Cell Cycle and Cell Division

History of cell division

- **Prevost and Dumas (1824) first** studied cell division. They described cleavage in the **fertilized egg of frog.**
- **Remak (1841) Found new cells** to **develop from pre-existing cells** while studying cleavage in eggs. Discovered **amitosis (1855)**
- **Rudolf Virchow (1855, 1859)** Postulated **cell lineage theory** that cells arise by division of **pre-existing ones-omni-cellulae-cellula.**
- **Strasburger (1873)** Found that new nuclei develop from **pre-existing ones.**
- **Boveri and Flemming** (1879-1880) Studied details of **somatic cell division.**
- **Flemming** (1882) Coined the term '**Mitosis'.**
- **Braur, Sutton, Van-Benden,** Studied the process of cell division before the formation
- **Strasbarger and Winiwater of gametes (meiosis)**(1887-1900)
- **Farmer and Moore (1905**) Coined the term **"Meiosis"**
- **Gregoire** Differentiated **Meiosis I and II**
- **Howard and Pele 1953** Described **cell cycle.**
- **Montegomery** Introduced the **term synapsis.**
- Montose J. Moses (1955) **Synaptonemal complex**

- **Cell division** is the process by which a mature cell divides and forms two nearly equal **daughter cells** which resemble **the parental cell in a number of characters.**
- The cell which undergoes division is called **mother cell** or **parent cell**. The newly formed cells are known as **daughter cells**.

- In **unicellular organisms**, cell division is the means of reproduction by which the mother cell produces two or more new cells. In **multicellular organisms** also, new individual develops from **a single cell**.

State	Phase	Abbreviation	Description
Resting	Gap 0	**G_0**	A phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	**G_1**	Cells increase in size in Gap 1. The *G_1 checkpoint* control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	**S**	DNA replication occurs during this phase.
	Gap 2	**G_2**	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The *G_2 checkpoint* control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Cell division	Mitosis	**M**	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (*Metaphase Checkpoint*) ensures that the cell is ready to complete cell division.

- The period required to complete one cell cycle is called **generation time,** e.g., 20 **minutes for bacterial cell**, **90 minutes in yeast, 19 hours in beans, 20 hours for onion** root tip cells, **22 hours for human cells growing in** culture etc.

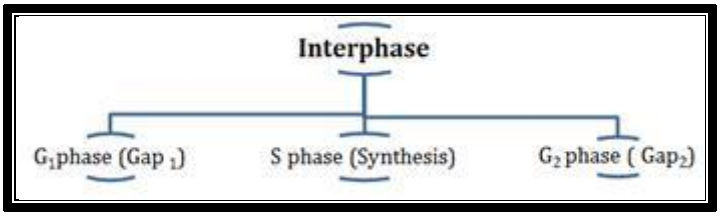
- **Howard** and **Pelc** (1953) have divided cell cycle into **four phases or stages : G1, S, G2 and M phase.** The **G1 phase, S phase and G2 phase** are combined to from the classical **interphase**.

cell division-hyperplasia
cell expansion - hypertrophy

Interphase:

- **Interphase** is the period from the end of one cell division to the **start of the next division.**
- The term **resting phase** is sometimes used for this phase, which is a misnomer, more accurately called **metabolic phase.**
- The phase is characterized by **high rate of metabolism involving both protein and nucleic acid** metabolism.
- In this phase the cell becomes enlarged in size due to high growth rate

✦ **Interphase** constitutes **the longest period of the cell cycle** and is sub divided into **three successive phases - <u>G_1, S and G_2</u>**



<u>I.G_1– phase (= Gap-I or First growth phase or post-mitotic gap phase)Post mitotic/Pre-DNA synthetic phase;</u>

✱ Intensive **cellular synthesis.**

✱ Synthesis of **rRNA, mRNA ribosomes and proteins**.

✱ **Metabolic rate is high.**

✱ Cells become **differentiated.**

✱ Synthesis of **enzymes and ATP storage.**

✱ Cell **size increases.**

✱ Decision for a **division in a cell occurs.**

✱ Substances of **G stimulates the onset of next S – phase.**

✱ Synthesis of **NHC protein(non-histone chromosomal proteins)carbohydrates, proteins, lipids.**

✱ Longest and most variable phase.

✱ Synthesis of enzyme, **amino acids, nucleotides** etc. but there is no change in DNA amount.

✱ After the M phase of **previous cell cycle, the daughter cells begin** G1 of interphase of new cell cycle. **G1 is a resting phase.**

✱ **It is called first gap phase**, since **no DNA synthesis** takes place during this stage; currently, G1 is also called **first growth phase**, since it involves synthesis of RNA, proteins and membranes which **leads to the growth of nucleus and cytoplasm** of each daughter cell towards their mature size .

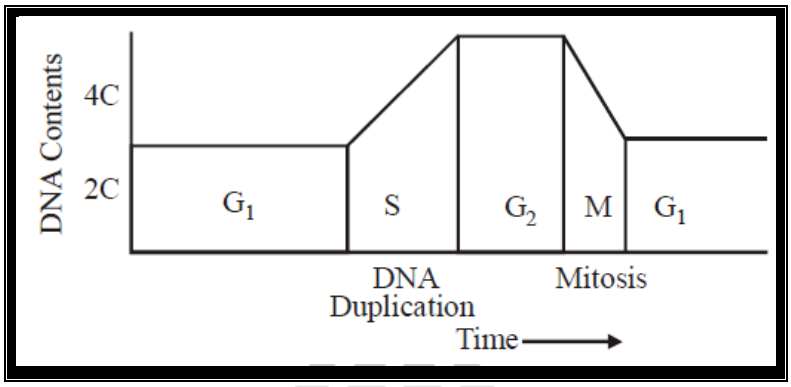
✱ **G_1 phase** corresponds to the interval between **mitosis and initiation of DNA replication**.

✱ During G1 phase, chromatin is **fully extended** and not distinguishable as discrete chromosomes with the light microscope. This is a **time of resumption of normal cell** metabolism which has slowed down during the previous cell division.

- Thus, G1 involves transcription of three types of RNAs, namely **rRNA, tRNA and mRNA** ; rRNA synthesis is indicated by the appearance of nucleolus in the interphase (G1 phase) nucleus.
- Proteins synthesized during **G1 phase regulatory proteins** which control various events of mitosis ; **enzymes (*e.g.*, DNA polymerase) necessary for DNA synthesis of the next stage** ; and **tubulin and other mitotic apparatus proteins**.
- Terminally differentiated somatic cells (end cells such as neurons and striated muscle cells) that no longer divide, are **arrested usually in the G1 stage**; such a type of G1 phase is called **G0 phase.**
- The new proteins are **translation** and RNA: **rRNA**, **tRNA** and **mRNA** transcription occurs during this phase. Also **Nucleotides**, **amino acids** and **ATPs** are formed. The **most variable phase** which **differs** in **time affecting** the **cell division** duration for each cell.
- It is the most variable as well as **longest phase of cell cycle during** which RNA and proteins are synthesized.
- In a specific point of G_1 a cell decides whether to start anew cycle or to withdraw from the cycle.
- This point is called **G_1 checkpoint**. The cell that leaves the cell cycle to remain in a resting stage is said to be in **Go state or quiscent phase (Lajtlia, 1963).**

Different stages of a mitotic cell cycle and their duration in hours.

Parts of cell cycle	Phases	Description of phases	Duration in hours: *Vicia faba*	Mouse L cells	Human HeLa cells
Interphase	G_1	Pre-DNA-synthesis phase	12	12	12
	S	DNA-synthesis phase	6	6–8	10
	G_2	Post-DNA synthesis phase	12	3–4	3
Mitosis	M	Mitotic phase	1	1	1



II.S-phase (= synthetic phase):

- DNA replicates and its amount becomes double (2C---→4C)
- Synthesis of histone proteins and NHC (**non-histone chromosomal proteins**).
- Euchromatin replicates **earlier than heterochromatin.**
- Each chromosome has **2 chromatids.**
- **S or synthesis phas**e marks the period during which **DNA synthesis or replication** takes place.
- **During this phase DNA synthesis or replication takes place**. As a result the DNA content per cell doubles. **DNA replicates and its amount becomes double**. If the initial amount of DNA is denoted as **2C then it increases to 4C.**
- However, the chromosome number remains the same **(Ploidy level remains the same).** Assume: the initial amount of **DNA as 2C**, then the DNA amount **increases to 4C**, and the cell has **2n number of chromosomes at G_1**, which remains the same even after **S-phase.**

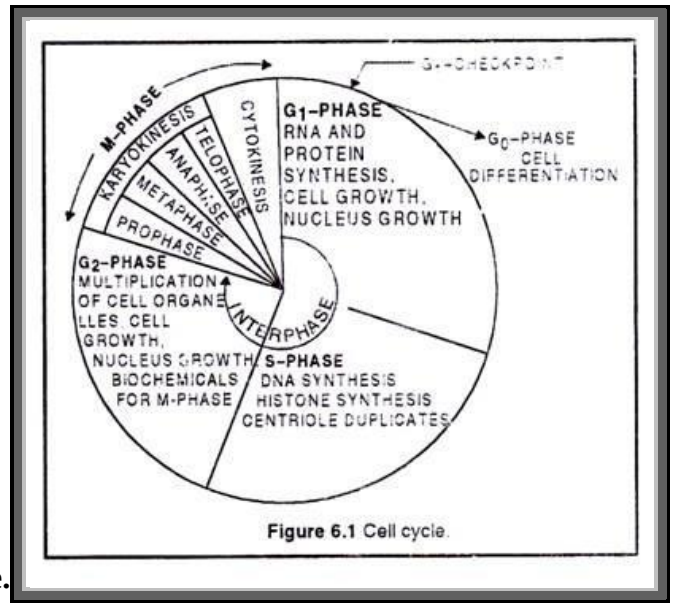


Figure 6.1 Cell cycle.

- Duplication of **centrioles in the cytoplasm.**
- **Histone proteins** are also synthesized in S-phase. This phase is called as the **invisible phase of the cell cycle** as the replicated chromosomes are invisible.
- S phase occupies roughly **35 to 45 per cent of cell cycle**

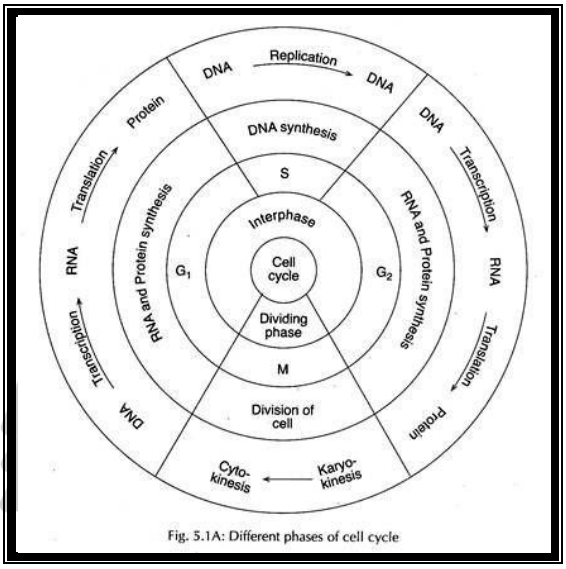


Fig. 5.1A: Different phases of cell cycle

III.G_2-phase (= Gap II or Second Growth Phase or Pre-mitotic phase). Post synthetic phase

- Mitotic spindle protein **(tubulin)** synthesis begins.
- Chromosome condensation **factor appears.**
- Synthesis of 3 types of **RNA, NHC proteins, and ATP molecule.**
- Repair of **damaged DNA occurs.**
- The cells that do not divide further exit G_1 phase to enter an inactive stage called **quiescent stage (G_0)** of the cell cycle. Cells in this stage remain metabolically active but no longer proliferate unless called on to do so depending on the requirement of the organism.
- In this phase, cell growth continues due to **synthesis of RNA and proteins**. However, **DNA synthesis stop**s. Cell organelles like **mitochondria and chloroplast** divide.
- This is a **second gap or growth phase or resting phase of interphase**. During G2 phase, synthesis of RNA and proteins continues which is required for cell growth. It may occupy

10 to 20 per cent time of cell cycle. As the G2 phase draws to a close, the cell enters the **M phase.**

- The phase just before the **mitosis (pre-mitotic gap phase)**. The cytoplasmic organelles multiply like **mitochondria, chloroplast and Golgi complex**.
- Transcription of RNA and then translation protein continues. Spindle tubulin synthesis and aster formation start.
- A cell contains double **the number (4C) of DNA present in the original diploid (2N) cell**. The cell is now prepared to enter into "**M" or Mitotic phase.**
- The main part is the synthesis of some **protein kinases used** in the regulation of cell division. **Kinases regulating** the cell cycle are called as **Cdks (cyclin-dependent kinases)** because they get activated after combination with the key protein called as **cyclin.**
- The kinase **enzyme along with cyclin** moves the cell cycle in the forward direction.
- **S-kinase** is capable of the **DNA replication** initiation after it combines with **S-cyclin.** After some time **S-cyclin gets destroyed and S-kinase loses** its activeness. The cell cycle in the **meristem cells** is with a special protein "**Cyclin and Cdks"** (discovered by **Nurse, T. Hunt & Hartmann** 2001 during the experiment on **yeast cell**). The cyclin protein triggers the DNA replication.

- **Generation time**: Period between 2 successive generation (range 8 hr – 100 days).
- Mitogens : Chemicals which enhance or stimulate cell division e.g. lymphokinase (in man)
- **Cell cycle duration**: 20 minutes in bacteria, 20 hrs in root tip of onion, 2-3 hrs in yeast, 24 hrs in man.
- **G_0 phase**: Cell only starts dividing when the period is favorable otherwise, it remain viable for months or years as such in G_0 phase.
- During the mitosis of He-La cells, the longest period is gap I phase or G_1.
- DNA replication occurs in S-phase.
- In a cell cycle the condensation of chromosome with visible centromere occurs during M-phase.
- Sequence in cell cycle is **G_1, S, G_2, M.**
- M-phase is of shortest duration of cell cycle.

- In G_2, the damaged DNA is repaired.
- Histone protein and RNA synthesis occurs in S-phase.
- Duplication of chromosome occurs at S- phase.

General Events of Interphase:

- The nuclear **envelope** remains intact. The chromosomes occur in the **form of diffused,** long, coiled and **indistinctly visible chromatin fibres.**
- The **DNA amount becomes double**. Due to accumulation of **ribosomal RNA (rRNA) and ribosomal proteins** in the nucleolus, the size of the **latter is greatly increased**.
- In animal cells, a daughter pair of centrioles originates near the **already existing centriole** and, thus, an interphase cell has **two pairs of centrioles.**
- In animal cells, net membrane **biosynthesis increases just before cell division** (mitosis). This extra membrane seems to be stored as blebs on the surface of the **cells about to divide**

Differences between G_1 and G_2 phases

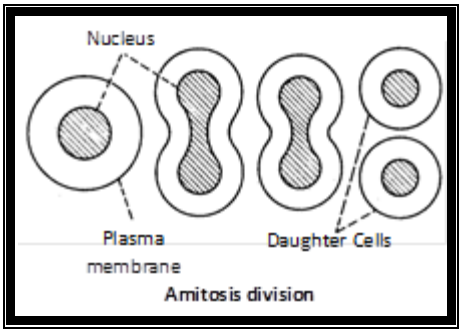
S.No.	G_1 phase	G_2 phase
1.	It is the first substage of interphase.	It is the last substage of interphase.
2.	Available factors determine its fate, entry in G 0, differentiation or continuity of cell cycle.	There is very little choice for the cell except to proceed further in cell cycle.
3.	Cell organelles do not increase in number.	Cell organelles increase in number.
4.	Cell grows in size but growth of nucleus is little.	Both cell and nucleus grow in size.
5.	It synthesizes RNAs, proteins and other biochemicals for cell growth and subsequent	It synthesizes RNAs, proteins and other biochemicals for spindle formation and M- phase division.

	replication of DNA.	

Divisional Phase: (= Mitotic phase) or M phase;

- It is the phase of **actual cell division.**
- It is divided into two phases - **karyokinesis** (division of nucleus) and **cytokinesis** (**division of the cytoplasm**).
- **Time period for G_1, S, G_2** and M-phase is species specific under specific environmental conditions e.g., **20 minutes for bacterial cel**l, 8-10 hours for intestinal epithelial cells, **onion root tip cells may take 20 hours**. 24 hours for human cells and 90 minutes for yeast cells to complete these phases of cell cycle.
- The **mitosis (Gr., mitos=thread)** occurs in the somatic cells and it is meant for the multiplication of cell number during embryogenesis **and blastogenesis of plants and animals.**
- Fundamentally, it remains related with the growth of an individual from **zygote to adult stage.**
- Mitosis starts at the culmination point of **interphase (G2 phase).** It is a short period of chromosome **condensation, segregation and cytoplasmic division**. Mitosis is important for replacement of cells lost to natureal friction (attrition), wear and tear and for **wound healing.**
- It is of three types – **amitosis**, **mitosis** and **meiosis**.

AMITOSIS



- **Amitosis (amitotic division, direct cell division)** is a direct division of nucleus with following cell division without formation of chromosomes and mitotic spindle apparatus.
- Amitosis was first described by the **R. Remak in 1841**.
- The term was proposed by **W. Flemming in 1882**.
- During amitosis the nucleus elongates and becomes **dumb-bell-shaped,** then it divides into **two nuclei, the cytoplasm constricts** into two parts, half going to each nucleus thus two daughter cells are forms.
- In this division, there is no differentiation of **chromosomes and spindle**. The nuclear envelope does not degenerate. The nucleus elongates and constricts in the **middle to form two daughter nuclei**. This is followed by a centripetal constriction of the cytoplasm to form two daughter cells.
- Examples : **Prokaryotes, protozoans, yeasts, foetal membrane of mammals, cartilage of mammals etc.**

ENDOMITOSIS

This is a **closed mitosis**. It is a process in which the number of chromosomes increases in the nucleus without destruction of **nuclear membrane, nucleolus and forming of mitotic spindle apparatus**. And then the division of cytoplasm is not occurred. (e.g. the cells of liver). **Endomitosis leads to polyploidy**

MITOSIS

- Mitosis produces genetically **identical cells**. The chromosomes undergo division and **replicate to form duplicates** which are **similar to mother cell chromosome number (equational division)**. The division is also called as **somatic cell division or equational division or indirect division.**
- **Mitosis** was coined by **Fleming in 1882.**

- Strasburger observed **mitosis in plants**. While **Boveri and Fleming** observed the same **in animals.**
- **Plant meristematic tissues (root and shoot tips)** and animal skin, bone marrow, even the embryonic developmental stages have the mitotic division.

Cause of mitosis:

- **Kern plasm theory: Hertwig** proposed **kern plasm theory**. According to this theory, mitosis occurs due to the disturbance in the **Karyoplasmic index (KI) or the nucleocytoplasmic ratio of the cell.**

V_n = Volume of the nucleus

V_c = Volume of cell

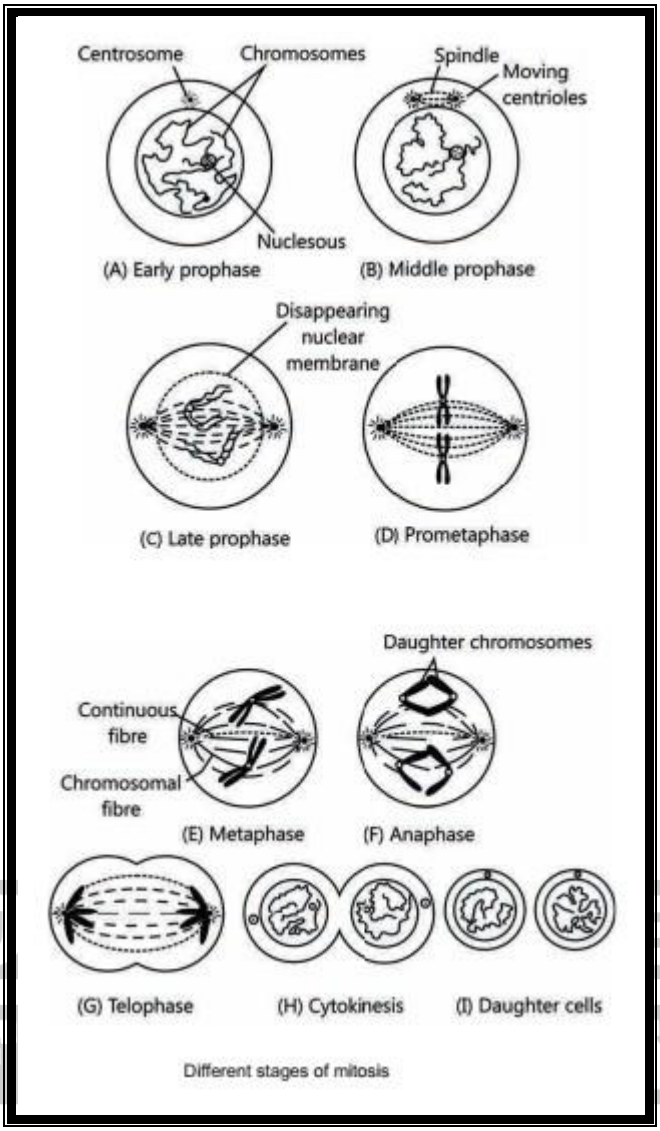
V_c-V_n= Volume of cytoplasm

Phases of Mitosis

- **Division phase** or **M-phase** or **mitotic phase** (duration 1hr) is the most dramatic period of the cell cycle.
- **Karyokinesis** – Division of the nucleus; and **Cytokinesis** – Division of cytoplasm.

Karyokinesis

Division of the nucleus occurs by sequential changes **(Indirect division) Karyokinesis** has 4 stages:



Different stages of mitosis

Duration of different phases of mitosis in certain plants and animals.

Organism	Duration in minutes			
	Prophase	Metaphase	Anaphase	Telophase
1. Mouse (spleen)	21	13	5	4
2. Grasshopper (neuroblasts)	102	13	9	57
3. Pea (root tip)	78	14.4	4.2	13.2
4. Onion (root tip)	71	6.5	2.4	3.8

1. Prophase (longest stage);

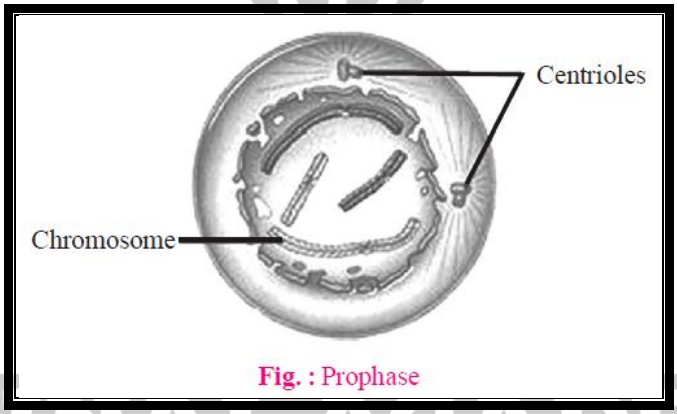


Fig. : Prophase

- ➔ Chromatin fibres thicken and shorter to form chromosomes which may overlap each other and appears like a ball of wool. **Spireme stage.**
- ➔ Each **chromosome divides longitudinally into 2 chromatids** which remain attached to centromere.
- ➔ Nuclear membrane starts disintegrating except in **dinoflagellates.**
- ➔ **Nucleolus starts** disintegrating.
- ➔ Cells become **viscous, refractive and oval in outline.**
- ➔ **Spindle formation** begins.
- ➔ Cell cytoskeleton, golgi complex, ER, etc. **disappear.**
- ➔ In animal cells, centrioles **move towards opposite sides.**
- ➔ **Lampbrush chromosomes** can be studied well.
- ➔ **Small globular structure (beaded)** on the chromosome are called **chromomeres.**
- ➔ Spindle is formed from **centriole (in animal cells) or MTOC** (microtubule organising centre) in plant cells successively called **astral and anastral spindle.**
- ➔ Chromatin threads get condensed to form **the chromosomes.**
- ➔ Centrioles get aligned towards the **opposite poles.**

- **Astral ray formation** from the proteins gelatinized around the **centrioles (initiation of the assembly of mitotic spindle).**
- Cells do not show **Golgi complexes, ER, Nucleolus and nuclear membrane at the end of the prophase.**
- Each prophase chromosome is **composed of two coiled filaments, the chromatids, which are the result of the replication of DNA during the S phase.**
- As **prophase progresses, the chromatids become shorter and thicker and two sister chromatids** of each chromosome are held together by a **special DNA-containing region**, called the **centromere or primary constriction**.
- During prophase, proteins of the **trilaminar kinetochores** (one for each chromatid) start depositing or organizing on the **centromere of each chromosome**.
- In the cytoplasm, the most conspicuous change is the formation of the spindle or mitotic apparatus.
- In the early prophase, there are **two pairs of centrioles**, each one surrounded by the socalled **aster** which is composed of microtubules radiating in **all directions.**
- **The two pairs of centrioles migrate to opposite poles** of the cell along with the **asters** and become situated in antipodal positions. Between the **separating centrioles** forms a spindle.
- The **microtubules of the spindle** are arranged like two cones base to base, broad at the centre or equator of the cell and narrowing to a point at **either end or pole**. **Mitotic spindle contains three main types of fibres** :

  1. **Polar fibres**, which extend from the two poles of the **spindle toward the equator**
  2. **Kinetochore fibres,** which attach to the kinetochores of centromeres of each mitotic chromosome and extend toward the poles
  3. **Astral fibres**, which radiate outward from the poles toward the periphery or cortex of cell. In cells of **higher plants,** however, spindle forms without the aid **of centrioles and lacks asters**

  Asters are not formed in plant cells as they do **not have centrioles**, hence called anastral cell division

- Lastly, during prophase, the **nucleolus gradually disintegrates**. **Degeneration and disappearance** of the **nuclear envelope marks the end of prophase**.
- Following two factors may be involved in this process:

1. **Enzymatic action** either by some mitochondrial enzymes ,**cytosolic MPF kinase or nuclear RNA (or ribozyme ; Burns and Bottino, 1989).**
2. **Physical action, physical** stress exerted by **microtubules which** become attached to the nuclear **envelope (Burns and Bottino, 1989).**

2. Metaphase:

➔ Chromosomes become maximally distinct size **can be measured.**

➔ **A colourless, fibrous, bipolar spindle** appears.

➔ Spindle fibre are made up of **97% tubulin protein and 3% RNA.**

➔ Chromosomes move towards equatorial plane of spindles called **congression** and become arranged with their arms directed towards pole and centromere towards equator.

➔ Spindle **fibres attach to kinetochores**.

➔ Metaphase is the best stage for studying chromosome morphology (structure, size, number).

➔ Spindle has two type of fibres

- **Continous fibre** (run from pole to pole).
- **Discontinous fibre** (between pole to centromeres).

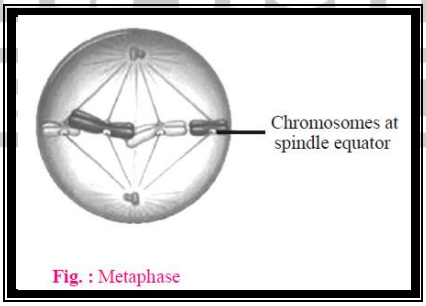
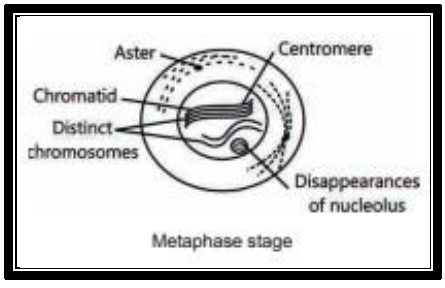


Fig. : Metaphase

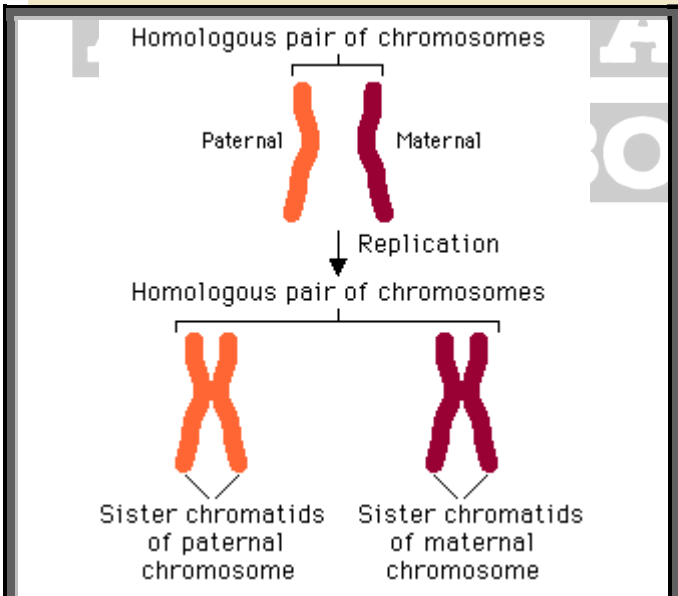
➔ The nuclear **envelope** is completely disintegrated which highlights the start of the second phase in mitosis.

➔ The **chromosomes spread** throughout the cytoplasm. Spindle fibres attach to the chromosomes at their **kinetochores.**



- ➔ The condensation of chromosomes is complete. This is the stage where **the morphology** of **chromosomes** is easily visible.
- ➔ The chromosome is compiled in **two sister chromatids**, held together with the centromere. Each **chromosome splits as per length upto the centromere** (the division of matrix of the chromosome). Thus, replicated chromatids are clearly visible at metaphase stage.
- ➔ **Chromosomes spilt up and arrange themselves on the equator to form metaphase** plate (equatorial plate). **Spindle fibres are microtubules.**
- ➔ **Chromosomal fibres,** (**discontinuous and run from pole to centromere**) and **supporting fibres**, (continuous and run from pole to pole), arranged in a cell. The centromere lies at the **equator with arms facing the poles.**
- ➔ At **metaphase, subunits (tubulin dimers)** are added to the plus end of a microtubule at the kinetochore and are removed from the minus end at the spindle pole. Thus, a **poleward flux of tubulin** subunits occurs, with the microtubules remaining stationary and under tension.

HOMOLOGOUS CHROMOSOME AND SISTER CHROMATID



HOMOLOGOUS CHROMOSOME-
These are chromosome pairs of approximately the same length, and centromere position.
Out of the pair one is inherited from the mother (maternal) and one from the father (paternal).

SISTER CHROMATID-
These are two identical chromatids connected by a centromere.

ARUNAI ACADEMY FOR PG TRB BOTANY

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3.Anaphase (smallest stage):

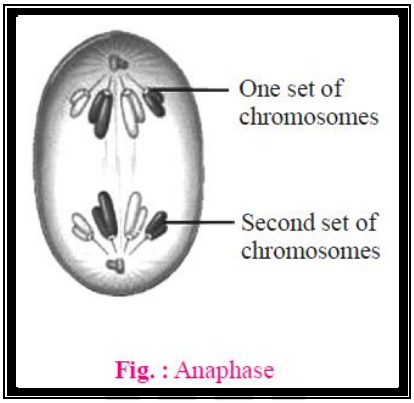
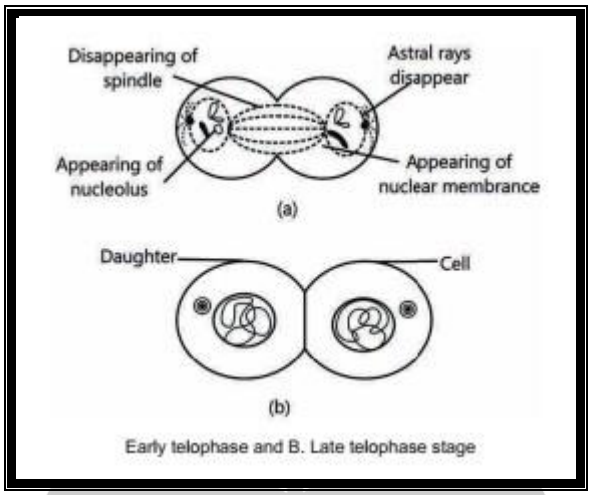


Fig. : Anaphase

- ➔ Centromere splits from the middle and **two chromatids gets separated.**
- ➔ Both the chromatids move towards opposite poles due to repulsive force called **anaphasic movement.**
- ➔ Anaphasic movement is brought about by the **repolymerisation of continuous fibres** and depolymerisation of chromosomal fibres. Formation and expansion of interzonal fibres.
- ➔ The centromere faces towards equator.
- ➔ Shape of chromosome is best studied at anaphase.
- ➔ The early anaphase has **interzonal fibres appearing at the equator**. Chromosome centromere splits lengthwise **(division of centromere).**
- ➔ Chromosomes double inside a cell during mitotic anaphase. Every chromosome has **one chromatid**.
- ➔ The **anaphase** begins abruptly with the synchronous splitting of each chromosome into its sister chromatids, called **daughter chromosomes**, each with **one kinetochore. Synchronous splitting of each centromere** during prophase is evidently caused by an increase in **cytosolic Ca2+**.
- ➔ In fact, **Ca2+-containing membrane vesicles** accumulate at spindle poles and release calcium ions to **initiate anaphase (Hapler and coworkers, 1980, 1987)**. Anaphase involves the following two steps :
    - **Anaphase A**:During it, there is **poleward movemen**t of chromatids due to shortening of the kinetochore microtubules. During their poleward migration, the **centromeres (and kinetochores)** remain foremost so that the chromosomes characteristically **appear U,V or J- shaped.**

- **Anaphase B:** It involves separation of poles themselves accompanied by the elongation of the **polar microtubules.** The **astral microtubules** also help in anaphase B by their attractive interaction with cell cortex.

4.Telophase (reverse of prophase)



Early telophase and B. Late telophase stage

➔ **Nuclear membrane, Nucleolus, Golgi complex and ER** now surround each of the chromosomal poles.

➔ The chromatin net is formed after the **chromosomes decondense**. Chromosomes lose their **individuality** which means the **individual chromosomes are not present**.

➔ Chromosomes reach the poles by the spindle fibers and form two groups.

➔ Chromosomes begin to uncoil and form a chromatin net.

➔ The nuclear membrane and nucleolus reappear.

➔ In animal cells, **astral rays and spindle fibres** completely disappear in **telophase**.

➔ The two centriole pairs organise **themselves into centrosomes.**

➔ In plant cells, the **spindle fibres disappear from near the poles** but remain intact **towards the equator.**

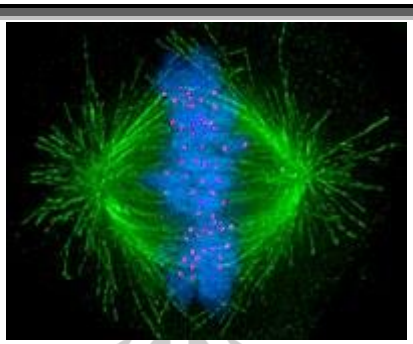


Image of the mitotic spindle in a human cell showing microtubules in green, chromosomes (DNA) in blue, and kinetochores in red.

Open mitosis: the nuclear envelope is **destroyed.**

Closed mitosis: the nuclear envelope is **not destroyed.**

Trunkal (stemal) mitosis:Parental cell is divided into **two daughter cells**; each of them is divided into two identical cells and so on. The model of mitosis is as **a trunk of the tree.**

Asymmetrical mitosis:Parental cell is divided into two daughter cells, and then one daughter cell is divided the same but another is not divided. (Ascaris)

Transforming mitosis: Parental cell is divided into two cells, but the daughter cells are never divided

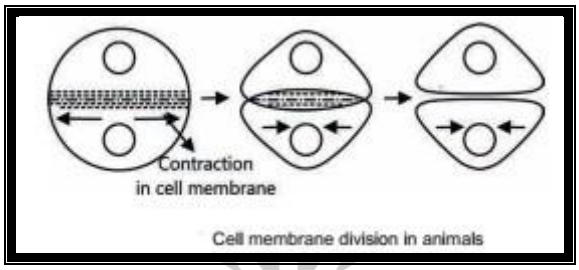
CYTOKINESIS

- **Cytokinesis** is the division of cell having undergone **karyokinesis to produce two daughter cells** each with a **daughter nucleus**. It begins in **mid anaphase** and is generally completed along with the completion of **telophase.**
- Both **DNA synthesis and mitosis** are **coupled to cytoplasmic divison**, **or cytokinesis-** the constriction of **cytoplasm into two separate cells.** During cytokinesis, the cytoplasm divides by a process, called **cleavage.**
- In plant cell, the **cytokinesis** occurs due to the formation of **phragmoplast** in centrifugal direction. The **phragmoplast is formed by golgicomplex, ER and pectin containing vesicles.**
- **Cytokinesis is by 2 methods:**

1.Cell furrow method / Cytokinesis in animals

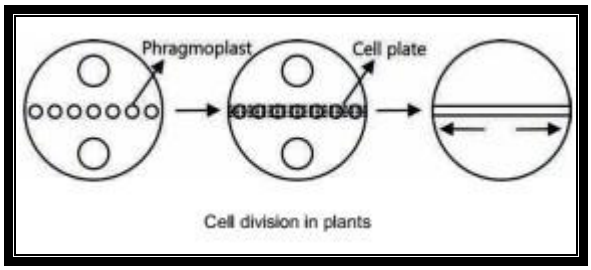
2.Cell plate method / Cytokinesis in plants

1.Cell furrow method / Cytokinesis in animals :



- It occurs through **constriction and furrow formation** in **the cell membrane**.
- A **mid body equator** is formed when **the microtubules** arrange in the middle while the **microfilaments** arrange in the **peripheral ring just below the plasma membrane**.
- The **cell organelles arrange** themselves at either side of the equator. The contraction occurs as the attraction occurs between **mid body and peripheral ring**, forming a furrow from the outside of the **cell to inside.**
- The **furrow formed deepens continuously and finally**, the **cell divides into two daughter cells**. The **cytokinesis in the animal cell occurs** in the **centripetal order**
- A dense **vesicular and fibrous structure** is formed in the **equatorial region of the spindle simultaneously** with its **depolymerisation**. It is called **mid body.**
- A **circular constriction or invagination appears** at the centre or equator and forms a furrow. **The constriction or furrow deepens** centripetally and divides the **mother cell into two daughter cells.**

2.Cell plate method / Cytokinesis in plants:



- Vesicles provided by **Golgi apparatus unite to form phragmoplasts**, which join to form the cell plate.

- **Cell plate** is **first laid down** in the centre and then proceeds towards periphery **(centrifugal plate-formation)**.
- **Cell wall materials** are now laid down on **both sides of cell plate** thus, forming two **daughter cells.**
- The cell plate **formation takes** place because **the constriction or even furrow** is not possible as the **cell wall is rigid.**
- Many **Golgi vesicles** and **spindle microtubules** arrange themselves on the equator and the cell has a **Phragmoplast.**
- It may also have the **deposits of fragments of ER. Golgi vesicles membranes** fuse and form a plate like structure which is called as **the cell plate.**
- Golgi vesicles then **secret pectates of calcium and magnesium**. The **cell plate modifies into the middle lamella.**
- The cytokinesis of **plant cells occurs in the centrifugal order** (**cell plate formation is from centre to periphery).**

TYPES OF MITOSIS

- **Anastral mitosis:** It is found in **plants in which spindle no aster has**.
- **Amphiastral mitosis:** It is found in animals in which spindle has two asters, one at each pole of the spindle. Spindle is barrel-like.
- **Intranuclear or Promitosis:** In this **nuclear membrane is not lost** and spindle is formed inside the nuclear membrane e.g. **Protozoans (Amoeba) and yeast**. It is so as centriole is present within the nucleus.
- **Extranuclear or Eumitosis:** In this **nuclear membrane** is **lost and spindle** is formed outside nuclear membrane e.g. in **plants and animals.**
- **Endomitosis:** Chromosomes and their **DNA duplicate but fail to separate** which lead to polyploidy e.g. in liver of man, **both diploid (2N) and polyploid cells (4N)** have been reported. It is also called **endoduplication and endopolyploidy**.
- **Dinomitosis** : In this, **nuclear envelope** persists but **microtubular spindle is not formed**. During movement, the **chromosomes are attached with nuclear membrane**, e.g., **dinoflagellates.**

MITOGENS AND MITOTIC POISONS

- The agents which stimulate **cell division** are called **mitogens**, e.g., cytokinins, some steroids, however, some chemicals inhibit cell division, these are called mitotic poisons, e.g., **azides, cyanides, colchicine, chalones, etc.**
- **Colchicine is an alkaloid** derived from Colchicum autumnale. It interferes with spindle formation and arrests **cell division at metaphase**. **Polyploidy** in plants can be induced by the **application of colchicine.**

- **Azides and Cyanides : Inhibit prophase.**
- **Colchicine : Inhibits spindle formation at metaphase.**
- **Mustard gas : Agglutinates the chromosomes.**
- **Chalones : These were first reported by Laurence and Bullough (1960). They are peptides and glycoproteins secreated by extracellular fluid of healthy cells and** inhibit cellular division.
- **Karyochoriosis : A type of mitosis in fungi in which is** intranuclear nucleus **divides by** furrow formation.

TOTAL NUMBER OF PG ASST BOTANY SELECTED FROM ARUNAI ACADEMY-PG TRB 2019

ARUNAI ACADEMY FOR PG TRB
BOTANY.DHARMAPURI.9500244679
Provisional Selection List-2019

SNO	ROLLNO	NAME	MARK	COMMUNITY	DOB	Gender	Selection Turn	Mark Position
1	19PG061803103	CHENNAMMAL.C	94	SC	08-Jun-87	Female	GT W	State 2nd Mark
2	19PG061500227	SUDHA M	91	MBC/DNC	10-Jun-88	Female	GT W	State 4th Mark
3	19PG061801079	GOVINDARAJU R	89	BC	30-Jun-76	Male	GT	State 6th Mark
4	19PG061801110	JAYARAMAN C	89	MBC/DNC	08-Jun-84	Male	GT	State 6th Mark
5	19PG061801154	SANJAIGANDHI P	88	MBC/DNC	05-Jun-80	Male	GT	State 7th Mark
6	19PG061801151	KATHIRVELU	88	MBC/DNC	16-Jun-85	Male	GT	State 7th Mark
7	19PG061801060	ARTHANAREESWARAN P	88	MBC/DNC	20-May-89	Male	GT	State 7th Mark
8	19PG061500237	SANGEETHA P	87	MBC/DNC	16-May-88	Female	GT WT*	State 8th Mark
9	19PG061807600	RAMESH A	86	MBC/DNC	05-May-83	Male	GT	State 9th Mark
10	19PG061300142	SAKTHIVEL K	86	SC	21-Jun-91	Male	GT	State 9th Mark
11	19PG061500224	SAMBAVI S	84	MBC/DNC	12-Apr-89	Female	GT SG*	State 11th Mark
12	19PG062107356	VASANTH K	84	ST	27-Apr-87	Male	ST	State 11th Mark
13	19PG061809260	KARTHIK M	83	SC	03-Jan-85	Male	SC	State 12th Mark
14	19PG061803107	POOVARASI S M	83	MBC/DNC	08-Sep-85	Female	MBC/DNC W	State 12th Mark
15	19PG061408534	REVATHI K	83	BC	15-May-90	Female	BC W	State 12th Mark
16	19PG061807557	VIVEKANANDAN G	82	SC	10-May-80	Male	SC	State 13th Mark
17	19PG061301575	THANGARASU M	82	BC	15-Jul-80	Male	BC	State 13th Mark
18	19PG061800195	MURUGAN T	82	SCA	10-Jun-82	Male	SCA	State 13th Mark
19	19PG062110427	SATHEESH KUMAR M	82	SC	06-May-86	Male	SC	State 13th Mark
20	19PG062105867	C.SUBASHINI	82	SC	19-Jun-86	Female	SC W	State 13th Mark
21	19PG063803521	JAYANTHI.A	81	BC	01-Jul-79	Female	BC W	State 14th Mark
22	19PG061500254	MAYIL M	80	MBC/DNC	25-Apr-80	Female	MBC/DNC W	State Mark***
23	19PG061812379	MUNIRAJU. M.	80	SC	14-Apr-89	Male	SC T*	State Mark***
24	19PG061803274	A ANITHA	79	BC	27-Nov-81	Female	Adi-Dravida, Tribal Welfare	State Mark***
25	19PG061808460	ANANDAKUMAR D	79	MBC/DNC	28-Jul-83	Male	MBC/DNC T*	State Mark***
26	19PG061812445	MANIVEL M	79	MBC/DNC	07-Jun-87	Male	MBC/DNC SG*	State Mark***
27	19PG062750215	SANKILI R	78	SC	20-May-73	Male	SC SG*	State Mark***
28	19PG061801066	MAHENDRAN T	78	ST	05-Jun-81	Male	ST	State Mark***
29	19PG062750161	SUGANTHI S	78	SC	03-Feb-83	Female	SC W	State Mark***
30	19PG061809264	K TAMILARASAN	77	MBC/DNC	15-Apr-83	Male	GT T	State Mark***
31	19PG061809820	MAHESWARAN M	76	ST	07-Jul-89	Male	ST T*	State Mark***

Physiology of Cell Cycle and Mitosis

☎ **Regulation of mitotic chromosome cycl**e. Mitotic chromosome cycles is found to be regulated by the following **three control factors (diffusible proteins)** :

➔ The **S-phase activator** that normally appears in the cytoplasm only during S-phase and **'switches on' DNA synthesis (Rao and Johanson, 1970).**

➔ The **M-phase promoting factor (MPF)** that is present only in **M-phase cytoplasm and causes chromosome condensation (Johanson and Rao, 1970)**.

➔ The **DNA-dependent M-phase** delaying factor that is present in S-phase cytoplasm and inhibits the process leading to onset of **MPF production**

☎ Each **successive step** depends on a preceding one **(all processes of chromosome cycle are linked together as dependent sequence).**

☎ the cell cannot pass through **mitosis until MPF has been produce**

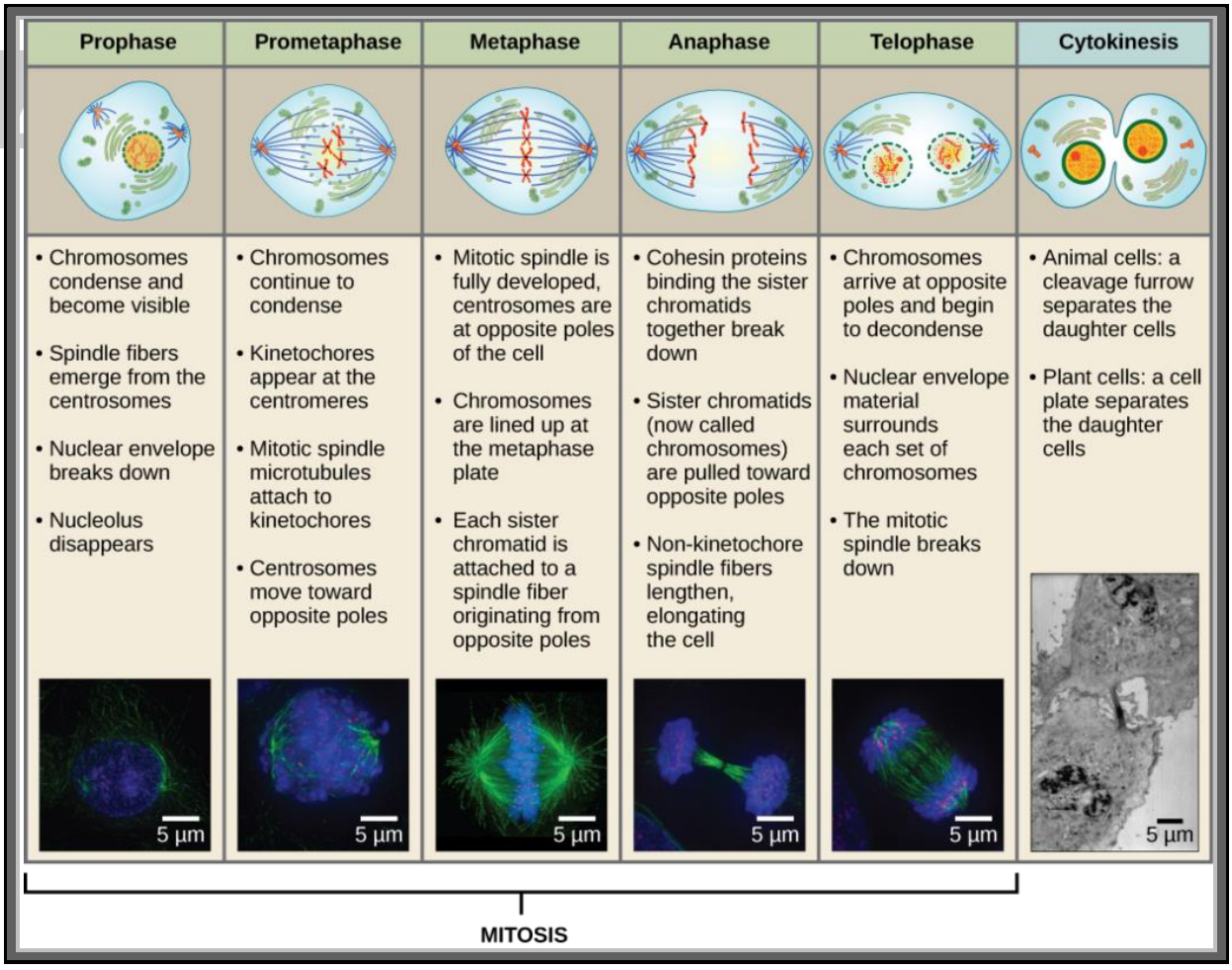
☎ **MPF cannot** be produced until the **M-phase-delaying-factor has disappeared**

☎ the **M-phase-delaying factor and S-phase** activator **cannot disappear** until DNA-**synthesis has ended**

☎ **DNA synthesis cannot end until all of the DNA** has replicated

☎ the **DNA cannot begin to replicate until DNA** rereplication block has been removed by passage through **mitosis into G1**

☎ A **cell cannot progress from mitosis into G1**, until the chromosomes have separated on the **mitotic spindle.**



Difference between animal and plant cells (Mitosis)

Animal cells	Plant cells
Centrioles present at **spindle poles**.	Centrioles lacking at **spindle poles.**
Asters are formed (amphiastral).	No asters are formed (anastral).
Cytokinesis by **furrowing of cytoplasm**.	**Cytokinesis** mostly by cell plate formation.
Furrow extends centripetally	Cell plate grows centrifugally.
Microfilament ring brings about cleavage.	Microfilaments have no role in cytokinesis.
Occurs nearly in **all tissues**.	Occurs mainly at **meristems.**
Cell becomes **rounded** and its cytoplasm more viscous at the time of mitosis.	Cell does **not change** form or nature at the time of mitosis.
Midbody is formed at the equator of the spindle.	Equator of the spindle changes into **phragmoplast.**
Intercellular spaces appear between the daughter cells.	Daughter cells remain adhered together by middle lamella.
Animal mitosis is controlled by certain **mitogen**s.	Plant mitosis is usually controlled by a hormone **cytokinin.**

Working of mitotic spindle during anaphase.

- **During anaphase A**, a surprisingly large force acts **on a chromosome** as it moves from the **metaphase plate to the spindle pole**. By **hydrodynamic analysis** it has been calculated that to **move a chromosome**, a force about 10-11 dynes is needed, and that the entire **displacement- from equator** to the pole of a **chromosome-may require** the use of about **30 ATP molecules**.
- As each chromosome moves **polewards**, its **kinetochore** microtubules disassemble, so that they have nearly disappeared **at telophase**. The site of subunit loss can be determined by **injecting labelled tubulin into cells during metaphase.** The labelled subunits are found to be added to the **kinetochore end of kinetochore microtubules** and then **lost as anaphase** A proceeds, indicating that **kinetochore "eats"** its way **poleward** along its microtubules at anaphase However, typically **microtubule** disassemble at **kinetochores**, poles or at both sites is probably necessery for **equator- to- pole movement**

Significance of Mitosis

- The **equational division** is a common division method for the **diploid** cells only. However, some **lower plants and social insects** which have **haploid** cells, also use **mitosis** for the division.
- The **significance** of this division is **essential** to **understand** in the **life** of an organism. Mitosis results in the **production of diploid daughter cells** which have identical genetic **chromosome number.**
- The **multicellular** organisms grow due to the mitosis. Cell growth often results in disturbing the usual ratio of the **nucleus and the cytoplasm**. Thus, the cell divides and restores the **nucleo cytoplasmic ratio**.
- A very significant contribution is that a **cell is repaired**. Best examples are the cells of the **upper epidermis layer**, cells of the gut lining, and **blood cells** being replaced constantly.
- It keeps **the chromosome number constant and maintains genet**ic stability in daughter cells. All the cells have similar **genetic constituents**.
- It provides new cells for **repair and regeneration and for healing of the wounds**. (e.g., cells of the **upper layer of the epidermis**, cells of the lining of the **gut, and blood cells).**
- It helps in asexual reproduction by **fragmentation, budding, stem cutting,** etc.
- Somatic variations when **maintained by vegetative propagation can play** an important role in speciation.

Difference between the Cytokinesis of Animal cell and Plant cell.

S. No.	Animal cell cytokinesis	Plant cell cytokinesis
1.	Centrioles are present at spindle poles.	Centrioles are absent at spindle poles.
2.	Asters are formed (amphiastral).	No asters are formed (anastral).
3.	Cytokinesis occurs by furrowing of cytoplasm.	Cytokinesis occurs mostly by cell plate formation.
4.	Furrow extends centripetally.	Cell plate grows centrifugally.
5.	Occurs nearly in all tissues.	Occurs mainly at meristems.
6.	Cell becomes rounded and its cytoplasm more viscous at the time of mitosis.	Cell does not change form or nature at the time of mitosis.
7.	Intercellular spaces appear between the daughter cells.	Daughter cells remain adhered together by middle lamella.
8.	Animal mitosis is controlled by certain mitogens.	Plant mitosis is usually controlled by a hormone cytokinin.

MEIOSIS

- The term **meiosis** was coined by **J.B. Farmer and Moore in 1905**.
- It was first demonstrated by **Van Beneden (1887)** but was described by **Winiwarter (1900). Gregoire** used the term **meiosis I and II.**
- Meiosis produces a **total of four haploid cells** from each original diploid cell.

- These haploid cells either become or give rise to gametes, which through union **(fertilization) support sexual reproduction and a new generation of diploid organisms**.
- It is found in special types and at specific period. It is reported in diploid germ cells of sex organs (*e.g.*, **primary spermatocytes of testes to form male gametes called spermotozoa and primary oocytes to form female gametes called ova in animals**) and in pollen mother cells (**microsporocytes**) of anther and **megasporocyte** of ovule of ovary of flowers in plant to form the **haploid spores**. The study of **meiosis in plants** can be done in **young flower buds.**
- Thus, meiosis is required to run the reproductive cycle of eukaryotes such as microorganisms **Chlamydomonas, Neurospora; bryophytes; plants and animals**. For example, the **reproductive cycle of Chlamydomonas includes** a long **haploid generation** and a short diploid generation which involves the **zygote formation**.
- The **zygote undergoes reduction division** (meiosis) resulting in the **formation of haploid** spores.
- In higher plants, however, **the reproductive cycle** includes a **long dominant diploid** and **multicellular generation** (called **sporophyte**) and a short, multicellular haploid generation, called **gametophyte generation**.
- The tiny gametophyte is nurtured in specialized **tissues of sporophyte**. Male and female haploid cells called **spores** are produced by **meiosis** in the **diploid (sporophyte) organism**.
- Spores grow into **multicellular male and female haploid (gametophyte) structures**, which through meiosis **produce haploid cells corresponding to the actual gamete**.
- In both **animals and plants, male and female gametes** unite during fertilization to produce a **zygote** in which the **diploid chromosome** number is restored.
- In **animals and simpler plants**, the zygote **matures to a new diploid** organism. In the **seed-producing plants**, development is arrested at an early **multicellular stage** as a **seed,** which may remain stable for long time before **germination permits** a continuation of growth. Thus, **reproductive cycle** includes alternation of two generations : **haploid and diploid and involves meiosis.**

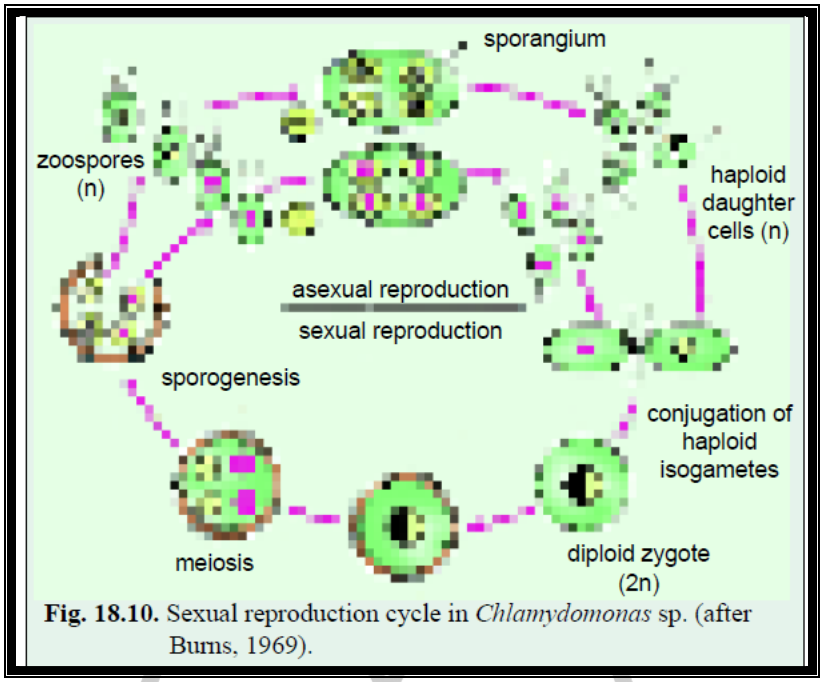


Fig. 18.10. Sexual reproduction cycle in *Chlamydomonas* sp. (after Burns, 1969).

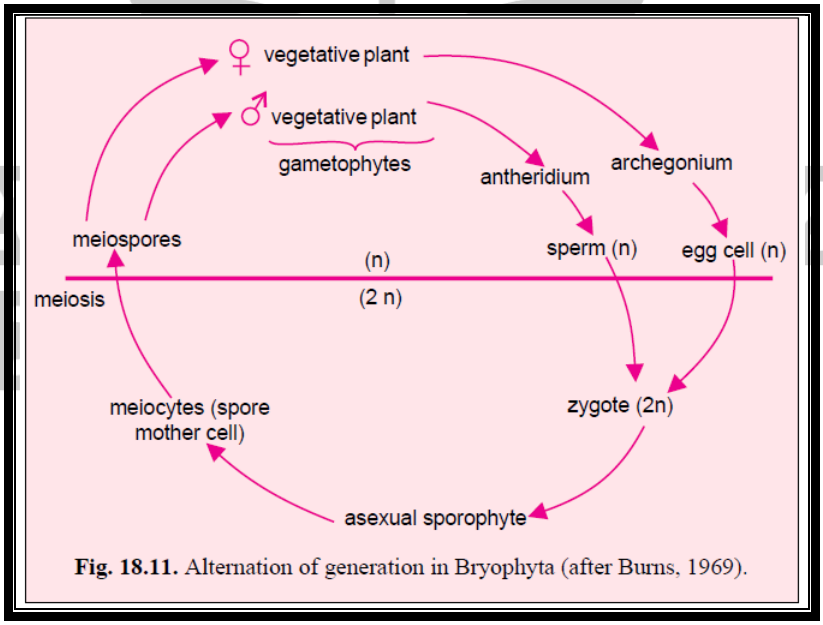


Fig. 18.11. Alternation of generation in Bryophyta (after Burns, 1969).

- It is a division that **occurs in mature diploid reproductive cells (2x)** in which the nucleus divides twice but **chromosome (DNA) replicates only once** to form four haploid cells, each having **half the number of chromosomes present** in **the parent cell**. As it causes a reduction in the **number of chromosomes**, it is known as **reduction division**. Meiosis in a **cell occurs only once**. The so **formed haploid** cells do not further undergo meiosis because there is **no synaptonemal complex** in haploid **genome.**

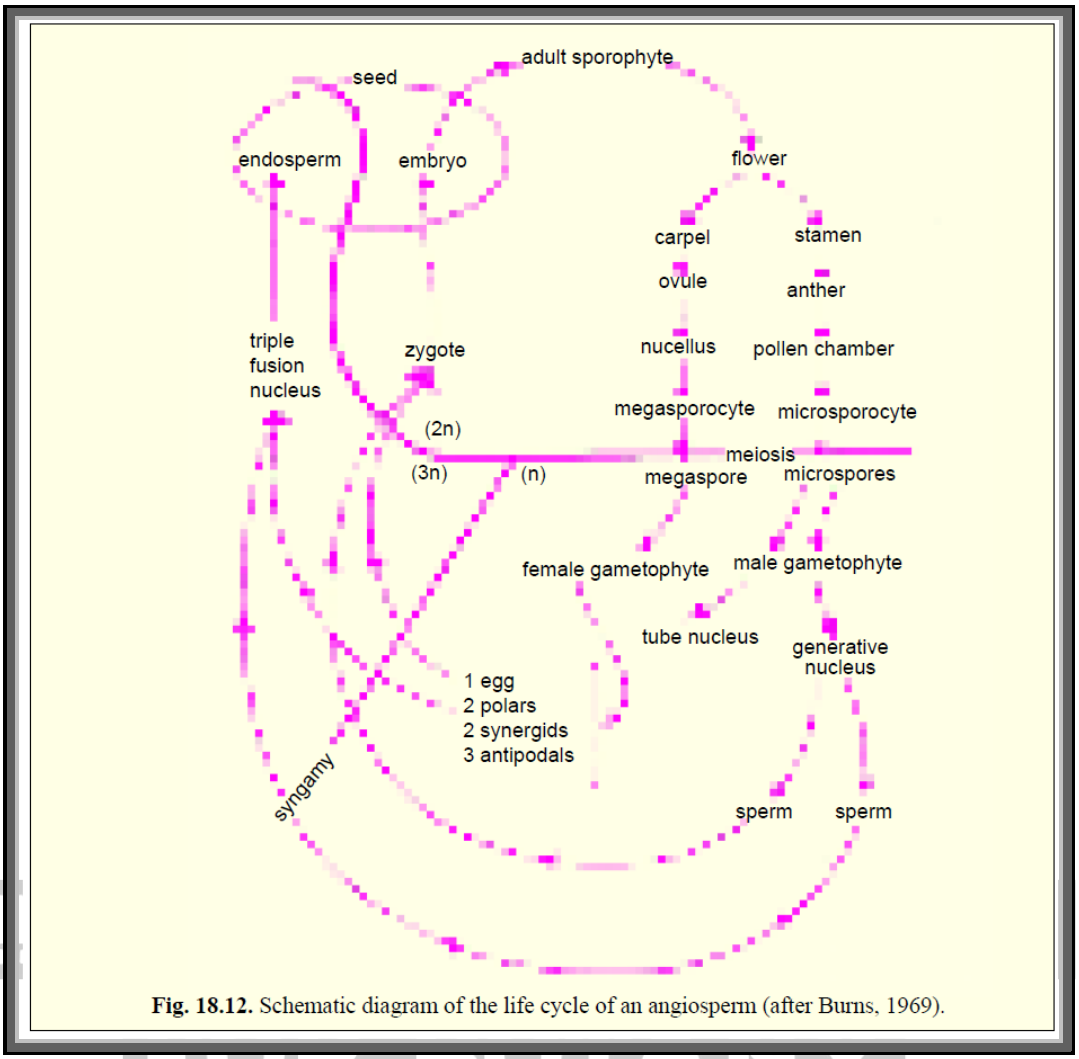
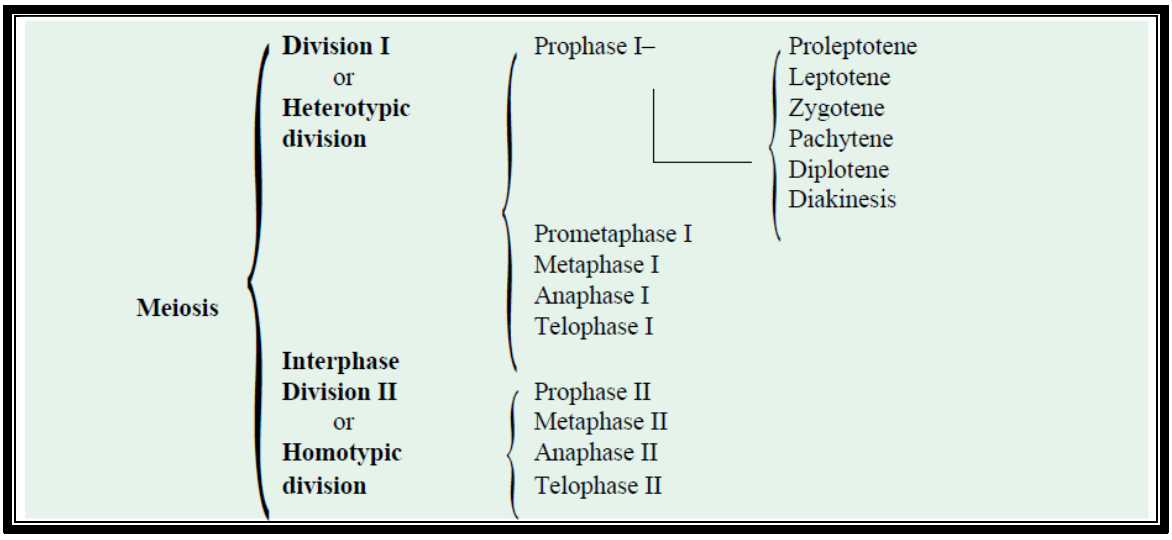


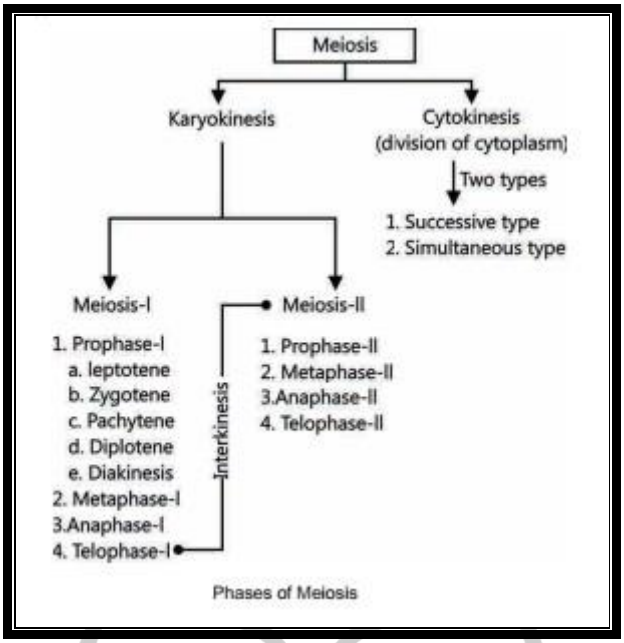
Fig. 18.12. Schematic diagram of the life cycle of an angiosperm (after Burns, 1969).

- It is reported in **diploid germ cells of sex organs** (e.g., **primary spermatocytes** of testes to form male gametes called **spermotozoa and primary oocytes** to form female gametes called ova in animals) and in **pollen mother cells (microsporocytes)** of anther and **megasporocyte of ovules of ovary of flowers in plant to form the haploid spores**. The **study of meiosis in plants** can be done in **young flower buds.**
- **Meiosis I** is **initiated after the parental chromosomes** have replicated to produce identical **sister chromatids during the S phase.**
- Meiosis involves **pairing of homologous chromosomes and recombination** between them.
- Four **haploid cells are formed at the end of meiosis II**.
- **Process of meiosis:** Meiosis is completed in two steps, **Meiosis I and Meiosis II**. Each of them is further divisible into different stages as in mitosis.

Phases of Meiosis

- **Meiosis I: Heterotypic division or reduction division.** It leads to the reduction in chromosome number to half in daughter cells. Division of **chromosome does not occur** in meiosis-I, only **segregation of homologous chromosomes takes place.**
- **Meiosis II: Homotypic division or equational division**. It does not lead to any change in **chromosome number**. Division of the **nucleus occurs twice**, however, the DNA replication and **chromosome division occur only once**.
- Both **the meiotic divisions** occur continuously and each includes the usual stages of the meiosis, viz, **prophase, metaphase, anaphase and telophase**.
- The prophase of **first meiotic division is very significant phase** because the most **cytogenetical events such as synapsis, crossing over**, etc., occur during this phase.
- The prophase is the **longest meiotic phase**, therefore, for the sake of convenience it is divided into six substages, viz., **proleptonema (proleptotene), leptonema (leptotene), zygonema (zygotene), pachynema (pachytene), diplonema (diplotene) and diakinesis**.





Phases of Meiosis

Meiosis I or Heterotypic Division or First Meiotic Division

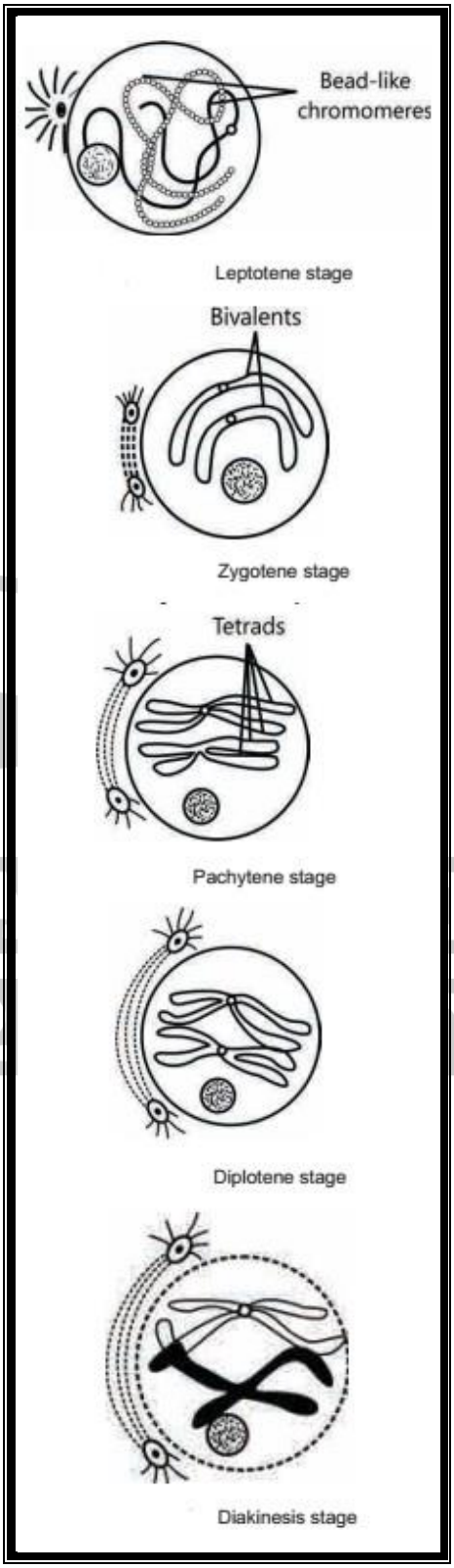
- It results in the formation of **two haploid cells from one diploid cell**. The daughter cells are, therefore, haploid but with **2n DNA content**. It is divided into four phases i.e., **prophase, metaphase, anaphase, telophase**.
- **Meiosis starts** after an **interphase** which is not very different from that of an **intermitotic interphase.**
- During the **premeiotic interphase DNA duplication** has occurred at the **S phase**.
- In the **G2 phase of interphase apparently** there is a decisive change that directs the cell toward **meiosis**, instead of toward **mitosis (Stern and Hotta, 1969)**.
- Further, in the beginning of the **first meiotic division the nucleus of the meiocyte starts** to swell up by **absorbing the water from the cytoplasm and the nuclear volume increases** about three folds. After these changes the cell passes to the **first stage of first** meiotic division which is known as **prophase**.

1) Prophase-I

- It is the longest phase of karyokinesis of meiosis. It is again divisible into five subphases **leptotene➔ zygotene➔ pachytene➔ diplotene ➔ diakinesis**.

Leptotene/Leptonema/ (bouquet stage)

- **Chromosomes** are long thread like with chromomeres (**linear series of darkly stained swollen areas**) on it.



- Chromosomes are long thread like with **chromomeres** on it.
- Volume of **nucleus increases.**
- Chromatin network has half chromosomes from **male and half** from female parent.

- Each **chromosome** has its similar structure or homologue known as **homologous chromosomes** derived from different **parents either paternal or maternal.**
- Chromatin threads are condensed so that they form chromosomes which are longest and **thinnest fibers**. There are bead like structures present on it called as **chromomeres**.
- All the **chromosomes** move **towards centrioles** in **the nucleus**, so **the group** of chromosomes in the **nucleus appears** like a **bouquet** in the animal cell. **(Bouquet stage).**
- The **centriole duplicates** and each daughter centriole migrates towards the opposite poles of the cell. On reaching at the poles, each **centriole duplicates** and, thus, each pole of cell possesses two centrioles of a **single diplosome**.
- **Leptonema chromosomes** have a definite polarization and form loops whose ends are attached to the **nuclear envelope at points near the centrioles**, contained within an **aster**.
- The **telomeric ends of all the chromosomes** converge towards one side of nuclear membrane; therefore they appear **horse shoe shaped**. Such **peculiar arrangement** is termed as **bouquet stage (in animals) and synzetic knot (in plants like Lilium).**
- **Lampbrush chromosome found in oocyte of amphibians** is seen in leptotene.

✱ **Chromosomes** become more **uncoiled and assume** a **long thread-like shape**. The two members of a pair are called **homologous pair.**

Zygotene/Zygonema/ Synaptotene/ (Zipper stage):

- In the **zygotene stage**, the **pairing of homologous chromosomes** takes place. The **homologous chromosomes** which come from the **mother (by ova) and father (by sperm)** are attracted towards each other and their pairing takes place
- **Pairing or "Synapsis"** of **homologous chromosomes takes place** in this stage.
- The synapsis begins at **one or more points along** the length of the homologous chromosomes. **Three types of synapsis** have been recognised.

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

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

3. Localized pairing or Random synapsis. The random type of **synapsis occurs at various points of the homologous chromosomes.**

- There is pairing of **homologous chromosomes (Synapsis).** The **pairs of homologous chromosomes** which are formed here are called as **Bivalents** or **Tetrads** and **spiralization** become shorter and **thicker** are clearly identified in **the next stage.**
- The **paired homologous chromosomes are joined by a roughly 0.2-µm thick**, protein containing framework called a **synaptonemal complex (SC)**. **Synaptinemal complex** is seen between the **homologous chromosomes** which were discovered by "***Moses*" (1953).**
- **Synaptonemal complex** along the whole length of the **paired chromosomes** and is usually anchored at **either end to the nuclear envelope**.
- **Synaptonemal complex** helps to stabilize the **pairing of homologous chromosomes** and to facilitate the **cytogenetical activity**, called **recombination or crossing over (occurring during pachynema)**.
- **Synaptonemal complex** is **not found in those organisms** in which **crossing over** does **not** occur (e.g., **the male fruitfly, Drosophila melanogaster (Burns and Bottino, 1989).**
- It has three thick **lines made up** of **DNA and proteins**. The complete **set helps** in the pairing of the DNA.
- Pairing of **homologous chromosomes in a zipper-like fashion.**

Pachytene/Pachynema (thick thread) (Tetrad stage):

- In the **tetrad**, two similar chromatids of the same chromosome are called **sister chromatids** and those of **two homologous** chromosomes are termed **non-sister chromatids.**
- **Crossing over exchange of segments** between **non-sister chromatids of homologous** chromosome occurs at this stage.
- It takes place by **breakage and reunion of chromatis** segments. Breakage called **nicking** is assisted by an enzyme e**ndonuclease** and **reunion termed annealing** is added by an **enzyme ligase**. **Breakage and reunion hypothesis** proposed by **Darlington (1937).**
- **Chromatids of pachytene** chromosome are attached with **centromere.**
- A tetrad consists of two sets of homologous chromosomes each with two chromatids. Each tetrad has **four kinetochore (two sister and two homologous).**

- A number of electron dense bodies about 100 *nm* in diameter are seen at irregular intervals within the centre of the **synaptonemal complex**, **known as recombination nodules.**
- In the **pachynema stage the pair of chromosomes** become twisted spirally around each other and **cannot be distinguished** separately.
- In the **middle of the pachynema stage each homologous chromosome spilts** lengthwise to **form two chromatids**.
- Actually, the **doubling of the DNA molecule strands** which is necessary for the subsequent **duplication of chromosomes** occurs earlier, before the beginning of **meiotic prophase**.
- Through the earlier part of the **meiotic prophase**, however, the **DNA molecule in each chromosome** behaves as a **single body**.
- In the **pachynema stage,** this is **now changed**, the **two chromatids** of each **chromosome containing half of the DNA** present in the **chromosome at start**, **become partially independent of one another**, although they still continue to be linked together by **their common centromere**.
- Each **synaptonemal pair** at this point is **commonly referred to as bivalent or dyads** because it consists of **two visible chromosomes**, or as a **quadrivalent or tetrad** because of the four visible **chromatids.**
- During **pachynema stage During pachynema stage** an important genetic phenomenon called **" crossing over"** takes place.
- The crossing over involves reshuffling, redistribution and mutual exchange of hereditary material of **two parents between two homologous chromosomes**.
- According to recent views, **one chromatid of each homologous chromosome of a bivalent** may divide transversely by the help of an **enzyme the endonuclease** which is **reported to increase** in the nucleus during this stage by **Stern and Hotta (1969).**
- After the **division of chromatids,** the interchange of chromatid segments takes place between the **nonsister chromatids of the homologous chromosomes**.
- The broken chromatid segments are united with the chromatids due to the presence of an **enzyme, ligase (Stern and Hotta, 1969)**.
- The **process of interchange of chromatin** material between one non-sister chromatid of each **homologous chromosome** is known as the **crossing over** which is accompanied by the **chiasmata formation**

- **Stern and Hotta (1969)** have reported that during the **pachytene and zygotene stage,** synthesis of **small amount of DNA takes** place. This **DNA amount** is utilized in the repairing of broken DNA molecule of the **chromatids during the chiasmata** formation and **crossing over.**
- The **nucleolus** remains **prominent up to this stage** and it is found to be associated with the **nucleolar** organizer region of the chromosome
- In the **tetrad, two similar chromatids** of the **same chromosome** are called **sister chromatids while chromatids** belonging to **different chromosomes of the homologous pairs** are termed as **non-sister chromatids.**
- **Crossing over** is exchange of segments between n**on-sister chromatids of homologous chromosomes occurs at this stage**.
- It takes place by **breakage and reunion of chromatid segments**. Breakage called **nicking** is assisted by an enzyme **endonuclease** and reunion termed annealing is done by an **enzyme ligase.**
- A **tetrad consists of two sets** of homologous chromosomes each with **two chromatids.**
- **Chromatids of pachytene** chromosomes are attached with **centromere**. Each tetrad has **four kinetochores (two sisters and two homologous).**
- A number of electron dense bodies about 100 nm in diameter are seen at irregular intervals within the centre of the **synaptonemal complex** known as **recombination nodules**. These are believed to be sites having **multienzyme recombinase complex** required for crossing over. This is an **enzyme-mediated process** and the enzyme is **recombinase**.
- Both the **chromatid**s in the chromosome are **clear and distinct** and now the pair or **bivalent** is found as a **tetrad**

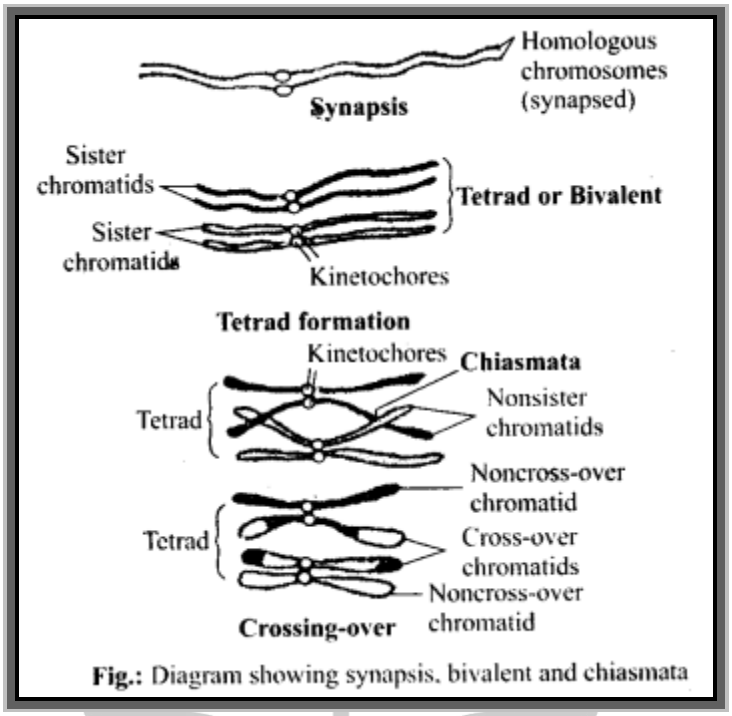


Fig.: Diagram showing synapsis, bivalent and chiasmata

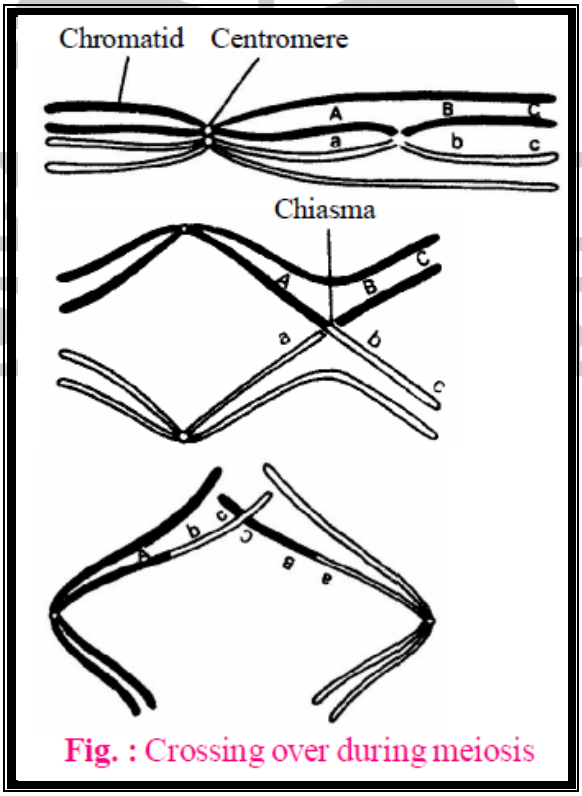


Fig. : Crossing over during meiosis

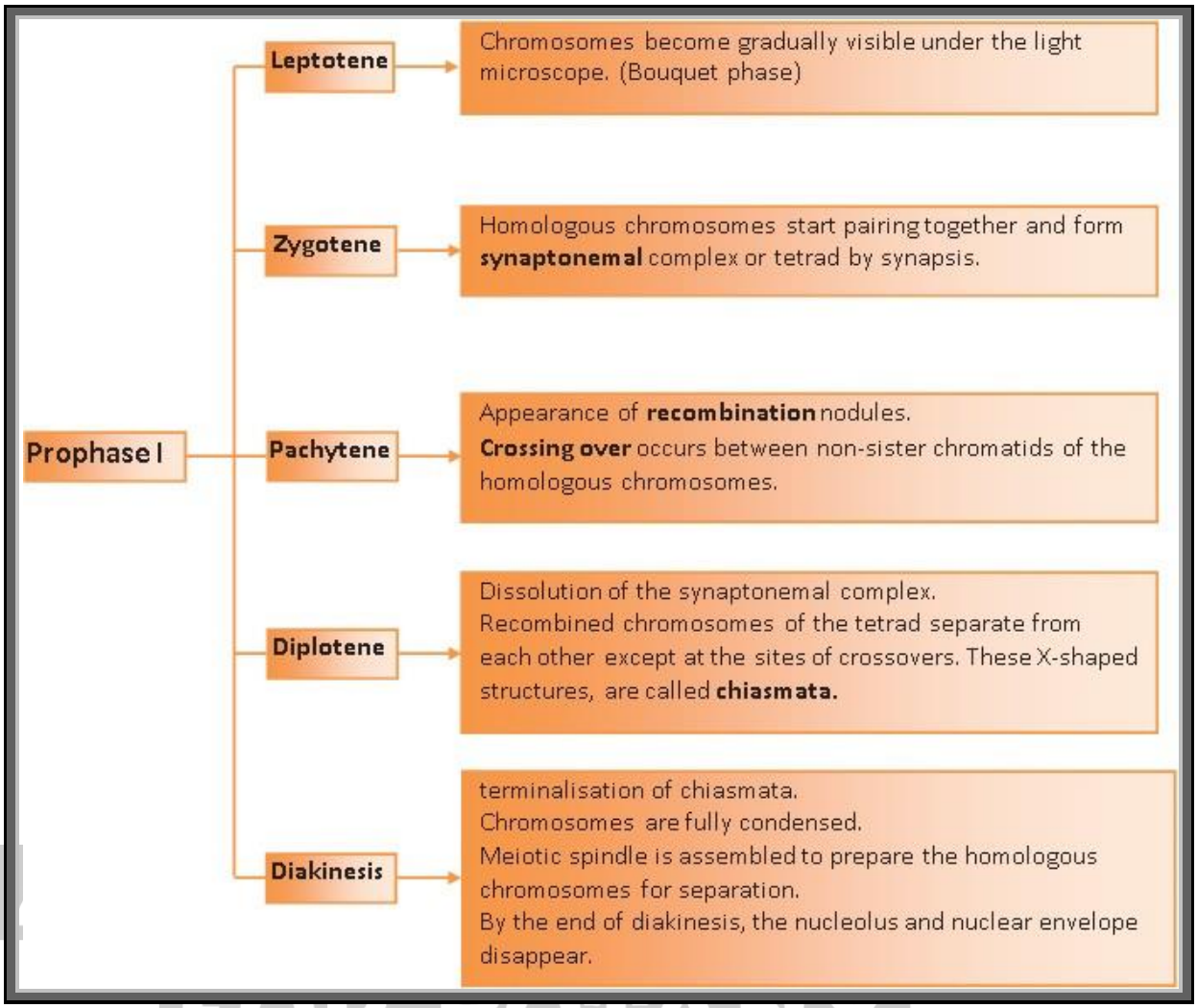
Diplotene/Diplonema

✦ At this stage, the paired chromosomes begin to **separate (desynapsis)** and **terminalisation starts**.

- The diplotene starts with the **dissolution** of the **synaptonemal complex**. There is also the tendency in the bivalent **recombined homologous chromosomes** to separate from each other while still joint at the cross-overs.
- These X-shaped structures **formed and Homologous chromosomes** move apart and remain attached to one another at specific points called **chiasmata**.
- The diplotene can last for months or years, in **some vertebral oocytes which** are called as **dictyotene**.
- **Cross** is formed at the place of crossing over between **non-sister chromatids.**
- At least **one chiasma is formed** in each bivalent.
- This stage remains as such for a **long time**. For example, all the **oocytes of human female reach the diplotene stage in the fifth month of foetus** and remain so for many years till ovulation is to occur.
- In oocytes **of many fishes, amphibians,** reptiles and birds, the **bivalents elongate and become converted into lampbrush chromosomes** for synthesis of specific biochemicals.

Diakinesis

- **Terminalization of chiasmata** takes place.
- The chromosomes get fully condensed and then the meiotic spindle assembles to prepare the **homologous chromosomes** which separate.
- When **diakinesis ends**, the **nucleolus disappears** and the **nuclear envelope breaks** down. **Diakinesis ends** and **metaphase starts.**
- **Chromosomes recondense** and **tetrad moves to the metaphase plate**.
- Formation of **spindle occurs.**
- When the **diakinesis of prophase-I** is completed then **cell enters into metaphase-I.**



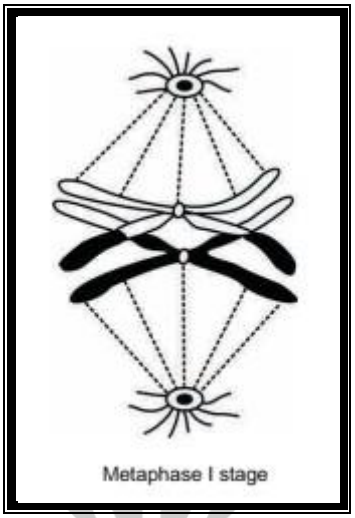
2).Metaphase I:

- **Bivalents** form **metaphase plate** after arranging on the equator of the cell such that the **centromeres** face the poles while arms face the **equator. Spindle fibres** now attach to the pair of **homologous chromosomes**.
- Each homologous chromosome has two **kinetochores and both the kinetochores** of a chromosome are joined to the chromosomal or tractile fibre of same side.
- There are in all **3 types of spindle fibres** in the cell:

  1. **Chromosomal / Kinetochore Spindle fibres**

  2. Supporting / Continuous Spindle fibres

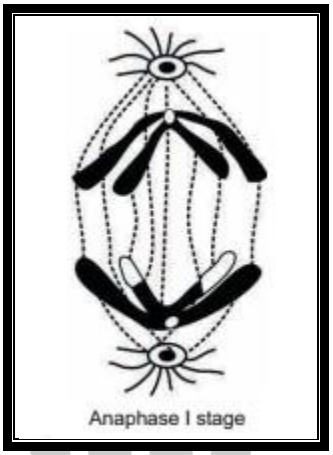
  3. Interzonal Spindle fibres.



Metaphase I stage

3. Anaphase I:

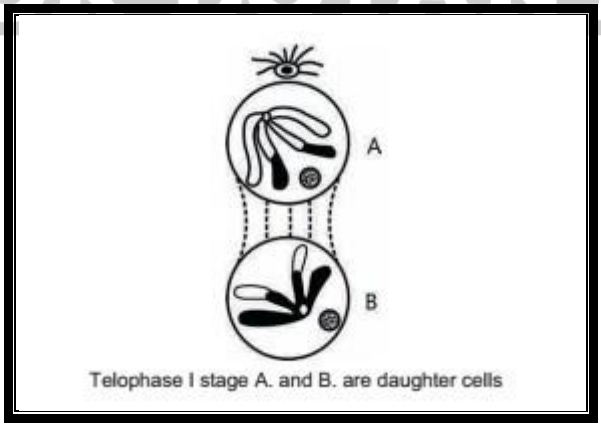
- It involves **separartion of homologous chromosomes** which start moving opposite poles so each tetrad is divided into **two daughter dyads**. So anaphase-I involves the reduction of chromosome number, this is called **disjunction.**
- The **shape of separating chromosomes** may be **rod or J or V-shape** depending upon the **position of centromere**.
- **Segregation of mendalian factors or independent assortment of chromosomes** takes place. In which the **paternal and maternal chromosomes** of each homologous pair segregate during **anaphase-I** which introduces genetic variability.
- There is a **contraction of chromosomal fibres** and expansion of **interzonal fibres**. The **homologous chromosomes** move towards the **opposite poles** after they **segregate** from each other.
- It involves **separation of homologous chromosomes** which start moving towards opposite poles so that each tetrad is divided into **two daughter dyads**. So, **anaphase-I** involves the **reduction of chromosome number**, this is called **disjunction**.
- **Segregation of Mendelian** factors or **independent assortment of chromosomes** takes place, in which the **paternal and maternal chromosomes** of each homologous pair segregate during **anaphase-I** thus, introducing genetic variability.
- **Anaphase I** has segregation or disjunction of **the homologous chromosomes**. There is **no division of centromere**.



Anaphase I stage

4).Telophase I:

- The **nuclear membrane and nucleolus reappear**. This is followed by the cytoplasm division or **the cytokinesis and two daughter** cells together are called as **diad** of cells.
- The **chromosomes** in some situations undergo some **dispersion**, and are thus fail to reach the extremely extended state of the **interphase nucleus**.
- Two daughter nuclei are formed but the **chromosome number** is half the chromosome **number of mother cell.**
- After **telophase I, cytokinesis** may or may not occur.
- In case of **Trillium telophase** I and interphase I do not occur and the anaphase I is followed by prophase II directly.



Telophase I stage A. and B. are daughter cells

- The connecting stage of the two **meiotic divisions** is called as **interkinesis** which is short in duration. DNA does not replicate in this stage. **Interkinesis** ends with the start of **prophase II**, which is **simpler than prophase I.**

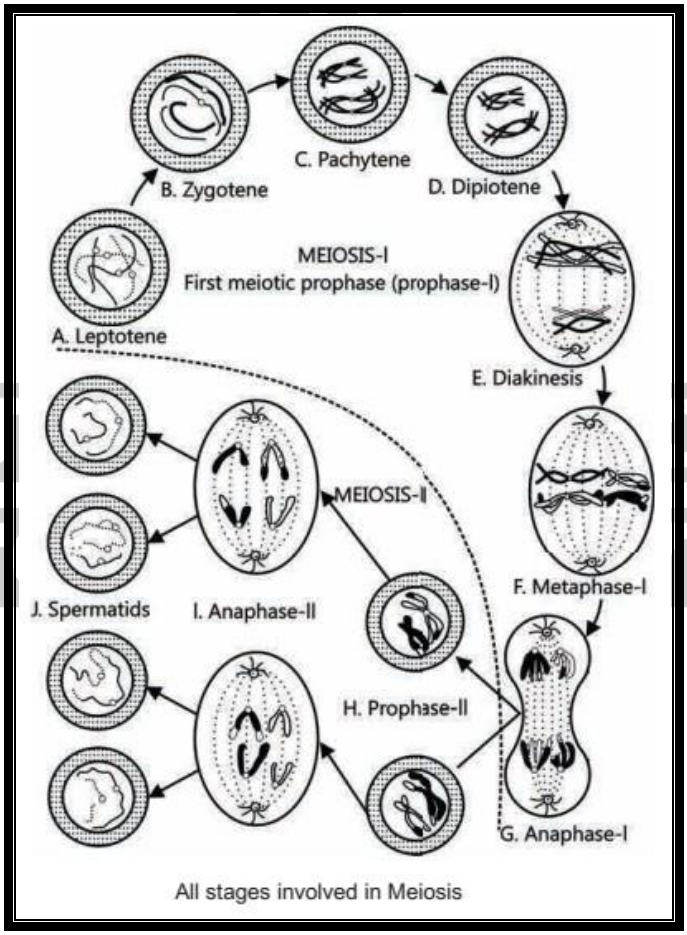
Interkinesis (Intrameiotic interphase)

- Generally there is **no interphase between meiosis-I and meiosis-II**.

- ✦ A brief interphase called **interkinesis, or intrameiotic interphase** occurs. There is no replication of **chromosomes because chromosomes** are already in replicated state.
- ✦ Centrosome may replicate in **animal cells.**

Significance of Meiosis-I

- ✦ It separates the **homologous chromosomes to reduce the chromosome** number to the haploid state, a necessity for sexual reproduction.
- ✦ It introduces variation by forming **new gene combinations** through crossing over and random assortment of **paternal and maternal chromosomes.**

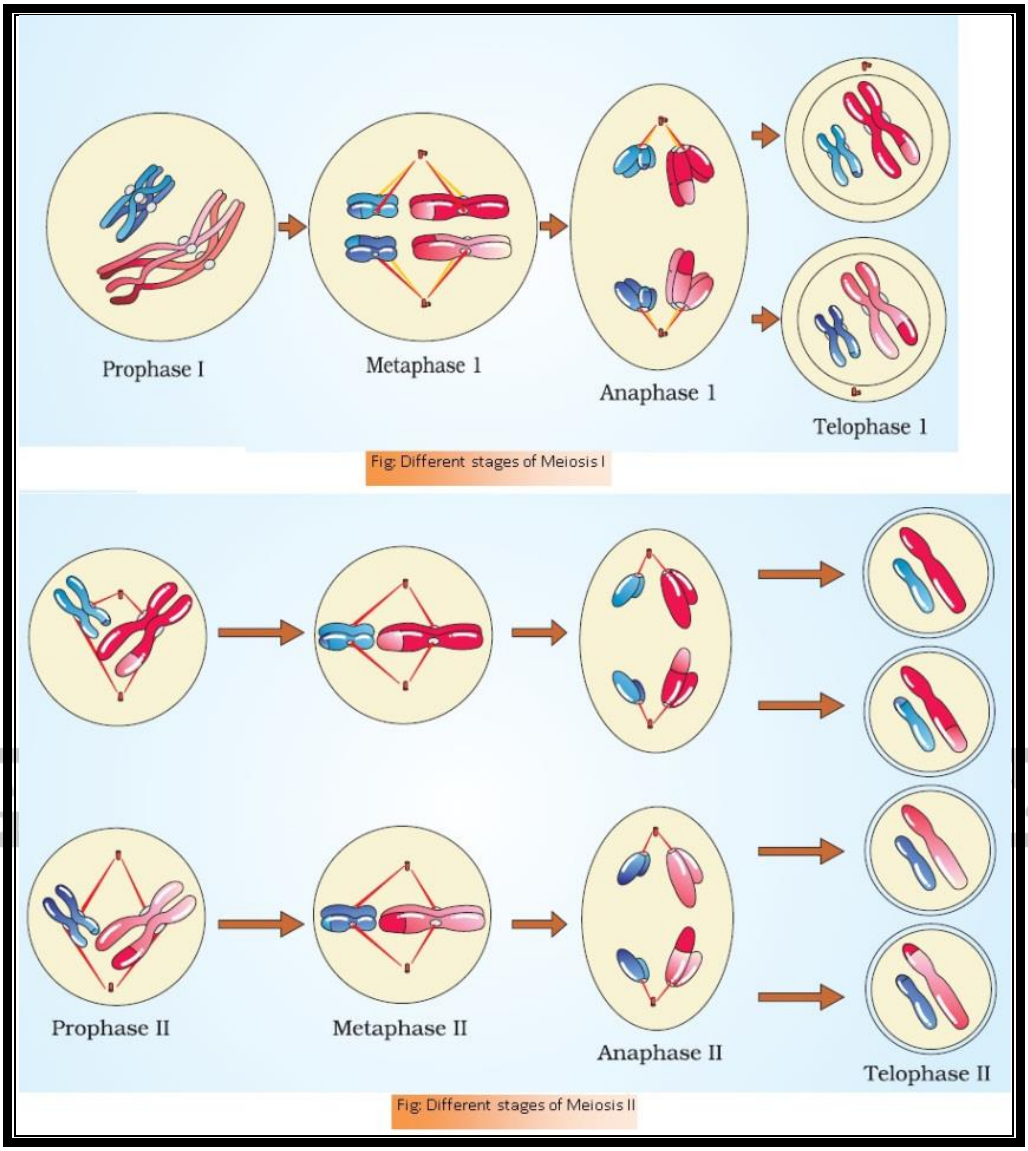


All stages involved in Meiosis

Meiosis-II or Homotypic or Second Meiotic Division

- It is also called **equational or homotypical division** because the number of **chromosomes remain same as after meiosis-I.**
- It involves the separation of **two chromatids of each chromosome** and their movement to separate cells.

- It is divided into four phases - **Prophase-II, Metaphase-II. Anaphase-II and Telophase-II.**



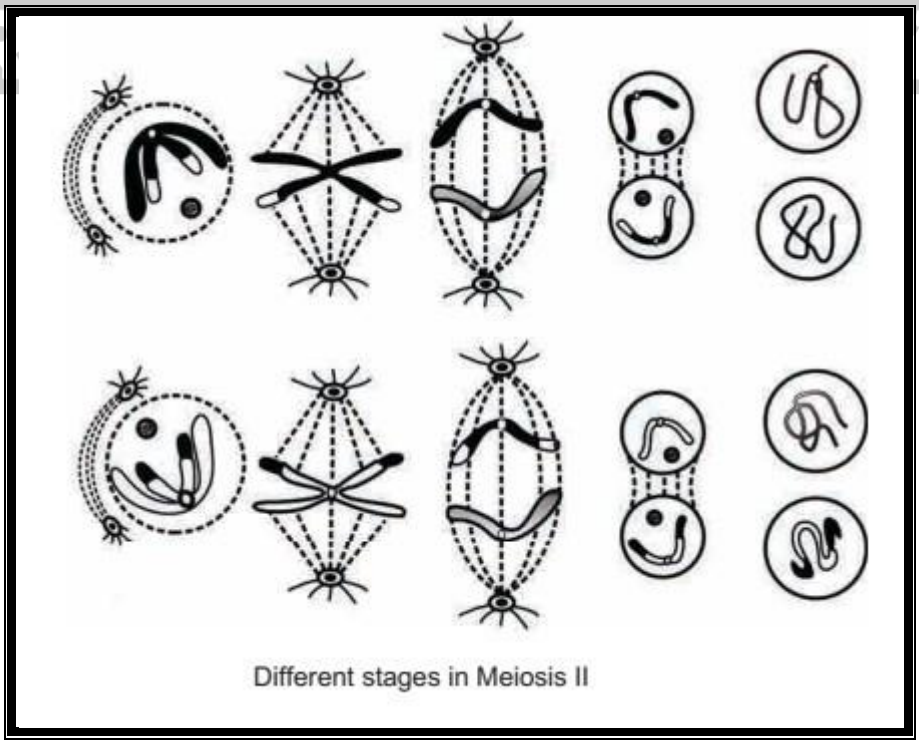
<u>Karyokinesis-II :</u> It involves the separation of two chromatids of each chromosome and their movement to separate cells. It is divided in four **phases, Prophase-II, Metaphase-II. Anaphase-II and Telophase-II.**

- ✦ **Almost all the changes** of **Karyokinesis-II resembles to mitosis which involves**.
- ✦ It starts just after end **of telophase I.**
- ✦ Each daughter **cell (nucleus) undergoes mitotic division**.
- ✦ It is exactly similar to mitosis.
- ✦ At the end of **process, cytokinesis** takes place.

- **Four daughter cells are formed** after completion.
- The **sister kinetochores of one chromosome are separated.**
- The four daughter cells **receive one chromatid each of the tetravalent.**
- **Centromere divide** at anaphase II.
- **Spindle fibres contract** at prophase II.

1).Prophase-II

- In contrast to **meiosis I, meiosis II** resembles a **normal mitosis.**
- The nuclear membrane **disappears by the end of prophase II.**
- The chromosomes **again become compact.**
- **Meiosis II is an intermediate step** which starts **immediately after cytokinesis**, and before the **chromosomes have** elongated fully.
- **Meiosis II is similar to a normal mitosis, in contrast to meiosis I**. The nuclear membrane disappears and **chromosomes are compact again at the end of this stage**.



Different stages in Meiosis II

2). Metaphase-II

- At this stage, the chromosomes align at the **equator and microtubules** from opposite poles of the spindle get attached to **kinetochores of sister chromatids**.
- The **chromosomes get aligned at the equator** while at the opposite poles the spindle **microtubules** are in close contact with the **kinetochores of the sister chromatids.**

3).Anaphase-II

- The **centromeres of all the chromosomes undergo longitudinal splitting.**
- It begins with the **simultaneous splitting of the centromere of each chromosome** (which was holding the sister chromatids together), allowing them to move towards **opposite poles of the cell**.
- The simultaneous **splitting** of the **chromosome centromere** occurs **(which was holding the sister chromatids together)**, which moves the **chromosomes** toward the **opposite poles** of the cell.

4) Telophase-II

- Meiosis ends with **telophase II**, in which **chromosomes** once again get enclosed by a nuclear envelope; **cytokinesis** follows **resulting in the formation of tetrad of cells - four haploid daughter cells.**
- **Cytokinesis-II** is always **present and occurs by cell furrow formation in animal cells** and **cell plate formation in plant cells.**
- The **two sets of chromosomes are again** enclosed in a **nuclear envelope** and **cytokinesis** begins. There is a **formation of tetrads (four haploid daughter cells).**

Cytokinesis-II :

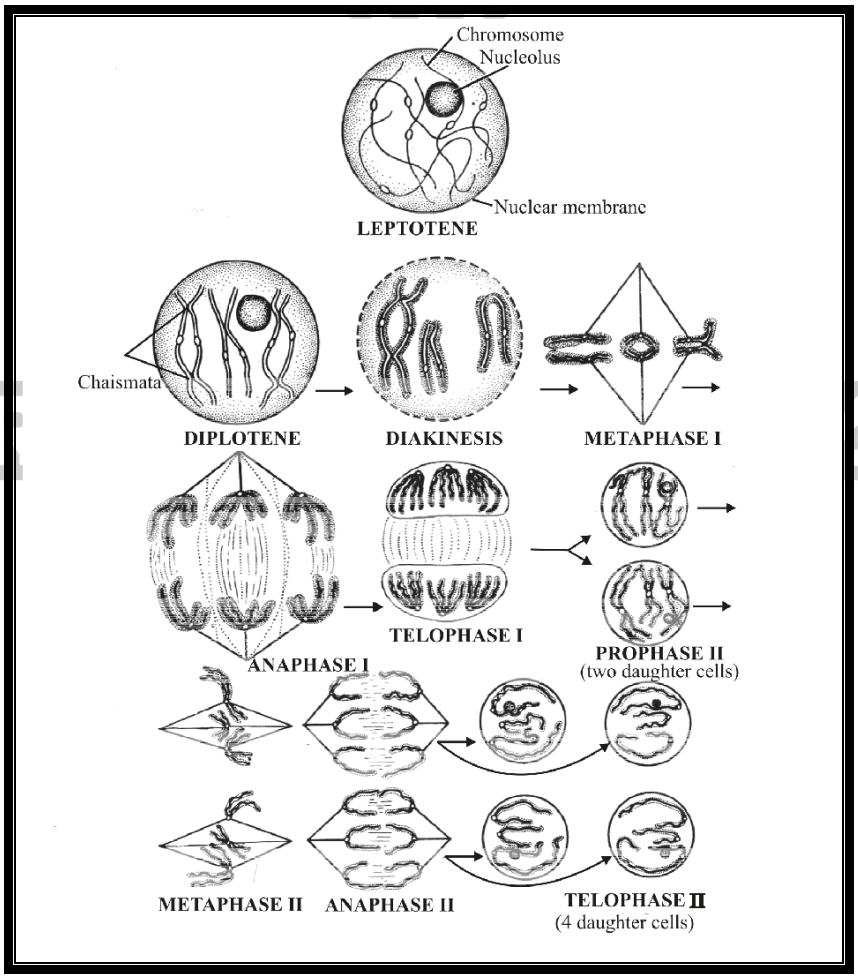
- It is **always present and occurs** by **cell furrow formation in animal cell** and cell plate formation in **plant cell.**
- So by **meiosis, a diploid parental cell divides** twice forming four haploid gametes or sex cells, each having half the **DNA amount than that of the parental cell** and one-fourth of **DNA present** in the cell at the **time of beginning of meiosis.**

Significance of Meiosis-II

- Constancy of **chromosome number in successive generation** is brought **by process.**
- Chromosome **number becomes half during meiosis**.

- It helps in **introducing variations and mutation**.
- It brings **about gamete formation.**
- It **maintains** the **amount of genetic informative material.**
- **Sexual reproduction** includes **one meiosis and fusion.**
- The **four daughter cells** will have different **types of chromatids.**
- It maintains the **amount of genetic material.**

Stages in Meiosis



Types of meiosis:

On the basis of **time and place, meiosis is of three types**

1. **Gametic/Terminal meiosis**
2. **Sporogenetic Meiosis/ Intermediate meiosis**

3. **Zygotic or Initial Meiosis**

- **Gametic/Terminal meiosis:** In many **protozoans, all animals and some lower plants;** meiosis takes place **before fertilization** during the formation of gametes. Such **meiosis** is described as **gametic or terminal**.

Gametic meiosis

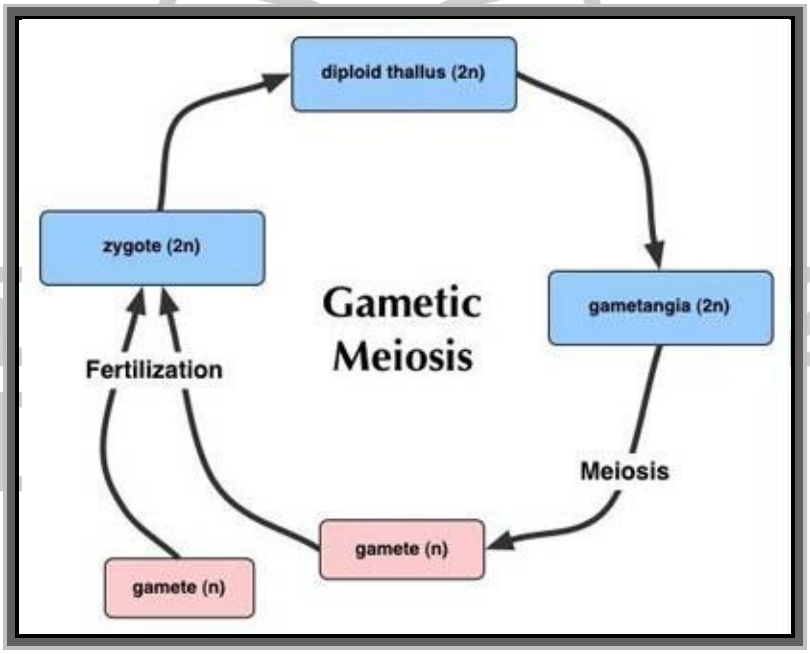
Meiosis occurs in the **gametangia.**

Vegetative phases are **diploid.**

Gametes are the only haploid cells. Characteristic of **most animals.**

This is the familiar life cycle type, **common in animals.**

Its not very common in **algae or fungi, never found in bryophytes or vascular plants**



- **Zygotic or Initial Meiosis:** In **fungi, certain protozoan groups**, **and some algae** fertilization is immediately followed by **meiosis in the zygote, and the resulting adult organisms are haploid**. Such a **meiosis is said to be zygotic or initial.** This type of life cycle with haploid adult and **zygotic meiosis** is termed the haplontic cycle.

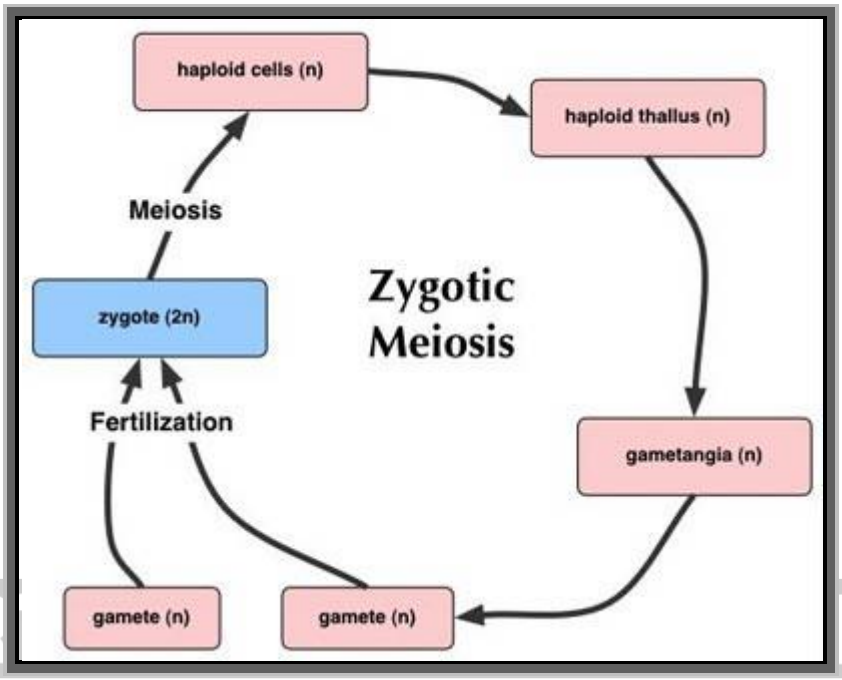
Zygotic meiosis.

Meiosis occurs in the **zygote.**

Vegetative phases are **haploid.**

Zygotes is the **only diploid cell**.

The most common type in **algae and fungi**



✦ **Sporogenetic Meiosis/ Intermediate meiosis**

- **Diploid sporocytes or spore mother cells of sporophytic plant**, undergo meiosis to form the haploid spores in the **sporangia.**
- **Haploid spore germinates to form haploid gametophyte** which produces the haploid gametes by mitosis.
- **Haploid gametes fuse to form diploid zygote** which develops into diploid sporophyte by mitotic divisions. e.g. in **higher plants like pteridophytes, gymnosperms and angiosperms.**

Sporic meiosis

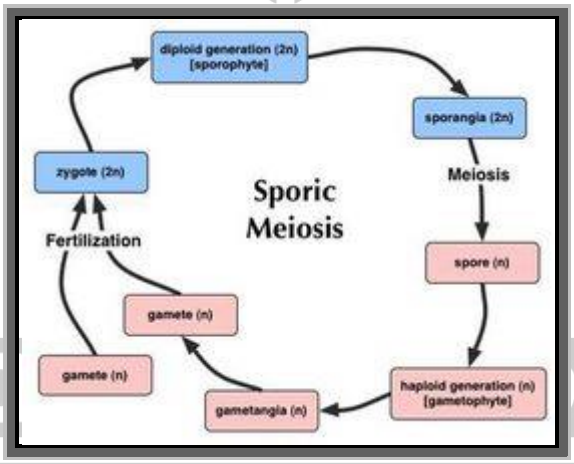
Meiosis occurs in **sporangia**

Haploid spores develop into **haploid vegetative stage (gametophyte generation)**

Diploid zygotes develop into diploid vegetative stage **(sporophyte generation)**

If the two generations are morphologically similar, the organism is said to have ***isomorphic*** **alternation** of generations. This type is **characteristic of some algae and a few fungi.**

If the two generations are morphologically different, the organism is said to have ***heteromorphic*** **alternation of generations**. This type is characteristic of **some algae, all bryophytes and all vascular plants**



Differences between Mitosis I and Meiosis II :

Meiosis I	Meiosis - II
1. It is heterotypic or reductional division.	1. It is homotypic or equational division.
2. The chromosomes remain in the replicated state.	2. The two chromatids of a replicated chromosome separate.
3. The number of chromosomes is reduced to half, *i.e.*, from diploid to haploid state.	3. The number of chromosomes remains the same, *i.e.*, from haploid to haploid state.
4. Crossing over occurs which makes the two chromatids of a chromosome different.	4. The genetically different chromatids of a chromosome are separated.
5. It is complicated and takes a long time.	5. It is simple and is of shorter duration.

COMPARISON BETWEEN MITOSIS AND MEIOSIS

Mitosis	Meiosis
1. Mitosis occurs continuously in the body or somatic cells.	1. Meiosis occurs in the germ cells (the cells of the testes or ovaries) during the process of gametogenesis.
2. The whole process completes in one sequence or phase.	2. The whole process completes in two successive divisions which occur one after the other.
Prophase	
3. The prophase is of short duration and includes no substage.	3. The prophase is of longer duration and it completes in six successive stages, *viz*., proleptotene, leptotene, zygotene, pachytene, diplotene and dikinesis.
4. The homologous chromosomes (paternal and maternal) duplicate into two chromatids. The two chromatids separate and form new chromosomes. Each daughter cell receives the daughter chromosome or chromatids of each homologous chromosome and, thus, having the chromosome number like the parental cells.	4. Out of two homologous chromosomes only one type of chromosome either maternal or paternal moves to the daughter cells. A daughter cell, thus, receives only a maternal or paternal chromosome of the homologous pair and the number of chromosomes remain half than the paternal cells.
5. No pairing or synapsis takes place between the homologous chromosomes.	5. Pairing or synapsis occurs between the homologous chromosomes.
6. Duplication of chromosomes takes place in the early prophase.	6. Duplication or splitting of chromosomes takes place in the late prophase (pachytene stage).
7. No chiasma formation or crossing over takes place.	7. Chiasma formation or crossing over takes place.
8. The exchange of the genetic material between the homologous chromosomes does not occur.	8. The exchage of the genetic material takes place between the non-sister chromatids of homologous chromosomes.
Metaphase	
9. The chromatids occur in the form of dyads.	9. The chromatids of two homologous chromosomes occur as the tetrads.
10. The centromeres of the chromosomes remain directed towards the equator and the arms of the chromosomes remain directed towards the poles.	10. The centromeres of the chromosomes remain directed towards the poles and the chromosomal arms remain directed towards the equator.
Anaphase	
11. The chromosomes are the monads, *i.e*., having single chromatid.	11. The chromosomes are the diads, *i.e*., having two chromatids and single centromere.
12. The chromosomes are long and thin.	12. The chromosomes are short and thick.
Telophase	
13. The telophase always occurs.	13. The first telophase is sometimes omitted.
Significance	
14. The chromosome number in each daughter cell remains the same like the parent cell.	14. In meiotic division the chormosome number is reduced to half in the daughter cells than the parental cells.
15. A diploid cell produces four diploid cells by a mitotic division.	15. A diploid cell produces four haploid cells by a meiotic division.

Difference between cell Mitosis and Meiosis

S.No	Characters	Mitosis	Meiosis
I. General			
(1)	**Site of occurrence**	Somatic cells and during the multiplicative phase of gametogenesis in germ cells.	Reproductive germ cells of gonads.
(2)	Period of occurrence	Throughout life.	During sexual reproduction.
(3)	Nature of cells	Haploid or diploid.	Always diploid.
(4)	Number of divisions	Parental cell divides once.	Parent cell divides twice.
(5)	Number of daughter cells	Two.	Four.
(6)	Nature of daughter cells	Genetically similar to parental cell. Amount of DNA and chromosome number is same as in parental cell.	Genetically different from parental cell. Amount of DNA and chromosome number is half to that of parent cell.
II. Prophase			
(7)	Duration	Shorter (of a few hours) and simple.	Prophase-I is very long (may be in days or months or years) and complex.
(8)	Subphases	Formed of 3 subphases :	Prophase-I is formed of 5

		early-prophase, mid-prophase and late-prophase.	subphases: leptotene, zygotene, pachytene, diplotene and diakinesis.
(9)	Bouquet stage	Absent.	Present in leptotene stage.
(10)	Synapsis	Absent.	Pairing of homologous chromosomes in zygotene stage.
(11)	Chiasma formation and crossing over.	Absent.	Occurs during pachytene stage of prophase-I.
(12)	Disappearance of nucleolus and nuclear membrane	Comparatively in earlier part.	Comparatively in later part of prophase-I.
(13)	Nature of coiling	Plectonemic.	Paranemic.
III. Metaphase			
(14)	Metaphase plates	Only one equatorial plate	Two plates in metaphase-I but one plate in metaphase-II.
(15)	Position of centromeres	Lie at the equator. Arms are generally directed towards the poles.	Lie equidistant from equator and towards poles in metaphase-I while lie at the equator in metaphase-II.
(16)	Number of	Two chromosomal fibre	Single in metaphase-I

	chromosomal fibres	join at centromere.	while two in metaphase-II.
IV. Anaphase			
(17)	Nature of separating chromosomes	Daughter chromosomes (chromatids with independent centromeres) separate.	Homologous chromosomes separate in anaphase-I while chromatids separate in anaphase in anaphase-II.
(18)	Splitting of centromeres and development of inter-zonal fibres	Occurs in anaphase.	No splitting of centromeres. Inter-zonal fibres are developed in metaphase-I.
V. Telophase			
(19)	Occurrence	Always occurs	Telophase-I may be absent but telophase-II is always present.
VI. Cytokinesis			
(20)	Occurrence	Always occurs	Cytokinesis-I may be absent but cytokinesis-II is always present.
(21)	Nature of daughter cells	2N amount of DNA than 4N amount of DNA in parental cell.	1 N amount of DNA than 4 N amount of DNA in parental cell.
(22)	Fate of daughter cells	Divide again after interphase.	Do not divide and act as gametes.

VII. Significance			
(23)	Functions	Helps in growth, healing, repair and multiplication of somatic cells. Occurs in both asexually and sexually reproducing organisms.	Produces gametes which help in sexual reproduction.
(24)	Variations	Variations are not produced as it keeps quality and quantity of genes same.	Produces variations due to crossing over and chance arrangement of bivalents at metaphase-I.
(25)	In evolution	No role in evolution.	It plays an important role in speciation and evolution.

Cell cycle checkpoint

- **Cell cycle is a sequential** step that taking place in a **cell leading to the accurate duplication of genetic materials (DNA),** precise separation of replicated genetic materials and passing them in to **two daughter cells.**
- The **process of cell cycle** is very critical in each cell, thus it operate strictly under strong surveillance to prevent any mistakes.
- This **strong surveillance system in the cell to monitor the cell cycle progression** itself is called **cell cycle checkpoints.**
- **Checkpoints** are surveillance mechanisms that **halt the progress of cell cycle** if
- Any of the chromosomal DNA is damaged or critical cellular processes, such as **DNA replication during S phase or chromosome alignment during M phase**, have not been

properly completed. Thus **cell cycle checkpoints** ensure that the various events in the cell **cycle progression occur** accurately and in correct order.

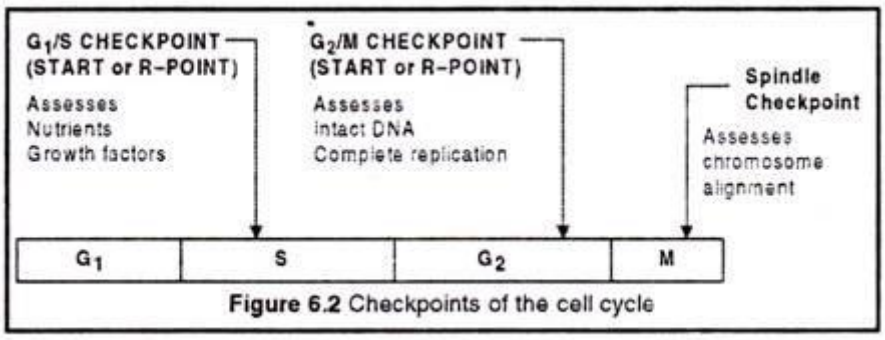


Figure 6.2 Checkpoints of the cell cycle

- In this **post we will discuss the three types of cell cycle checkpoints** that operate in eukaryotic cells **during cell cycle progression**.
- Progression of **cell cycle in eukaryotes** is highly regulated in certain points. These critical regulatory points of cell cycle are called **cell cycle checkpoints.**

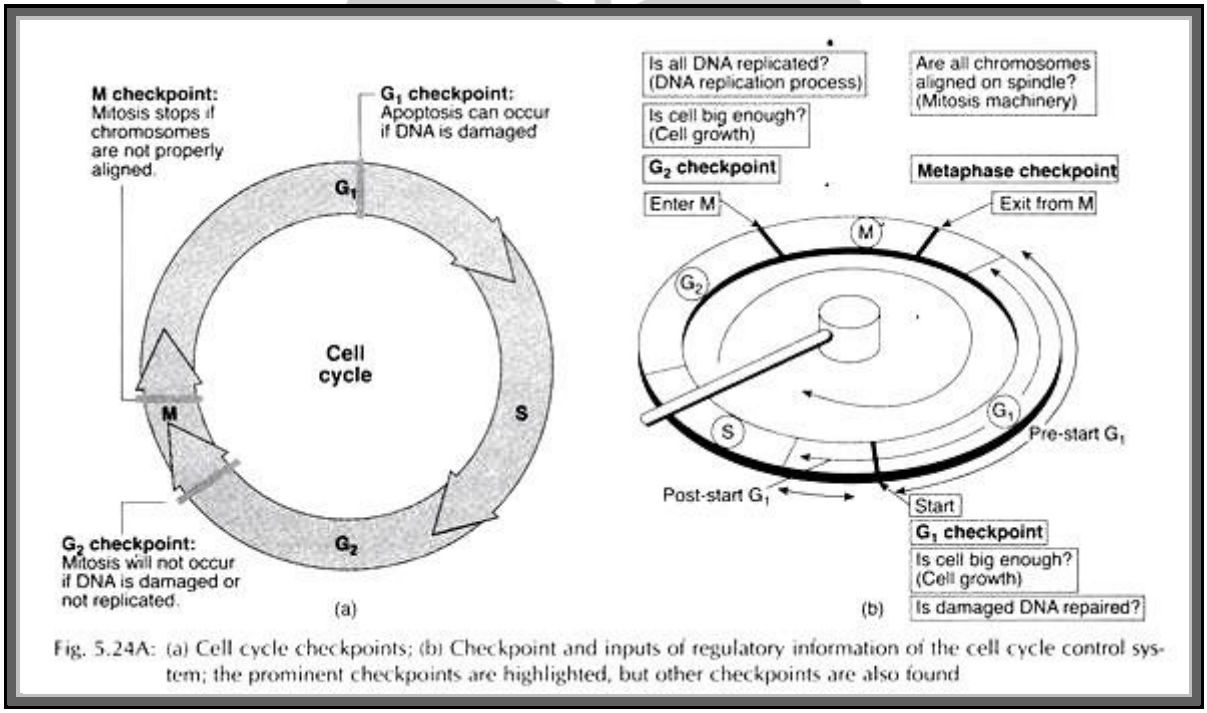
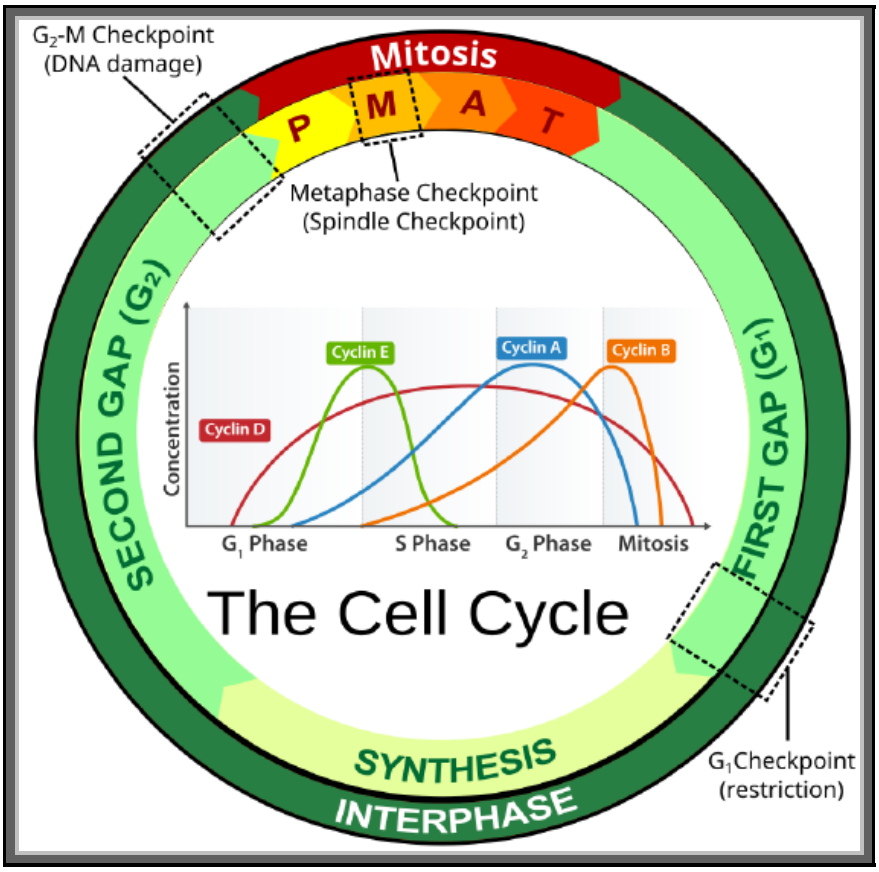


Fig. 5.24A: (a) Cell cycle checkpoints; (b) Checkpoint and inputs of regulatory information of the cell cycle control system; the prominent checkpoints are highlighted, but other checkpoints are also found



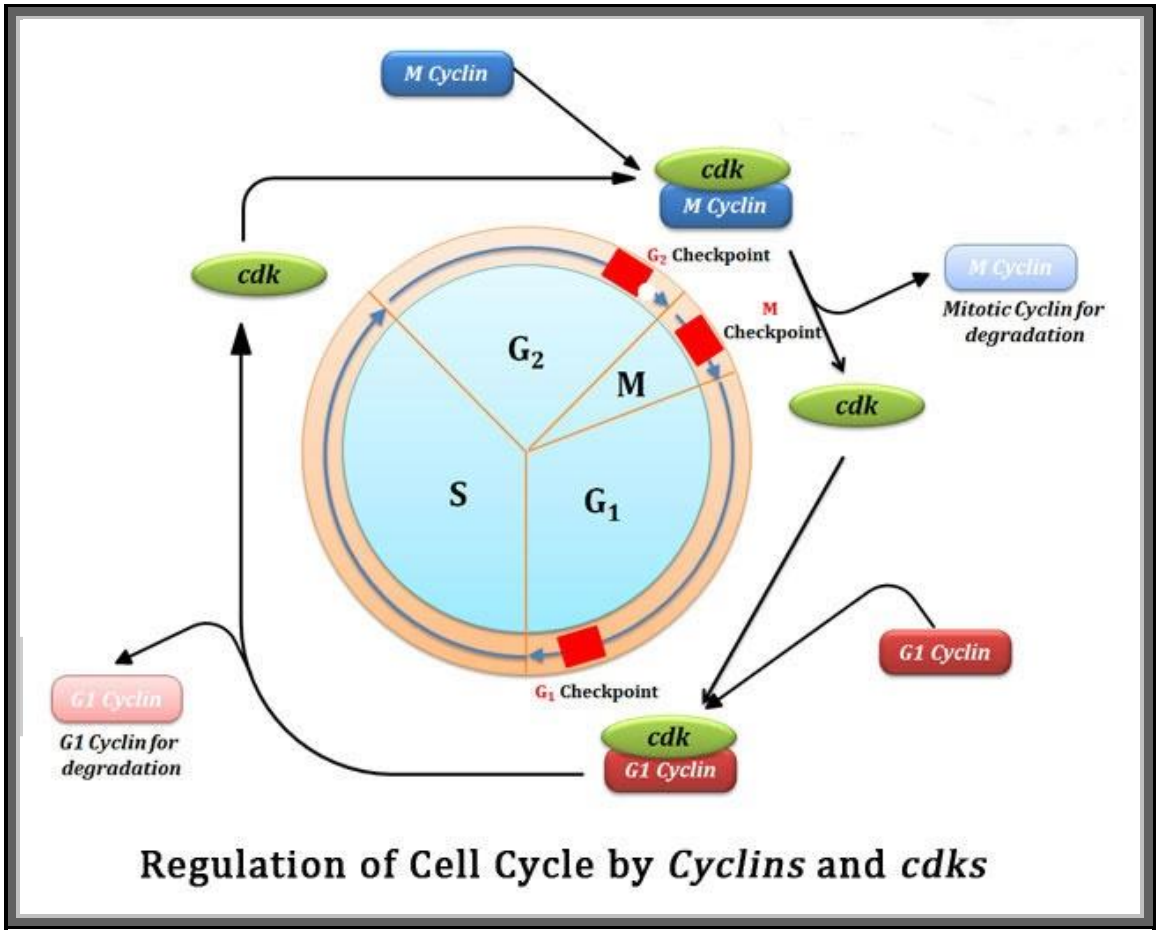
cyclins and cyclin dependent kinases (cdks)

Two categories of related proteins called **cyclins** and c**yclin-dependent kinases (cdks)** orchestrate the cell cycle checkpoint in **eukaryotic cells**. The cyclins are so named because their amount varies throughout the cell cycle. To be active, the **cyclin dependent kinases (cdks)** controlling the cell cycle must bind to a **specific cyclin**. Number and types of **cyclins and cdks** varies from species to species. The level of different cyclins and cdks are also different in different phases of cell cycle.

Cell cycle checkpoints ensure that:

- The nuclear **genome is intact** (without any mutation)
- The conditions are **appropriate for a cell to divide** (enough nutrients is there for the daughter cells)
- Genetic material is **replicated only once in a cell cycle**
- Genetic material is **completely replicated**

- **No mutations** occurred in the **replicated chromosomes**
- If mutations are occurred, these **mutations will be rectified by DNA repair system**
- **Chromosomes** are correctly oriented in the **metaphase plate**
- All chromosomes are correctly attached to the **spindle fibres**



Thus in the cell cycle there are three main types of checkpoints:

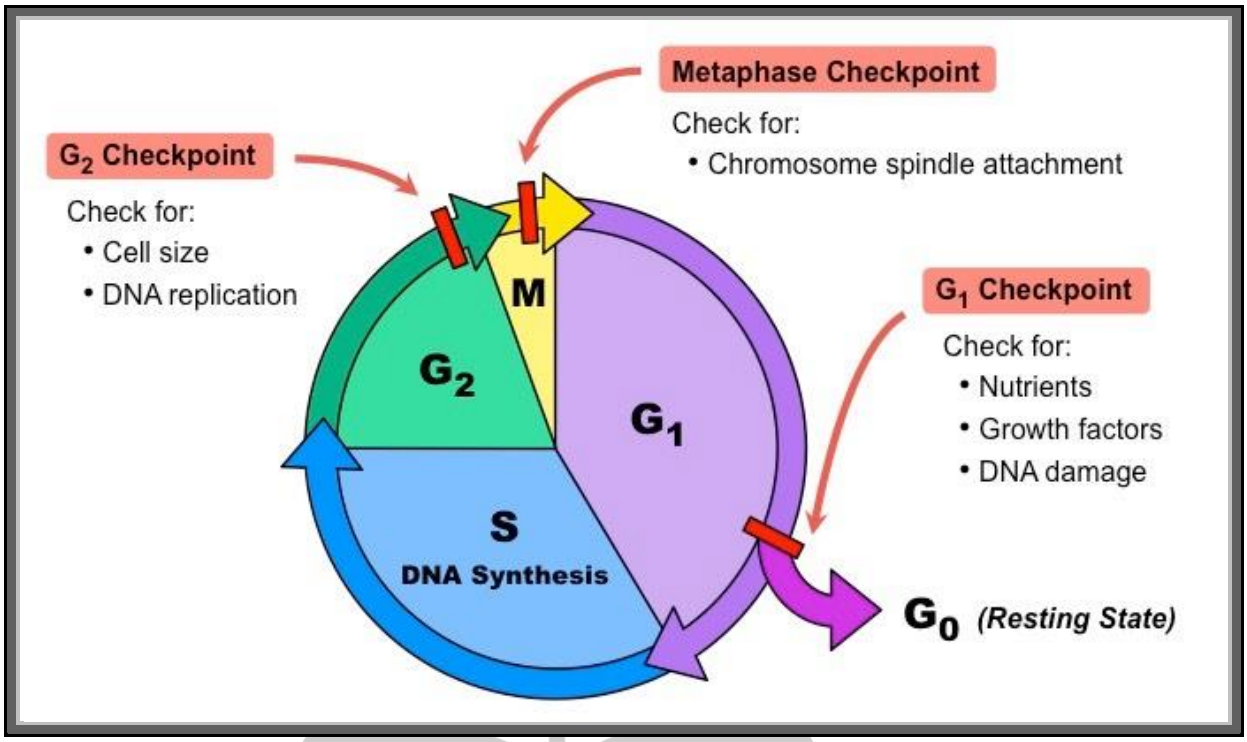
1. **DNA damage checkpoint or G_1/S Checkpoint or G1 checkpoint (restriction checkpoint)**
2. **DNA replication checkpoint or G_2/M Checkpoint or G2 checkpoint (G2-M DNA Damage Checkpoint) /**
3. **Spindle checkpoint or Metaphase (M) checkpoint (spindle assembly checkpoint)**

Usually the cell cycle is controlled at three main checkpoints G1/S, G_2/M and spindle checkpoint **(late metaphase)**

A large number of cell cycle mutants have been isolated for these checkpoints.

G_1/S Checkpoint or G1 checkpoint (restriction checkpoint)/ (DNA Damage Checkpoint):

- Transition from **G_1 phase (start),**
- It is **the primary checkpoint commonly known as 'start' (in yeast), restriction point** or R-point (in animals).
- This checkpoint assesses extracellular growth factors or mitogens and intracellular nutritional state. **Starvation, lack of mitogens** can halt the cell cycle at this point and the cell **enters the G_0-phase.**
- **G1 checkpoint** is also called as **restriction point**.
- G1 checkpoint operates at the end of **G1 phase of cell cycle**.
- G1 check points checks whether the conditions are **favorable for the cell to divide.**
- It also checks the **DNA for any damage** before it is going for a cycle of DNA replication in the next phase (S phase).
- If **DNA damage is detected, checkpoint proteins** will prevent the formation of active cyclin/cdk complexes.
- **Inhibition of cyclin/cdk** complex formation stops the progression of the cell cycle.
- The cells are then direct the **DNA repair mechanism** to rectify the DNA damage. If the environmental conditions are not good, the cell may enter into G0 phase. In **yeast cells,** G1 checkpoint is also called as **start point.**
- This **checkpoint blocks progression into S phase** by inhibition of **S-Cdk complex.** Damaged **DNA stimulates transcription** of many genes which encode the proteins that bind to S-Cdk, inhibits their activity and thus blocks the **entry into mitosis.**
- **In mammals, a protein of p^{53} gene** causes delay in entry of cells with damaged DNA into S phage and mutation in p^{53} gene, therefore, causes cancer due to increase in frequency of cancer **promoting genetic alterations**.
- The protein **p^{53} blocks the activity of Cdks and has been dubbed as 'Watchman'** because DNA damage is sensed by it

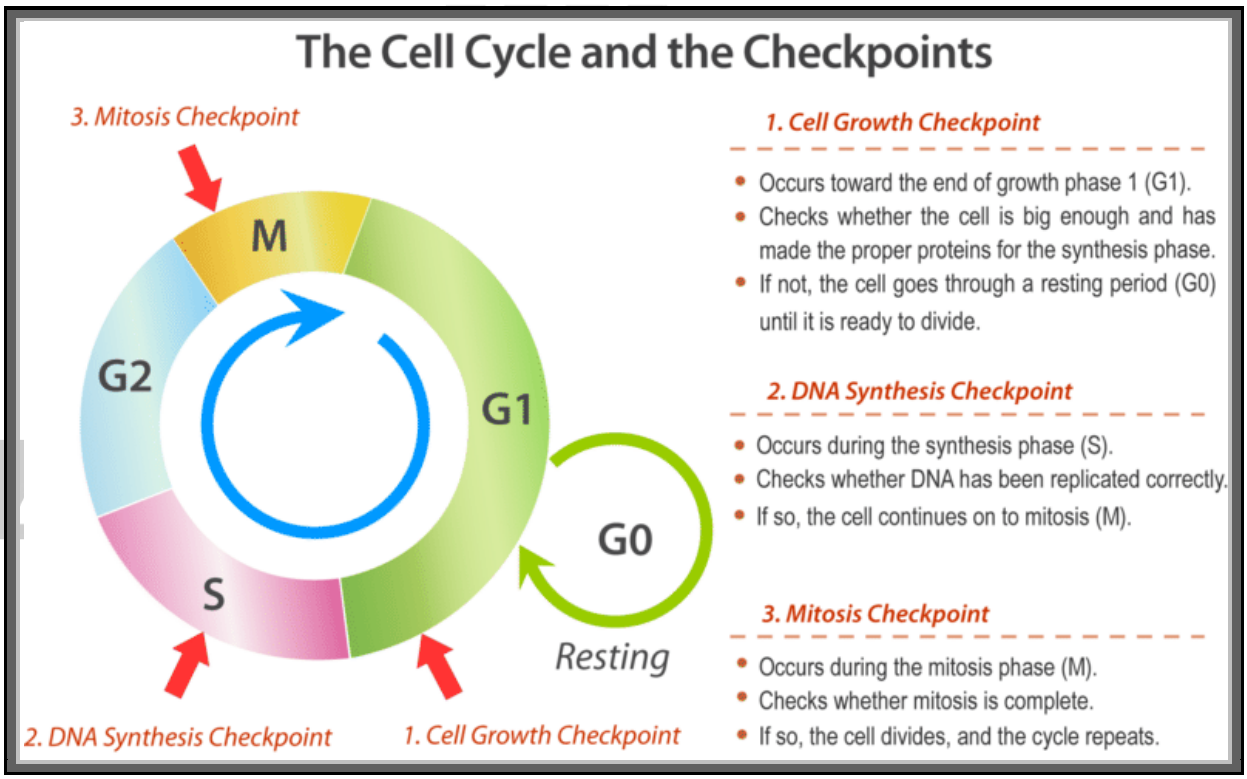


G2/M Checkpoint or G2 checkpoint (G2-M DNA Damage Checkpoint) /

(DNA Replication/DNA Damage Checkpoint):

- G2 is the second checkpoint which operates at the end of G2 phase. It is also called as **G2-M DNA damage checkpoint**.
- Transition from **G2 to M phase,**
- It ensures the success **of DNA-replication**. DNA-dam aging agents can stop the cycle at this point.
- **G2 checkpoint checks the DNA** for any damage that might be occurred during the DNA replication in the **previous cell cycle phase (S phase).**
- **G2 checkpoint** also ensures that the entire DNA has been replicated completely. Apart from this,
- **G2 checkpoint monitors** the levels of proteins and growth factors that are needed in the next phase **(M phase) of cell cycle**.
- If any of the above factors are **not satisfactory, the G2 check point** hold the cells at G2 phase and initiate machineries to **rectify the problems**.
- It is the controlling point involved in driving the cell **to the M phase.**
- This is triggered by **MPF (Maturation Promoting Factor or M-phase Promoting Factor)** which is a **cyclin-Cdk complex (Cdk – cyclin dependent kinase).**

- It promotes mitosis by **phosphorylating a variety of other protein kinases**.
- This checkpoint **monitors un replicated and damaged DNA** which delays mitosis until **DNA replication** is complete and **DNA damage** is repaired.
- Mitotic entry of G_1 cells is delayed in **yeast by the rad 9 gene.**
- The damaged DNA sends a signal to a series of protein kinases that blocks the **dephosphorylation** and activation of **M-Cdk, blocking entry into mitosis .**
- Normal cells treated with **hydroxyurea, inhibitor of DNA synthesis**, activates this checkpoint mechanism that arrests the cells in S phase, thus delaying mitosis.



Metaphase (M) checkpoint (spindle assembly checkpoint)

- It operates at the beginning of anaphase. It assesses whether **all chromosomes are attached to the spindle or not.**
- Passage through the above checkpoints is controlled by **specific protein kinases that take** part in phosphorylation and dephosphorylation. For the activity of protein kinases a number of protein stimulators are required which are called **cyclins.**
- The protein kinases are thus called **Cyclin-dependent Kinases (Cdks) or mitosis promoting factor (MPF).**

- Metaphase checkpoint is also called as **spindle assembly checkpoint**. It is the third and last cell cycle checkpoint in a cell cycle operates at the **end of M phase. Metaphase** checkpoint senses the integrity of the **spindle apparatus** in the cell.
- Spindle apparatus is involved in sorting of chromosomes during cell division. Correct orientation **of chromosomes in the metaphase** plate of cell is very essential for the proper segregation of chromosomes.
- If **chromosomes are not correctly attached to the spindle apparatus**, the metaphase checkpoint will stop **the cell cycle**. Thus, **M checkpoint prevents** cells from incorrectly sorting their chromosomes during division.
- It ensures that ail the **chromosomes are properly** attached to the spindle at the **metaphase plate before anaphase.**
- A signal to delay anaphase originates at **kinetochore** which inhibits attachment of spindle microtubule; this keeps the anaphase **promoting complex (APC)** in **an inactive state.**
- After attachment of all **kinetochores, the APC is activated**, triggering breakdown of **cyclin and inactivation of proteins** holding sister **chromatids together**.
- Mutants in **MAD and BUB genes** of **mammals and yeast inactivate** this spindle checkpoint .
- The effect of **colchicine** which **inhibits spindle** assembly demonstrate the presence of this checkpoint.

Control of the Cell Cycle:

- The cell cycle has **key checkpoints (inspection points)** at which feedback signals from the cell can trigger the next phase of **the cell cycle (green light)**. Other feedback signals can delay the next phase to allow for completion of the current phase (yellow or red light).
- Control occurs at three principal checkpoints: 1. **Cell growth (G1) checkpoint:** This **checkpoint makes the decision of whether the cell will** divide. 2**. DNA synthesis (G2) checkpoint:** DNA replication is checked at this point by DNA repair enzymes. 3. Mitosis checkpoint: This checkpoint triggers the exit from mitosis.

Importance of cell cycle checkpoints

- Checkpoint proteins delay the **cell cycle progression until problems** are fixed
- Checkpoint can prevent **cell division** when problems cannot be fixed

- They can induce apoptosis **(programmed cell death)** if the problems are so severe and cannot be repaired
- Cell cycle checkpoints accurately **maintain the genome of the organism**
- **Cell cycle checkpoint** ensure only one round replication of **DNA per cell cycle**
- If functions of checkpoint genes are **lost due to mutation**, leads to additional mutations and cancerous growth **initiate in the organ**
- Almost all **cancers are due to the improper** functioning of either one or many proteins involved in **cell cycle** regulation. (Eg. P53 – guardian of genome, a tumor suppressor gene)

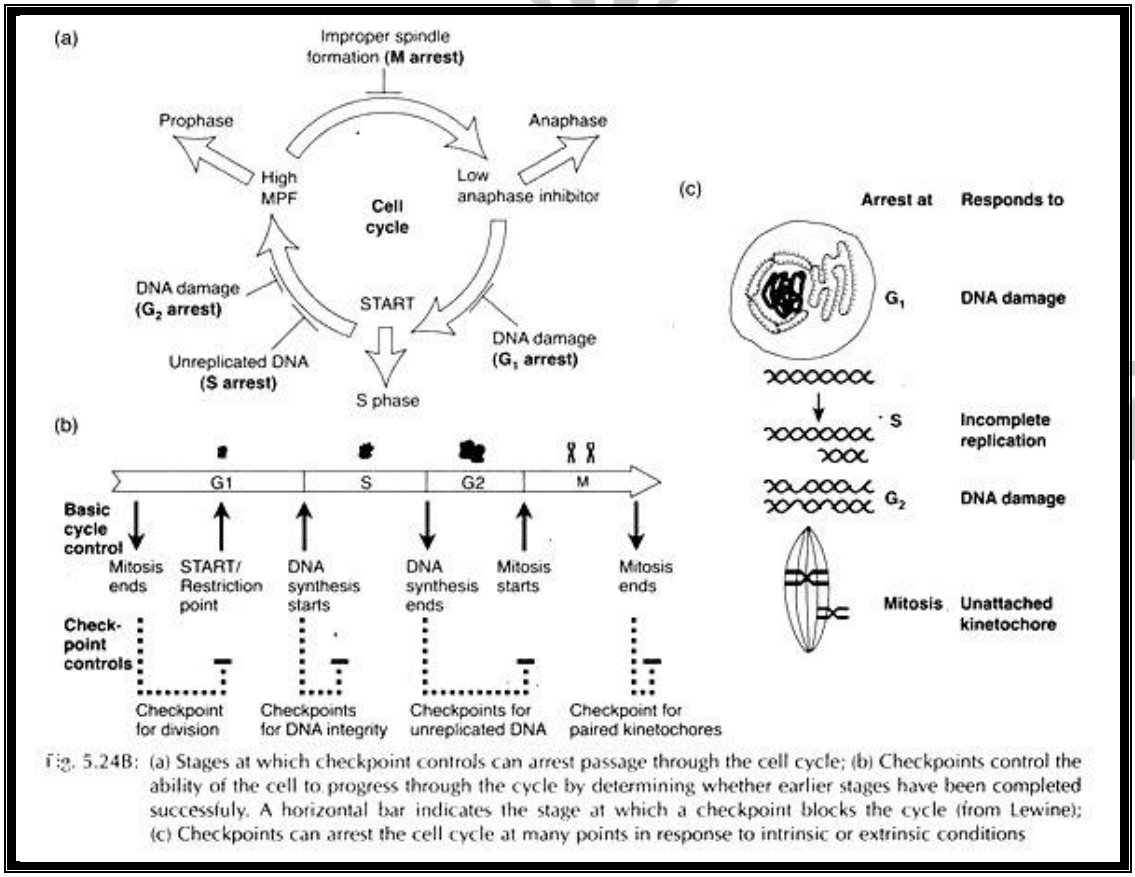


Fig. 5.24B: (a) Stages at which checkpoint controls can arrest passage through the cell cycle; (b) Checkpoints control the ability of the cell to progress through the cycle by determining whether earlier stages have been completed successfuly. A horizontal bar indicates the stage at which a checkpoint blocks the cycle (from Lewine); (c) Checkpoints can arrest the cell cycle at many points in response to intrinsic or extrinsic conditions

Regulation of cell cycle:

- The cell cycle is controlled by **regulator molecules t**hat either promote the process or stop it from progressing.

1. Positive regulation of cell cycle:

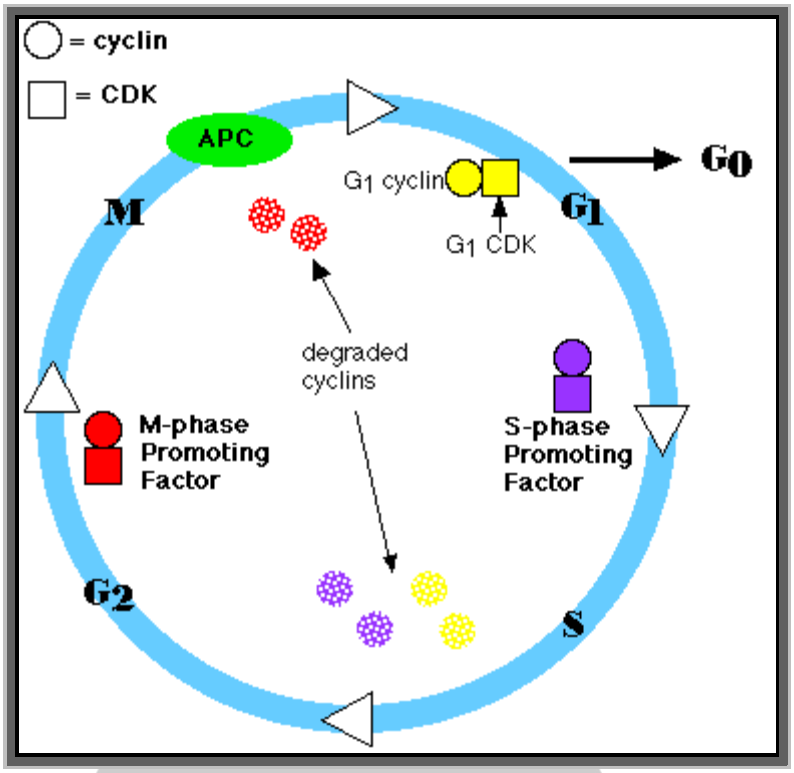
- Two groups of proteins; **cyclins** and **cyclin-dependent kinases** (Cdks), are responsible for **promoting the cell cycle**

i. Maturation promoting factor (MPF):

- **MPF** is composed of two protein complex; **cyclin and cyclin dependent kinase (cdc2p).**
- These two groups of proteins, called **cyclins and cyclin-dependent kinases (Cdks),** are responsible for the progress of the **cell through the various checkpoints.**

a. Cyclin:

- **Cyclins are cell-signaling molecules** that regulate the cell cycle
- There are four types of **cyclins proteins- A, B, D and E**
- The levels of the **four cyclin proteins (A,B,D,E)** fluctuate throughout the cell cycle in a predictable pattern
- **Cyclin B** is very important in **mitosis.**
- After the cell moves to the next **stage of the cell cycle**, the cyclins that were active in the previous stage are degraded.
- **Cyclins** regulate the cell cycle only when they are **tightly bound to Cdks.**
- To be fully active, the **Cdk/cyclin complex** must also be **phosphorylated** in specific locations.



b. Cyclin dependent kinases(CDKs):

- Cdks are **kinase enzymes that phosphorylate** other proteins or enzymes. **Phosphorylation** activates the protein by **changing its shape.**
- The **proteins phosphorylated by Cdks** are involved in **advancing the cell to the next** phase.
- **The levels of Cdk proteins** are relatively stable throughout the cell cycle; however, the concentrations of cyclin fluctuate and determine when **Cdk/cyclin complexes** form or not.
- The different **cyclins and Cdks** bind at specific points in the cell cycle and thus regulate different checkpoints.

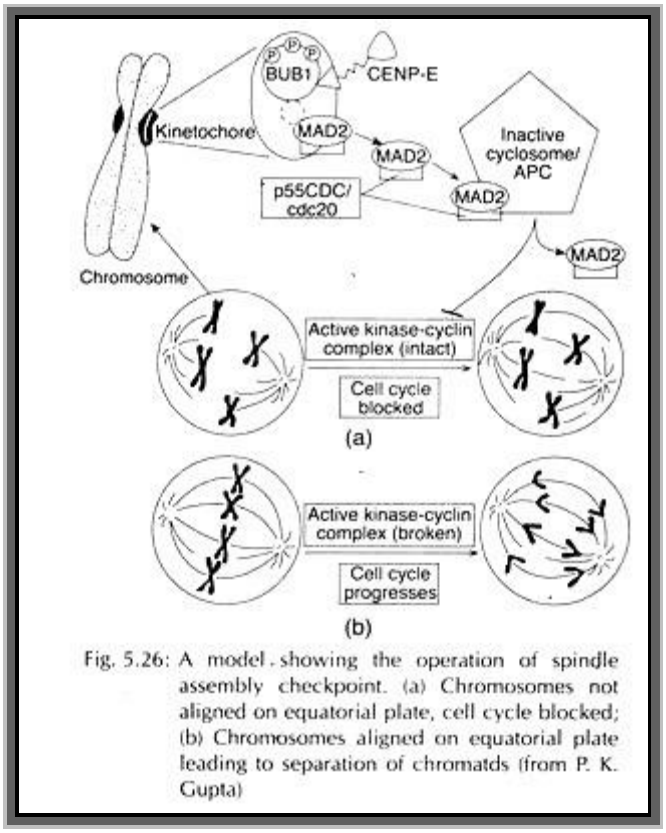


Fig. 5.26: A model showing the operation of spindle assembly checkpoint. (a) Chromosomes not aligned on equatorial plate, cell cycle blocked; (b) Chromosomes aligned on equatorial plate leading to separation of chromatds (from P. K. Gupta)

2. Negative regulation of cell cycle:

- Negative regulators halt the cell cycle.
- Negative regulatory molecules are **retinoblastoma protein (Rb), p53, and p21.**
- If negative regulator proteins are damaged or become non-functional then it results in uncontrolled cell division leading to tumor or cancer.

i. Retinoblastoma proteins:

- **Rb** are a group of **tumor-suppressor proteins** common in many cells.

ii. P53

- **P53** is a **multi-functional protein**. It is activated during G1 phase when there is DNA damage in the cell and cell employed the mechanism to repair the damage.
- When **damaged DNA is detected**, **p53 protein halts the cell cycle** and recruits enzymes to repair the DNA. If the DNA cannot be repaired, **p53 can trigger** apoptosis to prevent the duplication of damaged chromosomes.
- As **p53 levels** rise, the production of p21 is triggered.

iii. p21:

- **p21 enforces the halt in the cell cycle dictated** by p53 by binding to and inhibiting the activity of the Cdk/cyclin complexes.
- In case of **DNA damage condition or inadequate cell size**, more and more p53 and p21 are produced which halt the cell cycle and prevent the cell to enter S phase.
- These negative regulators are known as **tumor suppressor protein and gene** that codes for such proteins are called tumor suppressor gene.
- **Tumor suppressor** either halt the cell until repair or leads to apoptosis thus preventing damaged cell from division. If mutation occurs in tumor suppressor gene, then those negative regulator proteins lost the function to halt the cell cycle leading cancerous cell of **continuous growth and division.**

Importance of cell cycle checkpoints and regulation

- The cell cycle of each cell must be precisely **controlled** and timed to faithfully and reproducibly complete the developmental program in every individual. Each type of **cell in every** tissue must control its replication precisely for normal development of **complex organs** such as the brain or the kidney. In a normal adult, cells divide only when and where they are needed. However, loss of normal controls on cell replication is the fundamental defect in **cancer**.
- **Cell cycle** occurs with high accuracy and fidelity to assure that each daughter cell inherits the equal number of chromosome as of **parent cell.**
- **Chromosome replication and cell division** must occur in the proper order in every cell division. If a cell undergoes the events of **mitosis before the replication** of all chromosomes has been completed, at **Ieast one daughter cell will lose genetic information.**
- Similarly, if a **second round of replication occurs in one region of a chromosome** before cell division occurs, the genes encoded in that region are increased in number out of proportion to other. Therefore, **single round of DNA replication** occurs in a cell.

GLOSSARY

cell cycle checkpoint: mechanism that monitors the preparedness of a eukaryotic cell to advance through the various cell cycle stages

cyclin: one of a group of proteins that act in conjunction with cyclin-dependent kinases to help regulate the cell cycle by phosphorylating key proteins; the concentrations of cyclins fluctuate throughout the cell cycle

cyclin-dependent kinase: one of a group of **protein kinases** that helps to regulate the cell cycle when bound to cyclin; it **functions to phosphorylate** other proteins that are either activated or inactivated by phosphorylation

p21: cell cycle regulatory **protein that inhibits the cell cycle**; its levels are controlled by **p53**

p53: cell cycle regulatory protein that regulates cell growth and monitors DNA damage; it halts the progression of **the cell cycle in cases of DNA damage** and may induce apoptosis

retinoblastoma protein (Rb): regulatory molecule that exhibits negative effects on the cell cycle by interacting with a **transcription factor (E2F)**

Proterminal synapsis: the pairing in homologous chromosomes starts from the end and continues towards their centromeres.

Procentric synapsis: the homologous chromosomes start pairing from their centromeres and the pairing progresses towards the ends of the homologues.

Localized pairing or Random synapsis:synapsis occurs at various points of the homologous chromosomes.

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