Contents

CHAPTER

18

Cell Growth and Cell Division

(Cell Cycle, Mitosis and Meiosis)

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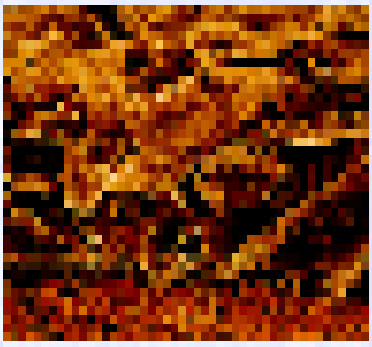

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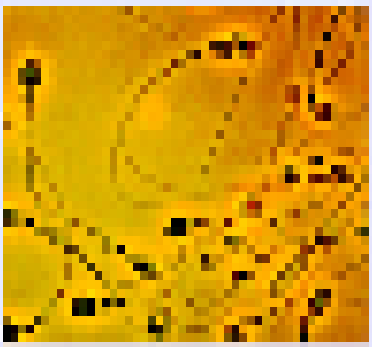


(c)

(a) Intestinal epithelial cells have a very rapid turnover; the entire gut lining is replaced every few days.

(b) SEM of human epidermis. Dead skin cells are constantly being lost and replaced by mitosis.

(c) Human sperms.

Growth— an increase in size or mass of a developing/living system— is an irreversible process that occurs at all organizational levels. Often, it is difficult to define, because, it is. multifactorial, that is, growth embodies following three interacting growth patterns : (1) **auxetic growth**— an increase in cell mass or **auxesis** ; (2) **multiplicative growth**— an increase in cell number due to cell division; and (3) **accretionary growth**— growth due to accumulation of extracellular products (accretion means increase by addition on the surface of the material of same nature as that is already present, *e.g.*, the manner of growth of crystal). Generally, when rate of anabolism (*i.e.*, photosynthesis, protein synthesis, etc.) far exceeds the rate of catabolism (*i.e.*, respiration), the growth of protoplasm (*i.e.*, auxetic growth) takes place.

CELL CYCLE AND MITOSIS

All cells are produced by divisions of pre-existing cell. Continuity of life depends on cell division. A cell born after a division, proceeds to grow by macromolecular synthesis, reaches a species-determined division size and divides. This cycle acts

as a unit of biological time and defines life history of a cell. **Cell cycle** can be defined as the entire sequence of events happening from the end of one nuclear division to the beginning of the next. The cell cycle involves the following three cycles (see **Albert** *et al.*, 1989).

1. Chromosome cycle. In it **DNA synthesis** alternates with **mitosis** (or karyokinesis or nuclear division). During DNA synthesis, each double-helical DNA molecule is replicated into two identical daughter DNA molecules and during mitosis the duplicated copies of the genome are ultimately separated.

2. Cytoplasmic cycle. In it **cell growth** alternates with **cytokinesis** (or cytoplasmic division). During cell growth many other components of the cell (RNA, proteins and membranes) become double in quantity and during cytokinesis cell as a whole divides into two. Usually the karyokinesis is followed by the cytokinesis but sometimes the cytokinesis does not follow the karyokinesis and results into the multinucleate cell, *e.g.*, cleavage of egg in *Drosophila*.

3. Centrosome cycle. Both of the above cycles require that the **centrosome** be inherited reliably and duplicated precisely in order to form the two poles of the mitotic spindle ; thus, centrosome cycle forms the third component of cell cycle.

Howard and **Pelc** (1953) have divided cell cycle into four phases or stages : G_1, S, G_2 and M phase. The G_1 phase, S phase and G_2 phase are combined to from the classical **interphase**.

1. G_1 Phase. After the M phase of previous cell cycle, the daughter cells begin G_1 of interphase of new cell cycle. G_1 is a resting phase. It is called **first gap phase**, since no DNA synthesis takes place during this stage; currently, G_1 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 (see **Maclean** and **Hall**, 1987).

During G_1 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, G_1 involves transcription of three types of RNAs, namely rRNA, tRNA and mRNA ; rRNA synthesis is indicated by the appearance of nucleolus in the interphase (G_1 phase) nucleus. Proteins synthesized during G_1 phase (1) regulatory proteins which control various events of mitosis ; (2) enzymes (*e.g.*, DNA polymerase) necessary for DNA synthesis of the next stage ; and (3) tubulin and other mitotic apparatus proteins.

G_1 phase is most variable as to duration (Table 18-1) ; it either occupies 30 to 50 per cent of the total time of the cell cycle or lacks entirely in rapidly dividing cells (*e.g.*, blastomeres of early embryo of frog and mammals). Terminally differentiated somatic cells (*i.e.*, end cells such as neurons and striated muscle cells) that no longer divide, are arrested usually in the G_1 stage; such a type of G_1 phase is called **G_0 phase.**

Table 18.1. 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

2. S phase. During the S phase or **synthetic phase** of interphase, replication of DNA and synthesis of histone proteins occur. New histones are required in massive amounts immediately at the beginning of the S period of DNA synthesis to provide the new DNA with nucleosomes. Thus, at the end of S phase, each chromosome has two DNA molecules and a duplicate set of genes. S phase

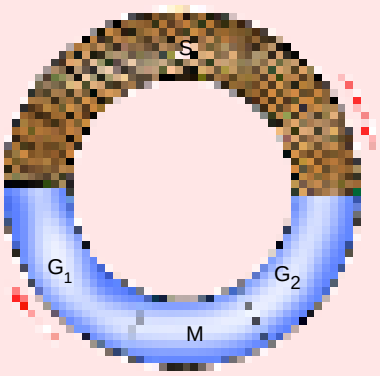


Fig. 18.1. The cell cycle or mitotic cycle, showing relative duration of phases (*e.g.*, interphase and mitotic phase) in a growing cell. S, synthesis of DNA ; G_1 the first gap or growth phase ; G_2, the second gap or growth phase ; and M, mitotic phase.

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.

4. M phase or Mitotic phase. The mitosis (Gr., *mitos*=thread) occurs in the somatic cells occupies roughly 35 to 45 per cent of cell cycle.

3. G_2 phase. This is a **second gap** or **growth phase** or resting phase of interphase. During G_2 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 G_2 phase draws to a close, the cell enters the M phase.

General Events of Interphase

The interphase is characterized by the following features :

The nuclear envelope remains intact. The chromosomes occur in the form of diffused, long, coiled and

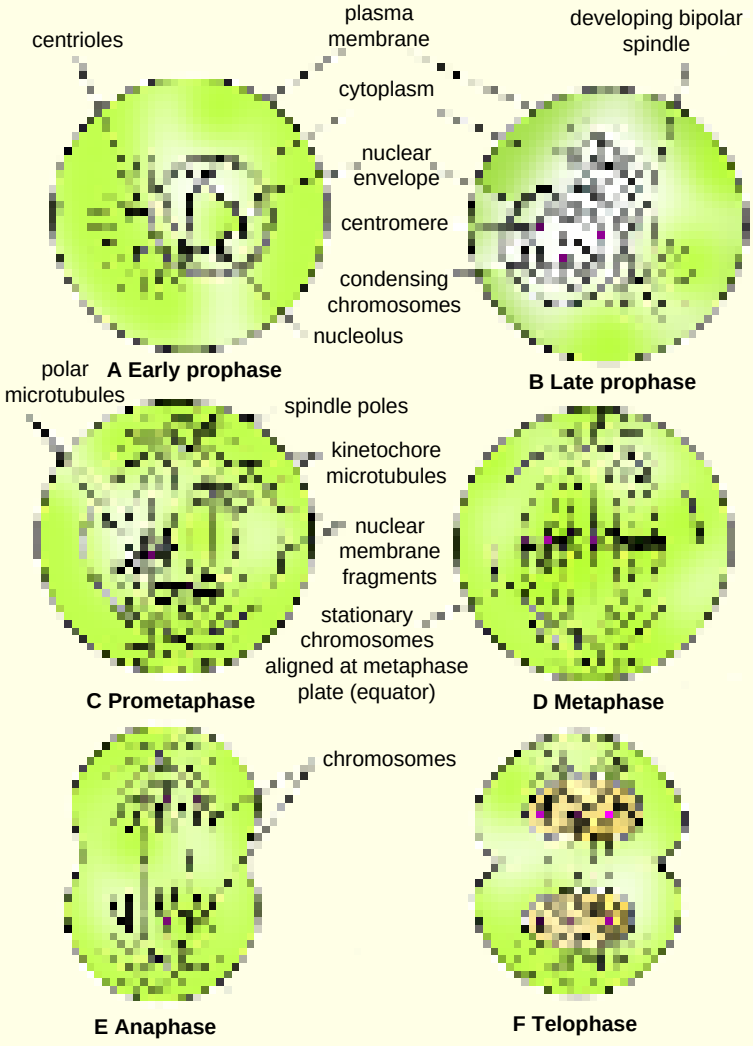


Fig. 18.2. Diagrammatic summary of mitosis in the animal cell (after Burns and Bottino, 1989).

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 (*i.e.*, G_2 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.

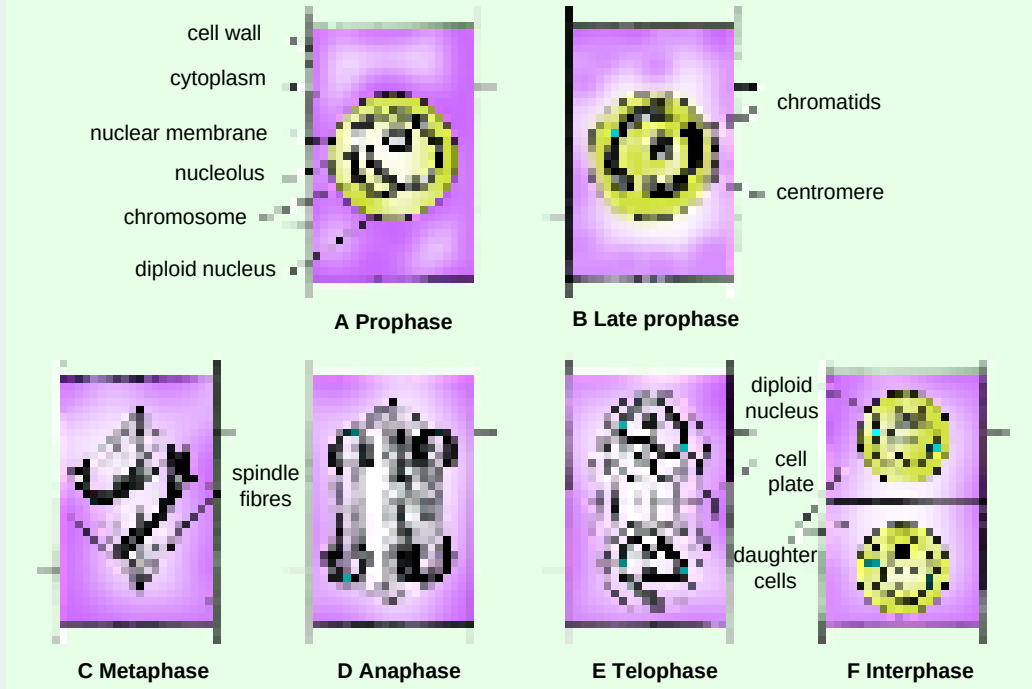


Fig. 18.3. Diagrammatic summary of mitosis in the higher plant cells.

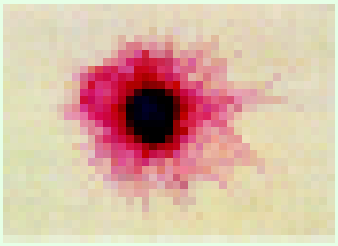
As a process, mitosis is remarkably similar in all animals and plants. It is a smoothly continuous process and is divided arbitrarily into following stages or phases for convenient reference (Fig. 18.2 and Fig. 18.3) :

1. Prophase. The appearance of thin-thread like condensing chromosomes marks the first phase of mitosis, called **prophase** (Gr., *pro*=before ; *phasis*=appearance). The cell becomes spheroid, more refractile and viscous.

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 (see **Darnell** *et al.*, 1986). Further, during early prophase, the chromosomes are evenly distributed in the nuclear cavity ; as prophase progresses, the chromosomes approach the nuclear envelope, causing the central space of the nucleus to become empty.

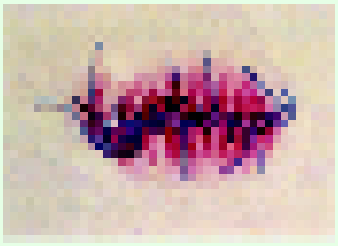
In the cytoplasm, the most conspicuous change is the formation of the spindle or **mitotic apparatus**. In the early prophase, there are two pairs of centrioles, each one surrounded by the so-called **aster** which is composed of microtubules radiating in all directions. The two pairs of centrioles migrate 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 (Fig. 18.7) : (1) **polar fibres**, which extend from the two poles of the spindle toward the equator ; (2) **kinetochore fibres**, which attach to the



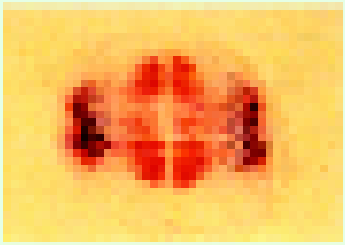
(a) Interphase in a cell of the endosperm (a food-storage organ in the seed) before mitosis begins: The chromosomes are in the thin, extended state and appear as a mass in the center of the cell. The microtubules extend outward from the nucleus to all parts of the cell.

(b) Late prophase: The chromosomes have condensed and attached to microtubules of the spindle fibers.

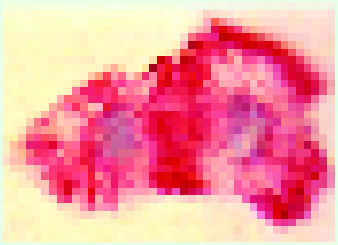


(c) Metaphase: The spindle fibers have pulled the chromosomes to the equator of the cell.

(d) Anaphase: The spindle fibers are moving one set of chromosomes to each pole of the cell.



(e) Telophase: The chromosomes have been gathered into two clusters, one at the site of each future nucleus.



(f) Resumption of interphase: The chromosomes are relaxing again into their extended state. The spindle fibers are disappearing, and the microtubules of the two daughter cells are rearranging into the interphase pattern.

kinetochores of centromeres of each mitotic chromosomes and extend toward the poles ; and (3) **astral fibres**, which radiate outward from the poles toward the periphery or cortex of cell. In cells of most higher plants, however, spindle forms without the aid of centrioles and lacks asters (Fig. 18.4).

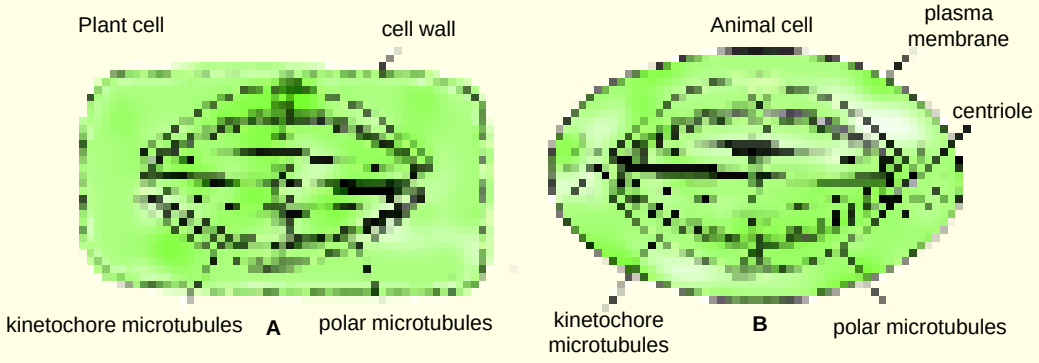


Fig. 18.4. Mitotic metaphase spindle structure in (A) a plant cell and (B) an animal cell. Higher plants lack centrioles and astral fibres (after Darnell *et al.*, 1986).

Lastly, during prophase, the nucleolus gradually disintegrates. Degeneration and disappearance of the nuclear envelope marks the end of prophase. This process is incompletely understood. However, following two factors may be involved in this process : 1. **Enzymatic action** either by some mitochondrial enzymes (see **Grant**, 1978), cytosolic MPF kinase (see **Alberts**, *et al*., 1989) or nuclear RNA (or ribozyme ; **Burns** and **Bottino**, 1989). 2. **Physical action**, *i.e.*, physical stress exerted by microtubules which become attached to the nuclear envelope (see **Burns** and **Bottino**, 1989).

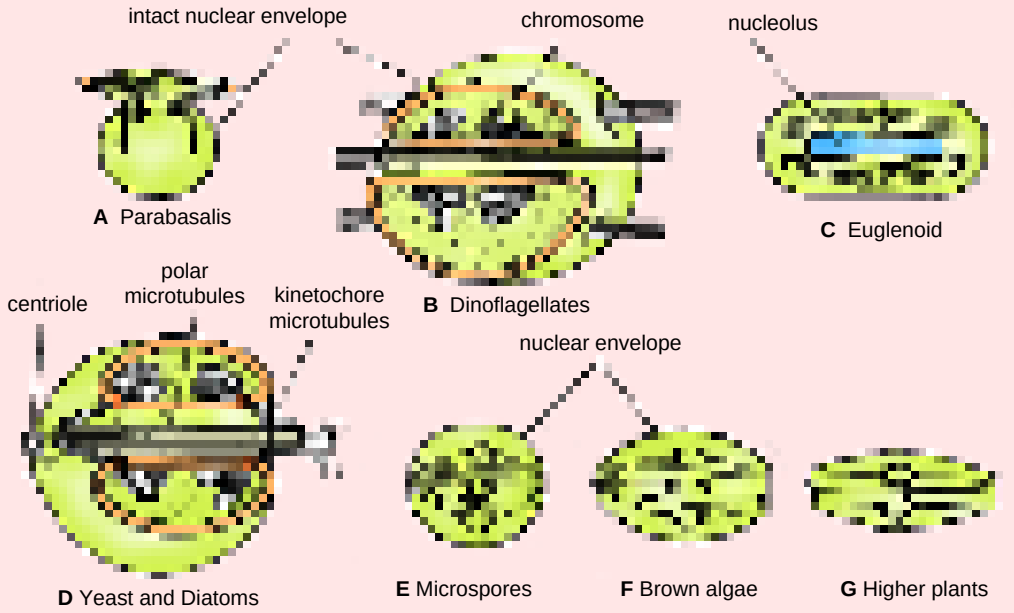
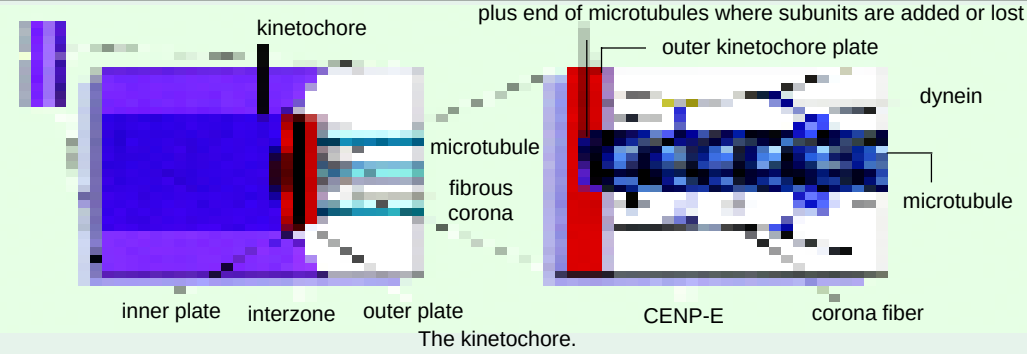


Fig. 18.5. Some variations of mitosis which have been observed during anaphase (after Albert *et al*., 1989).

There are variations available with respect to the dissolution of nuclear envelope and the nucleolus. In a number of primitive classes of plants and animals the nuclear envelope does not dissolve during mitosis (Fig. 18.5).

Prometaphase. The breakdown of nuclear envelope signals the commencement of prometaphase and enables the mitotic spindle to interact with the chromosomes. This stage is characterized by a period of frantic activity during which the spindle appears to be trying to



The kinetochore.

contain and align the chromosomes at the metaphase plate. In fact, at this stage the chromosomes are violently rotated and oscillated back and forth between the spindle poles because their kinetochores are capturing the plus ends of microtubules growing from one or the other spindle

pole and are being pulled by the captured microtubules. The kinetochores thereby act as a "cap" that tends to protect the plus end from depolymerizing, just as the centrosome at the spindle pole tends to protect the minus end from depolymerizing. Thus, sister chromatids become attached by their kinetochores to opposite poles ; balanced bipolar forces hold chromosomes on the metaphase plate (Fig. 18.6).

2. Metaphase. During metaphase (Gr.,*meta*=after; *phasis* =appearance) the chromosomes are shortest and thickest. Their centromeres occupy the plane of the equator of the mitotic apparatus (a region known as the **equatorial** or **metaphase plate**), although the chromosomal arms may extend in any direction. At this stage the sister chromatids are still held together by centromere and the kinetochores of the two sister chromatids face opposite poles ; this would permit proper separation in the next phase (anaphase).

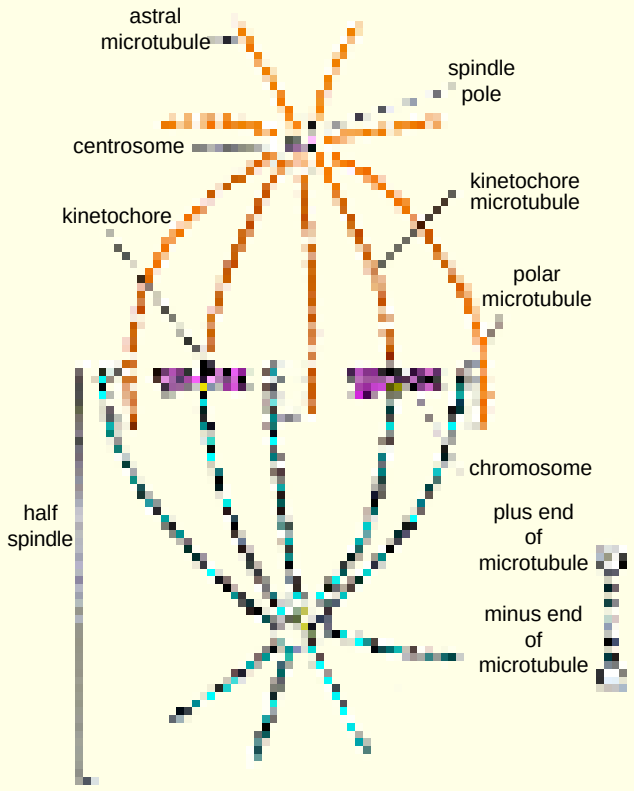


Fig. 18.6. The mitotic spindle at metaphae. The spindle is constructed from two half spindles, each composed of kinetochore, polar and astral microtubules. The polarity of the microtubules is indicated by the arrowheads (after Albert *et al*., 1989).

Metaphase occupies a substantial portion of the mitotic phase (see Table 18-2), as if the cell pause until all their chromosomes are lined up appropriately on the metaphase plate. 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 (Fig. 18.8A).

3. Anaphase. The anaphase (Gr., *ana*=up ; *phasis*=appearance) 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 Ca^{2+}. In fact, Ca^{2+}-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 :

(i) Anaphase A. During it, there is poleward movement 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.

(ii) 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.

Table 18.2. **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

4. Telophase. The end of the polar migration of the daughter chromosomes marks the beginning of the telophase ; which in turn is terminated by the reorganization of two new nuclei and their entry into the G_1 phase of interphase. In general terms, the events of prophase occur in reverse sequence during this phase. A nuclear envelope reassembles around each group of chromosomes to form two daughter nuclei. The mitotic apparatus except the centrioles disappears ; high viscosity of the cytoplasm decreases; the chromosomes resume their long, slender, extended form as their coils relax; and RNA- synthesis restarts causing the nucleolus to reappear.

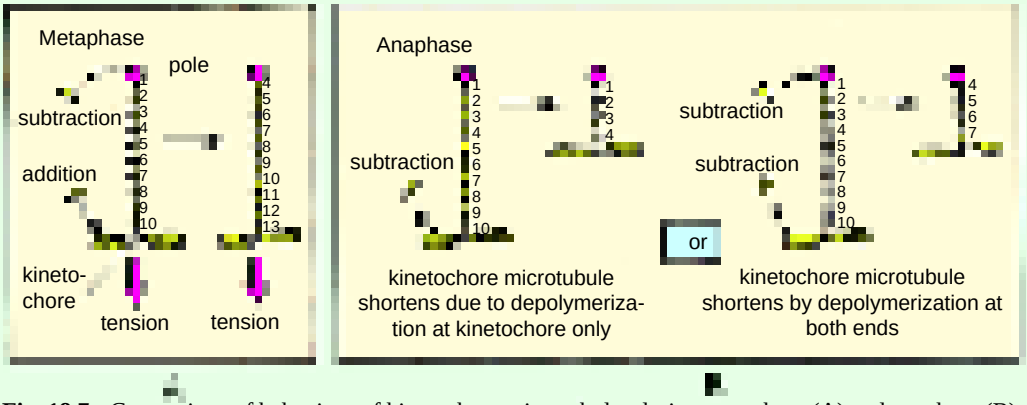
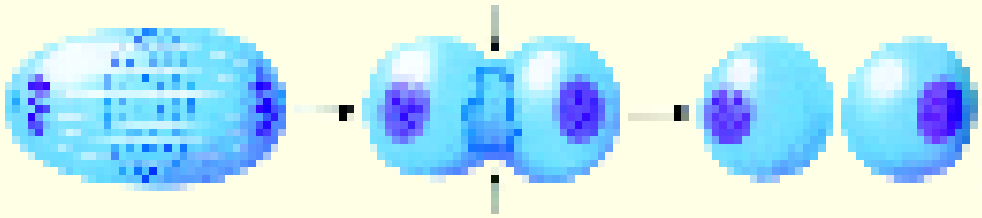


Fig. 18.7. Comparison of behaviour of kinetochore microtubules during metaphase (A) and anaphase (B). A—At metaphase, subunits are added to the plus end of a microtubule at the kinetochore and are removed from the minus end at the spindle pole. Thus, a constant poleward flux of tubulin subunits occurs, with the microtubules remaining stationary and under tension ; B—At the anaphase the tension is released, and the kinetochore moves rapidly up the microtubule, removing subunits from its plus end as it goes (BI). Its attached chromatid is thereby carried to a spindle pole. In some organisms, part of the chromatid movement is due to the simultaneous shortening of the microtubules (B II) (after Alberts *et al.*, 1989).

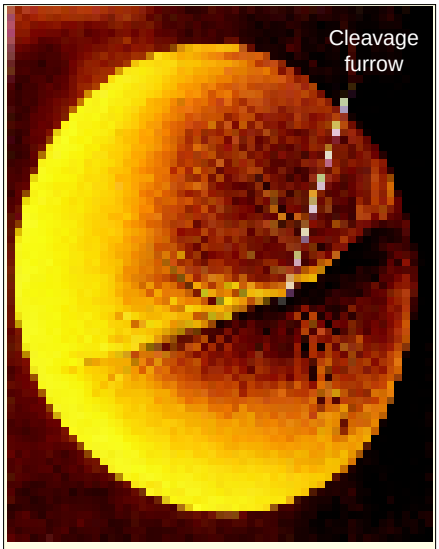
Cytokinesis

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**. The mitotic spindle plays an important role in determining where and when cleavage occurs. Cytokinesis usually begins in anaphase and continues through telophase and into interphase. The first sign of cleavage in animal cells is **puckering** and **furrowing** of the plasma membrane during anaphase. The furrowing invariably occurs in the plane of the metaphase plate, at right angles to the long axis of the mitotic spindle. A cleavage furrow tends to form midway between asters originating from two centrosomes in fertilized sand dollar eggs.

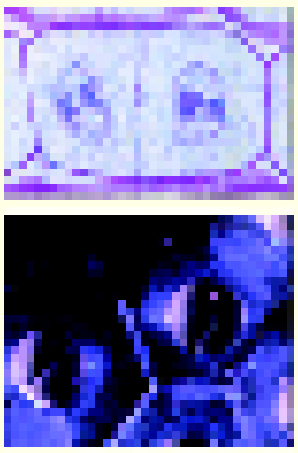


Cytokinesis in an animal cell.

Cleavage is accomplished by the contraction of a ring composed mainly of actin filaments. This bundle of filaments, called **contractile ring**, is bound to the cytoplasmic face of the plasma membrane by unidentified attachment proteins. The contractile ring assembles in early anaphase, once assembled, it develops a force large enough to bend a fine glass needle inserted into the cell. Evidently this force is generated due to muscle-like sliding of actin and myosin filaments in the contractile ring. The actin-myosin interaction pulls the plasma membrane down into a furrow. During a normal cytokinesis, the contractile ring does not get thicker as the furrow invaginates, suggesting that it continuouly reduces its volume by losing filaments. When cleavage ends, the contractile ring is finally dispensed with altogether and the plasma membrane of the cleavage furrow narrows to form the **midbody**, which remains as a tether (Tether means a rope for confining a beast within certain limits) between two daughter cells. The midbody contains the remains of the two sets of polar microtubules, packed tightly together with dense matrix material.



Cytokinesis in an animal egg.



A furrow develops. It pinches the cell membrane in. As the furrow deepens the animal cell divides into two.

Cytokinesis greatly increases the total cell-surface area as two cells form from one. Therefore, the two daughter cells resulting from cytokinesis require more plasma membrane than in the plant cell. Lastly, prior to cytokinesis, in M phase large membrane-bounded organelles such as Golgi apparatus and the endoplasmic reticulum break up into smaller fragments and vesicles ; this may ensure their even distribution into daughter cells during cytokinesis. (Note : For cytokinesis in plants see Chapter 5).

Physiology of Cell Cycle and Mitosis

Follwing aspects of cell cycle and mitosis need somewhat more detailed explanation :

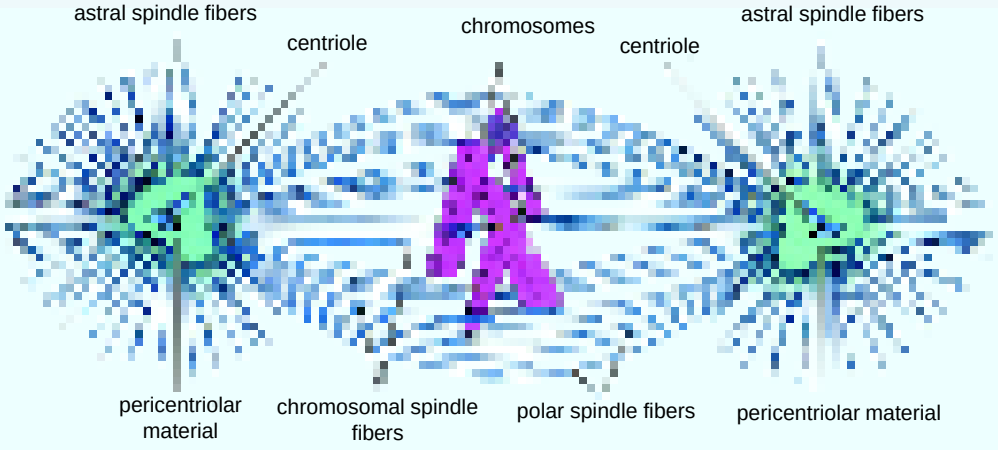
1. Regulation of mitotic chromosome cycle. Mitotic chromosome cycles is found to be regulated by the following three control factors (*i.e.*, diffusible proteins) : 1. The **S-phase activator** that normally appears in the cytoplasm only during S-phase and 'switches on' DNA synthesis (**Rao** and **Johanson**, 1970). 2. The **M-phase-**

promoting factor (**MPF**) that is present only in M-phase cytoplasm and causes chromosome condensation (**Johanson** and **Rao**, 1970). 3. The DNA-dependent **M-phase delaying factor** that is present in S-phase cytoplasm and inhibits the process leading to onset of MPF production.

The abrupt appearance and disappearance of these diffusible factors in the cytoplasm are landmark events in the cell cycle. The causal relationships among these factors guarantee that the events of the chromosome cycle will always occur in a fixed order, prohibiting such fatal accidents as chromosome condensation occurring in the middle of DNA synthesis. Each successive step depends on a preceding one (*i.e.* all processes of chromosome cycle are linked together as **dependent sequence**). Thus, (1) the cell cannot pass through mitosis until MPF has been produced ; (2) MPF cannot be produced until the M-phase-delaying-factor has disappeared ; (3) the M-phase-delaying factor and S-phase activator cannot disappear until DNA-synthesis has ended ; (4) DNA synthesis cannot end until all of the DNA has replicated ; (5) the DNA cannot begin to replicate until DNA re-replication block has been removed by passage through mitosis into G_1 ; and lastly (6) a cell cannot progress from mitosis into G_1, until the chromosomes have separated on the mitotic spindle.

MPF is a large-sized protein comprising two subunits– an inert subunit and a **kinase** subunit which can phosphorylate (and activate) the inert subunit (called **self-activation**) and other molecules (**Lohka** *et al.*, 1988). Thus MPF kinase directly phosphorylates several substances, including histone H1, thereby promoting chromosome condensation; and it may be through a cascade of phosphorylation that MPF triggers all the complex events of mitosis such as nuclear envelope breakdown and cytoskeletal change (*e.g.*, formation of mitotic spindle).

2. Dissolution and formation of nuclear envelope during mitosis. At least three parts of the nuclear envelope complex must be considered during its breakdown (at prophase) and reassembly (at telophase) : 1. outer and inner nuclear membranes; 2. the underlying nuclear lamina of lamin proteins and ; 3. the nuclear pores.



The mitotic spindle of an animal cell.

At prophase many proteins become phosphorylated by MPF and phosphorylation of nuclear lamins help regulate the disintegration and reconstruction of the nuclear envelope. The phosphorylation of the lamins occurs at many different sites in each polypeptide chain and causes them to disassemble, thereby disrupting the nuclear lamina. Subsequently, perhaps in response to a different signal (see Telophase), the nuclear envelope proper breaks up into small membrane vesicles. **Maul** (1977) has reported that in less than an hour (prophase to prometaphase) almost the entire 4000 pores disappear from the nuclear membranes of cultured mammalian cells. These pore complexes have been found on chromosomes during mitosis.

The sudden transition from metaphase to anaphase is thought to initiate dephosphorylation of many proteins, including histone H1 and the lamins, that were phosphorylated at prophase. Shortly thereafter, at telophase, nuclear membrane vesicles associated with the surface of individual chromosomes are fused to re-form the nuclear membranes which partially enclose clusters of chromosomes before coalescing to re-form the complete nuclear envelope (Fig. 18.8). During this process the presynthesized nuclear pores reassemble and the dephosphorylated lamins reassociated to form the nuclear lamina ; one of the lamina protein (lamin B) remains with the nuclear membrane fragments throughout mitosis and may help in nuclear assembly.

3. Role of cytoskeleton in mitosis. It has been said that the chromosomes in mitosis are like the corpse at a funeral : *they provide the reason for the proceedings but play no active part in them* (see **Alberts** *et al.,* 1989). The active role is played by their distinct cytoskeletal structures that appear short-termly in M phase. The first to form is a bipolar **mitotic spinde**, composed of microtubules and their associated proteins ; it is meant for poleward migration of daughter chromosomes during mitosis. The second cytoskeletal structure required in M phase in animal cells is a **contractile ring** of microfilaments and myosin that forms slightly later just beneath the plasma membrane ; it is meant for cytokinesis of the cell. The third cytoskeletal component is **meshwork** of intermediate filaments that surrounds the interphase nucleus ; it elongates during mitosis to enclose the two daughter nuclei and finally divides in half by the cleavage furrow.

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

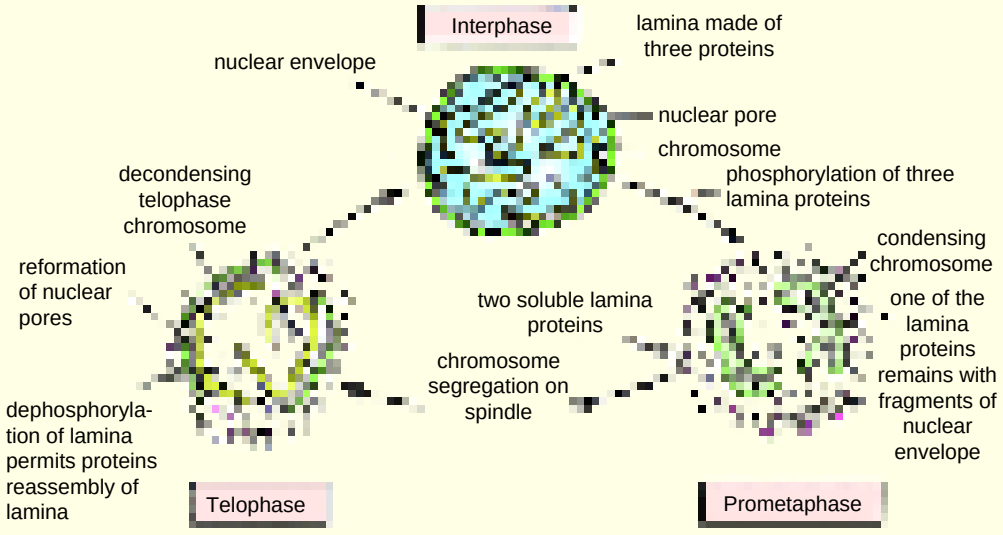


Fig. 18. 8. Schematic view of the nuclear-envelope cycle that occurs during mitosis (after Alberts *et al.*, 1989).

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 (Fig. 18.7) However, typically microtubule disassemble at kinetochores, poles or at both sites is probably necessery for equator- to- pole movement.

Further, the mechanism by which kinetochore of the chromosome moves up the spindle during anaphase A is still unknown. However, the following three models throw some light on it :

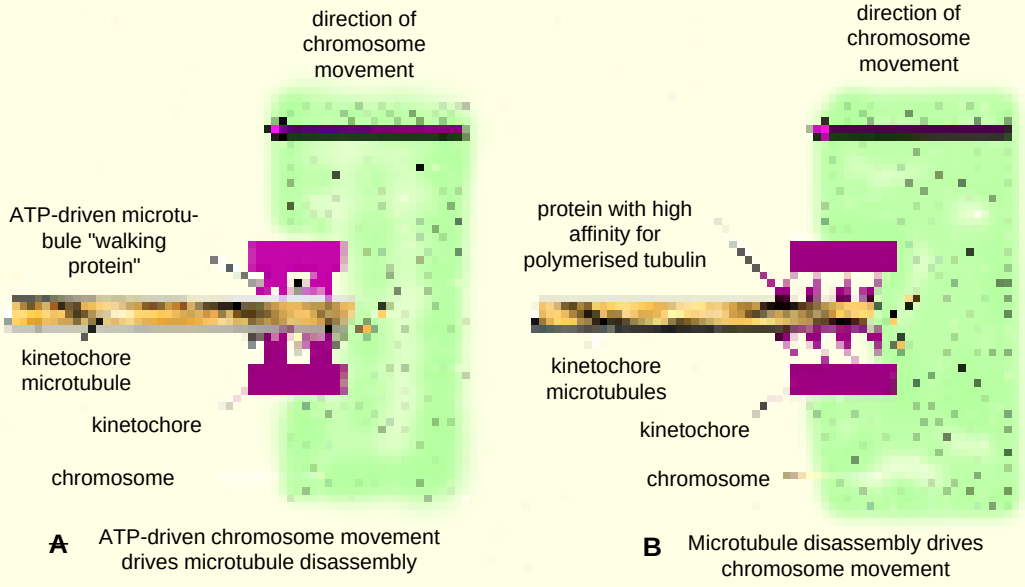


Fig. 18.9. Two alternative models to explain the generation of poleward force by the kinetochore for its chromosome during anaphase. A—Microtubule-walking proteins that resemble dynein or kinesin are part of the kinetochore, and they use the energy of ATP hydrolysis to pull the chromosome along its bound microtubules; B—Chromosome movement is driven by microtubule disassembly : as tubulin subunits dissociate, the kinetochore tends to slide poleward in order to restore its binding to the walls of the microtubule (After Alberts *et al.*, 1989).

1. The kinetochore hydrolyzes ATP to move along its attached microtubule, with the plus end of the microtubule depolymerizing as it becomes exposed (Fig. 18.9A). 2. The depolymerization of the microtubule itself causes the kinetochore to move passively to optimize its binding energy on the microtubule (Fig. 18.9B). 3. A system of elastic protein filaments might connect the kinetochore to the pole and pull the kinetochore steadily poleward. Thus, in this case, microtubules merely regulate movements of the chromosomes.

In mammalian cells, anaphase B begins shortly after the chromatids have begun their voyage to the poles and stops when the spindle is about 1.5 to 2.0 times its metaphase length (15 times increase in certain protozoa). Thus, anaphase B increases the distance between two spindle poles and in contrast to anaphase A, is accompanied by the polymerization of polar microtubules at their plus ends. Further, the polar microtubules from each half-spindle overlap in a central region near the spindle equator (*e.g.*, diatoms). During anaphase B these two sets of antiparallel polar microtubules appear to slide away from each other in the region of overlap. Dynein-like force generating protein may be involved in such a directed movement of chromosomes.

Significance of Mitosis

The mitosis has the following singificance for living organisms :

1. The mitosis helps the cell in maintaining proper size.

2. It helps in the maintenance of an equilibrium in the amount of DNA and RNA in the cell.

3. The mitosis provides the opportunity for the growth and development to organs and the body of the organisms.

4. The old decaying and dead cells of body are replaced by the help of mitosis.

5. In certain organisms, the mitosis is involved in asexual reproduction.

6. The gonads and the sex cells depend on the mitosis for the increase in their number.

7. The cleavage of egg during embryogenesis and division of blastema during blastogenesis, both involve mitosis.

MEIOSIS AND REPRODUCTIVE CYCLE

The term meiosis (Gr., *meioum*=to reduce or to diminish) was coined by **J.B. Farmer** in 1905. 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. 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 (*i.e.*, meiosis) resulting in the formation of haploid spores (Fig. 18.10). 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.

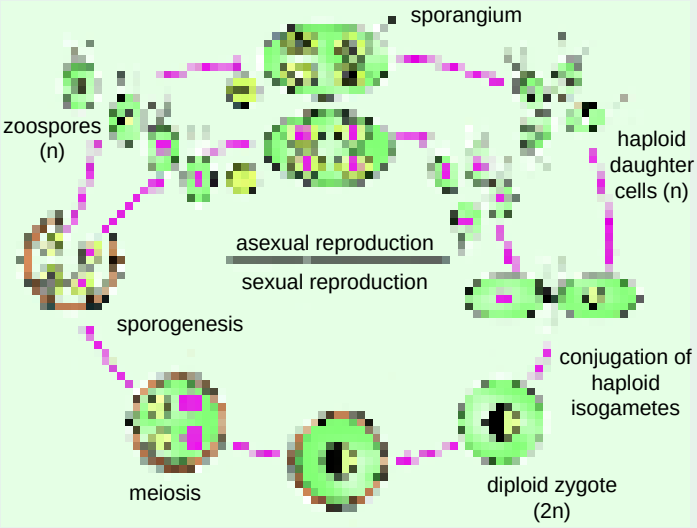


Fig. 18.10. Sexual reproduction cycle in *Chlamydomonas* sp. (after Burns, 1969).

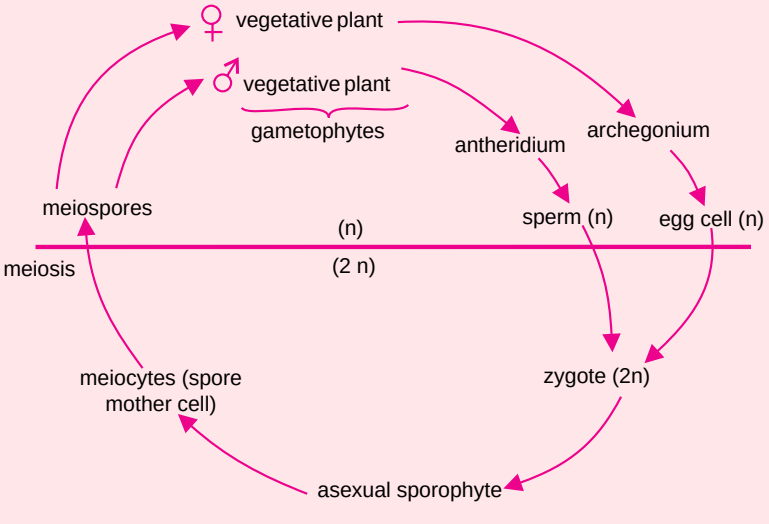


Fig. 18.11. Alternation of generation in Bryophyta (after Burns, 1969).

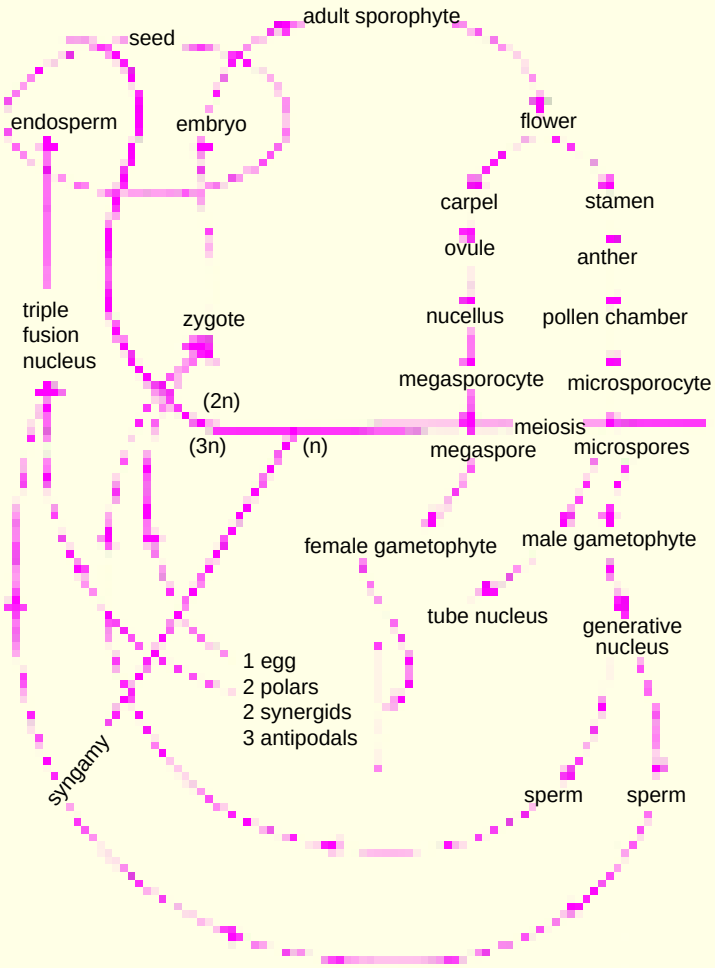


Fig. 18.12. Schematic diagram of the life cycle of an angiosperm (after Burns, 1969).

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. 18.11, Fig. 18.12 and Fig. 18.13).

Kinds of Meiosis

Meiosis occurs in the germ cells of sexually reproducing organisms. In both plants and animals, germ cells are localized in the gonads. The time at which meiosis takes place varies among different organisms, and on this basis the process can be classified into : **terminal, intermediate** or **initial**.

1. Terminal meiosis. It is also called **gametic meiosis** and is found in animals and a few lower plants. In terminal meiosis, the meiotic division occurs immediately before the formation of gametes or **gametogenesis** and will be discussed in detail in Chapter 20.

2. Intermediary or sporic meiosis. It is the characteristic of flowering plants. This meiosis takes place at some intermediate time between fertilization and the formation of gametes. It is also involved in the production of microspores (in anthers) and megaspores (in ovary or pistil) or in **microsporogenesis** and **megasporogenesis**, respectively.

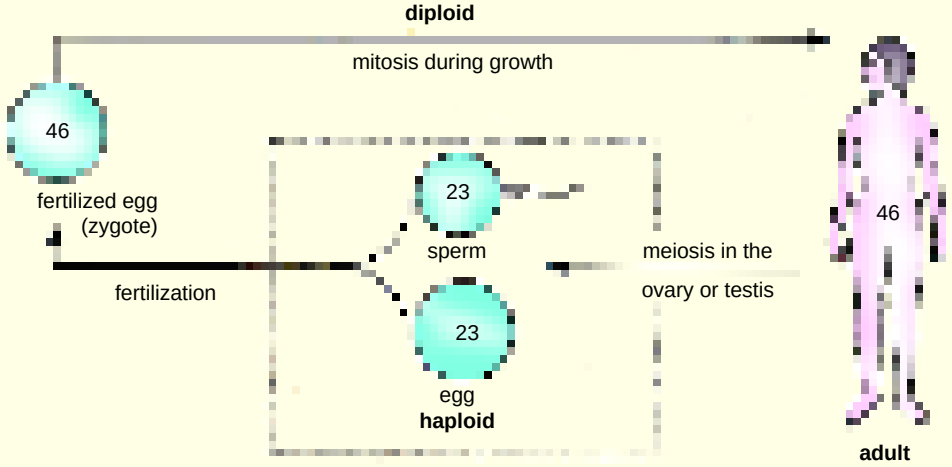


Fig. 18.13. Life cycle of a higher animal (human being).

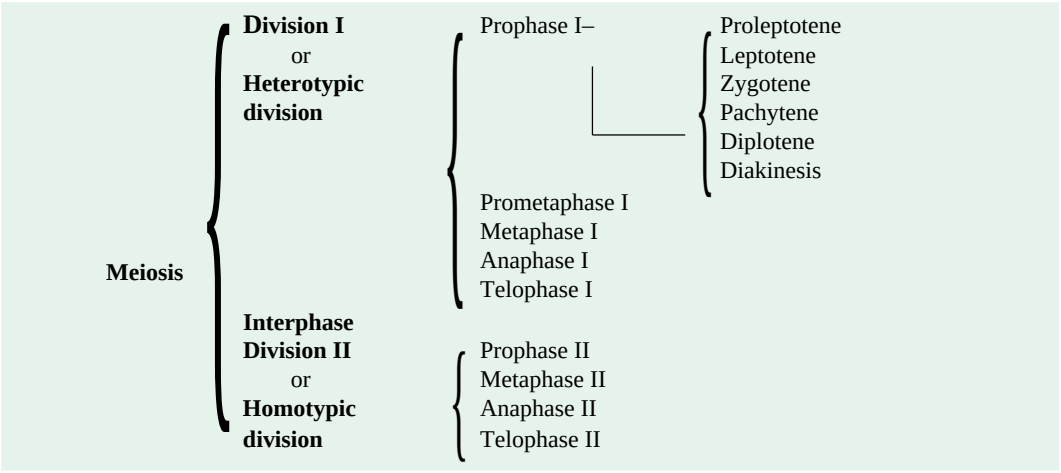
3. Initial or zygotic meiosis. It occurs in some algae, fungi, and diatoms. Meiotic division occurs immediately after fertilization; in this case, only the egg is diploid.

Meiocytes. The cells in which meiosis takes place are known as **meiocytes**. The meiocytes of gonads are called **gonocytes** which may be **spermatocytes** in male and **oocytes** in female. The meiocytes of the plant sporangium are called **sporocytes** (*i.e.*, **microsporocytes** and **megasporocytes**).

Process of Meiosis

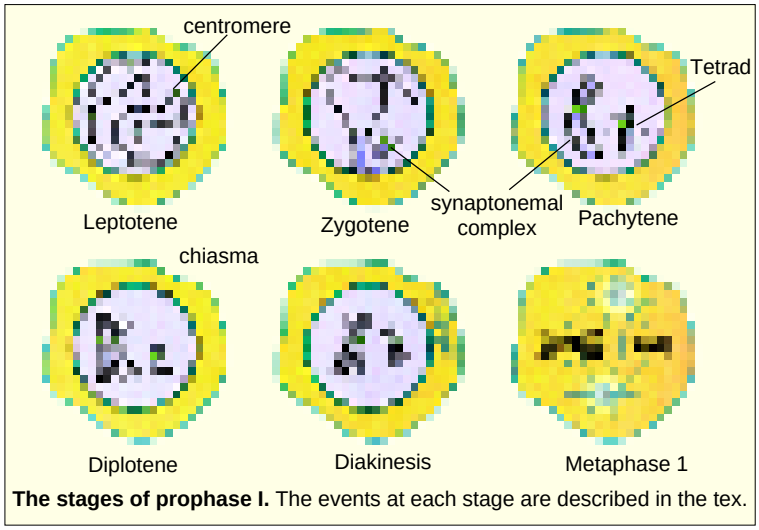
Meiosis superficially resembles two mitotic divisions without an intervening period of DNA replication. The first meiotic division includes a long prophase in which the homologous chromosomes become closely associated to each other and interchange of hereditary material takes place between them. Further, in the first meiotic division the reduction of chromosome number takes place and, thus, two haploid cells are resulted by this division. The first meiotic division is also known as the **heterotypic division**. In the second meiotic division the haploid cell divides mitotically and results into four haploid cells. The second meiotic division is also known as the **homotypic division**. In the homotypic division pairing of chromosomes, exchange of the genetic material and reduction of the chromosome number do not occur.

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. The successive meiotic substages can be represented as follows :



Heterotypic Division or First Meiotic Division

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 G_2 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.



The stages of prophase I. The events at each stage are described in the tex.

Prophase I

The first prophase is the longest stage of the meiotic division. It includes following substages :

1. Proleptotene or Prolepto-nema. (Gr., *pro*=before; *leptas*= thin; *nema*= thread). The proleptotene stage closely resembles with the early mitotic prophase. In this stage the chromosomes are extremely thin, long, uncoiled, longitudinally single and slender thread-like structures.

2. Leptotene or Leptonema. In the leptotene stage the chromosomes become more uncoiled and assume a long thread-like shape. The chromosomes at this stage take up a specific orientation inside the nucleus; the ends of the chromosomes converge toward one side of the nucleus, that side where the centrosome lies (the **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.

3. Zygotene or Zygonema. (Gr., *zygon*=adjoining). In the zygotene stage, the pairing of homologous chromosomes takes place. The homologous chromosomes which come from the mother

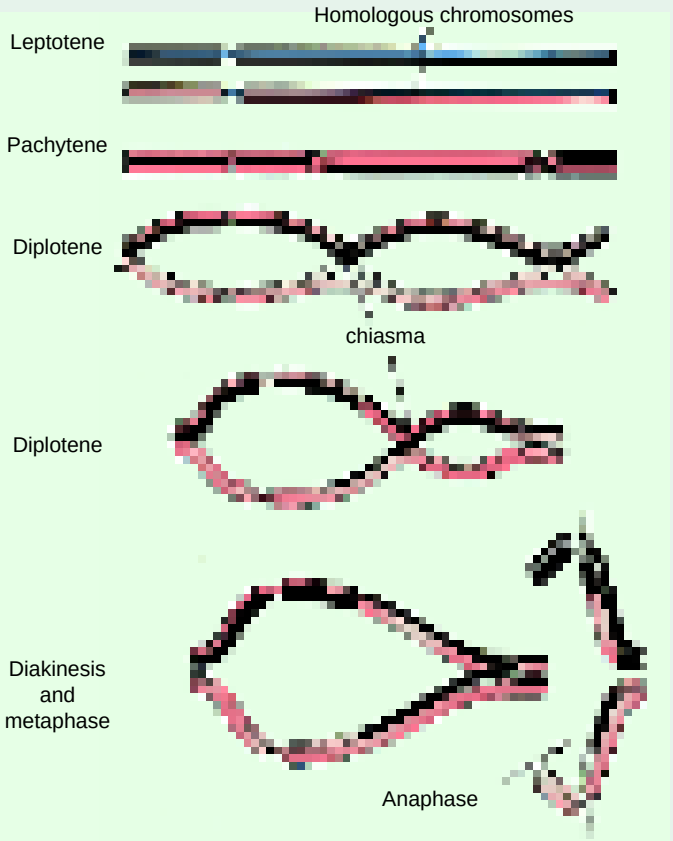


Fig. 18.14. Diagram of chromosomal exchange in the four-strand stage and of terminalization during first meiotic division.

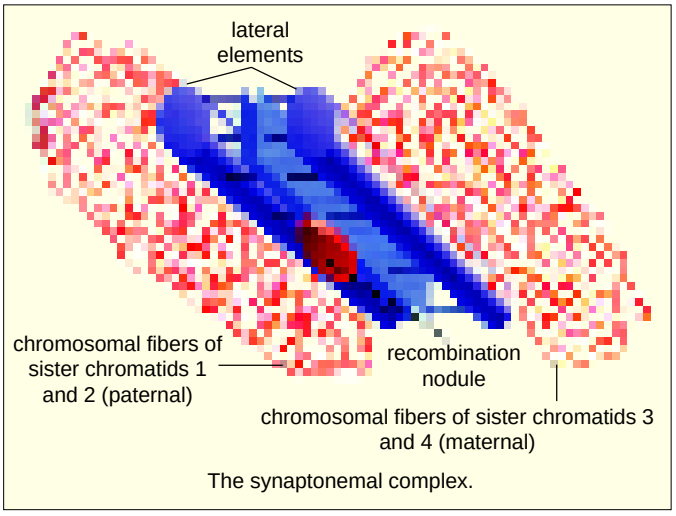
(by ova) and father (by sperm) are attracted towards each other and their pairing takes place. The pairing of the homologous chromosomes is known as **synapsis** (Gr.,*synapsis*=union). The synapsis begins at one or more points along the length of the homologous chromosomes. Three types of synapsis have been recognised.

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

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

(iii) Localized pairing or Random synapsis. The random type of synapsis occurs at various points of the homologous chromosomes.

The pairing of the homologous chromosome is very exact and specific (*i.e.*, alignment of chromosomes is exactly gene-for-gene). The paired homologous chromosomes are joined by a roughly 0.2-µm thick, protein-containing framework called a **synaptonemal complex** (**SC**). This complex extends along the whole length of the paired chromosomes and is usually anchored at either end to the nuclear envelope. SC helps to stabilize the pairing of homologous chromosomes and to facilitate the cytogenetical activity, called **recombination** or **crossing over** (occurring during pachynema). SC is not found in those organisms in which crossing over does not occur (*e.g.*, the male fruitfly, *Drosophila melanogaster* ; see **Burns** and **Bottino**, 1989).



The synaptonemal complex.

4. Pachytene or Pachynema. (Gr., *pachus*=thick). 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.

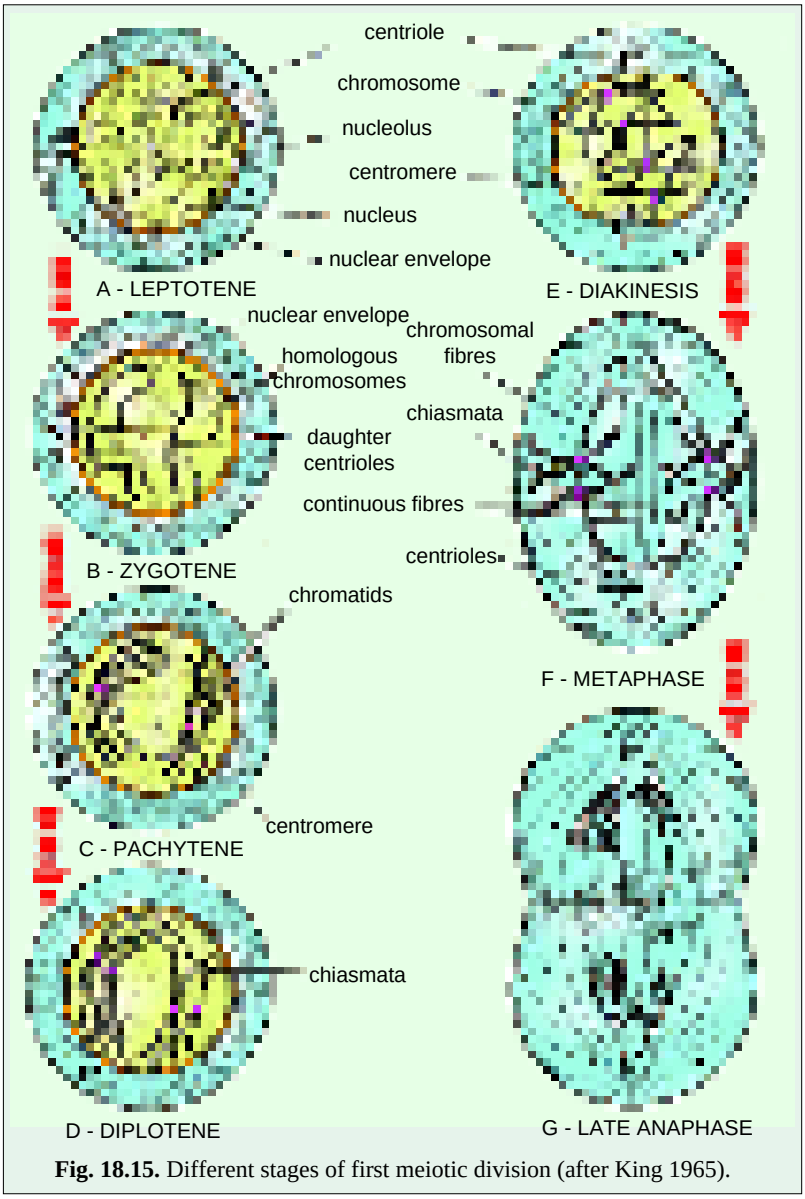


Fig. 18.15. Different stages of first meiotic division (after King 1965).

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 non-sister 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.

5. Diplotene or Diplonema. In diplonema, unpairing or **desynapsis** of homologous chromosomes is started and chiasmata are first seen. At this phase the chromatids of each tetrad are usually clearly visible, but the synaptonemal complex appears to be dissolved, leaving participating chromatids of the paired homologous chromosome physically joined at one or more discrete points called **chiasmata** (singular, **chiasma**; Gr., *chiasma*= cross piece). These points are where crossing over took place. Often there is some unfolding of the chromatids at this stage, allowing for RNA synthesis and cellular growth.

6. Diakinesis. In the diakinesis stage the bivalent chromosomes become more condensed and evenly distributed in the nucleus. The nucleolus detaches from the nucleolar organizer and ultimately disappers. The nuclear envelope breaks down. During diakinesis the chiasma moves from the centromere towards the end of the chromosomes and the intermediate chiasmata diminish. This type of movement of the chiasmata is known as **terminalization**. The chromatids still remain connected by the terminal chiasmata and these exist up to the metaphase.

Prometaphase

In the prometaphase the nuclear envelope disintegrates and the microtubules get arranged in the form of spindle in between the two centrioles which occupy the position of two opposite poles of the cell. The chromosomes become greatly coiled in the spiral manner and get arranged on the equator of the spindle.

Metaphase I

Metaphase I consists of spindle fibre attachment to chromosomes and chromosomal alignment at the equator. During metaphase I, the microtubules of the spindle are attached with the centromeres of the homologous chromosomes of each tetrad. The centromere of each chromosome is directed towards the opposite poles. The repulsive forces between the homologous chromosomes increase greatly and the chromosomes become ready to separate.

Anaphase I

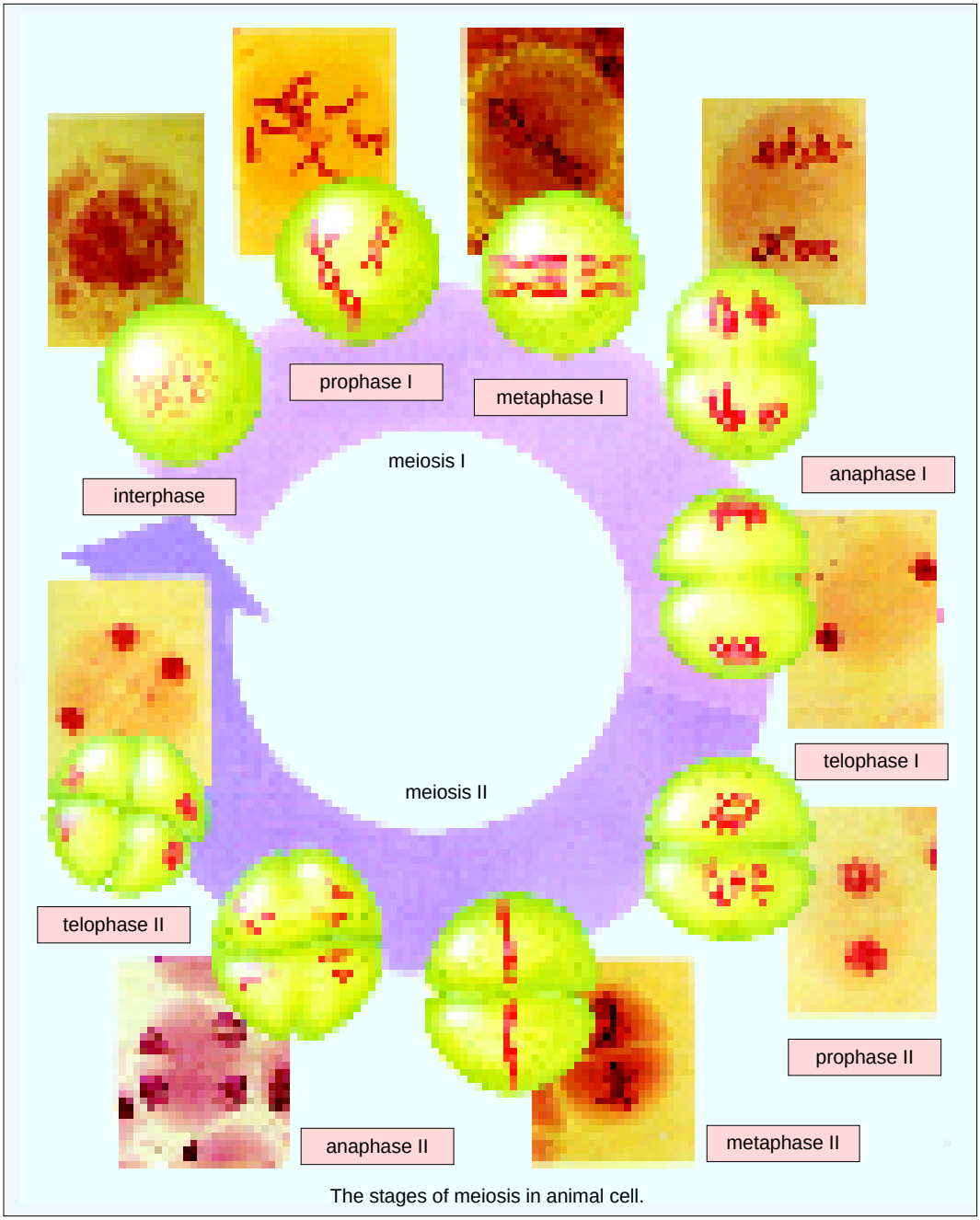
At anaphase I homologues are freed from each other and due to the shortening of chromosomal fibres or microtubules each homologous chromosome with its two chromatids and undivided centromere move towards the opposite poles of the cell. The chromosomes with single or few terminal chiasma usually separate more frequently than the longer chromosomes containing many chiasmata.

The actual reduction and **disjunction** occurs at this stage. Here it should be carefully noted that the homologous chromosomes which move towards the opposite poles are the chromosomes of either paternal or maternal origin. Moreover, because during the chiasma formation out of two chromatids of a chromosome, one has changed its counterpart, therefore, the two chromatids of a chromosome do not resemble with each other in the genetical terms.

Telophase I

The arrival of a haploid set of chromosomes at each pole defines the onset of telophase I, during which nuclei are reassembled. The endoplasmic reticulum forms the nuclear envelope around the chromosomes and the chromosomes become uncoil. The nucleolus reappears and, thus, two daughter chromosomes are formed. After the karyokinesis, cytokinesis occurs and two haploid cells are formed.

Both cells pass through a short resting phase of interphase. During interphase, no DNA replication occurs, so that chromosomes at the second prophase are the same double-stranded



The stages of meiosis in animal cell.

structures that disappeared at the first telophase. In case of *Trillium* telophase I and interphase I do not occur and the anaphase I is followed by prophase II directly.

Homotypic or Second Meiotic Division

The homotypic or second meiotic division is actually the mitotic division which divides each haploid meiotic cell into two haploid cells. The second meiotic division includes following four stages.

Prophase II

In the prophase second, each centriole divides into two and, thus, two pairs of centrioles are

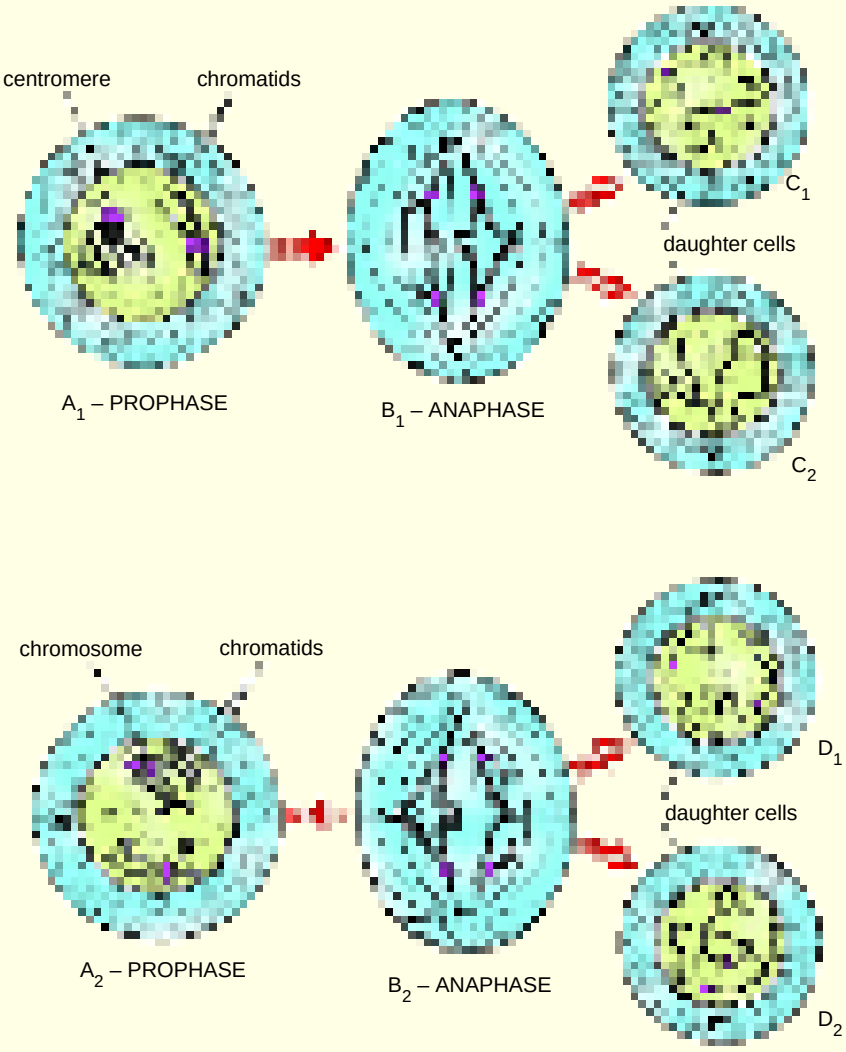


Fig. 18.16. Different stages of second meiotic division (after King, 1965).

formed. Each pair of centrioles migrates to the opposite pole. The microtubules get arranged in the form of spindle at the right angle of the spindle of first meiosis. The nuclear membrane and the nucleolus disappear. The chromosomes with two chromatids become short and thick.

Metaphase II

During metaphse II, the chromosomes get arranged on the equator of the spindle.The centromere divides into two and, thus, each chromosome produces two monads or daughter chromosomes. The microtubules of the spindle are attached with the centromere of the chromosomes.

Anaphase II

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

Telophase II

The chromatids migrate to the opposite poles and now known as chromosomes. The endoplas-

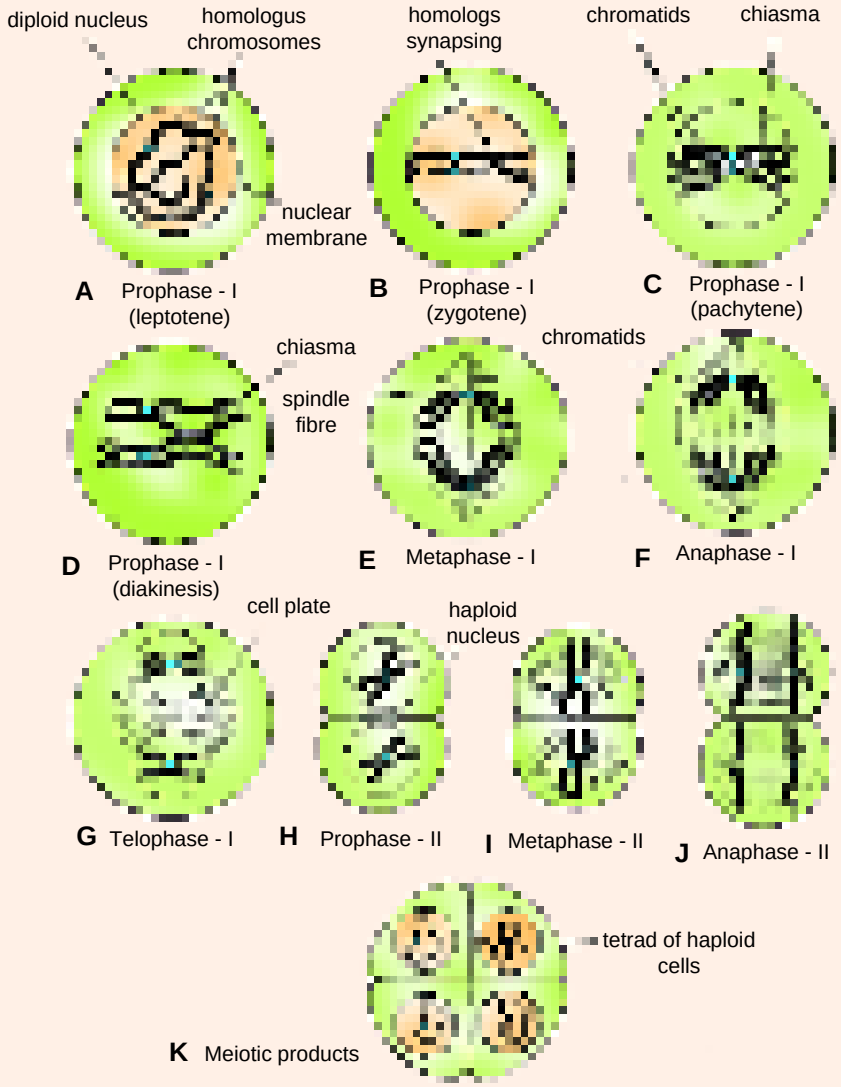


Fig. 18.17. Diagrammatic representation of meiosis in a plant cell showing one pair of homologous chromosomes (2n=2).

mic reticulum forms the nuclear envelope around the chromosomes and the nucleolus reappears due to synthesis of ribosomal RNA (rRNA) by rDNA and also due to accumulation of ribosomal proteins.

After the karyokinesis, in each haploid meiotic cell, the cytokinesis occurs and, thus, four haploid cells are resulted. These cells have different types of chromosomes due to the crossing over in the prophase I.

SIGNIFICANCE OF MEIOSIS

The meiosis has the greatest significance for the biological world because of its following uses :

Meiosis produces egg and sperms.

1. The meiosis maintains a definite and constant number of the chromosomes in the organisms.

2. By crossing over, the meiosis provides an opportunity for the exchange of the genes and, thus, causes the genetical variations among the species. The variations are the raw materials of the evolutionary process.

Thus the meiosis is a peculiar taxonomic, genetical and evolutionary process.

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.

REVISION QUESTIONS

1. What is the cell division ? How many types of cell division occur in living organisms ? Discuss the use and biological significance of each type of cell division.
2. Define the terms : cell cycle and mitosis. Name the stages of cell cycle. Which is usually the longest stage? What are the major features of each mitotic phase ?
3. What basic activities occur during mitosis ? How does mitosis differ in animal and plant cells ?
4. What biochemical events take place in cells before visible cellular division occurs ? Compare the cytogenetic view of chromatin in interphase, in mitosis, and in meiosis.
5. Describe the behaviour and presumed role of centrioles during mitosis.
6. What is meiosis ? Describe the major features of each meiotic phase. Also discuss, why is meiosis needed for the production of gametes.
7. Summarize the event of the first meiotic prophase.
8. Which phases of meiosis are the same as the corresponding mitotic phase and which are different ? In what ways do they differ ?
9. Describe various roles of cytoskeleton during mitosis ; stress upon the function of mitotic spindle during anaphase A and anaphase B.
10. Describe the role of the microtubules in chromosome movement during mitosis and meiosis.
11. What is cytokiness ? Describe the process of cytokinesis in animal and plant cells.
12. What is a division furrow and contractile ring ? What influences appear to be instrumental in establishing contractile ring ?
13. If one creates two spheres out of one, additional surface would be needed. In cell division, where does this additional surface come from.
14. Describe the process of cell plate formation. What are plasmodesmata and how are they formed?
15. Write short notes on the following :

    (i) Auxetic growth ; (ii) Cell cycle ;
    (iii) G_0 phase ; (iv) Mitotic spindle ;
    (v) MPF ; (vi) Synaptonemal complex.