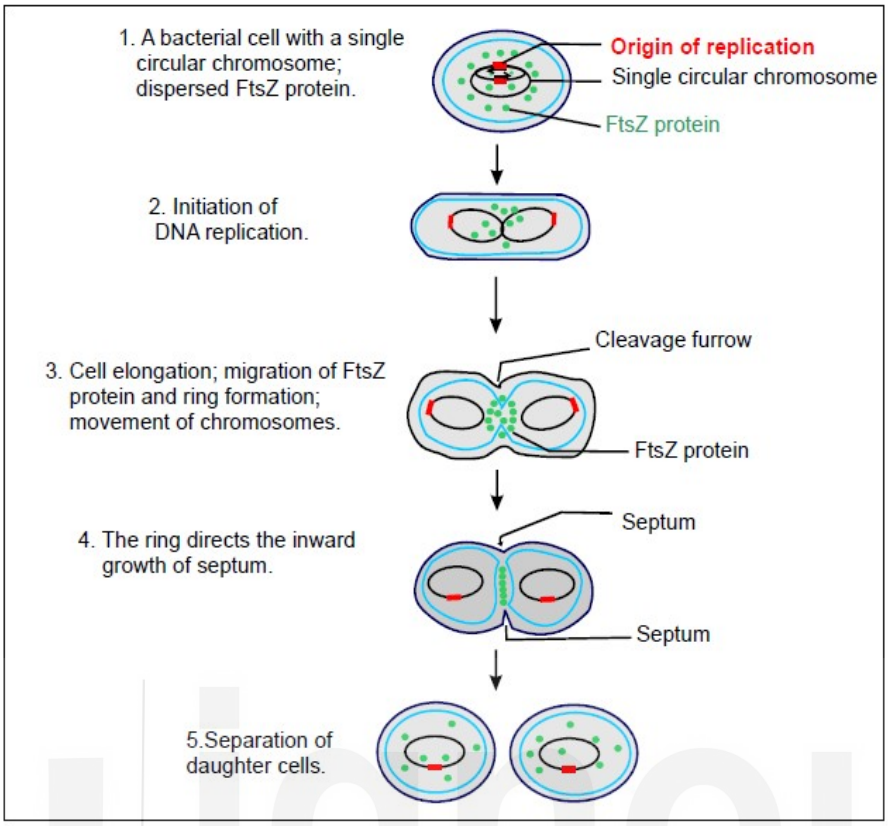


Fig.11.1: Binary fission in prokaryotes.
(Source: https://bio.libratexts.org/Bookshelves/Microbiology)

SAQ 1

Name the process by which a prokaryotic cell divides?

11.4 MITOSIS

Walther Flemming
(1843-1905)

Mitosis (comes from the Greek word *mitos* meaning "thread") is the major form of eukaryotic cell division, taking place essentially in somatic cells. Mitotic cell division was first observed by **Walther Flemming in 1878**. He described the whole process in his book Zell-substanz, Kern und Zelltheilung (Cell-Substance, Nucleus, and Cell-Division), which was published in 1882. His contribution was useful for later work in meiosis and the chromosomal theory of inheritance. He was however unable to verify the proposed sequence of events and it took a long time before it became possible.

The single most important factor on which our progress depended has been advancements in light microscopy that made observation of chromosome movements in live cells feasible and still continues to enhance our ability to understand the dynamics of the process. Now we have information on the molecular machinery of cell division and the control system that determines not only the need to divide but also its step by step progress through the cycle.

The mitotic cell division comprises of two main phases, Karyokinesis (Greek word *Karyon* meaning nucleus, *kinesis* meaning movement) and cytokinesis

(Greek word *Kytos* meaning cell and *kinesis* meaning movement) that results in two identical daughter cells starting from a single cell. In most cells karyokinesis is followed by cytokinesis. Each round of cell division is preceded by a preparatory or interphase and together these ordered series of cyclical events constitute the cell cycle (Fig.11.2). Both interphase and mitosis are further subdivided into stages based on certain characteristic events that help to define them. It is worth keeping in mind that the division into distinct phases is for convenience and in a dividing cell one phase continuously follows the other. Now let us begin with the interphase (a preparatory phase) of the cell cycle.

Walther Flemming was the first to study chromosomes and provide an insight into chromosome movements during mitosis. He could observe thread like structures during cell division which he called' mitosen' and was able to correctly deduce the sequence of cell division cycle. Much of what we know today about mitosis originated with Flemming's observations. He saw that chromosomes were "doubled" when they appeared in prophase, and thus resolved the issue of chromosomal partitioning between daughter cells. He coined the term mitosis in 1882 to describe this process.

11.4.1 Interphase

During interphase cells grow and stockpiles material enough for two cells. It is the period between mitosis. The time a cell spends here is variable depending on the cell type. Most cells spend approx.95% of the cell cycle time in interphase with the notable exception of early embryonic cells where they lack the usual gap phases and a short S phase alternates with the M phase. In a cell cycle lasting 24 hrs, mitosis is completed in less than 2hrs and the rest of the time is spent in interphase. Although in biochemical terms it represents a very active phase but under the light microscope distinct chromosomes are not visible as they are decondensed and diffuse to allow for transcription and occupy distinct territories in the nucleoplasm.

Generally cell biologists divide interphase into two gap phases (G_1 and G_2) and a synthetic or S phase (Fig. 11.2). In most cells the duration of the G_1 is the longest and that is primarily responsible for the enormous variation in the length of the cell cycle. The interphase also has checkpoints (G_1/S or G_2 /M) to ensure that preceding event is completed before embarking on the next step and to check that DNA is undamaged before entering mitosis. The molecular aspects of the control system will be discussed in unit 12.

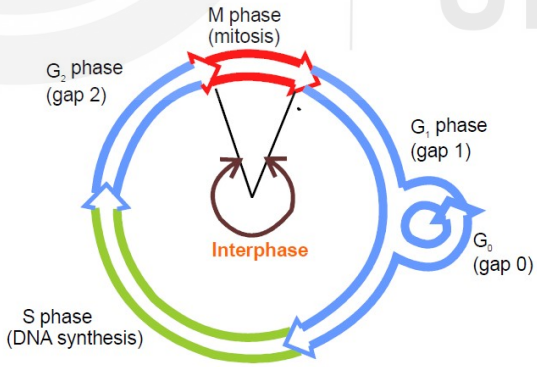


Fig. 11.2: The Cell division cycle.

G_1 Phase: G_1 or Gap phase1 is the first stage of interphase in which cells grow and resumes RNA and protein synthesis after exiting mitosis. It is the primary growth phase of the cell cycle. The chromosomes become highly decondensed and relaxed. The duration of G_1 phase varies considerably among different types of cells. The replication of the microtubule organizing

centre (MOTC) or centrosome begins at G_1 and completes at G_2 but they remain close together in a single centrosomal complex.

S Phase: The G_1 phase is followed by S or synthetic phase in which chromosomal DNA is replicated. Each chromosome produces two daughter DNA strands which recruit additional histones and other proteins to form sister chromatids. At this stage individual chromosomes are still indistinguishable. The sister chromatids are encircled by a multi protein complex called **cohesin (a protein "glue")** that are members of the structural maintenance of chromosome (SMC) family of proteins. At the end of S phase the cells contain double the amount of DNA but the number chromosomes remain unchanged.

G_2 Phase: G_2 or Gap phase 2 is another period of growth. There is an increase in transcription and protein synthesis that essentially prepares the cell for the mitotic phase. The number of organelles like mitochondria grows and divides to double their numbers. Before embarking on mitosis the cells ensure that damages in DNA are detected and repaired and duplication is complete especially of late replicating sequences. The cells start storing energy reserves. The process of chromosome condensation begins at this stage and continues till metaphase.

11.4.2 Mitotic Phase

Mitosis separates the replicated sister chromatids**.** It is divided into six phases namely prophase, prometaphase, metaphase, anaphase, telophase and cytokinesis. One of the most dramatic outcomes of a cell's entry into mitosis is the **condensation of chromosomes** that reaches the climax at metaphase. The individual chromosomes can be seen under the light microscope. During mitosis transcription is repressed.

Prophase

The chromosomes begin to condense and appear as thin thread like structures as prophase progresses. A group of SMC proteins called **condensins** bind to each sister chromatid at multiple points to facilitate condensation of chromatin. The process of condensation continues till metaphase. At this stage each duplicated chromosome consists of **two sister chromatids** that are attached at the centromere (Fig.11.3).

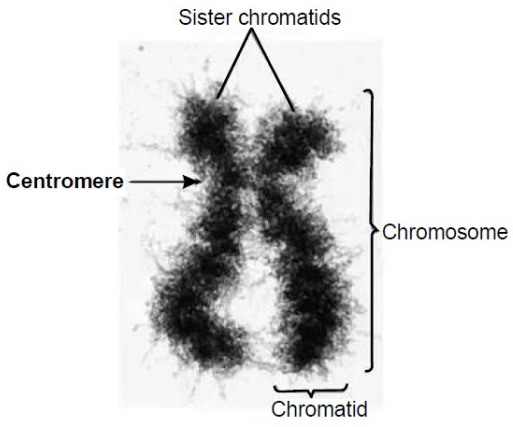


Fig.11.3: Sister chromatids attached at the centromere.

The individual sister chromatids are resolved by the removal of cohesin from most parts of the chromosome except centromere that remains to hold them together. The centromere is characterised by the presence of highly repetitive DNA sequences that serve as binding sites for proteins. The kinetochore is assembled on the outer surface of the centromere on each chromatid and it links the sister chromatids to the spindle apparatus. **A bipolar mitotic spindle** starts forming (Fig.11.4a). In animal cells the spindle grows in opposite direction from the replicated pair of centrosomes; each of which has two centrioles made up of microtubules. It was **Theodor Boveri** who observed spindle fibers radiating from structures present at the poles that are now recognised as centrosomes. He also noted that each centrosome has two rod like bodies and observed that these bodies duplicate before the chromosomes are visible as distinct structures. In addition, the fragmentation of ER and Golgi apparatus into small vesicles occurs whereas others like mitochondria, lysosomes and peroxisomes remain intact.

Prometaphase

The fragmentation of nuclear envelope into vesicles marks the beginning of prometaphase. This allows the spindle microtubules emanating from opposite poles to locate and ultimately attach to the kinetochore of each sister chromatid (Fig.11.4b). The kinetochores are assembled only on one face of each sister chromatid at its centromere. At this stage the microtubules show dynamic behaviour and they rapidly assemble and disassemble. The arrangement of the chromosomes at this stage is called **bi-orientation** as the sister chromatids are attached to microtubules from opposite poles of the spindle. They keep moving back and forth in an attempt to align at the centre or equator of the cell and are firmly held by cohesin.

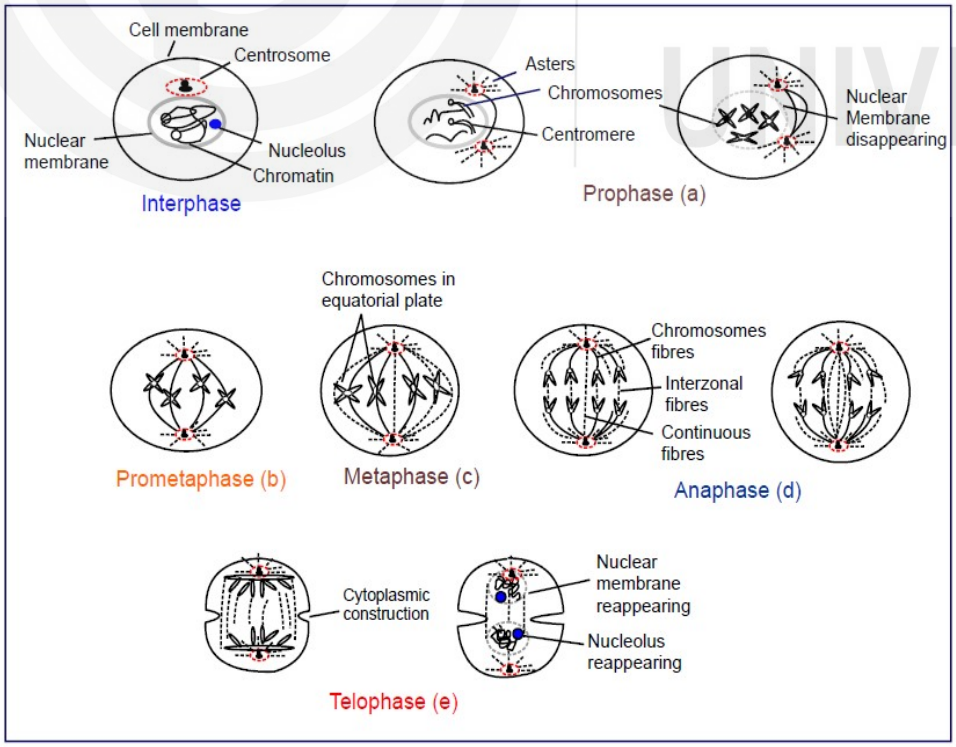


Fig.11.4: Stages of Mitosis in animal cells.

Metaphase

The chromosomes are maximally condensed at this stage and they are aligned at the metaphase plate, held by paired kinetochores and associated with microtubules from opposite poles. The metaphase chromosomes are very useful for cytogenetic studies as they are clearly visible. Metaphase–Anaphase transition is critically monitored in eukaryotic cells by spindle assembly checkpoint mechanism that ensures that all chromosomes are aligned at the equator of the spindle before progressing to anaphase (Fig.11.4c). The polar fibers continue to extend from the poles to the centre of the cell. The microtubules (MT) are classified functionally into astral, kinetochore and polar group of fibers (Fig.11.5). The astral MT radiates outside; the kinetochore MT extends from the centrosome to the kinetochore of the chromosome and the polar MT extend beyond the chromosomes.

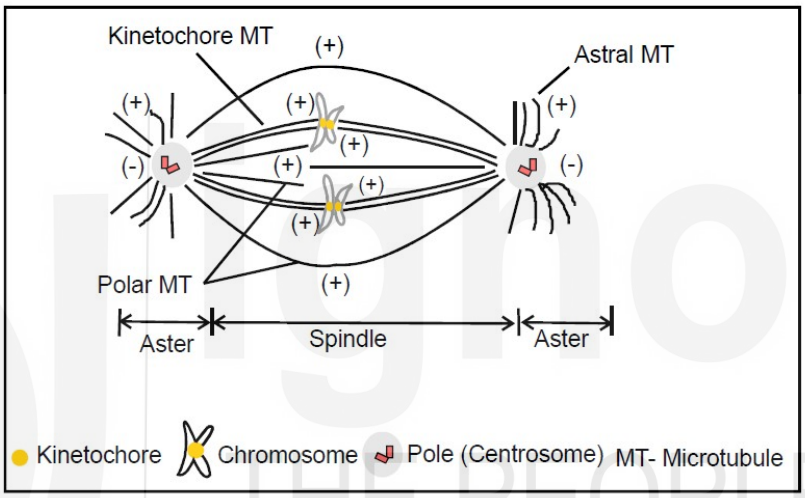


Fig.11.5: Mitotic spindle.

Anaphase

The separation of sister chromatids begins at anaphase. It is almost a synchronous separation and represents the shortest stage of mitosis. The separation occurs due to the breakdown of a cohesin subunit, encircling the sister chromatids by a cysteine protease **separase.** Each centromere then splits to separate the sister chromatids. The movement of sister chromatids to the poles is assisted by another round of dynamic behaviour of microtubules. The movement of the chromosomes to the opposite poles of the spindle is known as **anaphase movement**. It is dependent both on the disassembly of tubulin subunits and energy requiring motor proteins. At the beginning of anaphase the kinetochore microtubules shorten, and the chromosomes move towards the spindle poles. Subsequently the spindle poles separate as other microtubules emerging from the centrosome move past each other. The involvement of motor proteins is implicated in generating the force for pulling the chromosomes. During anaphase movement the chromosomes assume different shapes depending on the position of the centromere in the chromosome. Some may look like 'V', 'L', 'J' or I shaped (Fig.11.4d). At the end of anaphase each separated sister chromatid is an independent chromosome.

Telophase

It is the final stage of mitosis and by this time all chromosomes have arrived at the spindle poles (Fig.11.4e). Many events that follow are essentially the reversal of changes that were made at the beginning of the cycle. The chromosomes become decondensed and resume transcription, the nucleolus reappears and vesicles fuse to reform the nuclear membrane, ER and the Golgi body. The kinetochore and associated microtubules disappear and each cell has a centrosome that nucleates the growth of microtubules (MT). The dynamic behaviour of MT is modified. The organelles that were doubled are roughly distributed equally to both cells. Each daughter cell receives a copy of the duplicated genetic material.

Cytokinesis

In many cells cytokinesis follows telophase. It is the division of cytoplasm that results in two daughter cells with identical genetic material. In animal cells, an actin based **contractile ring** is transiently formed in a plane perpendicular to the axis of the spindle (Fig.11. 6a) that deepens to form a furrow as the actin filaments slide past each other in the mid portion of the spindle. The constriction progresses inwards until the opposing surfaces finally meet and fuse in the centre to divide the cytoplasm into two cells. The contraction of the ring requires the motor protein myosin.

In plant cells (Fig.11.6b), presence of a rigid cell wall prevents formation of a cleavage furrow. Instead they form a membrane partition called **cell plate** in their interior. The first sign of cell plate formation are visible towards the end of anaphase/ early telophase. It is positioned at right angles to the spindle apparatus. The membrane partition continues to grow outward until it reaches the cytoplasmic face of the plasma membrane, fuses with it forming two cells. The cell wall material is then laid down in the new membranes. The space between the cells deposits pectins and other polysaccharides and proteins to form the middle lamella. The Golgi apparatus plays a dominant role in the synthesis and trafficking of complex polysaccharides.

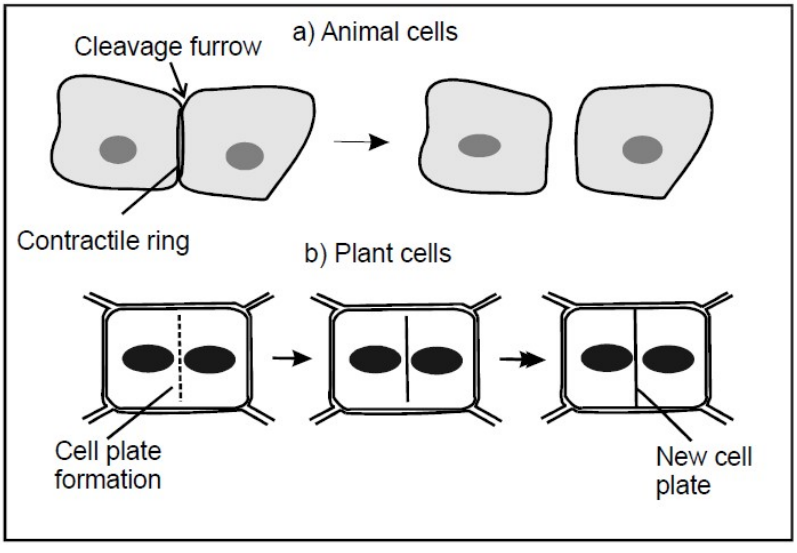


Fig.11.6: Cytokinesis in (a) animal cells and (b) plant cells.

During development of mitotic cell division is often **asymmetric** and the two daughter cells are of unequal size. For instance, when stem cells differentiate to form different kinds of lineage specific cells, they divide asymmetrically by pushing all cell fate determining components to just one of the daughter cells.

11.4.3 Significance of Mitosis

- Each mitotic cycle produces two genetically identical daughter cells, although not always identical in their cytoplasmic content.
- Mitosis leads to growth of the organism through an increase in the number of somatic cells.
- All multicellular organisms originate from a fertilized egg that divides by mitosis to form all the cells.
- Both haploid and diploid cells can divide by mitosis.
- The repair of cellular damage; continuous renewal of some cell types and even our ability to defend ourselves depend on mitosis.

SAQ 2

a) Enlist two differences between plant and animal cell mitosis.

b). What would happen if a cell continues to grow without dividing?

c). Highlight the characteristics of the following phases:

i) G_1 phase ii) S phase iii) G_2 phase iv) G_0 phase

11.5 MEIOSIS

Wilhelm A Oscar Hertwig
(1849-1922)

Edouard van Beneden
(1846- 1910)

Early investigators recognised the need for a special mechanism by which chromosome number can be halved prior to gametic fusion such that chromosome number of a species remains constant through successive generations. This was finally resolved with the discovery of meiosis in the germ cells of sexually reproducing plants and animals.

The word meiosis is derived from the Greek word meaning **'diminution'** as the process results in daughter cells with half the number of chromosomes. This term was coined in 1905 by J.B. Farmer and J.E.S. Moore.

Meiosis was first discovered in 1876 by a German biologist **Oscar Hertwig** in sea urchin eggs. He also observed fusion of eggs and sperms. In 1883 the process was described at the chromosome level in roundworm *Ascaris* eggs by a Belgian cytologist, **Edouard Van Beneden**. He observed differences in the number of chromosomes between gametes and other cells in the organism. He came up with the basic essence of meiosis in 1887 that gametes contain half the number of chromosomes and fertilization restores their chromosome number.

In this type of division, a diploid mother cell gives rise to four haploid daughter cells after two rounds of successive chromosome segregation events that is preceded by only one round of DNA replication in the S phase. These two multistep division stages are called **meiosis I** (reductional division) and **meiosis II** (equational division) (Fig.11.7).

In sexually reproducing organisms there are variations in their life cycles and hence the differences in the time spent between haploid and diploid phases and stage at which meiosis occurs.

11.5.1 Meiosis I

Meiosis I is the most important phase of meiosis and the cell spends most of the time here. At the end of this phase the two daughter cells have half the number of chromosomes. Each cell is genetically non identical because of crossing over between homologous chromosome at prophase I and later independent assortment of chromosomes at anaphase I due to their random alignment at metaphase I. In the beginning of division, a meiosis specific cohesin can be detected along the length of the chromosome. It is also present on the chromosome arms of homologs at the chiasmata that help in holding the homologs firmly at their ends.

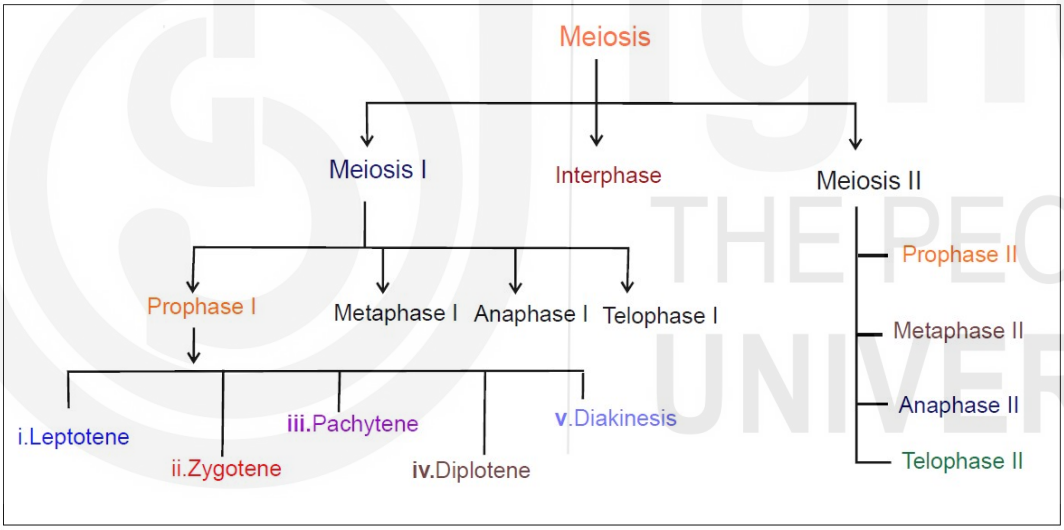


Fig.11.7: Overview of Meiosis I and II.

Prophase I is very complex and it is subdivided into five sub phases (Fig.11.7). The characteristics of each of them are described below from (i) to (v).

(i) Leptotene (thin thread)

In leptotene thread-like chromosomes are distinctly visible and they have a beaded appearance along their length. As leptonema progresses the chromosomes become more condensed and the homologous chromosomes begin to come in close proximity to each other. The terminal portions (telomeres) of the chromosomes attach to the nuclear membrane in many eukaryotes and the chromosomes bear resemblance to a ‘bouquet of flowers’. Such an arrangement may facilitate the alignment of chromosomes.

(ii) Zygotene

The homologous chromosomes that are held close to each other start pairing **(synapsis).** A **synaptonemal complex** is formed between the paired homologues. It is believed to function as a scaffold to allow crossing over between the non-sister chromatids. Each pair of synapsed chromosome is called a bivalent or tetrad. Essentially, they include two homologues (bivalent) with two sister chromatids each (tetrad). Zygonema ends with the completion of pairing.

(iii) Pachytene (thick thread)

The chromosomes continue to thicken and shorten. The synaptonemal complex has dense granules at some places along its length that harbor enzymes to assist in recombination. They are called **recombination nodules**. Although genetic exchange (crossing over) between non sister chromatids takes place during pachytene but the exchanges do not become apparent under the microscope immediately (Fig.11.8).

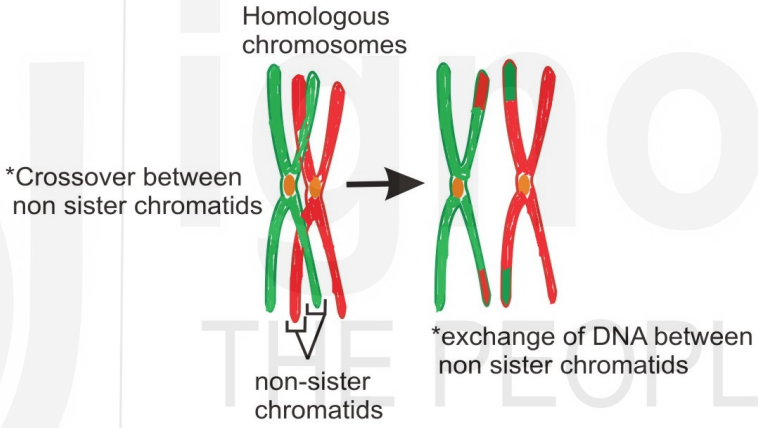


Fig.11.8: Physical exchange between non-sister chromatids.

(iv) Diplotene (double thread)

It is marked by separation of homologous chromosomes in a bivalent. They are held together at points of physical change **(chiasma**) and these chiasmata can be considered as vestiges of the actual exchange process. The points of exchange appear like the alphabet 'X'. The chromosomes become shorter and thicker and the chiasma begins to moves toward the chromosome ends (**terminalization)** and the chromosomes assume different shapes (round, cross, number eight, etc).The synaptonemal complex disappears at this stage.

(v) Diakinesis

The chromosomes continue condense and shorten. Towards the end of diakinesis, spindle formation is initiated and the nuclear envelope breaks down. The nucleolus disappears.

Metaphase I

The homologous chromosomes start arranging at the equatorial plane of the spindle. The alignment of homologous chromosomes is **random** at the metaphase plate. The chiasmata between homologues are responsible for

holding them as bivalents and play an important role in chromosome alignment and segregation. In many organisms it is known that each pair has at least one chiasma and in longer chromosomes their number is generally limited to two or three.

The pair of homologous chromosomes is linked to kinetochore microtubules emerging from opposite poles. The kinetochores of sister chromatids fuse and function as one unit and hence are attached to microtubules emanating from the same spindle pole (**mono-orientation**). This is in stark contrast to what happens at mitosis (bi-orientation).

Shugoshin is a conserved eukaryotic protein that is required for the persistence of centromeric cohesion during meiosis I such that homologous chromosomes separate and move to the poles. This is achieved by protecting the integrity of cohesin complex. More specifically it helps indirectly in preventing Rec8 cleavage by separase.

Anaphase I

The homologous chromosomes move to the opposite poles each with two chromatids. This is possible because centromeres are not split and only cohesin between sister chromatids is released and chiasmata break. At the centromere, cohesin is protected from the action of separase by another protein called **shugoshin** (meaning 'guardian spirit' in Japanese).The dynamics of MT is as in mitosis. This movement is termed as anaphase movement. As chromosomes separate during anaphase I, the two poles receive an assorted combination of maternal and paternal chromosomes due to the random alignment of homologues at metaphase I (Fig.11.9).

Telophase I

The chromosomes have reached the poles. The changes are relatively less dramatic as compared to mitosis and they are also quite variable. In some organisms the nuclear membrane reforms around each nucleus; the nucleolus reappears and the chromosomes become thin and elongated while these changes are not so evident in others. The sister chromatids are not genetically identical. Telophase may or may not be followed by cytokinesis into two daughter cells with half the number of chromosomes than the mother cell (1N and 2C).

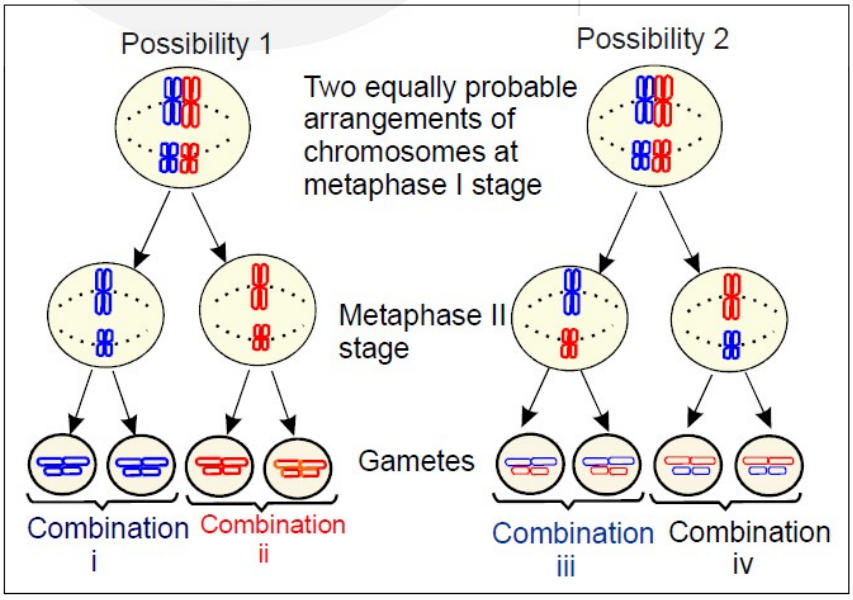


Fig. 11.9: The random alignment of homologous chromosomes at metaphase-I.

11.5.2 Meiosis II

The second meiotic division is similar to mitosis except that the haploid cells have chromosomes with two non identical sister chromatids linked at the centromere (Fig.11.10). The outcome of meiosis II is chromatid segregation and the daughter cells will be 1N with 1C DNA content as compared to the replicated mother cell (4N and 4C).

Prophase II

The chromosomes become visible. The spindle apparatus is reformed and the nuclear membrane breaks down. The nucleolus disappears.

Metaphase II

The chromosomes have condensed and each chromosome has two sister chromatids joined at the centromere. They align at the equatorial plane of the spindle assisted by kinetochore MT emanating from the opposite poles of the spindle that bind to both sides of the kinetochore of each sister chromatid.

Anaphase II

The centromeres split and sister chromatids move to opposite pole due to the shortening of MT and the pulling action of polar MT. At anaphase II sister chromatids separate because by this time shugoshin is degraded and cohesin is no longer protected from the action of separase.

Telophase II

The chromosomes have reached the poles and start elongating. The nuclear membrane reforms and nucleolus reappears. The last event is cytokinesis that gives rise to four genetically non identical haploid cells.

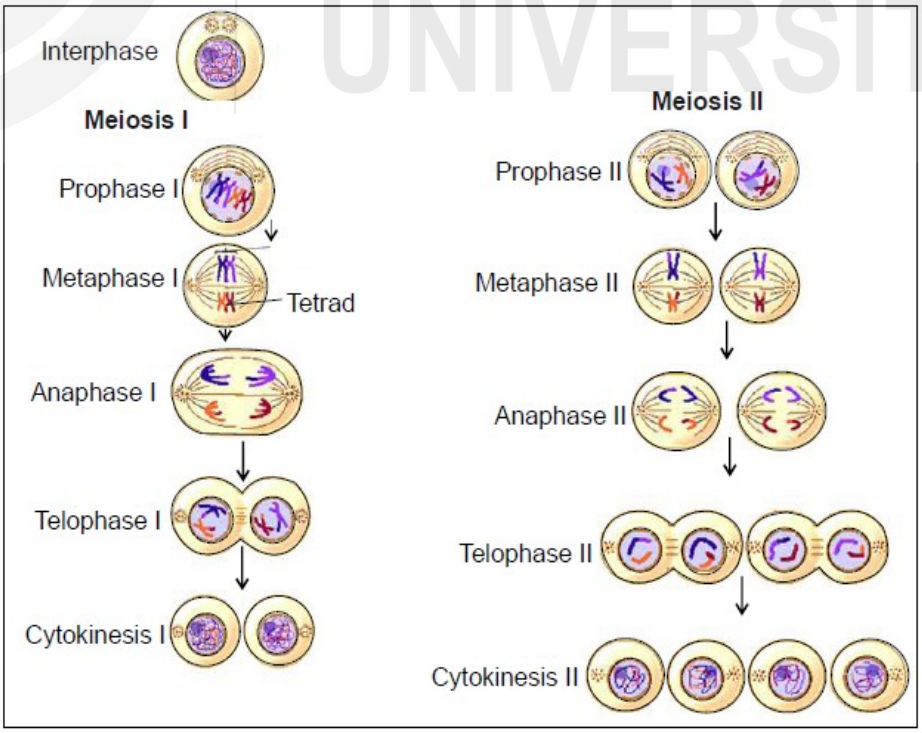


Fig.11.10: Meiosis in animal cells. (Source : Gerald Karp, Cell and Molecular Biology)

11.5.3 Significance of Meiosis

- Meiosis ensures that gametes receive only haploid set of chromosomes.
- It provides a means of maintaining constancy of chromosome number in a particular species following gametic fusion.
- It is mechanism to generate genetic variability by crossing over and independent assortment of homologous chromosomes.
- New combinations provide survival advantage and raw material for evolution.

We end the subject of cell division by comparing in table 11.1 the two types of division cycles operative in eukaryotes.

Table 11.1: Characteristics of mitosis and meiosis

S.No.	Mitosis	Meiosis
1.	Mitosis is an equational division cycle that results in the separation of sister chromatids.	Meiosis has two division cycles in which homologous chromosomes (reduction division) separate at meiosis I and the second is an equational division.
2.	Two genetically identical daughter cells like the parental cell are produced per cycle.	Four genetically non identical haploid cells are produced per cycle, starting from a diploid cell. At times all products may not be recovered.
3.	The daughter cells are competent to divide again by mitosis. Both haploid and cells with higher ploidy can divide by mitosis.	The haploid products of meiosis cannot undergo another meiotic division although they can divide mitotically.
4.	Each round of cell division is preceded by DNA replication in the S phase that maintains the ploidy of descendent cells.	DNA is replicated prior to meiosis as in mitosis with the difference that the chromosome number is halved as it is segregated into four cells.
5.	It normally occurs in somatic cells. All somatic tissues in an organism are formed by mitosis and it helps in the renewal and repair of tissues throughout life.	Only specialised cells in sexually reproducing organisms divide by meiosis. The generation of haploid germ cells is a way to maintain constancy in chromosome number following fertilization.
6.	The homologous chromosomes do not pair during mitosis.	During prophase-I homologous chromosomes pair and exchange genetic material by crossing over.
7.	The cells derived from mitotic divisions generally do not show genetic variation and changes that take place during the life of these cells are not transmitted to the next generation.	Genetic variation in meiosis is created both by crossing over at prophase I and random alignment of homologous chromosomes at metaphase I. These events provide the raw material for evolution.

SAQ 3

Fill in the blanks:

(i) A human cell undergoing meiosis will have ……..chromosomes at the end of meiosis I and ……. chromosomes after completing meiosis.

(ii) The cells become haploid at …….. of meiosis.

(iii) The condensation of the chromosome region containing rRNA genes results in the disappearance of ……

(iv) The number of bivalents in a corn cell (2n=20) at the beginning of meiosis I are …….

v) The progression through the cell cycle is regulated at ……..

11.6 SUMMARY

- All cells arise by the division of preexisting cells. The basic process in all organisms involves DNA replication, segregation of replicated genetic material and division of the cytoplasm into two daughter cells.
- In prokaryotes cell division results in an increase in the number of organisms whereas in multicellular organisms it is the process by which an organism is formed and their cell number are maintained through life.All living organisms have developed one or more ways to divide. The complexity of the process has no doubt increased as we evolved from simple to more complex organisms. In this unit three types of cell division processes are discussed. Most prokaryotes have a single circular DNA and divide by binary fission.
- Cell division in eukaryotes is more complex than prokaryotes and can occur either by mitosis (equational division) or meiosis (reduction division). The former cycle operates in vegetative or somatic cells while the latter occurs only in specialised reproductive cells to produce haploid gametes.
- The cell cycle in eukaryotes is divided into stages through which a cell passes sequentially. The two major subdivisions are interphase and division *per se.* The interphase is a preparatory phase in which a cell grows and prepares to divide. In most cases nuclear division is followed by cytokinesis.
- Each mitotic cycle produces two genetically identical daughter cells, although not always identical in their cytoplasmic content. Both haploid and diploid cells can divide by mitosis. The repair of cellular damage; continuous renewal of some cell types and our ability to defend ourselves depend on cell division.
- In sexually reproducing organisms, a diploid mother cell gives rise to four haploid daughter cells after two rounds of consecutive division cycles that

are preceded by a single round of DNA replication. These two multistep stages are called **meiosis I** (reductional division) and **meiosis II** (equational division). Meiosis provides a means of maintaining constancy of chromosome number in a particular species following gametic fusion. It is also a mechanism to generate genetic variability by crossing over and independent assortment of homologous chromosomes.

- The cell cycle control system ensures that the cycle pauses at checkpoints and moves ahead only when the preceding event is completed.

11.7 TERMINAL QUESTIONS

1. Enlist the stages of mitosis and tabulate the major events that take place at each stage. Why it is called an equational division?
2. Explain how chromosomes are segregated to the pole during anaphase.
3. What is the difference between sister chromatids and homologous chromosomes?
4. Describe the structure and role of synaptonemal complex.
5. State the significance of cell division.
6. How does anaphase I of meiosis differ from anaphase of mitosis?
7. A human cell in G_1 has 46 chromosomes. How many chromosomes and DNA molecules will be there per cell as the cell completes a round of (a) mitosis (b) meiosis?
8. An analysis of dividing cells in a plant root tip and recording the number of cells in each stage showed that 100 cells were in interphase; 20 in prophase; 5 in metaphase; 6 in anaphase and 2 in telophase. If the cycle completes in 24 hours, what is the average duration of each stage in the cell cycle?
9. Briefly describe the role of the following proteins /enzymes in cell division:

   a) Cohesin b) Separase c) Tubulins

   d) Shugoshin e) Condensin

11.8 ANSWERS

Self-Assessment Questions

1. Binary fission
2. a) i) In plant cells the mitotic spindle is assembled without centrioles and they lack distinct centrosomes. The spindle also lacks astral MT. The

centrosomes in animal cells have two centrioles which are nucleation sites for MT and they have astral MT in addition to polar and kinetochore MT.

ii)

Plant cells divide by forming a cell plate at right angles to the mitotic spindle. The membrane partition continues to grow outward till it reaches the cytoplasmic face of the plasma membrane and fuses with it resulting in two cells. The cell wall material is then laid down in the new membranes. In animal cells, an actin based contractile ring is transiently formed in a plane perpendicular to the axis of the spindle that deepens to form a furrow as the actin filaments slide past each other in the mid portion of the spindle. The constriction progresses **inwards** until the opposing surfaces finally meet and fuse in the centre of the cell to divide the cytoplasm into two cells.

(iii)

In plants mitosis is restricted to meristems whereas in animals it can occur throughout the body.

b).

If a cell grows without dividing then its surface to volume ratio decreases because the increase in volume is more than surface area. This difference will in turn affect nutrient uptake and waste disposal.

c).

i) **G_1:** It is the primary growth phase. The cell prepares for DNA replication by synthesizing the enzymes, building blocks and other proteins. The replication of the microtubule organizing centre (MOTC) or centrosome begins at G_1 and completes at G_2 but they remain close together in a single centrosomal complex. In eukaryotes the major checkpoint lies late in G_1.

ii) **S:** It is the phase of DNA replication.

iii) **G_2:** It is another period for growth and prepares the cell for the mitotic phase. Before entering mitosis, the cell ensures that DNA replication has completed and DNA damage if any is repaired. The number of organelles like mitochondria grows and divides to double their numbers. The process of chromosome condensation begins at this stage and continues till metaphase.

iv) **G_o:** It is a non-dividing or resting phase. During this phase cells generally have a constant size. Most cells in an organism are in G_o. These cells can revert back to a cycling phase.

3. i) 23; 23 ii) Anaphase I iii) Nucleolus
   iv) 10 v) Checkpoints

Terminal Questions

1.

Stages of mitosis	Major Events
Prophase	• Chromatin condenses into discrete chromosomes • Fragmentation of ER and Golgi apparatus. • spindles form at opposite poles of the cell
Prometaphase	• Nuclear envelope begins to disintegrate • Nucleoli disappear
Metaphase	• Microtubules continue to extend from the poles to the center of the cell. • Chromosomes move randomly until their centromeres attach to the spindle. • Chromosomes are held at the metaphase plate.
Anaphase	• The sister chromatids separate at the centromere following the breakdown of cohesin. • The chromosomes move to the poles at opposite ends of the cell.
Telophase	• The nuclear envelope reappears around the separated chromosomes at each pole.. • Chromosomes are decondensed • Nucleolus reappears.

Mitosis is also known as equational division because it produces two daughter cells that are genetically identical to the mother cell.

2.

The separation of sister chromatids marks the beginning of anaphase. It is almost a synchronous separation and represents the shortest stage of mitosis. This separation occurs due to the breakdown of a cohesin subunit, encircling the sister chromatids by a protease **separase.** Each centromere then splits to separate sister chromatids. The movement of sister chromatids to the poles is assisted by another round of dynamic behaviour of microtubules **(anaphase movement)**.

It is dependent both on the disassembly of tubulin subunits and motor proteins. At the beginning of anaphase the kinetochore microtubules shorten, and the chromosomes move towards the spindle poles. Subsequently the spindle poles separate as other microtubules emerging from the centrosome move past each other. The involvement of motor proteins is implicated in generating the force for pulling the chromosomes.

3.

Homologous chromosomes are a pair of chromosomes that are alike in structure and size but they are not completely identical. A member of every homologous pair is inherited from each parent. Sister chromatids represent two copies of a chromosome that are held together at the centromere. Each chromatid has a single DNA molecule as a result of DNA replication prior to mitosis or meiosis.

4.

A **synaptonemal complex** is formed of protein filaments which are formed between paired homologous chromosomes. It is believed to function as a scaffold to facilitate crossing over between non-sister chromatids. The synaptonemal complex has dense granules at some places along its length that harbor enzymes to assist in recombination. They are called **recombination nodules.**

5.

The main aim of cell division is to keep genetic information consistent between generations. At the same time cells growing too large, face adversities in uptake and disposal of materials. These factors thus limit the growth of cells to a particular threshold beyond which it divides, distributing its contents equally to give rise to identical daughter cells.

Other than this, a cell needs to divide to replace dead or damaged cells / to heal a wound and for defense. Multicellular organisms grow and develop both by increasing their size and cell number through cell division. In unicellular forms cell division is a means of increasing the number of organisms.

6.

During anaphase I of meiosis, homologous chromosomes separate and move to opposite poles, while the sister chromatids remain attached at their centromeres. But during anaphase of mitosis the centromere splits and the sister chromatids separate completely. This results in reduction of chromosome number to half in meiosis I and no change in mitosis.

7. a) Mitosis: **46** b) Meiosis: **23**

8. **Step 1**: Calculate the proportion of cells in each stage of the cell cycle. This is equal to the number of cells in a given stage divided by the total number of cells counted.

Total number of cells analysed = 100+20+5+6+2=**133**

Interphase =100/133=0.751

Prophase =20/133 =0.15

Metaphase = 5/133=0.037

Anaphase = 6/133=0.045

Telophase= 2/133=0.015

Step 2: The average duration of each stage can be deduced by multiplying the proportion of cells in each stage by the time required to complete the cell cycle.

Interphase= 0.751 x 24 = 18 hrs

Prophase = 0.15 x 24 = 3.61 hrs

Metaphase = 0.037 x 24 =0.90 hr (or 54 min)

Anaphase= 0.045 x 24 = 1.08 hr (or 64.9 min)

Telophase = 0.015 x 24 = 0.361 (or 21.65 min)

9. a) **Cohesin**

It is a multi protein complex that holds sister chromatids together. The separation of sister chromatids in anaphase II of meiosis and anaphase of mitosis occurs only after its breakdown.

b) **Separase**

It is a cysteine protease that degrades a subunit of cohesin, dissociating it from the chromatids and bringing about separation of sister chromatids during anaphase.

c) **Tubulins**

Tubulins are globular proteins that polymerise to form microtubules. They represent an important class of cytoskeletal proteins that are required for chromosome segregation and cellular transport.

d) **Shugoshin**

It is required for the persistence of centromeric cohesion during meiosis I such that homologous chromosomes separate and move to the poles. This is achieved by protecting the integrity of cohesin complex.

e) **Condensin**

They are a group of SMC proteins that bind to each sister chromatid at multiple points to facilitate their condensation.

11.9 SUGGESTED READINGS

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2. www.nature.com /scitable/ cell division: stages of mitosis/ Clare O'Conner
3. Molecular Cell Biology, 6th edition, Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell. 2000

4. Molecular Biology of the Cell: The Problems Book, by John Wilson and Tim Hunt, Garlend Science, 2002
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UNIT 12

REGULATION OF CELL DIVISION

Structure

12.1 INTRODUCTION

In the previous Unit you have learnt about cell division process in eukaryotes and prokaryotes. The simplest version is seen in prokaryotes. It is called binary fission. In eukaryotes most cells divide by mitosis that segregates the replicated genetic material equally between daughter cells. The second form of cell division is meiosis that is restricted to specialised cells and it is responsible for forming haploid gametes. The cell division cycles are complex multistep processes and the cell must stringently monitor to achieve the desired outcome. Therefore, this unit deals with the regulation of cell division in eukaryotes.

In this unit, you will learn about the molecular machinery of cell cycle control system and how various events are coordinated in time and space. You will be introduced to the concept of 'checkpoints' where progress of ongoing process is monitored and accordingly the cell is either allowed to move ahead or paused to complete it.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ explain how does the cell cycle control system works;
- ❖ describe the cell cycle checkpoints and restriction point;
- ❖ highlight the regulatory molecules of cell cycle;
- ❖ indicate the role of cyclins and cyclin-dependent kinases;
- ❖ explain the role of cyclin expression cycle and;
- ❖ understand the relevance of CDK inhibitors in cell cycle control.

12.2 CELL CYCLE CONTROL- A HISTORICAL PERSPECTIVE

A eukaryotic cell division cycle as described in unit 11 essentially has two main phases: interphase and mitosis and the success of the whole process are heavily dependent on coordinating different events (Fig.12.1). We have already discussed that cells must replicate DNA before entering mitosis (or meiosis) and they stockpile material sufficient for two cells. The study of the cell division cycle stages under the microscope made it clear that dividing cells follow a definite course. Yet it was only in the 1970s that we began to get clues about the existence of a control system from the work of independent groups that used different approaches in a variety of biological systems.

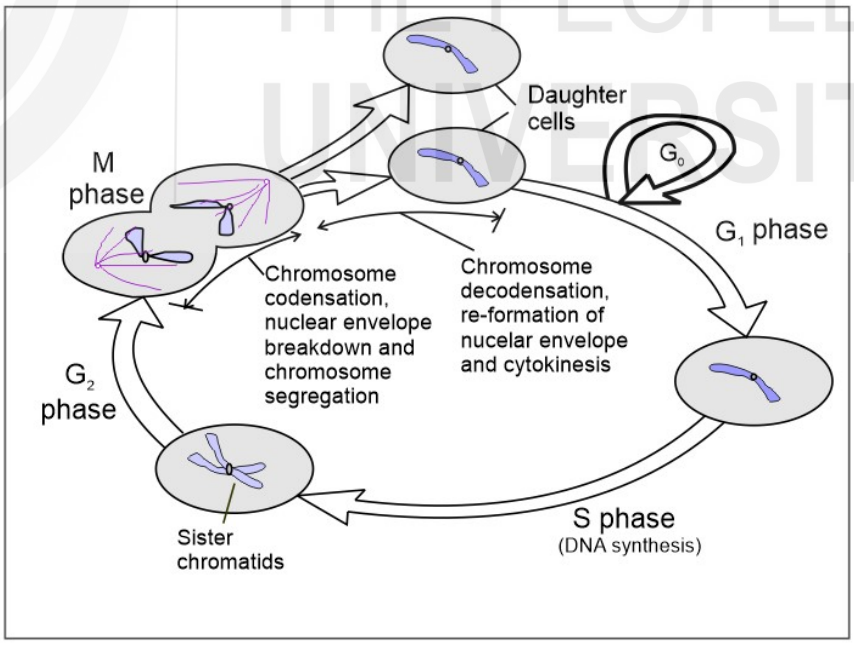

Fig. 12.1: Major events in a eukaryotic cell cycle.

Our current understanding of the cell cycle control has emerged largely from the study of model experimental systems such as *Schizosaccharomyces pombe* (fission yeast), *Saccharomyces cerevisiae* (budding yeast), frog eggs and mammalian cells in culture. One of the earliest evidence for the existence

of a positive regulator of mitosis came from studies in a slime mold *Physarum polycephalum (1966)*. When plasmodia at different stages of the cell cycle were fused the cell that was not yet dividing can be induced prematurely to enter mitosis. The level of this factor was shown to fluctuate during the cell cycle, peaking at mitosis.

In the early 1970s microinjection experiments conducted in the frog *Xenopus* and cell-cell fusion of human cells supported the existence of a soluble regulator of cell division. **Y. Masui and L. Dennis Smith** independently found that frog oocytes undergoing meiosis contain a substance capable of inducing an unfertilized immature oocyte to divide and become an egg. They called this uncharacterized substance '**maturation promoting factor' (MPF**) because meiosis is often referred to as maturation. Similarly, **Potu Rao and Robert Johnson** in 1970 conducted a series of cell-fusion experiments in which they fused a somatic cell in one phase with a cell in another phase of the cell cycle.

Look at Fig 12.2 in which two cells in different stages of cell division have been fused. These experiments demonstrated that an interphase cell (S phase) can be driven into mitosis if it is fused with a mitotic cell, irrespective of the fact whether it has replicated its DNA or not. Similarly a cell in G1 immediately enters S phase when fused with a cell in S phase. These experiments demonstrated that positive factors may drive entry into S and M phases. Subsequently it was shown that the soluble regulator of mitosis and meiosis is the same substance (maturation promoting factor = M-phase promoting factor).

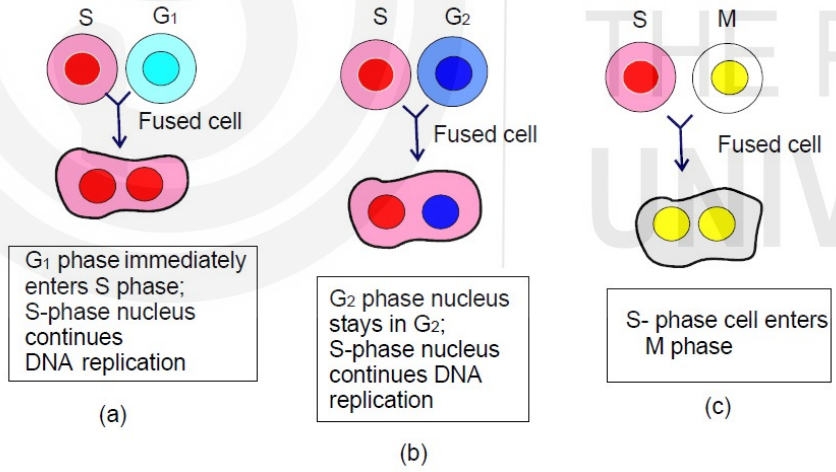

Fig.12.2: Cell fusion experiments. (Modified Image, Source: M. Cooper The Cell-Molecular Approach)

The next step was to find out the molecular components of cell cycle control system.

Leland H. Hartwell from the University of Washington used genetic approach in budding yeast and began to pick up yeast mutants that failed to progress through the cell cycle at specific points. He succeeded in arranging these mutants in accordance with the step they were stuck and elucidated the normal sequence in which the cell division cycle (Cdc) genes function. He could demonstrate in some cases that initiation of some steps depended on

the completion of the preceding step and introduced the concept of 'checkpoint' that delay cell cycle progression until the previous event was completed.


Leland H. Hartwell

Sir Paul Nurse and his colleagues did similar studies with fission yeast. One of the genes, cdc2 was found to be crucial for entry into mitosis. Based on the sequence cdc2 proteins have been identified as protein kinases. He also established that these kinases are highly conserved among eukaryotes.

In 1988 Manfred J .Lohka and James L. Maller succeeded in isolating small amount of MPF and showed that it consists of two proteins and there were indications that one of the proteins was cdc2. Many other teams then independently confirmed this idea.


Tim Hunt

The next question to answer was why MPF was active in mitosis and not at interphase. It was known that the concentration of cdc2 protein remains constant throughout the cell cycle so the characterization and the role of the other component of MPF for its possible involvement in activation had to be looked into.


Sir Paul Nurse

In the early 1980s**Timothy Hunt** from the University of Cambridge was investigating with his students changes in the levels of proteins following fertilization in sea urchin eggs. They found that unlike other newly synthesized proteins, there was one protein that showed a dramatic decline at each mitotic cycle and accumulated again during interphase (Fig.12.3). He named it **cyclin** based on its oscillatory behavior. His group also demonstrated that cyclin disappears at the end of mitosis due to an increase in the rate of degradation while at interphase the pattern reversed. It suggested that cyclin may be the protein that regulated MPF activity.

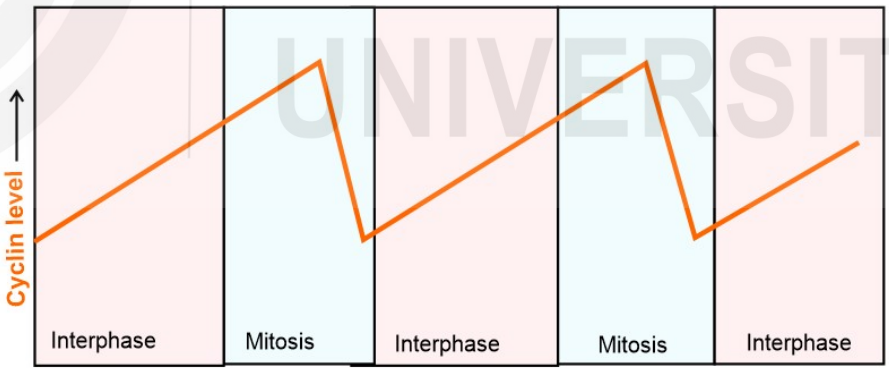

Fig.12.3: Oscillations in the levels of cyclin in sea urchin eggs.
(Modified Image, Source: M. Cooper The Cell- Molecular Approach)

The abrupt degradation of cyclin at mitosis allows the cell to exit mitosis. It soon became clear that association of cyclin with cdc2 protein is not sufficient to activate MPF. One of the proteins identified by genetic methods was named cdc25 and the rate of its accumulation rather than cyclin determines when cdc2 becomes active and the cell enters mitosis in certain cells like fly embryos and fission yeast.

Leland H. Hartwell, Timothy Hunt and Paul Nurse shared the 2001 Nobel Prize in Physiology or Medicine for their discoveries on the key regulators of

the cell cycle. Today it is known that cyclin is indeed the second component of MPF and the cell cycle control system is essentially a **kinase based machine**.

The complexity of the control system and the variety of external signals to which a multicellular organism can respond is much more than yeast. In case of mammalian cells, it is known that they need positive factors such as growth factors to override the negative influence on cell division. In addition simple unicellular eukaryotes (yeast) and multicellular organisms have developed mechanisms to sense and delay entry into mitosis until DNA replication is completed and DNA damage, if any is repaired.

The control system in some organisms such as *Xenopus* early embryos is driven by an autonomous oscillator or clock that operates even in the absence of the nucleus. The frog egg contracts at the onset of mitosis and the contraction disappears during interphase. These periodic contractions occurring every 30min coincide with the surge in MPF activity. In other cells the timing of the cell cycle depends on the interplay of positive and negative regulators (Fig.12.4). The progression depends on the completion of the earlier events and it is delayed if DNA is damaged or not completely replicated.

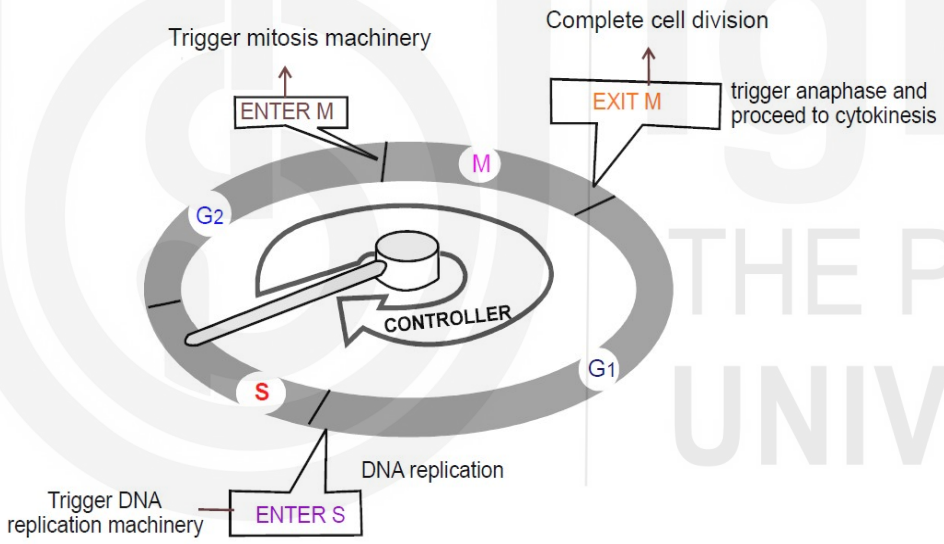

Fig.12.4: The controller rotates at each phase of cell cycle. (Modified image, Source: Essential Cell Biology, Garland Science, 2010)

The cell cycle also regulates cell number which is critical for development and maintenance of a multicellular organism. Each mitotic cycle ensures accurate segregation of duplicated chromosomes, starting with either haploid or diploid cells as most chromosome imbalances are not tolerated. The cell number can be altered by either inducing cell proliferation or through exit from the cell cycle and by programmed cell death. These two fundamental processes determining cell death and renewal will be dealt in Unit 13.

You have already studied that the cell division cycle progresses in a given sequence. To avoid errors during the cell cycle, the cell has internal control system that operates through checkpoints and monitors the completion of an ongoing event before moving ahead.

Let us begin by describing the cell cycle checkpoints.

12.2.1 Cell Cycle Checkpoints

The control system is regulated by brakes that can delay progression of the cell cycle at specific checkpoints, if cellular conditions are unfavorable or DNA is damaged / incompletely replicated. This helps to enhance the overall fidelity (accuracy) of the process. The breaks allow the cell to monitor completion of a preceding event before proceeding further. They also allow the control system to be regulated by signals from the environment and internal signals.

The three major cell cycle checkpoints are enlisted below and some of the components they monitor are highlighted in Fig.12.5.

1. The G_1 checkpoint at G1/S transition**,**

2. The G_2 checkpoint at G2 / M transition and

3. The M / spindle checkpoint is at the transition from metaphase to anaphase.

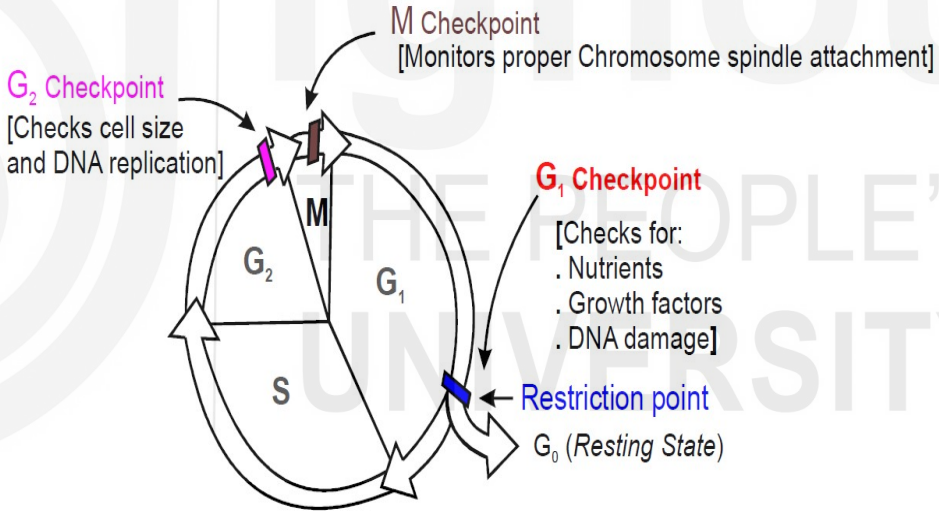

Fig. 12.5: Cell cycle checkpoints and restriction points.

1. G_1 checkpoint (G_1/S)

The replication of genetic material is one of the most crucial stage of the cell cycle and hence the major checkpoint in eukaryotic cells lies late in G_1 (G_1/S) and it controls the entry to S phase. This regulatory point is called **Restriction (R) Point** in animal cells and **START** in yeast. The cell integrates both external and internal signals before taking a decision to divide. Some of these factors include growth factors, nutrient availability, cell size and above all the intactness of the genome. The cells will remain in G_1 until it has built the requisite level of enzymes / proteins necessary for DNA replication. Once a cell crosses this checkpoint it will replicate and complete a round of division.

A cell can also undertake an alternative course before reaching the G_1/S checkpoint. In response to signals many cells enter a non dividing G_0 or quiescent phase for variable periods, ranging from days to years. During this phase they generally have a constant size. These cells have the potential to revert back to a cycling phase with changing situations. Some cells like most neurons remain indefinitely in G_o while on the other extreme are stem cells that never enter G_o state.

2. **G_2 Checkpoint (G_2/M)**

At this regulatory point, a cell monitors favorable conditions to enter the mitotic phase. The cell will not proceed to mitosis if DNA replication is not complete. The G2 / M checkpoint also ensures that all chromosomes have accurately replicated and DNA is free of damage.

3. **M Checkpoint**

Another important cell cycle checkpoint called M checkpoint or spindle attachment checkpoint is present at the end of metaphase. It is the key control point to monitor the alignment of all chromosomes at the metaphase plate, held in place by spindle MT emanating from both poles. This is a prerequisite condition for an equal chromosomal distribution to two daughter cells. The failure to attach even one duplicated chromosome is sensed at this stage and further progression is delayed by blocking the inactivation of MPF.

SAQ 1

a) Fill in the blanks:

(i) The cell cycle control system is regulated by brakes that can delay progression of the cycle at specific ……..

(ii) The requisite level of enzymes / proteins necessary for DNA replication is assessed at ……phase.

(iii) DNA integrity and completion of DNA replication are monitored at …… checkpoint.

(iv) Once a cell crosses ---------- checkpoint, it will complete a round of mitosis. The attachment of chromosomes to spindle microtubules is assessed at ….. …….

b) Complete the missing details in the table below.

Regulatory molecules	Scientist(s)	Experimental model
Cyclin		
Maturation promoting factor (MPF)		
Cyclin-Dependent Kinase		

(CDK)		

12.3 REGULATORY MOLECULES OF THE CELL CYCLE

Despite the internally controlled checkpoints, the cell cycle control system coordinates the cell cycle as a whole by two groups of intracellular molecules. These regulatory molecules either trigger cell cycle phase transition (positive regulation: Cyclin/CDKs)) or halt the cycle (negative regulation: p53). They may act directly or indirectly by influencing the activity or production of other regulatory proteins.

Let us proceed to discuss one by one the positive and negative regulators of the cell cycle and how they coordinate the cell cycle events.

12.3.1 Cyclins and Cyclin Dependent Kinases (CDKs)

The cell cycle control mechanism is based on positive regulators namely **cyclins** and **cyclin dependent kinases (CDKs).** The cyclin dependent kinases (CDKs) are a family of serine / threonine kinases. They are relatively low molecular weight proteins (34-40 Kda). As its name implies, the functional activity of CDKs depends on the regulatory protein, cyclin. They are the heterodimeric protein kinases with a **regulatory subunit** (cyclin) and **catalytic subunit** (kinase).

In 1982, Timothy Hunt discovered cyclin protein while studying the cell cycle in sea urchin eggs. He named them cyclins because their concentration varies periodically during the cell cycle. These proteins form a complex with CDK and become active only after further modifications.

Multicellular organisms have multiple Cdks and cyclins that assist in complex signal integration. The expression profile of cyclins is indicative of their role in specific phases of the cell cycle. For instance, cyclinB-Cdk1complex peaks at late G_2 to assemble the maturation / mitosis promoting factor (MPF) that allows the cell to advance from G_2 to M phase. The cyclins are degraded either by exposure of PEST sequences at their C terminus (cyclin C, D, E, and F) or by after acquiring a polyubiquitin tag (cyclin A and B). In higher eukaryotes, approximately 20 different Cdks and Cdk-related proteins and four major classes of cyclins have been characterized. Among them only four classes of cyclins/CDKs are required for eukaryotic cell cycle (Fig. 12.6).

These complexes are:

1. G_1 Cyclin E / CDK2 complex promotes G1/S phase transition and also initiates the duplication of DNA and centrosome,
2. S Cyclin A1 / CDK2 initiates S phase (DNA replication),
3. G_2 Cyclin A/ CDK1 allows the progression from G_2 to M phase and cyclin B/ CDK1 is required to coordinate the reorganization of the cytoskeleton.

4. Cyclins D1, 2 or 3 / CDK6 or CDK4 regulate late G_1 (start point or restriction point) to S phase transition.

Other cyclin dependent kinases like cdk7-cyclinH and cdk5-cyclinG are needed in response to DNA damage or to bring about Cdk activation.

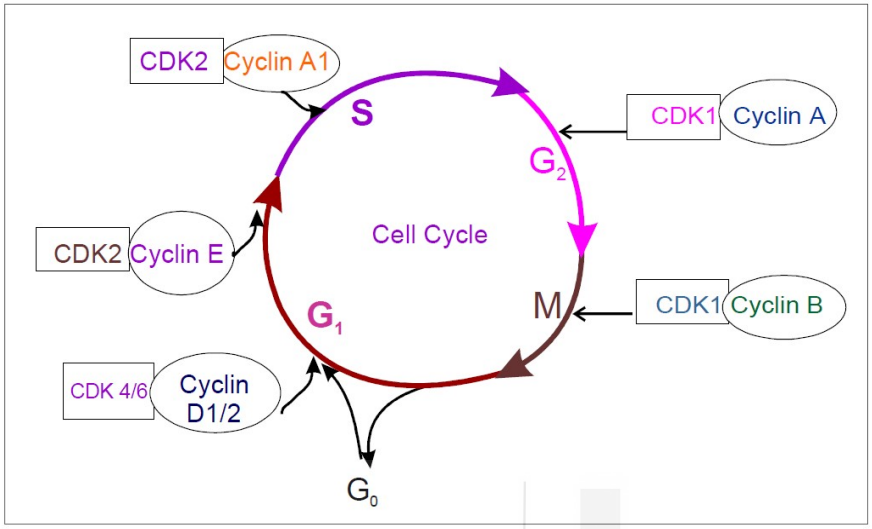

Fig.12.6: CDK/cyclin complexes active at different cell cycle stages.

Let us learn how cyclin-dependent kinases are activated. Most cyclin-dependent kinases associate with one or more cyclins to form cyclin-CDKs complexes. The resultant complex generally requires further modifications to become active (Fig 12.7). Once activated, they phosphorylate multiple protein targets and subsequently the cyclin subunit is degraded, rendering them ineffective.

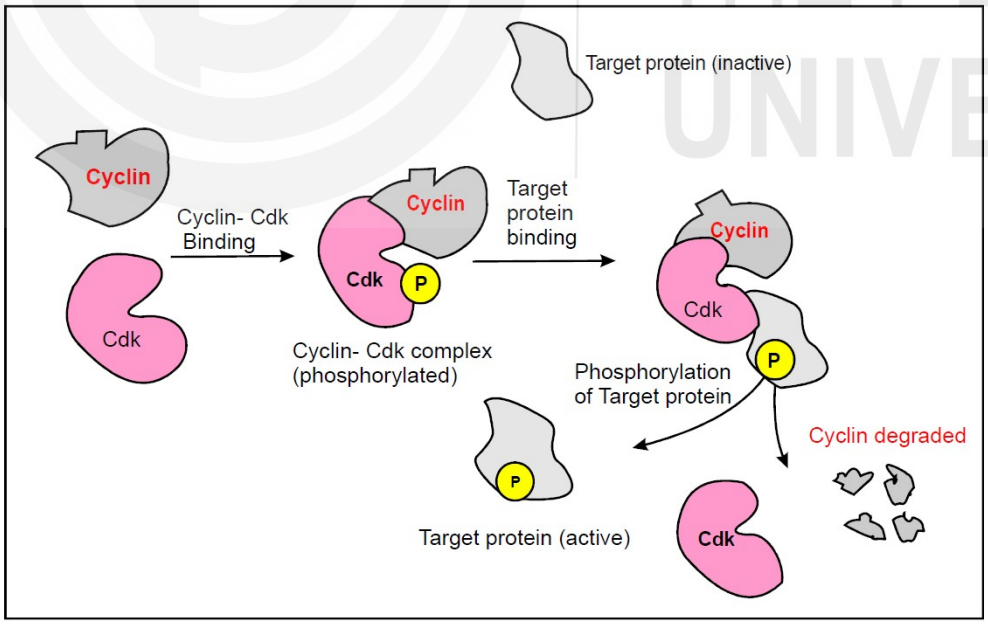

Fig. 12.7: Cyclin/CDKs complex activity.

Now we will take the example of **maturation-promoting factor** (**MPF**) to understand how cyclins and Cdks assemble and become functionally competent after specific phosphorylation(s). MPF is a protein dimer of cyclin B

and CDK1 (Fig.12.8).The formation of specific Cdk / cyclin complexes is controlled by cell cycle dependent cyclin synthesis and degradation.

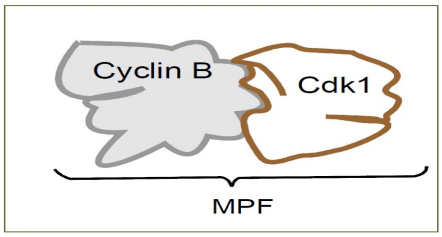

Fig.12.8: Structure of MPF.

In general, the activity of Cdks is regulated by **four molecular mechanisms** by virtue of multiple regulatory sites depicted in Fig.12.9a.

The first level of regulation involves the association of cyclins with their specific Cdks. As cyclins are cyclically synthesised and degraded, the association occurs only at defined phases of the cell cycle. **Second, Cdk/ cyclin complexes** is activated when a specific threonine residue is phosphorylated by an enzyme called CAK (for Cdk-activating kinase), which is itself a complex of **Cdk (Cdk1) and cyclin B**. Third and Fourth are the sites for binding of CDK inhibitors and inhibitory phosphorylation, respectively. The consensus sequence for phosphorylation of a CDK is [S/T*] PX [K/R], where S/T* is the phosphorylated serine or threonine residue, P is proline, X is any amino acid, K is lysine, and R is arginine.

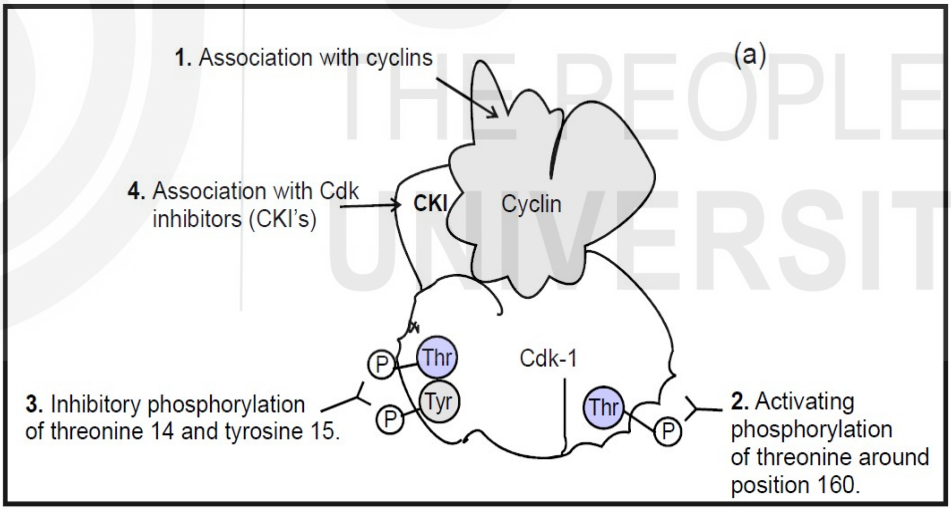

Fig. 12.9a: Regulatory sites of Cyclin/Cdk1 complex.
(Source: M. Cooper: The Cell: A Molecular Approach)

It is clear from Fig.12.9 that the activity of MPF is regulated by both phosphorylation and dephosphorylation and cyclin binding only generates a pre MPF that is incompetent to act on its targets**.** The binding of cyclin B during interphase is essential for the action of two kinases that phosphorylate Cdk1. One of kinase adds phosphate to a tyrosine residue that blocks its kinase activity while the second kinase phosphorylates a specific threonine residue. Unless phosphate from tyrosine hydroxyl is removed the activating influence of threonine phosphorylation will not be visible. The purpose of all this complexity is to prevent **premature activation of MPF**. The removal of

phosphate is catalysed by a phosphatase that is activated only when a cell is ready to enter mitosis. The activation of MPF is very rapid due to a positive feedback mechanism in which MPF activates the phosphatase by phosphorylation. Hence it is the assembly of cyclins; activation and then disassembly (at metaphase / anaphase boundary) of the cyclin –Cdk complexes are pivotal events of the cell cycle control system.

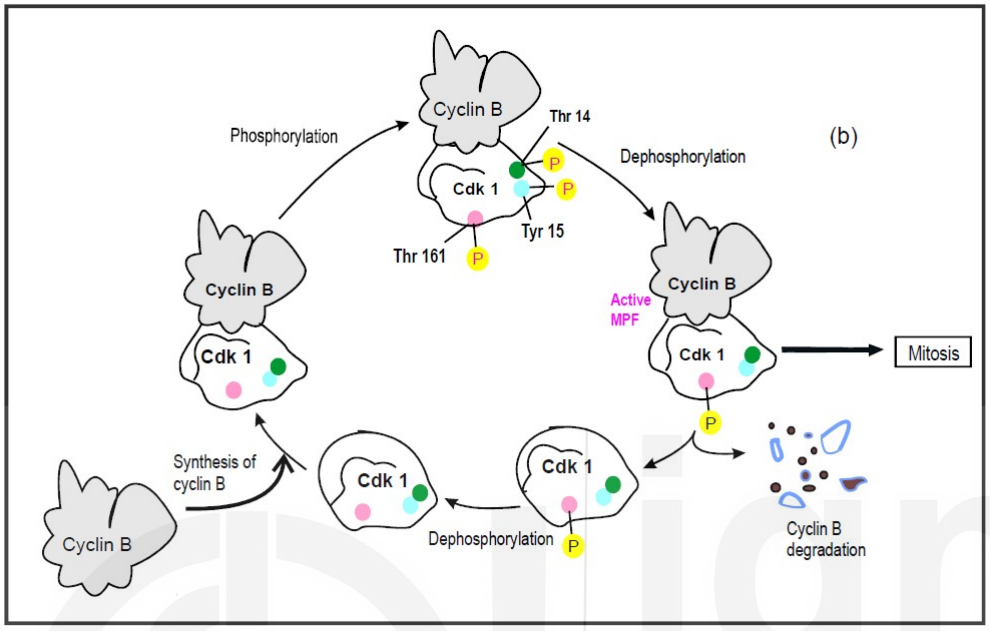

Fig. 12.9b: Molecular mechanism of MPF regulation *(Source*: M. Cooper: The Cell: A Molecular Approach)

The activation of MPF brings about changes in the cell necessary to enter mitosis. Some of the well known targets of MPF are condensins, lamins, histone H1 and microtubule associated proteins (MAP). The effects produced either directly or indirectly include breakdown of the nuclear envelope, condensation of chromosomes and changes in the behaviour of microtubules. In addition to driving cell cycle progression to M phase, MPF (cyclin/CDK complex) induces the degradation of cyclin by activating the **anaphase-promoting complex/ cyclosome** (APC/C) late in metaphase. It is an **E3 ubiquitin ligase** that attaches a polyubiquitin tag on specific protein regulators including mitotic cyclins and securin that are then directed for degradation to peptides by a large multiprotein assembly called proteasome (26S). APC also plays a similar role in meiosis II but it requirement in meiosis I depends on the organism.

APC controls anaphase entry, progression and exit from mitosis. It is a large complex that has at least 11 core subunits in vertebrates. Its activity is regulated during mitosis by two activators, Cdc20 and Cdh1. APC-cdc20 is responsible for degradation of cyclin B and securin. Let us see how the key event of anaphase – sister chromatid separation, is initiated by an active APC. Prior to anaphase the activity of separase is inhibited by a protein, **securin** that associates to form an inactive complex. At anaphase entry, APC degrades securin to relieve separase inhibition resulting in the cleavage of the cohesin subunits (Fig.12.10).

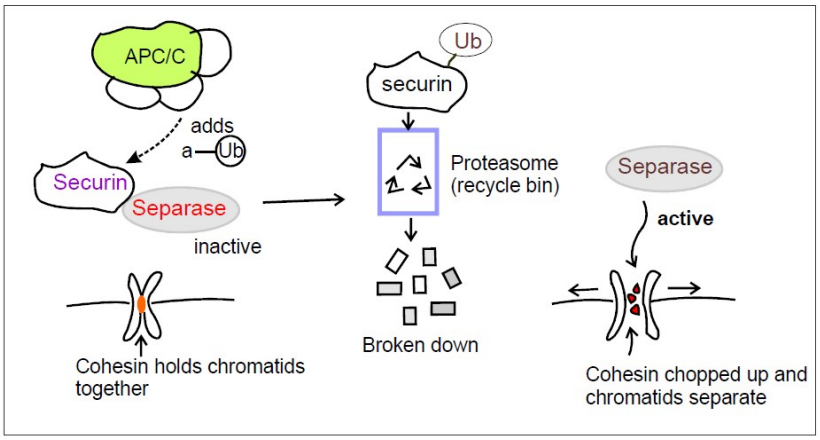

Fig. 12.10: Cyclin degradation by APC/C (A ubiquitin-proteasome pathway).

Now the multi protein cohesin complex will no longer hold sister chromatids together marking entry to anaphase. The degradation of the regulatory subunit of MPF by APC begins at metaphase and continues throughout mitosis and G1 phase. This inactivates Cdk1and induce multiple processes like reformation of the nuclear envelope, chromosome decondensation, disassembly of the mitotic spindle and cytokinesis. The events listed are essentially the reversal of changes initiated during or before the beginning of mitosis. Finally, the role of CDK inhibitors will be covered separately.

12.3.2 Cyclin expression cycle

In eukaryotes multiple cyclins participate during the cell cycle. Each of these cyclins peak at different time periods and function as regulatory partners of specific cell division kinases (CDKs) for that part of the cell cycle (Fig.12.11). Both cyclins and CDK must be present at higher concentrations for cells to divide. The rise and fall in the levels of cyclins allows one complex to complete its job and for the other to take over. Unlike cyclins, the level of CDKs remains relatively constant. The activation and inactivation of cell cycle kinases depends on the concentration of cyclins which have direct correlation with the checkpoints of the cell cycle. Thus, cyclin and Cdk are master components of the control system that ensure and promote cell cycle progression in correct order and allow the next phase transition to occur only when the preceding one has been accurately completed.

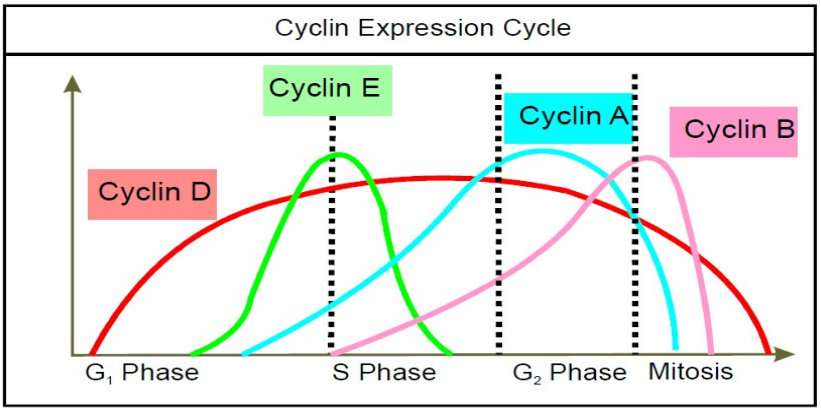

Fig. 12.11: The expression profile of Cyclins.

12.3.3 CDK inhibitors

In addition to the positive regulators (CDK/Cyclin complexes) discussed above, there are a number of CDK inhibitors known which halt or delay cell cycle progression. They are low molecular weight proteins that are grouped into two families; the **Ink4family** (p15, p16, p18, p19) and **the Cip/Kip family** (p21, p27, p57). They all target specific cyclin/CDKs complexes thereby affecting the normal cell cycle progression (Fig.12.12).

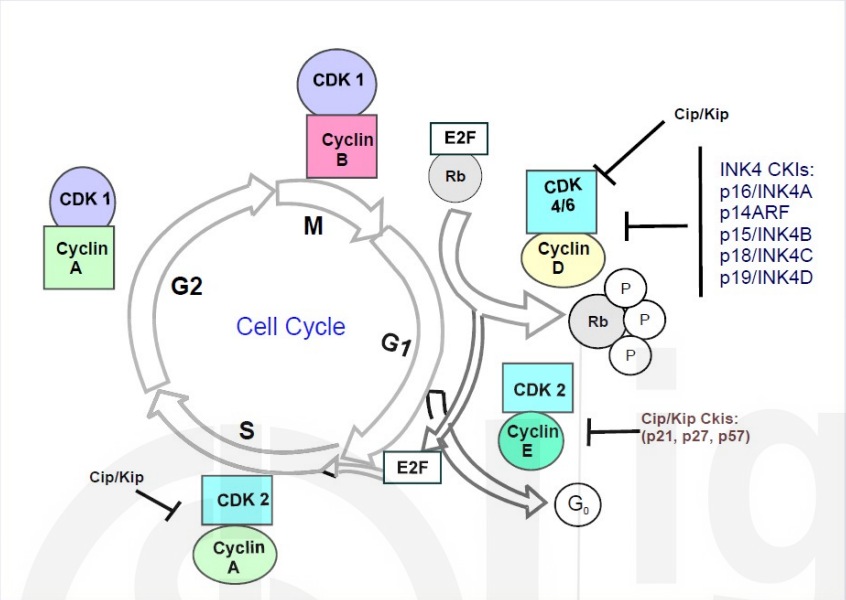

Fig. 12.12: CDK inhibitors and their targets.(Source: Lodish, Molecular Cell Biology)

Mammalian cells for instance restrain cells with damaged DNA from entering S phase by accumulating the protein **p53**. It is a tumor suppressor protein and is often referred to as “the guardian” of the genome. An increase in p53 arrests the cycling cells at G1checkpoint. The protein works at multiple levels to ensure that cells do not pass on damaged DNA to the daughter cells. The p53 protein triggers the synthesis of a Cdk inhibitor, p21 by binding to its promoter. The inhibitor binds tightly to Cdk / cyclin D complexes and inhibits them (Fig.12.13).

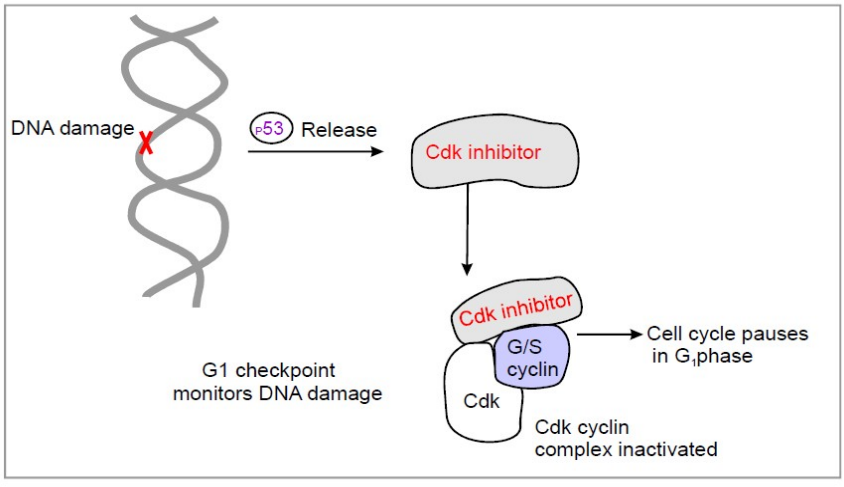

Fig. 12.13: Cell cycle arrest in response to DNA damage.

As you know cyclin D-kinase complex are the first to integrate extracellular signals to the cell cycle machinery and their inhibition will halt cell cycle progression and allow time for repair. The inhibition of the CDK leads to a decrease in Rb (retinoblastoma protein) phosphorylation, keeping it in an active state. In its active state Rb sequesters proteins including transcription factors (E2F) needed for entry into S phase. The other roles of p53 in the overall coordination are activating DNA repair enzymes and inhibiting PCNA (Proliferating cell nuclear antigen) dependent DNA polymerase. The importance of both p53 and Rb in controlling the replication of damaged DNA can be appreciated by the fact that they are mutated in many types of cancers accounting for the accumulation of mutations over time.

SAQ 2

Indicate whether the following statements are true (T) or false (F):

(i) Cyclins are activated and degraded during cell cycle. [ ]

(ii) The CDK1/cyclin A promotes M phase to G1transition. [ ]

(iii) Cyclin dependent kinases promote the duplication of centrosomes and DNA during interphase and mitosis. [ ]

(iv) The activation of Cyclin/CDK complex depends on phosphorylation [ ]

(v) The CDK inhibitors target specific cyclin/CDKs complexes and delay normal cell cycle progression. [ ]

(vi) The cell cycle is regulated by a mix of positive and negative regulators. [ ]

12.4 SUMMARY

After studying this unit, you have learned that:

- The study of the cell division cycle stages under the microscope made it clear that dividing cells follow a definite course. Our current understanding of the cell cycle control has emerged largely from the study of model experimental systems such as *Schizosaccharomyces pombe* (fission yeast), *Saccharomyces cerevisiae* (budding yeast), frog eggs and mammalian cells in culture.

- The cell fusion experiments were instrumental in providing the earliest evidence for the existence of a positive regulator of mitosis. The level of this factor was shown to fluctuate during the cell cycle, peaking at mitosis. Later microinjection experiments in *Xenopus* reported a soluble substance capable of inducing immature oocytes to divide and become an egg. This substance was therefore called ‘maturation promoting factor’ or MPF. It has been shown to be required for both mitosis and meiosis.

- The control system is a kinase-based machine. The activation of these kinases occurs only at particular phases of the cell cycle. The cell division kinases are called CDK as they are complexed with cyclins. The activity of kinases is further controlled by phosphorylation. MPF is an example of a

cyclin –kinase complex that controls entry to mitosis. As the name suggests the levels of cyclins fluctuate during the cell cycle.

- The control system is regulated by brakes that can delay progression of the cycle at specific checkpoints if cellular conditions are unfavorable or DNA is damaged / incompletely replicated. This enhances the overall fidelity of the process.
- The three major checkpoints are G_1, G_2 and spindle checkpoint. The replication of genetic material is one of the most crucial stage of the cell cycle and hence the major checkpoint in eukaryotic cells lies late in G_1 (G_1/S) and it controls the entry to the S phase. This regulatory point is called **Restriction (R) Point** in animal cells and **START** in yeast. The cell integrates both external and internal signals before it takes a decision to divide.
- A cell can also undertake an alternative course before reaching the G_1/S checkpoint. In response to signals many cells enter a non-dividing G_0 or quiescent phase for variable periods ranging from days to years.
- In addition to the positive regulators (CDK/Cyclin complexes) there are a number of CDK inhibitors known which halt or delay cell cycle progression. They belong to Ink4 family (p15, p16, p18, p19) and Cip/Kip family (p21, p27, p57). They all target specific cyclin/CDK complexes.

12.5 TERMINAL QUESTIONS

1. Explain the cell fusion experiments that predicted the existence of a positive regulator of cell division.
2. What are the major checkpoints in the cell cycle? Highlight the role of each in the fidelity (accuracy) of the process.
3. What will be effect of a base substitution that changes tyr15 to phe15 in cdc2?
4. Indicate the role of following proteins in the control of cell division cycle:

   a) Cyclin b) CDKs c) CAK d) Securin e) Cdk inhibitors
5. Explain how the APC/C degrades mitotic cyclins.
6. Describe the structure, activation and role of MPF.
7. Explain cyclin expression cycle.

12.6 ANSWERS

Self-Assessment Questions

1. a)

i. Check points. ii. G1. iii. G2/M.

iv. G2 v. The M/ spindle checkpoint

b)

Regulatory molecules	Scientist(s)	Experimental model
Cyclin	Timothy Hunt	Sea urchin eggs
Maturation promoting factor (MPF)	Y. Masui & L. Dennis Smith Potu Rao & Robert Johnson	Frog oocyte Somatic cells
Cyclin-Dependent Kinase (CDK)	Timothy Hunt	Sea urchin eggs

2.

(i) True (ii) False (iii) False
(iv) True (v) True (vi)True

Terminal Questions

1. Cell fusion experiments were conducted by the Potu Rao and Robert Johnson to demonstrate the existence of soluble factor in cell cycle control. These experiments demonstrated that an interphase cell (S phase) can be driven into mitosis if it is fused with a mitotic cell, irrespective of the fact whether it has replicated its DNA or not. Similarly a cell in G1 immediately enters S phase when fused with a cell in S phase. These experiments demonstrated that positive factors may drive entry into S and M phases (refer to Fig.12.2). Subsequently it was shown the soluble regulator of mitosis and meiosis is the same substance (maturation promoting factor = M-phase promoting factor).

2. The control system is regulated by brakes that can delay progression of the cell cycle at specific checkpoints, if cellular conditions are unfavorable or DNA is damaged / incompletely replicated. This helps to enhance the overall fidelity (accuracy) of the process. The major checkpoints in the cell cycle are:

   a. The G_1 checkpoint at G1/S transition. The replication of genetic material is one of the most crucial stage of the cell cycle and hence the major checkpoint in eukaryotic cells lies late in G_1 (G_1/S) and it controls the entry to the S phase. The cell integrates both external and internal signals before it takes a decision to divide.

   b. The G_2 checkpoint at G_2/M transition: The G_2/ M checkpoint also ensures all chromosomes have accurately replicated and DNA is free of damage.

   c. The M / spindle checkpoint is at the metaphase to anaphase transition. It is the key control point to monitor the alignment of all chromosomes at the metaphase plate, held by spindle MT from both poles. This is a prerequisite condition for an equal chromosomal distribution to daughter cells.

3. The tyrosine hydroxyl (tyr15) of cdc2 is phosphorylated in pre MPF. It is an inhibitory phosphorylation**.** Unless phosphate from tyrosine 15 is removed the activating influence of threonine phosphorylation will not be visible. The purpose of inhibitory and activating phosphorylation is to prevent premature activation of MPF. If a base substitution mutation changes tyr15 to phe, phosphorylation at this position is not possible. It will lead to an early activation of MPF and the cell will enter mitosis before the completion of preceding events.

4. a) **Cyclins:** Cyclins are proteins whose concentration varies periodically during the cell cycle. They are regulatory subunit of CDKs. These proteins form a complex with CDKs at specific times during the cell cycle, allowing further modifications of the kinase and their activation. In higher eukaryotes four major classes of cyclins have been characterized.

   b) **CDK:** The cyclin dependent kinases (CDKs) are a family of serine/threonine kinases. CDKs are relatively low molecular weight proteins (34-40 Kda). The functional activity of CDKs depends on a regulatory protein, cyclin. In higher eukaryotes, approximately 20 different Cdks and Cdk-related proteins are known. Among them only four classes of cyclins/CDKs are required for eukaryotic cell cycle.

   c) **CAK:** They are Cdk-activating kinases (CAK) that activate the Cdk/ cyclin complexes by phosphorylating specific threonine residue of the kinase. CAK is also a complex of a Cdk (Cdk7) and cyclin H and MAT1. It can activate CDK1, CDK2, CDK4 and CDK6. Along with other proteins CAK is also part of the general transcription factor TFIIH.

   d) **Securin:** Securin is a protein involved in control of the metaphase-anaphase transition and anaphase onset. It is initially present in the cytoplasm and binds to separase, a protease that degrades the cohesin rings, linking sister chromatids. At anaphase entry, APC degrades securin that relieves separase inhibition resulting in the cleavage of the cohesin subunits.

   e) CDK inhibitors: CDK inhibitors are responsible for delaying the cell cycle progression. They are low molecular weight proteins that are grouped into two families; the Ink4family (p15, p16, p18, p19) and the Cip/Kip family (p21, p27, p57). They all target specific Cyclin/CDKs complexes thereby affecting the normal cell cycle progression. Mammalian cells for instance restrain cells with damaged DNA from entering the S phase by accumulating the protein p53 that in turn triggers the synthesis of a Cdk inhibitor, p21.The inhibitor binds tightly to Cdk / cyclin D complexes.

5. Anaphase-promoting complex/ cyclosome (APC/C) is a large multi protein complex of 11–13 subunit proteins. Functionally it is an **E3 ubiquitin ligase** that attaches a polyubiquitin tag on mitotic cyclins. The latter are targeted for degradation into peptides by a large multiprotein complex called proteasome (26S).

6. Maturation-promoting factor (MPF) is a dimer of cyclinB-Cdk1 complex. MPF must be activated for the cell to enter M phase. The association of the cyclin subunit unit with the kinase makes it accessible to enzymes that modify it by phosphorylation. Some of the phosphorylation is activating (Thr-161) while others are inhibitory (Thr-14 and Tyr-15). The inhibitory phosphate prevents premature activation of MPF and is removed in late G2. Active MPF phosphorylate specific serine and threonine residues of target proteins.

The roles of MPF include:

- It triggers the formation of mitotic spindle by phosphorylating MAPs that change the behaviour of microtubules.
- It promotes chromatin condensation by phosphorylating condensins.
- It phosphorylates nuclear lamins (A, B and C that leads to depolymerisation of the nuclear lamina and breakdown of nuclear envelope into small vesicles.
- It is responsible for phosphorylation of GM130, which leads to the fragmentation of Golgi body and ER.

7. In eukaryotes, multiple cyclins participate during the cell cycle. Each of these cyclins peak at different time periods and are relevant as regulatory partners of specific cell division kinases (CDKs) for that part of the cell cycle. Both cyclins and CDK must be present at higher concentrations for cells to divide. The rise and fall in the levels of cyclins allows one complex to complete its job and for the other to take over. Unlike cyclin, the level of CDKs remains relatively constant. The activation and inactivation of cell cycle kinases depends on the concentration of cyclins which have direct correlation with the checkpoints of the cell cycle. Thus, cyclins and Cdks are master components of the control system that ensure and promote the cell cycle progression to proceed in correct order. The profile of different cyclins constitutes the expression cycle (Fig.12.11b).

12.7 SUGGESTED READINGS

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4. Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell. (2016). Molecular Cell Biology, VIII Edition, W. H. Freeman and Company; New York: ISBN-10: 1-4641-8339-2.
5. Gerald Karp. Cell and Molecular Biology Concepts and Experiments, 6th Edition, John Wiley & Sons, Inc.

UNIT 13

CELL DEATH AND CELL RENEWAL

Structure

13.1 INTRODUCTION

You have studied in units 11 and 12 about cell division in prokaryotes and eukaryotes and the factors that determine and control cell cycle progression in eukaryotes. It is a feature of normal cells to undergo senescence after dividing for a limited number of generations. Cell death and renewal are stringently balanced in multicellular organisms in order maintain cell number in tissues. The cells of the immune system are also screened for potential auto reactivity.

This unit deals with cell death and cell renewal. The cellular strategies for detection, molecular components of signaling cascade that activate the

apoptotic program and necrosis will be dealt in detail. You will also learn about cell proliferation/renewal; cell transformation and an indispensable technique used to sort cells – FACS (Fluorescence activated cell sorters).

Expected Learning Outcomes

After studying this unit, you should be able to:

- define the relevance of cell death and renewal;
- indicate the key players in apoptosis;
- explain the apoptotic pathways and cellular changes;
- describe necrosis;
- differentiate between apoptosis and necrosis;
- highlight the changes that accompany cell transformation and;
- describe the principle and working of FACS and its applications.

13.2 PROGRAMMED CELL DEATH (PCD)

Cell death is an essential part of normal development and continues through adulthood. Investigators engaged in studying embryonic development were the first to realise that cell death is not always bad for the body; in most cases it is necessary. The term programmed cell death (PCD) was introduced in 1964 by **Lockshin and Williams**, to emphasise that cell death during development is not an accidental outcome. In reality cell death is at least 20 times faster than mitosis.

Our present understanding of apoptosis has developed over the last 50 years although its role in embryonic development was recognised much earlier. It was not until the 1972 paper of **John F. R. Kerr, Andrew H. Wyllie, and Alastair R. Currie** the importance of apoptosis during the life time of an organism became acceptable. They introduced the term **apoptosis** (Greek, meaning **"falling off"**) in their paper to describe this morphologically distinct form of cell death.

These investigators also proposed that inappropriate initiation or inhibition can contribute to the onset of many diseases. It is now known that aberrant regulation of PCD or cell proliferation is a factor in many diseases including neurodegenerative diseases, autoimmune disorders and cancer. The cells that commit suicide are sacrificing themselves for the greater good of the organism and it is not a failure of homeostasis.

There is increasing evidence in support of other forms of PCD such as necroptosis. The molecular pathways and signals that activate one in favor of the other are only beginning to be understood and are they mutually exclusive routes also need to be clearly deciphered.

13.3 APOPTOSIS

Apoptosis is a major form of PCD that is best understood in molecular terms. It is an ongoing process by which damaged, transformed, infected and unnecessary / aged cells are removed. It is dependent on genetically encoded signals or activities within the cell and therefore called PCD.

The designation “programmed” refers to the fixed intracellular death pathway followed by dying cells regardless of the initial trigger or whether the characteristic features of apoptosis accompany the process. There are many documented examples of physiological apoptosis in all multicellular organisms. During metamorphosis a tadpole loses its tail before becoming a frog; the immune and the nervous system in vertebrates develops through an initial overproduction of cells followed by erasing them in large numbers as these systems take final shape and activated lymphocytes die at the end of an immune response. Certain cells that undergo apoptosis tend to persist for variable periods, for instance, lens of the eye. They replace their cell contents with crystallin as they die.

The basic understanding of mammalian apoptotic mechanism is drawn from the molecular pathway of PCD dissected in *Caenorhabditis elegans* (Horvitz, 1999). Out of 1090 somatic cells in the adult worm, 131 cells undergo apoptosis at specific times during development. It has served as a useful model system for studying the core components of apoptotic death machinery.

The beauty of the system lies in its simplicity. Only three gene products (*C.elegans* death or CED 3, 4 and 9) are essential for apoptosis. The caspase enzyme is encoded by CED-3 and its activation is under control. Normally the adaptor protein, CED-4 can bind simultaneously to both CED-9 and CED-3. The binding of CED-9 (anti apoptotic) is essential for cell survival as it prevents CED-4 from activating CED-3.

Apoptotic signals cause the dissociation of CED-9 and allow CED-3 activation. The CED3-CED4 complex constitutes the **worm apoptosome**. It exists as a zymogen and is activated by self cleavage. Subsequently a proapoptotic member, EGL-1 protein of the Bcl-2 family was added to this list. The basic mechanism worked out in worms is evolutionarily conserved and mammalian homologues for all four proteins are known.

13.3.1 Morphological Changes

A number of morphological changes during apoptosis can be observed under the electron microscope. The cell shrinks and moves away from its neighbours; condensed chromatin appears as distinct blobs near the nuclear membrane, cytoplasm shrinks, pseudopodia retract and plasma membrane (PM) ‘blebs’ (Fig. 13.1). These blebs keep forming and disappearing. Initially no detectable changes occur in mitochondria, ER or the Golgi body. During apoptosis a number of **apoptotic bodies** are generally formed by budding that are packed with organelles with or without nuclear fragments.

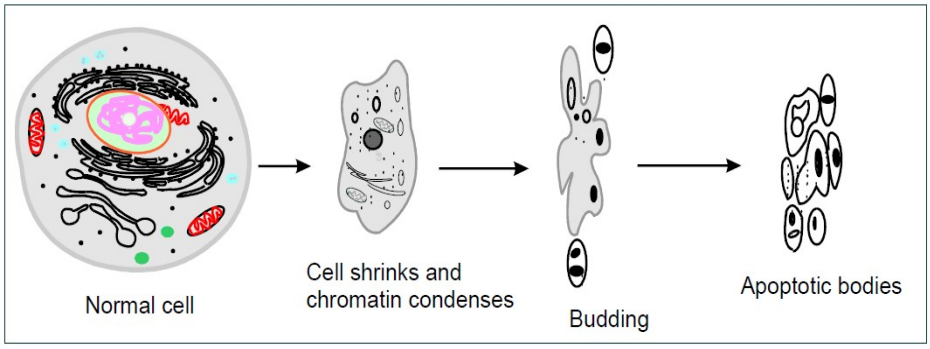

Fig. 13.1: Morphological changes during apoptosis.

13.3.2 Biochemical Changes

The most dramatic molecular change induced during apoptosis is inter-nucleosomal DNA cleavage that produces a ladder like pattern on gels. The activated caspases relieve the inhibition of DNA cleaving enzymes. The cross-linking of proteins by transglutaminase is another feature of apoptotic cells.

The release of chemoattractants and randomization of phosphatidyl serine (PS) between the external and internal leaflets of the plasma membrane (PM) facilitates their detection and clearance by phagocytes. The clearance of apoptotic bodies is highly efficient and does not incite an inflammatory response. It is an energy dependent quite process of removing dyeing cells.

13.3.3 Major Players in Apoptosis

In metazoans apoptotic cell death program are initiated by signals originating either within the cell or from outside. Irrespective of initial trigger all signaling pathways converge to common evolutionarily conserved death effecter machinery. The major players fall into four broad classes that are discussed below.

(a) **Caspases** are cysteine proteases that are synthesised as zymogens and upon activation they specifically and efficiently cleave on the carboxyl side of a specific aspartic acid residue. They are generally classified into initiator and effector caspases. The former promotes apoptosis by cleaving and activating other caspases and the latter is responsible for the cellular changes associated with apoptosis. Some initiator caspases like caspase 8 have a death effecter domain (DED) while others like caspase 9 have a caspase recruitment domain (CARD) that allows them to have homotypic interactions with adaptors with similar domains.

(b) **Adaptor proteins** as the name suggest link the caspases with the upstream regulators of apoptosis. FADD (Fas associating death domain) is an adaptor protein that links the receptor with caspase.

(c)**Tumor necrosis factor (TNF) receptor** superfamily members bring about multiple effects that range from cell proliferation to cell survival and even cell death. The ligands for this receptor family are structurally related membrane bound proteins that are generally active as trimers. Some members such as CD95 (Fas) have a cytoplasmic death domain (DD) enabling them to interact

with adaptor proteins and induce the apoptotic program. Therefore, these receptors are also called **death receptors.**

(d) **Bcl-2 family** members can either inhibit or induce apoptosis and they are related to the CED-9 protein of *C.elegans*. Bcl-2 was its first identified member in a B-cell lymphoma, promoting cell survival. The proteins have one or more regions having sequence similarity with Bcl-2 (BH homology). Many members have a conserved C-terminal transmembrane domain that localises them to different membranes.

13.4 PATHWAYS OF APOPTOSIS

Next, we will focus on the extrinsic and intrinsic pathways of apoptosis. As the name suggests the two pathways differ in the source of signals for inducing PCD. The extrinsic pathway is initiated by extracellular stimuli like Fas ligand (FasL) while the stimulus for intrinsic pathway is generated within the cell such as oxidative stress. The subsections below describe the two signaling cascades by taking specific examples. The discussion ends with a brief account of regulators of apoptosis. You will note that the multi step activation cascade leads to an enormous amplification of the initial signal.

13.4.1 Extrinsic Pathway (Death Receptor Pathway)

The extrinsic pathway of apoptosis is induced by ligation of transmembrane death receptors (DD) with extracellular ligands such as FasL (Fig.13.2).

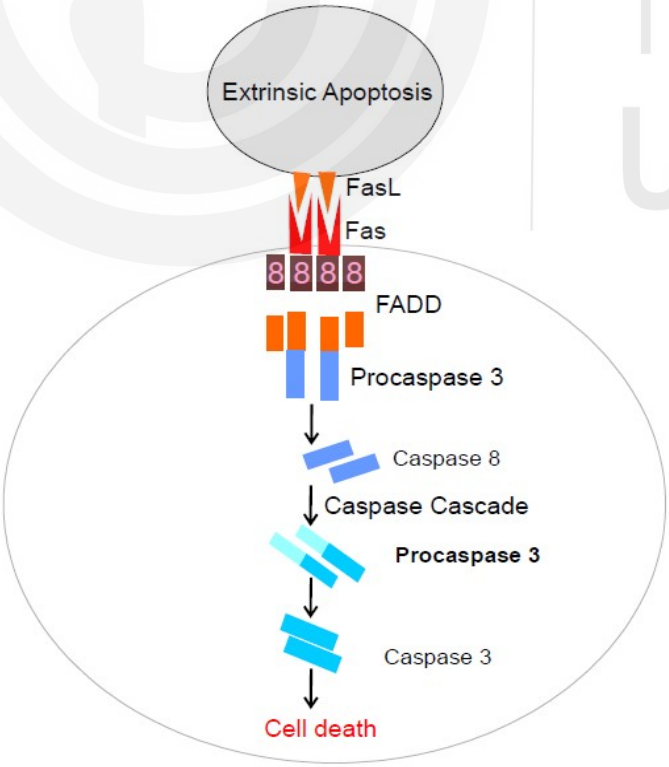

Fig.13.2: The extrinsic pathway. (Credit: https://commons.wikimedia.org/wiki/File.Extrinsic_apoptosis)

The first step in Fas (CD95) signaling is binding of homotrimeric FasL resulting in trimerisation of the receptor. The clustered death domains (DD) of Fas then bind to DD of the adaptor protein, FADD. The recruitment of the adaptor also facilitates linking to initiator procaspase-8 through a death effector domain (DED) present in both in FADD and initiator caspases. The net result in an increase in the local concentration of procaspases and at this point they undergo self cleavage. The activated enzyme is released from the receptor complex and they are ready to cleave effector / executioner caspases (caspase-3,-6 or -7). The effector caspases act by cleaving a variety of proteins that are required to maintain cellular integrity and activate enzymes that promote cell death. Some of their well characterised targets are (i) an inhibitor of caspase activated DNase (ICAD), (ii) Bcl-2 proteins and (iii) Lamins.

13.4.2 Intrinsic (Mitochondrial) Pathway

The central role of mitochondria in the activation of the intrinsic pathway of apoptosis has been established beyond doubt. In general, a cell under stress due to hypoxia; DNA damage; accumulation of ROS or in the absence of extracellular survival signals is induced to commit suicide.

The most important step is the release of cytochrome c from mitochondria which ultimately activates caspases (Fig. 13.3). The members of the Bcl-2 family have a dominant role in controlling the release of cytochrome c. The proapoptotic factors (Bax, Bok) bind to the mitochondrial membrane that directly causes the release cytochrome c from the mitochondria.

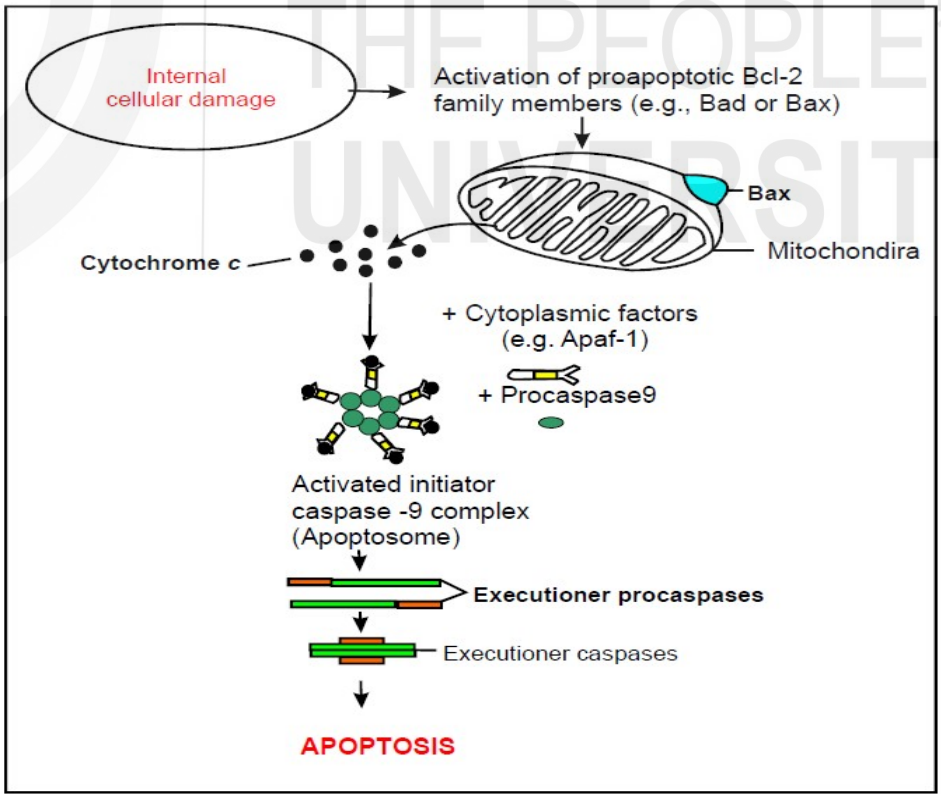

Fig. 13.3: The intrinsic pathway. *(Source*: Gerald Karp: Cell and Molecular Biology: Concepts and Experiments)

Bax forms heterodimers with Bcl-2 and functions as an apoptotic activator. The mitochondrial membrane swells and becomes more porous. In the cytoplasm it binds an apoptotic protease activating factor (Apaf-1), stimulating its oligomerisation. The oligomer in turn recruits the initiator procaspase-9 via its caspase recruitment domain (CARD) forming the vertebrate mitochondrial **apoptosome.** The procaspase-9 undergoes self cleavage to form activated caspase-9. It is now freed to activate effector caspases-3, 6, or 7.

The effector caspases of both pathways converge to bring about stereotyped morphological and biochemical changes. There is evidence of **crosstalk** between both pathways. For instance the activation of Fas pathway (death receptor pathway) can also result in mitochondrial damage by caspase-8 mediated cleavage of proapoptotic protein, Bid. The active Bid fragment (tBid) **sequesters** Bax.

It oligomerises and increases mitochondrial membrane permeability facilitating the release of apoptogenic proteins like cytochrome c, engaging the intrinsic pathway.

13.4.3 Regulators of Apoptosis

A genetic program as powerful as apoptosis needs to be stringently controlled for survival of a multicellular organism. As expected there is more than one way to regulate both pathways.

You have already learned that levels of pro- and anti-apoptotic proteins are important in deciding the fate of cell. One of the earliest identified anti apoptotic protein was **Bcl-2 (B-cell lymphoma 2)** in a human lymphoma. Since then many more proteins have been added to this family. These mediators function by physical interaction. The anti apoptotic members hold the proapoptotic members from signaling to the downstream proapoptotic members.

The p53 protein regulates both cell cycle progression and programmed cell death. In normal cells its levels are kept low and it dramatically rises in response to DNA damage. It is a transcription factor that is required to transcribe many genes including cyclin dependent kinase (Cdk2) inhibitor, p21 and for inducing apoptosis, if damage is irreparable. The inhibitor delays cell cycle progression, allowing time for DNA repair. The regulation of caspases is equally important in maintaining homeostasis. One of the simplest ways is by maintaining them as inactive precursors that can be activated in response to apoptotic stimuli. The next level of regulation is by inhibiting active caspases. These proteins belong to different protein families that include **Inhibitor of apoptosis proteins (**IAP) family and viral encoded family of proteins.

The IAPs are endogenous inhibitors of apoptosis that regulate cellular apoptosis by direct caspase inhibition. Interestingly they block substrate access without docking onto the substrate binding sites of the enzyme. All IAPs have one or more BIR (baculovirus IAP repeat) domain. An example of an IAP is the human **XIAP** (X-linked Inhibitor of apoptosis) that inhibits caspases 3, 7 and 9.

The other category of caspase inhibitors are **virus encoded proteins**. Two well known examples are the cowpox virus **CrmA** (cytokine response modifier A) and baculovirus**p35** protein. CrmA can inhibit both serine and cysteine proteases. The p35 inhibition results from mechanism based irreversible inactivation of the enzyme and they have broad specificity.

Other inhibitors like c-Flip bind to the death domain of DRs and procaspase-8 to inhibit its activation and induction of apoptosis.

SAQ 1

a. Match the items in Column I with Column II:

Column I	**Column II**
1) TNF-α	a) Apoptosis
2) Bcl-2	b) Caspase-8, -9
3) Intrinsic Pathway	c) anti-apoptotic protein
4) Programmed cell death	d) a protein with DD and DED domain
5) XIAP	e) Caspase-3, -7
6) Initiator caspases	f) Cytokine
7) Executioner caspases	g) Cytochrome c
8) FADD	h) Caspase inhibitor

b. Fill in the blanks:

i. The death receptor pathway is also known as ……

ii. The molecular components of apoptotic pathway were first described in ……

iii. The binding of FasL induces ……..of Fas receptors.

iv. FADD and TRADD are associated with …… pathway of apoptosis.

v. …… is a complex of Apaf-1, procaspase-9 and cytochrome c.

vi. ……..is an example of tumor suppressor gene.

c. Answer briefly the following questions.

1. What are proapoptotic and anti-apoptotic factors? Give one example of each.
2. Highlight the significance of apoptosis by taking any two examples.

13.5 NECROSIS

Necrosis (Greek “nekros,” for corpse) is less orderly process of cell death so it is more appropriately called an accidental cell death. Necrotic death occurs when a cell is severely damaged due to physical injury, inadequate blood supply (ischemia), oxygen deprivation, and in granulomas.

The morphological changes that accompany necrosis are swelling of the cell and organelles; organelles become dysfunctional, cell membrane disintegrates and ruptures. These effects are due to uncontrolled entry of fluid into the cell. In contrast to apoptosis, there is no inter nucleosomal DNA cleavage, however there may be random DNA degradation in later stages. Cell death is caspase independent. The release of cell contents invariably invokes an **inflammatory response** as they are sensed by circulating white cells (Fig 13.4). Such a response is like a double edged sword that on one hand helps to limit the spread of infection and clear debris while on the other hand the pro inflammatory mediators released by white cells can damage close by normal tissue. At times excessive damage to normal tissue may account for the pathological symptoms.

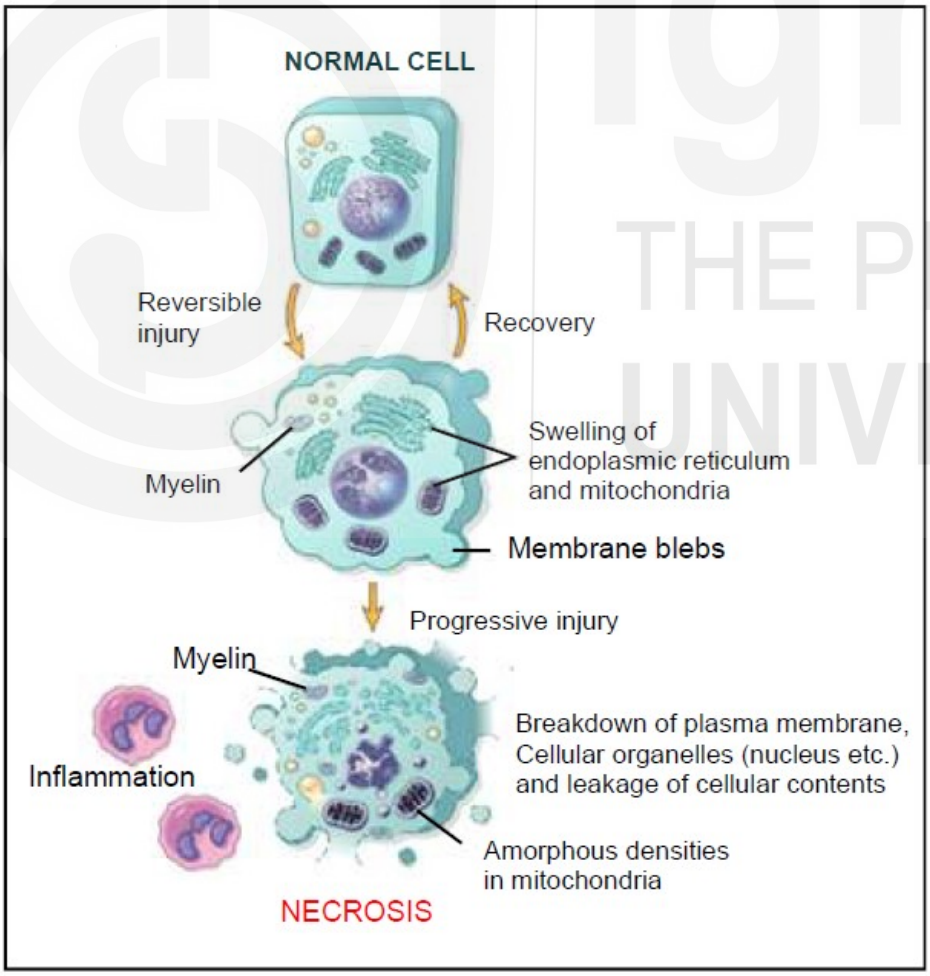

Fig. 13.4: Stages in necrotic cell death. (Courtesy: Suanders-Elsevier, Inc. 2010)

13.5.1 Necroptosis

In contrast to regulated and active apoptotic cell death, necrosis has long been viewed as passive unregulated process triggered by excessive stress. Recent studies have suggested this may not always be true. Some cells undergoing necrosis have a form of PCD which comes in different flavors. One of them described here is called **necroptosis.** It is dependent on cellular signaling pathways that initiate necrosis in response to specific stimulus rather than 'by accident'. **Programmed necrosis** is a type of cell death which is characterized by necrosis like features such as swelling of organelles, an increase in cell volume, cell lysis and disintegration of the cell membrane. It is a form of inflammatory cell death. It can be activated by ligands of death receptors (TNF) but like necrosis it is caspase independent. This form of cell death is mainly observed under conditions when caspases fail to be activated. In fact, the initiation of apoptosis may actively suppress necrosis as activated caspases cleave and inactivate proteins required for programmed necrosis. In the preceding sections you have learnt about the changes that accompany PCD and necrosis. Before proceeding further we will summarise the characteristics of both forms of cell death in table 13.1.

Table 13.1: Characteristics of apoptosis and necrosis

Characteristic	Apoptosis	Necrosis
Role	To maintain Homeostasis / embryonic development	Impairs homeostasis
Induction	Natural / internal mostly	Injury / infection
Type	Physiological /Pathological	Pathological process
DNA fragmentation	Inter nucleosomal cleavage (DNA Ladder on gels)	Random degradation
Nucleus	Chromatin condensation and margination	Moderate chromatin condensation.
Membrane Integrity	Maintained / membrane blebbing seen	Compromised early / become leaky
Mitochondria	May appear normal or mildly swollen.	Swollen
Inflammation	**No**	**Yes**
Pattern	Individual cell are induced to commit suicide.	A group of cells are affected.
Cell Volume	Cell shrinks	Cells swell including organelles
Cell Fragmentation	Yes; apoptotic bodies formed that are phagocytosed and quietly cleared.	No; cell lysis occurs and debris is cleared by phagocytes.
Energy dependence	Yes; active process	No; passive process

SAQ 2

a) State whether the following statements are true (T) or false (F):

i. Necrosis is an example of PCD. ☐

ii. Necrosis is usually a caspase independent process. ☐

iii. Random degradation of DNA occurs in apoptosis. ☐

iv. Inflammation is a hallmark of necrotic death. ☐

v. Pyknotic nucleus appears in necrotic cell death. ☐

b) What is necroptosis? In what way it is different from necrosis?

13.6 CELL PROLIFERATION/RENEWAL

You are already familiar with cell division and cell cycle control from the preceding units of this block. Now let us discuss about cell renewal. Cell renewal is interplay of proliferation, maintenance, pleuripotency and regenerative ability. During cell renewal some cells remain undifferentiated (stem cells) whereas others differentiate.

Stem cells (SC) are unspecialized cells capable of self renewing themselves and to develop into more specialised cells (differentiation). They are classified into embryonic stem (ES) cells, tissue specific or adult stem cells and induced pluripotent stem (iPS) cells. They differ in their stemness. The ES cells are obtained from inner cell mass (ICM) of the blastocyst. They are pluripotent and are capable of producing every cell type in the body except placenta and umbilical cord. The adult stem cells on the other hand are more specialised than ES cells. They are found in adult tissues and function as a repair system by maintaining normal turnover of regenerative tissues such as blood, skin and internal epithelial linings. These cells are multipotent and lineage restricted. Finally the iPS cells have been engineered in the laboratory by converting tissue specific cells into cells that behave like ES cells.

Stem cells have long term self renewal capability that makes them different from progenitor cells having limited self renewing ability. In addition progenitor cells are lineage restricted. The vast majority of differentiated cells in adults are not capable of proliferation. Whenever there is injury to these cells, they are replaced by the proliferation of less differentiated cells derived from self-renewing stem cells. In most tissues SCs self-renewal is coupled with regeneration of tissues. Some differentiated cells such as skin fibroblasts and epithelial cell of liver and pancreas can exit the G_0 phase to resume proliferation as and when the need arises.

There are variations in frequency of stem cell self-renewal. The small intestinal lining undergoes rapid turnover. To sustain this rate, mouse intestinal stem cells (ISCs) undergo nearly continuous self-renewal and divide at least

once a day. On the other hand mouse hematopoietic stem cells (HSC) responsible for producing nearly a billion circulating blood cells every day divides about once a month during normal homeostasis. This variation is essential for homeostasis as reduction in their numbers can result in tissue atrophy or premature ageing and if they divide rapidly without a proper differentiation balance it can lead to abnormal development and even cancer.

Two models have been put forward to explain stem cell renewal. In the asymmetric model mitosis will result in polarization and unequal segregation of cellular content **(Fig. 13.5a**) whereas in a symmetrical division, there is an equal distribution of all cell components to the daughter cells (**Fig. 13.5b**).

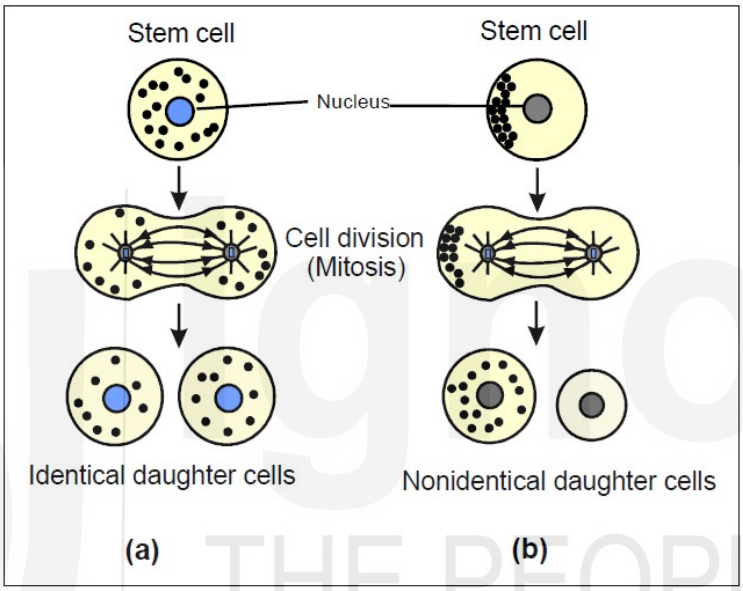

Fig. 13.5: Models of stem cell renewal: a) Symmetric model and b) Asymmetric model.

The ability of SCs to repair damaged tissue is indicative of their enormous potential in clinical medicine. They can be used to replace or regenerate tissues lost due to injury, burns and for the treatment of diseases like diabetes, heart disease, Parkinson's or Alzheimer's disease. In some cases, use of stem cells derived from adult tissues may be the optimal approach for such stem cell therapies, although embryonic stem cells is likely to provide a more versatile approach. One of the well established clinical application of adult stem cells is bone marrow transplantation (BMT) for treatment of a variety of cancers, thalassemia and some immunodeficiency conditions. The HSC therapy gained prominence after the pioneering research work of McCulloch and Till in the 1960s. They showed that these rare cells can be coaxed to give rise to all kinds of blood cells. Stem cells are now routinely grown and made to differentiate into specialised cells in culture for use in medical therapies.

13.6.1 Balance between Cell Death and Renewal

Two predominant physiological processes that control tissue homeostasis in an adult organism are cell division and cell death. Self-renewal is dependent

on proliferation along with the maintenance of both multi-/pleuripotency and tissue regenerative potential. In a fully formed organism cell death and renewal are balanced by strategically maintained stem cells populations in tissues that routinely replace cells lost by various injuries, aging or other abnormalities. Similarly the development of an organism from a fertilized egg happens by interplay of proliferation, differentiation and programmed cell death.

SAQ 3

a.

i) What is a stem cell?

ii) What is the difference between stem and progenitor cells?

b. Fill in the blanks:

i) ……..… and …… are two models of stem cell renewal.

ii) Stem cells can be used for treatment of……

iii) Stem cell self-renewal is coupled with …… of tissues.

iv) The two physiological processes that control tissue homeostasis are …… and ……..

v) A reduction in stem cell proliferation can lead to ……

13.7 SALIENT FEATURES OF TRANSFORMED CELL

In order to study any cell type a constant supply of such cells is required. As normal cells in culture do not survive long they are generally transformed into immortal cell lines (Fig.13.6).

Types of Cell Lines and transformed cells

A Cell Line or cell strain may be finite or continuous depending upon whether it has a limited life span or is immortal in culture.

Finite Cell Lines have limited life span and go through limited number of cell generations (usually 20-80). These cell lines exhibit the property of contact inhibition, density limitation and anchorage dependence. They grow as a monolayer in culture. Their growth rate is slow with doubling time around 24-96 hours. The cells derived from primary cultures of normal cells are finite cell lines.

Continuous Cell Lines are cell lines transformed under *in vitro* culture conditions and they can divide indefinitely. The Cos-1 cell line is derived from African green monkey kidney cells transformed by SV40.They show absence

of contact inhibition and anchorage dependence and can grow either in monolayer or suspension form. The growth rate is rapid and doubling time is 12-24 hours.

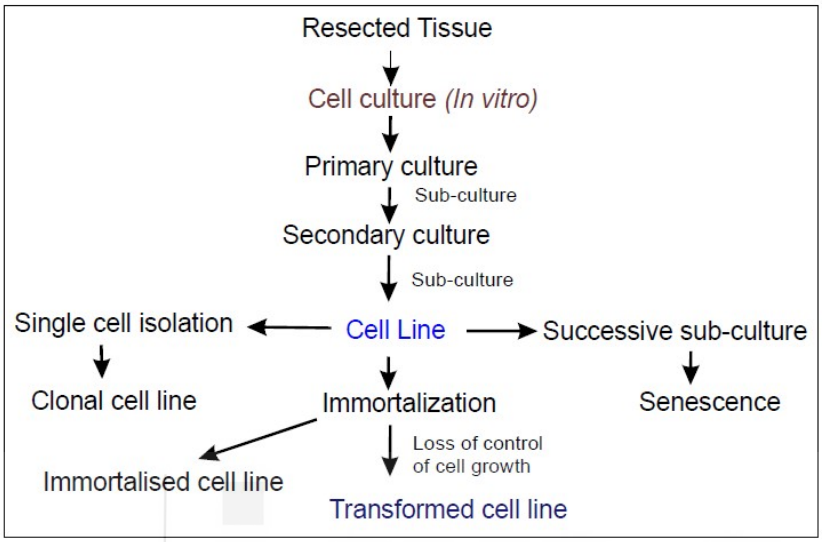

Fig. 13.6: A generalized scheme for cell line development.

In prokaryotes the term **transformation** refers to a heritable change due to uptake of naked DNA followed by recombination. In eukaryotes this is called transfection. Transformation in eukaryotes is reserved to describe DNA alterations that result in a tumor cell phenotype. The features of transformed cells include:

- Loss of contact inhibition.
- Many of them proliferate in the absence growth factors.
- Immortality.
- Do not depend on anchorage for growth.
- Abnormal nuclear morphology.
- Variations in both chromosome number (aneuploids or polyploids) and structure (translocations, inversions). They accumulate mutations over time.
- They are very efficient in the uptake of glucose.
- Some express proteins that reflect their origin.
- When injected into a suitable host they can form tumors.
- Many of them secrete proteases such as plasminogen activator.

The transformation of cells depends on changes in their genetic makeup and / epigenome. In most cases it involves multiple mutations in key genes controlling cell cycle progression. Some of them can occur spontaneously but more often they are induced by mutagens. They can be physical (ionizing and non ionizing radiations; prolonged irritation and burns), chemical (EMS, N-mustard) or biological agents (DNA and retroviruses), although all mutations do not lead to cell transformation. Therefore those that cause transformations

are called **carcinogens.** We all have genes that if mutated can cause transformation either due to over expression (cellular protooncogenes become oncogenes) or the loss of function of tumor suppressor genes such as p53 or retinoblastoma (Rb) to suppress cell cycle progression. The changes in the epigenome are not due to mutations in DNA sequence but due to modifications in DNA that in turn affects the expression of genes.

Some of the commonly used cell lines are HeLa cell line from human cervical carcinoma, CHO from Chinese hamster ovary and Cos-1 cell line from monkey kidney. They have been extremely useful in vaccine development, virus cultivation, immunological studies and basic research in cell and molecular biology

SAQ 4

a. Name any two agents that can cause cell transformation.

b. Indicate whether the following statements are true or false:

i) Transformed cells generally divide more rapidly than normal cells. ☐

ii) Normal cells do not have contact inhibition. ☐

iii) Finite cells lines have unlimited life span. ☐

iv) Cell transformation may lead to cancer. ☐

13.8 FLUORESCENCE ACTIVATED CELL SORTING (FACS)

The Leonard Arthur Herzenberg developed a cell sorter that did fluorescence based cell sorting**.** His group coined the term FACS for this innovation in 1972. It is a specialized type of flow cytometry. FACS is one of most widely used technique to sort and count the number of cells in suspension.

13.8.1 Instrumentation

Fluorescence activated cell sorters are flow cytometers that can sort in a short period of time fluorescently labeled cells from mixed cell population into two or more containers based on specific fluorescent characteristics of each cell. The essential components of all flow cytometers are fluidics, optics (excitation and collection), an electronic network (detectors) and a computer (Fig.13.7).

The **fluidics** is responsible for directing the liquid containing particles to the focused light source. It includes the sheath fluid (generally PBS) and pressurized lines. Cells are passed precisely through the illumination region using hydrodynamic focusing. The injection rate of cells into the laser beam can be varied depending on the nature of analysis.

Leonard Arthur Herzenberg (1931-2013)

Herzenberg was the professor of Genetics at Stanford University School of Medicine. He was a member of the Stanford community for more than 50 years. For his role in the development of the first FACS he was honoured with the Kyoto Prize in 2006 (Japan's equivalent of the Nobel Prize). Earlier in 2004 he received the special Novartis prize in immunology for making fluorescent labelled antibodies for tagging cells for FACS or other studies. The motivation for developing a cell sorter was to have a system for counting immunofluorescent cells with ease as his eyes get tired of counting cells under the microscope.

The **excitation optics** consists of a laser and lenses to focus light source onto the cells while the collection optics transmits scattered or fluorescent light of the particle to an electronic network. An optical bench holds excitation and collection optics in fixed position. There is a variety of laser configurations in flow cytometers based on the fluorochromes to be excited. The **collection optics** includes a collection of the lens to collect emitted light and a set of optical mirrors and filters that sort and direct specified wavelengths of light to optical detectors.

The optical signals generated are converted to proportionate voltages by photodetectors. They are generally photodiodes or photomultiplier tubes (PMT) and their use depends on the sensitivity required. Certain parameters like voltage pulse height, area and width are analysed. Then analog signals are amplified and converted into digital signals by an analog to digital convertor for computer processing. The digital data can be displayed as plots or histograms.

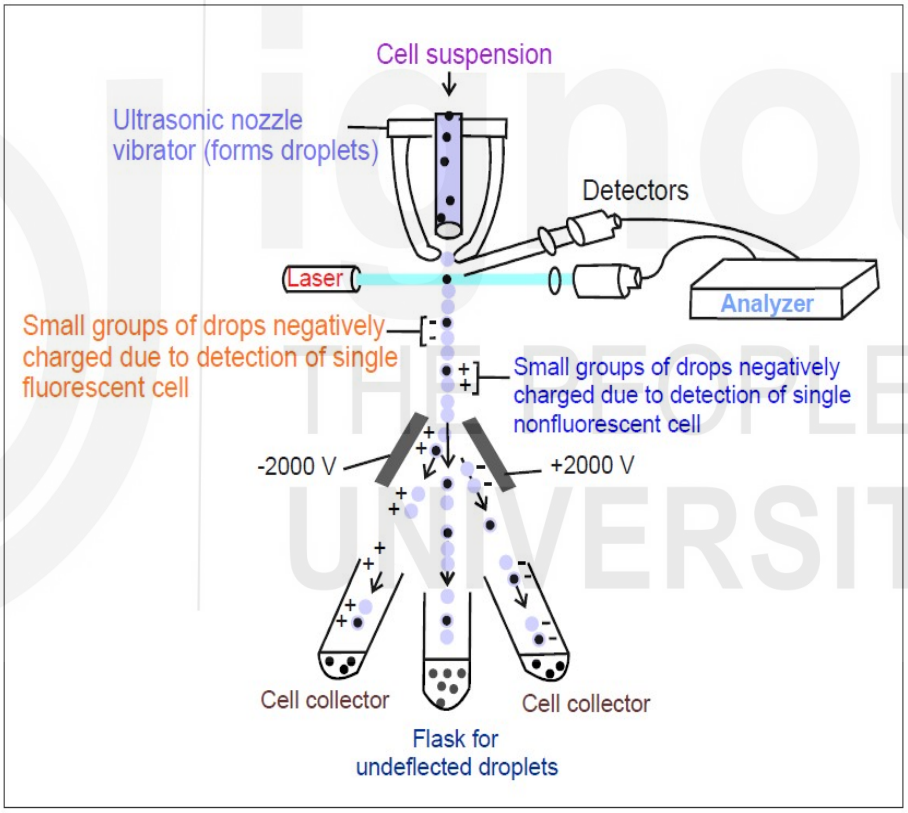

Fig. 13.7: FACS Unit Scheme.

13.8.2 Fluorochromes in Flow Cytometry

In Block 1you have studied the principle of fluorescence and about some commonly used fluorochromes and their properties. As the number of intrinsically fluorescent compounds in a cell is limited they are stained with fluorescent probes to make them visible. In flow cytometry fluorochromes are used to label proteins (generally antibodies), nucleic acids and reporter molecules. The most commonly used fluorochrome for antibodies are FITC and phycobiliproteins. Many dyes can stain nucleic acids such as propidium

iodide (PI); 7-AAD and DAP1. The green fluorescent protein is a commonly used reporter molecule.

13.8.3 Methodology

(a) The fluorochrome conjugated cells suspended in a buffer are counted as they pass through the laser beam. The cell suspension is diluted appropriately and its flow rate is carefully controlled to ensure that one cell passes through the flow cell at any given time. Light detectors positioned around the flow cell can detect any perturbation to the laser beam as each cell passes through.

(b) The path of light through a cell depends on the size and internal composition of the cell. As light interacts with the cell and its internal components, it deviates from its initial direction (scattering).

(c) A flow cytometer is equipped with number of different detectors which measure changes in the beam of light as it passes through the cell. Different characteristics of a cell can be analyzed based on light scattering (Fig.13.8):

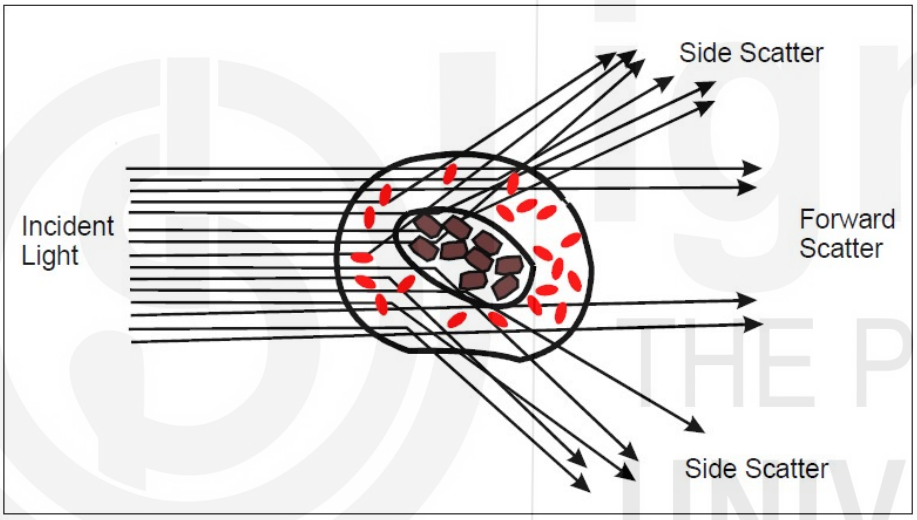

Fig. 13.8: Light scattering – FSC and SSC.

i) **Forward scatter (FSC**) is detected as light which passes relatively straight through the cell. The degree of forward scatter is proportional to the cell surface area or size.

ii) **Side scatter (SSC**) is detected as light which deviates significantly from the straight passage through the cell. It is a measurement of mostly refracted and reflected light which is collected 90^{o} to the laser beam. SSC light is proportional to the degree of internal complexity and granularity of the cell.

iii) By using their FSC / SSC characteristics, a population of cells can be divided into sub populations.

iv) Data analysis is the most important parameter for biological experiments. What is needed is to selectively show or 'gate' cells of interest. The parameters set up will help in excluding unwanted results like dead cells or cellular debris.

13.8.3.1 Data Display

The signals produced by fluorochromes are collected by detectors. The output of the detectors can be displayed in a number of different ways:

(a) **Single Channel**: It means when we are using a single fluorochrome or antibody tagged with fluorochrome and a single filter a population of cells may appear as peaks. Fig.13.9 depicts a typical single channel detection results using fluorescently labeled (propidium iodide) DNA.

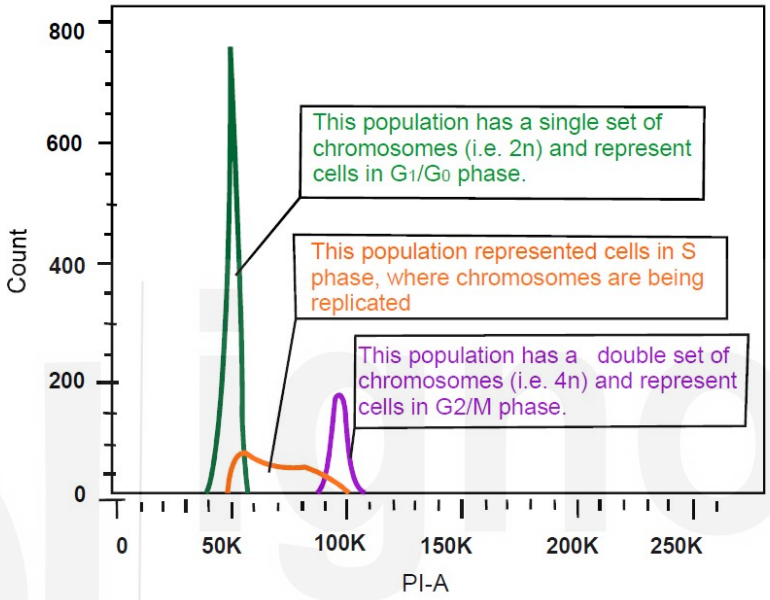

Fig. 13.9: Single channel detection result.

(b) **Dual Channel**: It is a plot of output signals from one detector with filter for a single wavelength, against that of a second detector with filter for a different wavelength on the same graphical representation. It means we are using two different fluorochromes of different wavelength to study the same sample. In Fig. 13.10 a population of immune cells has been labeled with antibodies against the two different membrane proteins, CD3 (conjugated to FITC, 488 Blue/519 Green) and CD19 (conjugated to Phycoerythrin 488 Blue/570 Yellow). If we consider two different fluorochrome as A and B then there will be four quadrants in the graph representing those that are A^+B^+, A^+B^-, A^-B^+ and A^-B^-.

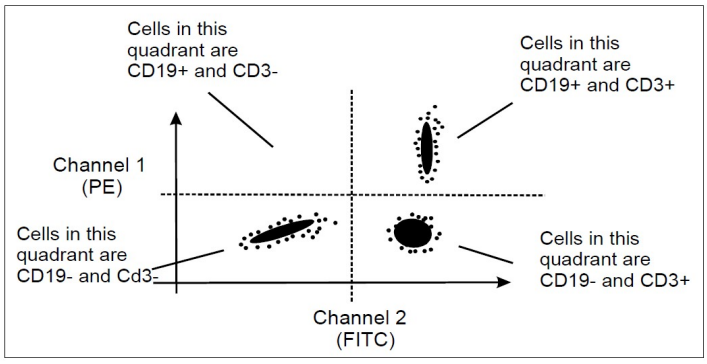

Fig. 13.10: Dual channel detection result.

13.8.4 Applications

- **Quantitative assessment of apoptotic cells:** The cells undergoing apoptosis can be assessed rapidly by detecting specific apoptotic markers like plasma membrane changes (externalized PS with fluorescently labeled Annexin-V and 7-aminoactinomycinD); DNA fragmentation with propidium iodide fluorescence; and active effector caspase-3 (with active caspase-3 specific labeled monoclonal antibody).
- **Detection of intracellular proteins:** The cells are fixed and permeabilised before addition of fluorescent–conjugated antibody against an intracellular protein like cytokines. The treated cells are then analyzed by flow cytometry.
- **Phenotypic characterization of cells:** It is possible to identify and determine quantitatively the subsets of cells in a mixed population**.** The phenotypic characterization **of** blood cells is one such example for which antibodies against specific cell surface markers are generally used in combination to label differentially specific subsets of blood cells.
- **Tool for studying and diagnosing diseases:** It is possible to diagnose and know the progression of a disease/ response to medication by using fluorescently labeled antibodies against proteins unique to a pathogen or self proteins expressed only in specific diseases.
- This technique has many applications in medicine especially in transplantation, hematology, tumor immunology and chemotherapy, prenatal diagnosis, genetics and sperm sorting for sex pre selection.

Recent advancements in flow cytometry technology include the discovery of better fluorochromes such as Quantum dots (fluorescent nanocrystals) and the development of spectral and microfluidic flow cytometers with improved resolution. These instruments are also small sized (portable) and cost effective. They require very small volumes to detect especially cells that are present in low numbers.

SAQ 5

Fill in the blanks.

i) …….. is a widely used technique for counting apoptotic cells.

ii) The term FACS was coined by ……..

iii) ……. and ……..are examples of fluorescent dyes.

iv) ……..occurs when a beam of light is deflected by internal components of the cell.

13.9 SUMMARY

In this unit, you have studied that:

- Cell death is an essential part of normal development and continues through adulthood. PCD plays a key role both in the maintenance of adult tissues and during embryonic development.
- For long it was believed that cell death occurs either by apoptosis (Greek, meaning “falling off”) or necrosis. Now there is increasing evidence in support of other forms of PCD viz., necroptosis, pyroptosis, autophagic cell death, etc
- Apoptosis is a major form of PCD and is better understood in molecular terms. The basic understanding of the mammalian cells apoptotic mechanism is drawn from the mechanism of PCD dissected in nematode *Caenorhabditis elegans*. It occurs quietly without inciting an inflammatory response.
- A group of cysteine proteases (caspases) are activated in response to signals emerging from both within the cell and outside. They fall into two categories namely, initiator (caspase-8, -9) and executioner (caspase-3,-6, -7). Apoptosis is stringently regulated by a combination of pro-and anti-apoptotic proteins.
- Necrosis is less orderly process of cell death. It may be more appropriately called an accidental cell death. It occurs when a cell is severely damaged due to physical injury, inadequate blood supply (ischemia), oxygen deprivation, and in some infections. It is invariably accompanied by an inflammatory response.
- Cell renewal involves proliferation, maintenance, pleuripotency and regenerative ability. During cell renewal some cells remain undifferentiated (stem cells, SC) whereas others differentiate. In most tissues, SC self-renewal is coupled with regeneration of tissues. A lot of variation exists in the frequency of renewal of stem cell populations in different niches. Two models have been put forward to explain stem cell renewal. The ability of stem cells to renew and differentiate offers great potential in repair of damaged tissues, cancer, surgery etc. BM transplantation is frequently used for replenishing the hematopoietic system.
- A cell line or cell strain may be finite or continuous depending upon whether it has a limited life span or is immortal in culture, respectively. Continuous Cell lines are transformed under in vitro culture conditions.
- Leonard Arthur Herzenberg developed a cell sorter that did fluorescence based cell sorting. It is a specialized type of flow cytometry. The essential components of all flow cytometers are fluidics, optics (excitation and collection), an electronic network (detectors) and a computer.
- FACS finds applications in quantitative assessment of apoptotic cells, detection of intercellular proteins, phenotypic characterization of cells, etc.

13.10 TERMINAL QUESTIONS

1. What is programmed cell death (PCD)? Indicate the differences between apoptosis and necrosis?
2. Differentiate between intrinsic and extrinsic pathways of apoptosis.
3. Describe the role of Bcl-2 family members in apoptosis.
4. Explain the role of cell renewal in homeostasis.
5. What is pleuripotency?
6. Give two applications of stem cell technology.
7. What are the characteristics of a transformed cell?
8. Describe the principle of FACS and its applications in cell biology.

13.11 ANSWERS

Self-Assessment Questions

1.

a. 1--- f; 2--c; 3--g; 4-a; 5—h; 6---b; 7---e; 8---d

b. i. Extrinsic pathway; ii. *C. elegans*; iii. Trimerzation;

iv. TNFR1 pathway; v. mitochondrial apoptosome; vi. p53

c. 1. Proapoptotic factors / proteins like Bax & Bad promote apoptosis.

Anti apoptotic factors block apoptosis and promote cell survival such as Bcl-2.

2. The importance of apoptosis is best assessed in situations where it occurs in- appropriately: (a) If auto reactive lymphocytes are not efficiently removed in the thymus by apoptosis; the incidence of autoimmune diseases is much higher. (b) During development multicellular organisms acquire their final form by quietly eliminating selected cells.

2.

a. i) False; ii) True; iii) False; iv) True; v) True

b. Necroptosis is programmed necrosis that morphologically looks like necrosis. It is dependent on cellular signaling pathways for its activation. Necrosis is an ‘accidental death’ accompanied by cell and organelle swelling; random fragmentation of DNA; breakdown of the plasma membrane; release of cell contents and inflammation.

3.

a. i) Stem cells are undifferentiated pluripotent cells that can either renew themselves or differentiate into any type of cell / have long term self renewing

capability / they vary in their stemness; The ES cells are pluripotent, whereas adult stem cells are multi potent.

ii) Progenitor cells have limited self renewal capacity/ can differentiate into limited type of cells (like pro T cells)

b. i) Asymmetric and symmetric; ii) Leukemia, etc; iii) Regeneration;

iv) Cell death and Cell renewal; (v) Tissue atrophy/premature ageing.

4.

a. i) Physical agent: X-rays; ii) Chemicals: EMS

b. i) True; ii) False; iii) False; iv) True

5.

i) FACS; ii) L.A. Herzenberg; iii) FITC and Phycoerythrin; iv) Scattering

Terminal Questions

1. PCD is a genetically controlled cell death cascade that is activated in response to a variety of signals. Refer to table 1 in section 13.4 for differences between apoptosis and necrosis.

2.

Extrinsic pathway	**Intrinsic pathway**
Activated in response to external stimuli / FasL, TNF-α etc	Internal stimuli / Oxidative stress
Cell surface DR (Fas, TNFR1) sense the death signals.	No involvement of DR.
Initiator caspase is caspase-8.	Caspase-9 is the initiator caspase.
Mitochondria are not directly involved.	Mitochondria play a dominant role. It releases cytochrome c.
Receptor ligation recruits pro caspase 8 with the help of adaptor protein that activates the executioner caspases like the apoptosome.	Apoptosome formation following cytochrome c release to the cytoplasm.

3. Bcl-2 family encodes multiple proteins which are either proapoptotic (Bad, Bax, etc) or anti apoptotic (Bcl-2, Bcl-xL, etc) members. //Their balance ultimately decides the fate of a cell // role in regulating apoptosis.

4. Cell renewal is indispensable in multicellular organisms/ helps to replace dying cells and maintain tissue homeostasis/ failure of cell renewal leads to tissue atrophy & survival will be at stake.

5. Pleuripotency refers to the ability of a cell to differentiate into any of the three germ layers.
6. Applications of stem cell technology include :(i) Bone marrow transplantation / A sample of the patients stem cells are separated before giving radiation/chemo-therapy and then put back into the system after the therapy. (ii) Treatment of thalassemia, multiple myeloma, etc.
7. Refer to section 13.7 for characteristics of transformed cells.
8. FACS is based on flow cytometry in which cells are tagged with fluorescent labeled antibodies or dyes specific for a cell surface or intracellular molecule and then sorted according to the presence, absence or quantity of label in a cell. It provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest.

13.12 SUGGESTED READINGS

2. The Cell: A Molecular Approach (2018) 7e. Geoffrey M. Cooper and Robert E. Hausman. Publisher: Oxford University Press. ISBN-9781605357461.
3. Horvitz, H. R. (1999). Genetic control of programmed cell death in the nematode *Caenorhabditis elegans,* Cancer Res. 59, 1701s-1706s.
4. Fuchs E, Chen T, (2013). A matter of life and death: self-renewal in stem cells. EMBO Rep. 14(1): 39-48.
5. Kerr, J. F. R., Wyllie, A. H. and Currie, A. R. (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J. Cancer. 26: 239-257.