Shree H. N. Shukla College of Science,
(Affiliated to Saurashtra university)
Nr. Lalpari Lake, B/H Marketing Yard, Rajkot-360 003

F.Y. B.Sc. (Sem. II) (CBCS)

BIOCHEMISTRY

[201]: CELL BIOLOGY

Unit 3
Cell Cycle, Cell Renewal and Cell Death

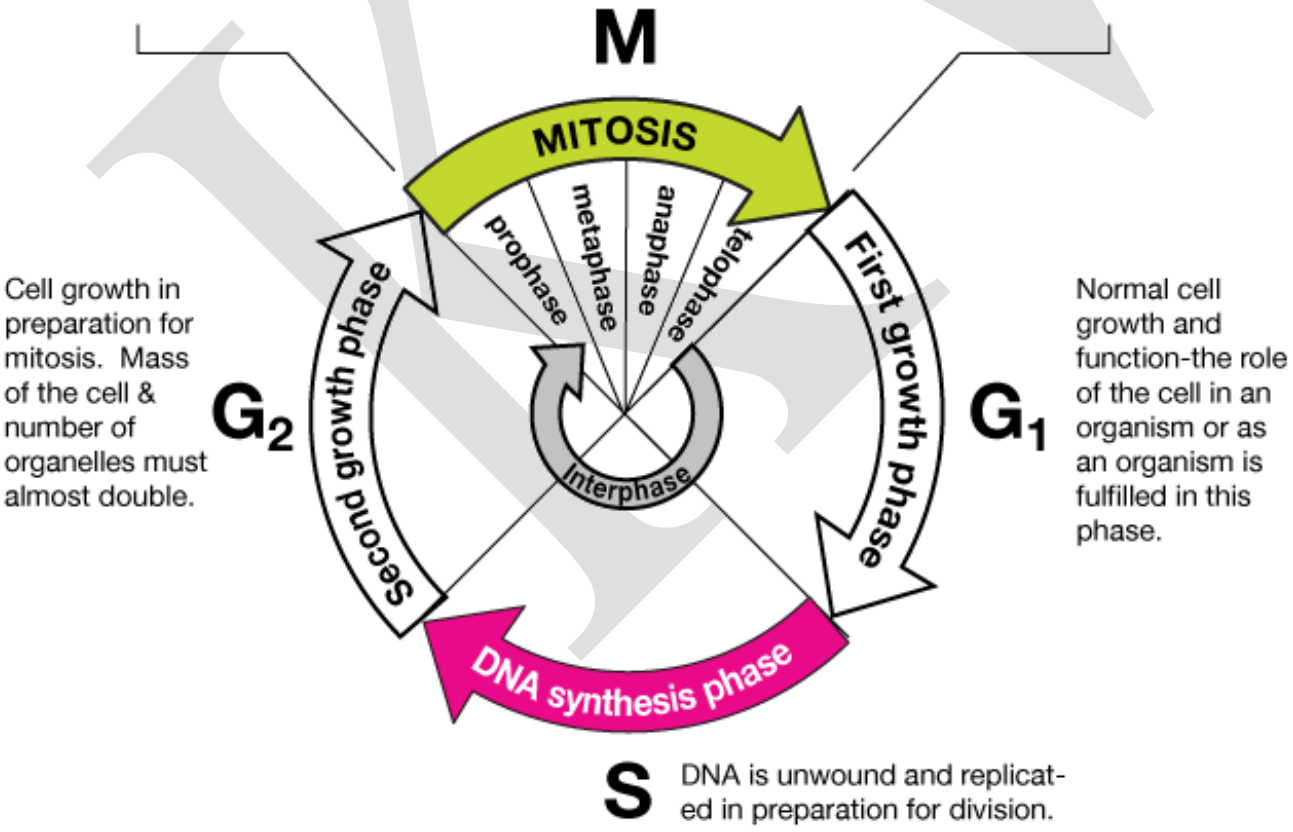
Prepared By

KISHAN VACHHANI

OVERVIEW OF EUKARYOTIC CELL CYCLE AND CHECKPOINTS

- The most basic function of the cell cycle is to duplicate the vast amount of DNA in the chromosomes and then segregate the copies into two genetically identical daughter cells.
- These processes define the two major phases of the cell cycle. Chromosome duplication occurs during *S phase* (S for DNA *s*ynthesis), which requires 10–12 hours and occupies about half of the cell-cycle time in a typical mammalian cell.
- After S phase, chromosome segregation and cell division occur in *M phase* (M for *m*itosis), which requires much less time (less than an hour in a mammalian cell).
- M phase comprises two major events:
  - Nuclear division, or *mitosis*, during which the copied chromosomes are distributed into a pair of daughter nuclei;
  - Cytoplasmic division, or *cytokinesis*, when the cell itself divides in two.

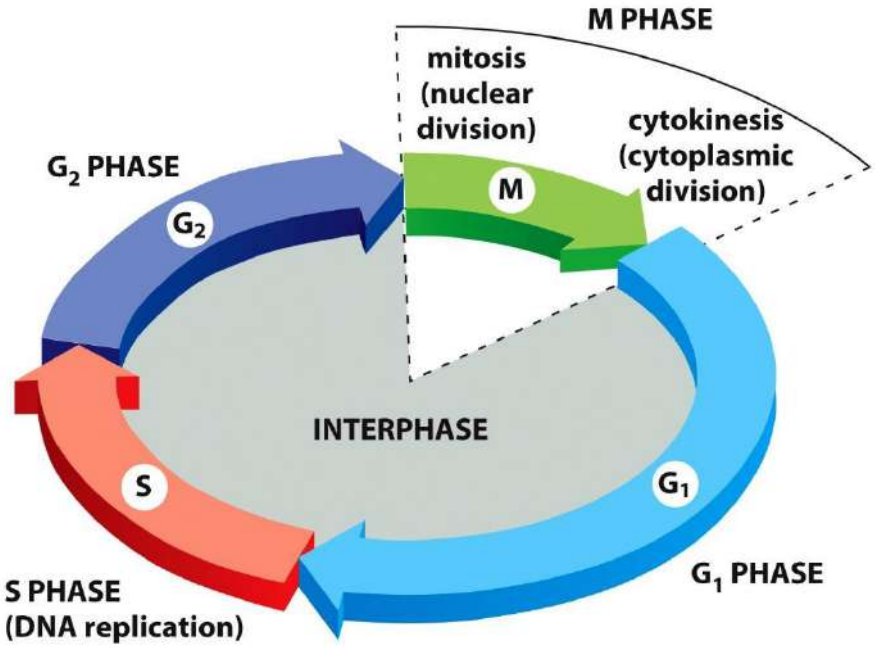
Prophase	Metaphase	Anaphase	Telophase
DNA coils into chromosomes, tightly-coiled DNA that is visible in a light microscope.	Microtubules bind to centromeres of chromosomes (point where sister chromatids attach).	Sister chromatids are pulled apart, each moving toward a new daughter cell.	Reverse of prophase, new nuclear membranes form, cytokinesis begins.

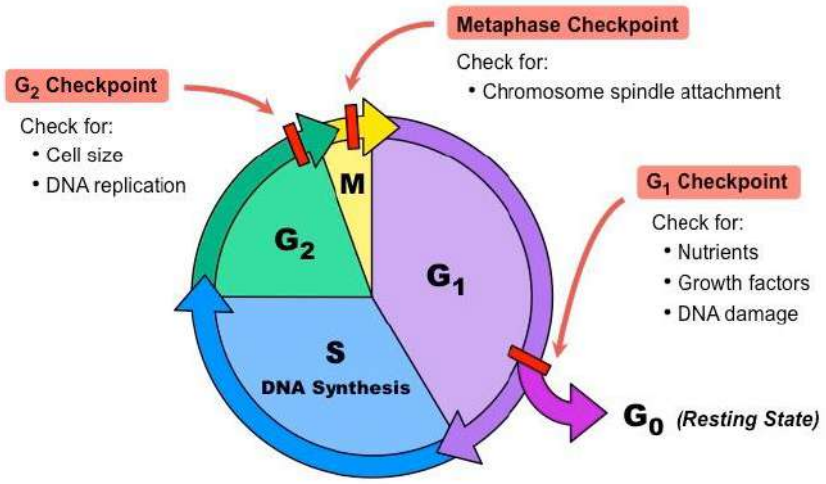


- At the end of S phase, the DNA molecules in each pair of duplicated chromosomes are intertwined and held tightly together by specialized protein linkages.
- Early in mitosis at a stage called *prophase*, the two DNA molecules are gradually disentangled and condensed into pairs of rigid, compact rods called sister chromatids, which remain linked by *sister-chromatid cohesion*. When the nuclear envelope disassembles later in mitosis, the sister-chromatid pairs become attached to the *mitotic spindle*, a giant bipolar array of microtubules.
- Sister chromatids are attached to opposite poles of the spindle and, eventually, align at the spindle equator in a stage called *metaphase*.
- The destruction of sister-chromatid cohesion at the start of *anaphase* separates the sister chromatids, which are pulled to opposite poles of the spindle. The spindle is then disassembled, and the segregated chromosomes are packaged into separate nuclei at *telophase.*
- Cytokinesis then cleaves the cell in two, so that each daughter cell inherits one of the two nuclei.

The Eukaryotic Cell Cycle Usually Consists of Four Phases

- Most cells require much more time to grow and double their mass of proteins and organelles than they require to duplicate their chromosomes and divide. Partly to allow time for growth, most cell cycles have *gap phases*—a G_1 phase between M phase and S phase and a G_2 phase between S phase and mitosis.
- Thus, the eukaryotic cell cycle is traditionally divided into four sequential phases: G_1, S, G_2, and M. G_1, S, and G_2 together are called interphase.
- In a typical human cell proliferating in culture, interphase might occupy 23 hours of a 24-hour cycle, with 1 hour for M phase.
- Cell growth occurs throughout the cell cycle, except during mitosis.
- The two gap phases are more than simple time delays to allow cell growth. They also provide time for the cell to monitor the internal and external environment to ensure that conditions are suitable and preparations are complete before the cell commits itself to the major upheavals of S phase and mitosis. The G_1 phase is especially important in this respect. Its length can vary greatly depending on external conditions and extracellular signals from other cells.





- If extracellular conditions are unfavourable, for example, cells delay progress through G_1 and may even enter a specialized rest in state known as *G_0* (G zero), in which they can remain for days, weeks, or even years before resuming proliferation.
- Indeed, many cells remain permanently in G_0 until they or the organism dies. If extracellular conditions are favourable and signals to grow and divide are present, cells in early G_1 or G_0 progress through a commitment point near the end of G_1 known as Start (in yeasts) or the restriction point (in mammalian cells). We will use the term Start for both yeast and animal cells. After passing this point, cells are committed to DNA replication, even if the extracellular signals that stimulate cell growth and division are removed.

CHECKPOINTS

- The goal of controlling any cyclic process is to adjust the duration of the cycle to allow sufficient time for all events to occur. Our way to achieve a more flexible and sensitive regulation of cycle is simply tom let the completion of each phase the cycle trigger the beginning of the next phase. Three principal checkpoints control the cell cycle in eukaryotes.

G_1 checkpoint:

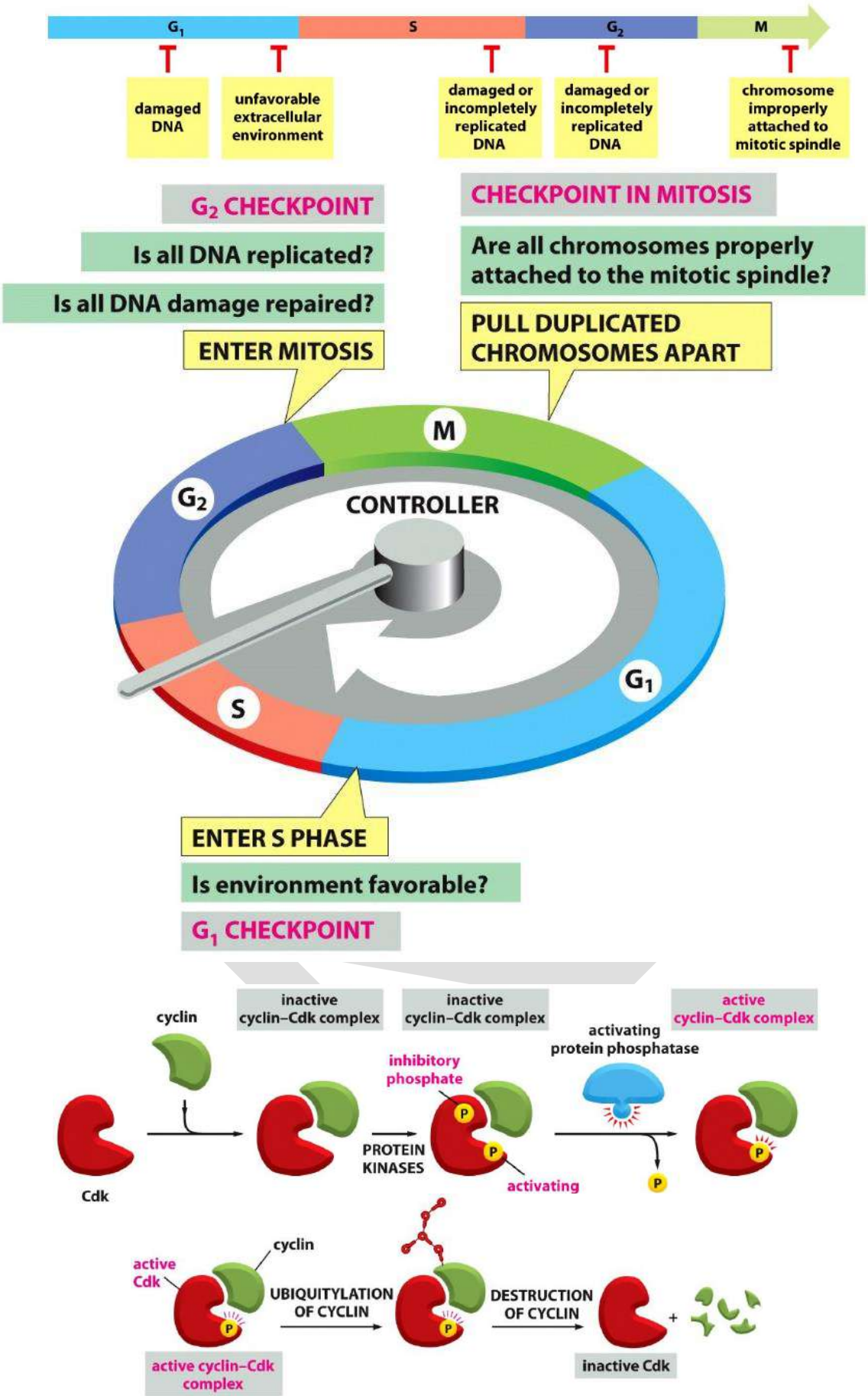
- Located near the end of G_1, just before entry into S phase. This checkpoint makes the key decision of whether the cell should divide, deadly division or enter a resting stage.
- Yeast, where researcher's first studied this checkpoint, it is called START, if conditions are favourable for division, the cell begins to copy its DNA initiating S phase.
- The G_1 checkpoint is where the more complex eukaryotic typically arrest the cell cycle if environmental conditions make cell division impossible, or if the cell passes into G_0 for an extended period.

G_2 checkpoint:

- The second checkpoint, which occurs at the end of G_2 triggers the start of M phase. If this checkpoint is passed, the cell initiates the many molecular processes that signal the beginning of mitosis.

M checkpoints:

- Occurring at metaphase, the third checkpoint triggers the exit from mitosis and cytokines is and the beginning of G_1.



This is how cyclin and Cdk are activated and deactivated after end of each phase.

PROCESS OF MITOTIC CELL DIVISION AND ITS PHYSIOLOGICAL SIGNIFICANCE

S PHASE

- The linear chromosomes of eukaryotic cells are vast and dynamic assemblies of DNA and protein, and their duplication is a complex process that takes up a major fraction of the cell cycle.
- Not only must the long DNA molecule of each chromosome be duplicated accurately—a remarkable feat in itself—but the protein packaging surrounding each region of that DNA must also be reproduced, ensuring that the daughter cells inherit all features of chromosome structure.
- The central event of chromosome duplication—DNA replication—poses two problems for the cell.
- First, replication must occur with extreme accuracy to minimize the risk of mutations in the next cell generation. Second, every nucleotide in the genome must be copied once, and only once, to prevent the damaging effects of gene amplification.
- We discuss the sophisticated protein machinery that performs DNA replication with astonishing speed and accuracy. In this section, we consider the elegant mechanisms by which the cell-cycle control system initiates the replication process and, at the same time, prevents it from happening more than once per cycle.

MITOSIS

- Following the completion of S phase and transition through G_2, the cell undergoes the dramatic upheaval of M phase. This begins with mitosis, during which the sister chromatids are separated and distributed (*segregated*) to a pair of identical daughter nuclei, each with its own copy of the genome.
- Mitosis is traditionally divided into five stages—*prophase*, *prometaphase*, *metaphase*, *anaphase*, and *telophase*—defined primarily on the basis of chromosome behaviour as seen in a microscope.
- As mitosis is completed, the second major event of M phase—cytokinesis— divides the cell into two halves, each with an identical nucleus.
- From a regulatory point of view, mitosis can be divided into two major parts, each governed by distinct components of the cell-cycle control system.
    - First, an abrupt increase in M-Cdk activity at the G_2/M transition triggers the events of early mitosis (prophase, prometaphase, and metaphase). During this period, M-Cdk and several other mitotic protein kinases phosphorylate a variety of proteins, leading to the assembly of the mitotic spindle and its attachment to the sister-chromatid pairs.
    - The second major part of mitosis begins at the metaphase to-anaphase transition, when the APC/C triggers the destruction of securin, liberating a protease that cleaves cohesin and thereby initiates separation of the sister chromatids.
- The APC/C also promotes the destruction of cyclins, which leads to Cdk inactivation and the dephosphorylation of Cdk targets, which is required for all events of late M phase, including the completion of anaphase, the disassembly of the mitotic spindle, and the division of the cell by cytokinesis.
- In this section, we describe the key mechanical events of mitosis and how M-Cdk and the APC/C orchestrate them.

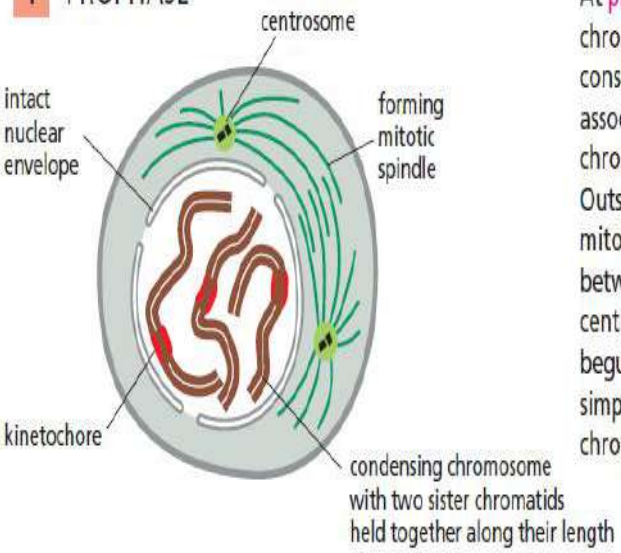
M-Cdk Drives Entry into Mitosis

- One of the most remarkable features of cell-cycle control is that a single protein kinase, M-Cdk, brings about all of the diverse and complex cell rearrangements that occur in the early stages of mitosis.
- At a minimum, M-Cdk must induce the assembly of the mitotic spindle and ensure that each sister chromatid in a pair is attached to the opposite pole of the spindle.
- It also triggers *chromosome condensation*, the large-scale reorganization of the intertwined sister chromatids into compact, rodlike structures.
- In animal cells, M-Cdk also promotes the breakdown of the nuclear envelope and rearrangements of the actin cytoskeleton and the Golgi apparatus.
- Each of these processes is thought to be initiated when M-Cdk phosphorylates specific proteins involved in the process, although most of these proteins have not yet been identified.
- M-Cdk does not act alone to phosphorylate key proteins involved in early mitosis. Two additional families of protein kinases, the *Polo-like kinases* and the *Aurora kinases*, also make important contributions to the control of early mitotic events.
- The Polo-like kinase Plk, for example, is required for the normal assembly of a bipolar mitotic spindle, in part because it phosphorylates proteins involved in separation of the spindle poles early in mitosis.
- The Aurora kinase Aurora-A also helps control proteins that govern the assembly and stability of the spindle, whereas Aurora-B controls attachment of sister chromatids to the spindle, as we discuss later.

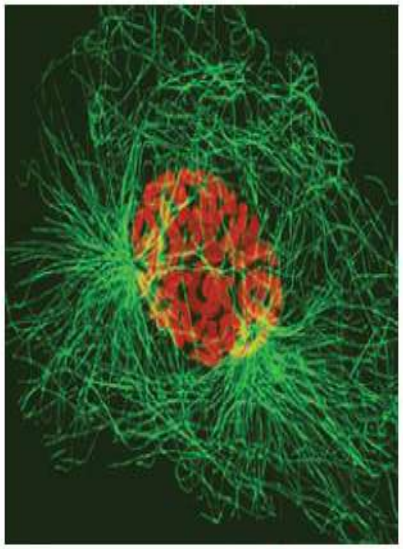
SIGNIFICANCE OF MITOTIC CELL DIVISION

- The mitosis helps the cell in maintaining proper size,
- It helps in the maintenance of equilibrium in the amount of DNA and RNA in the cell.
- The mitosis provides the opportunity for growth and development organs and the body of the organisms.
- The old decaying and dead cell of body are replaced by the help of mitosis.
- In certain organisms, the mitosis is involved in asexual reproduction.
- The gonads and the sex cells depend on the mitosis for the increase in their numbers.
- The cleavage of egg during embryogenesis and division of blastogenesis, mitosis is important for sexual reproduction indirectly.
- It allows the sexual reproducing organisms to grow and develop from single cell into a sexual mature individual.
- This allows organisms to continue to reproduce through the generations.
- It maintains genetic stability within the population of cells derived from same parent cell.
- It helps the growth and tissue repair.
- It helps the growth and replacement of dead and worn-out cells.
- It is a means of reproduction in lower organisms.

1 PROPHASE



At prophase, the replicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have begun to move apart. For simplicity, only three chromosomes are drawn.



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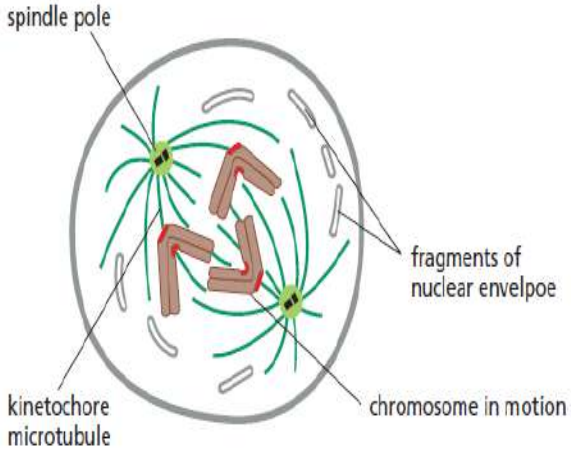
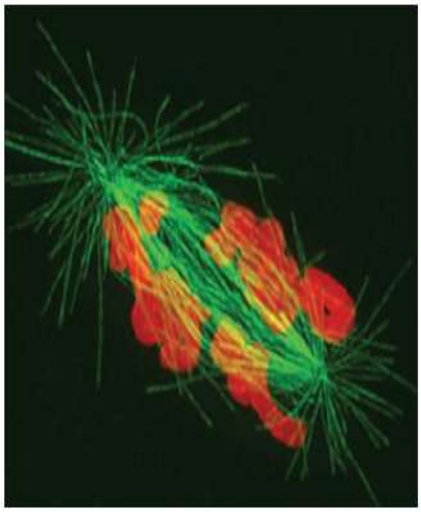
2 PROMETAPHASE



Prometaphase starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.



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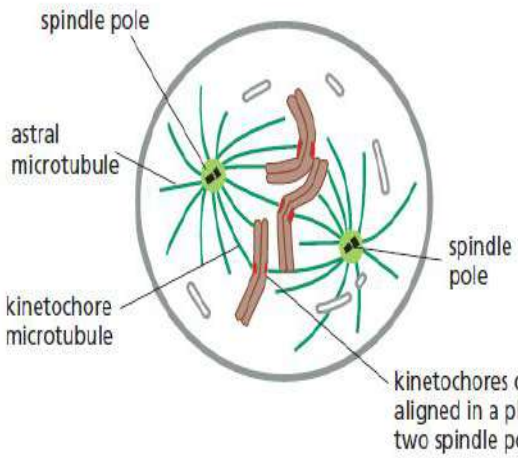
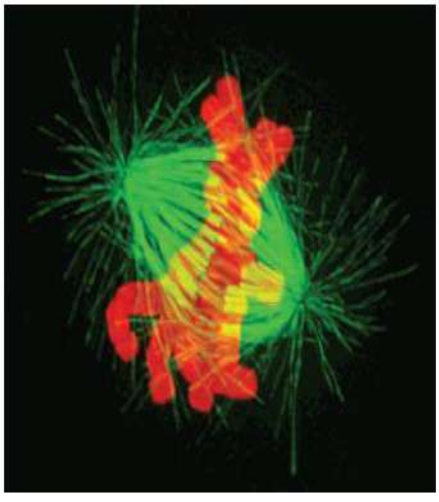
3 METAPHASE



At metaphase, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The paired kinetochore microtubules on each chromosome attach to opposite poles of the spindle.



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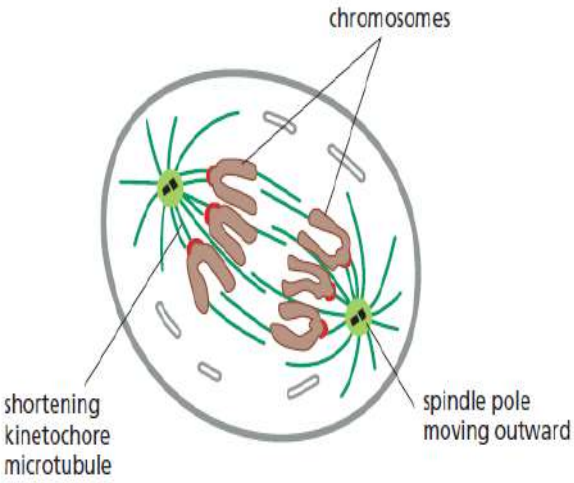
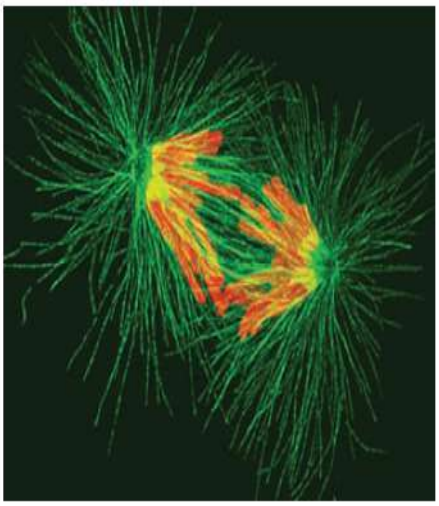
4 ANAPHASE



At anaphase, the sister chromatids synchronously separate, and each is pulled slowly toward the spindle pole it is attached to. The kinetochore microtubules get shorter, and the spindle poles also move apart, both contributing to chromosome segregation.



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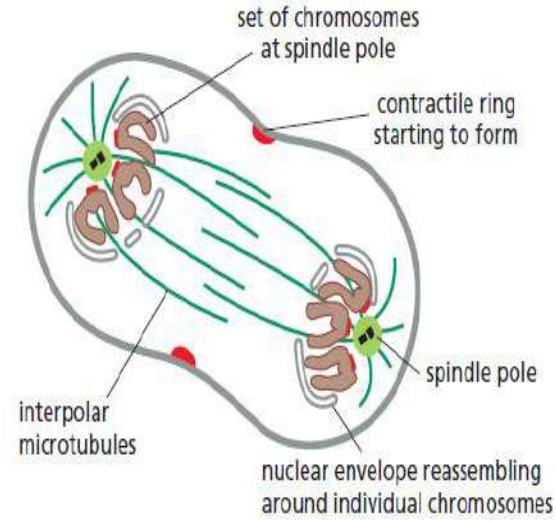
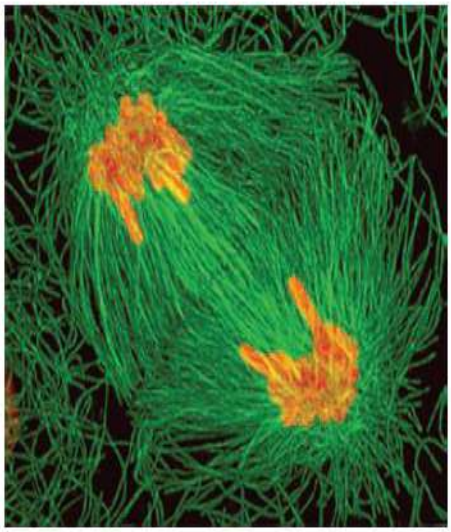
5 TELOPHASE



During telophase, the two sets of chromosomes arrive at the poles of the spindle. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with the assembly of the contractile ring.



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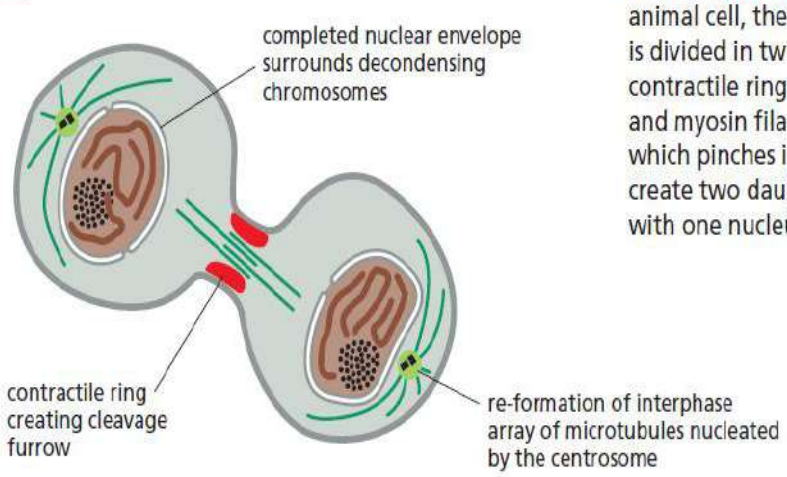
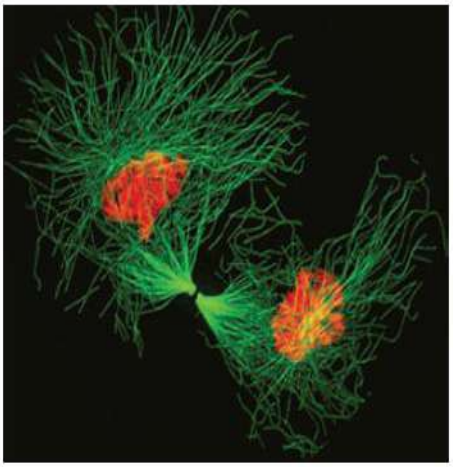
6 CYTOKINESIS



During cytokinesis of an animal cell, the cytoplasm is divided in two by a contractile ring of actin and myosin filaments, which pinches in the cell to create two daughters, each with one nucleus.



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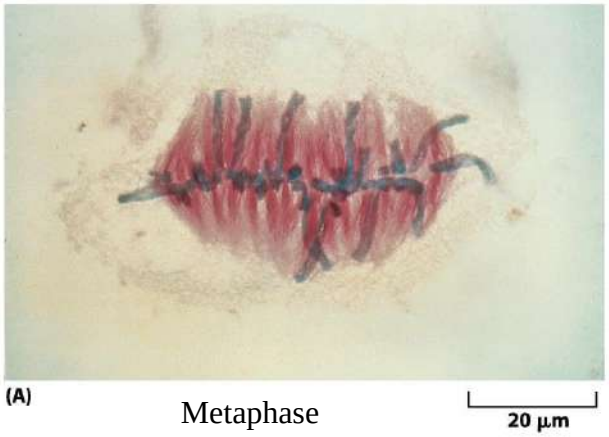
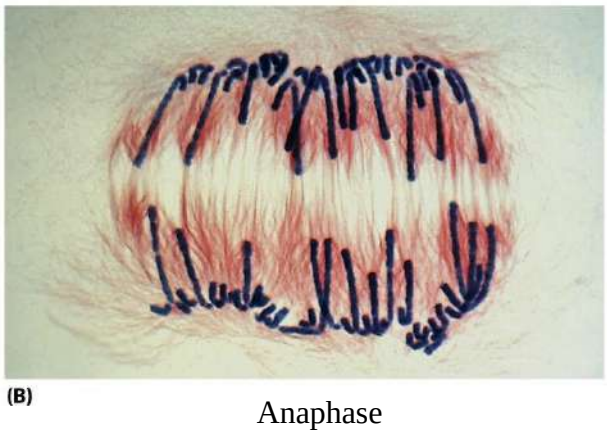
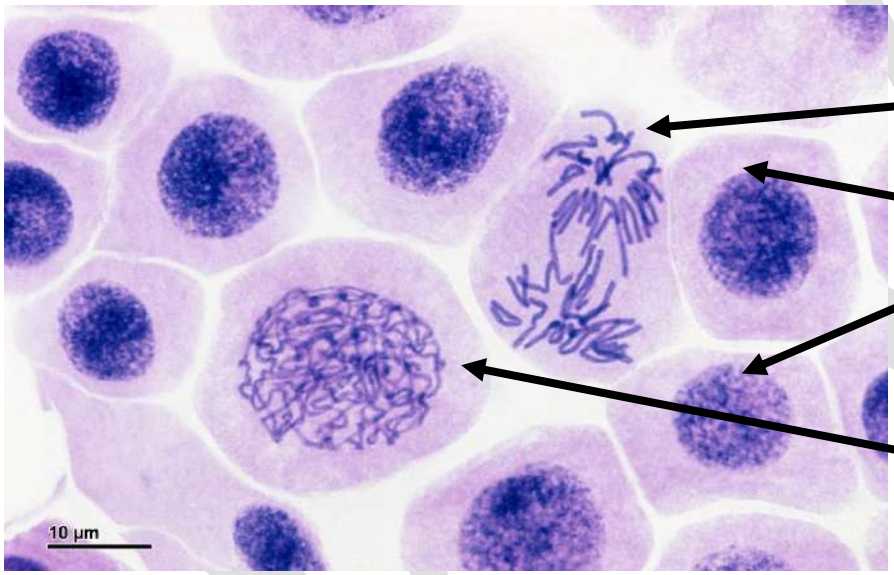
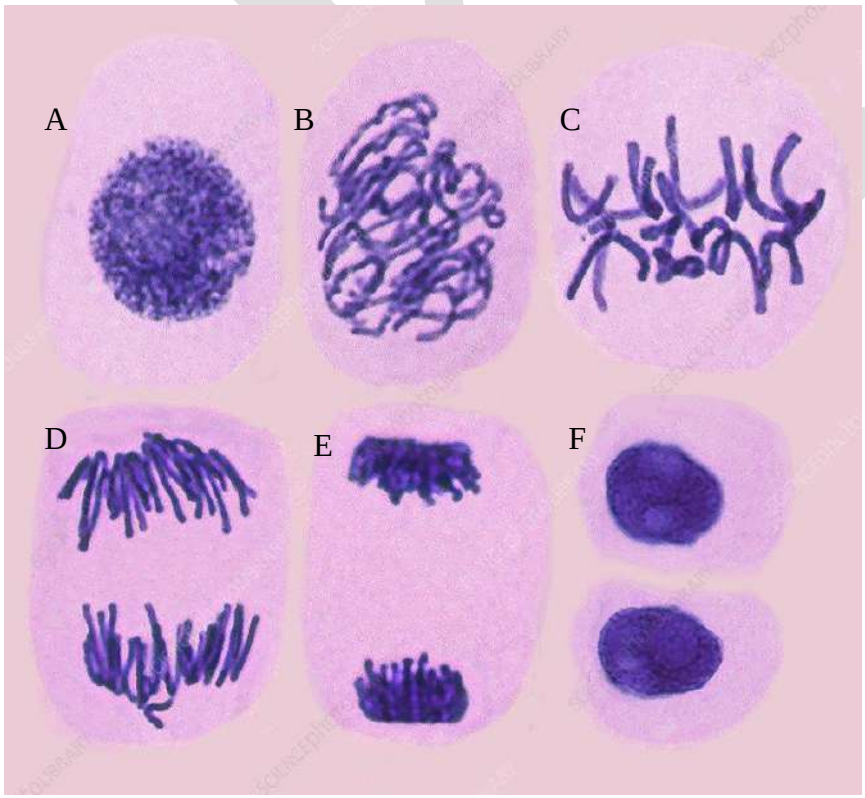


Metaphase



Anaphase





A Interphase
B Prophase
C Metaphase
D Anaphase
E Telophase
F Cytokinesis

MEIOSIS

- Most eukaryotic organisms reproduce sexually: the genomes of two parents mix to generate offspring that are genetically distinct from either parent.
- The cells of these organisms are generally *diploid*: that is, they contain two slightly different copies, or *homologs*, of each chromosome, one from each parent.
- Sexual reproduction depends on a specialized nuclear division process called *meiosis*, which produces *haploid* cells carrying only a single copy of each chromosome.
- In many organisms, the haploid cells differentiate into specialized reproductive cells called *gametes*—eggs and sperm in most species.
- In these species, the reproductive cycle ends when a sperm and egg fuse to form a diploid *zygote*, which has the potential to form a new individual.
- In this section, we consider the basic mechanisms and regulation of meiosis, with an emphasis on how they compare with those of mitosis.

Meiosis Includes Two Rounds of Chromosome Segregation

- Meiosis reduces the chromosome number by half using many of the same molecular machines and control systems that operate in mitosis.
- As in the mitotic cell cycle, the cell begins the meiotic program by duplicating its chromosomes in meiotic S phase, resulting in pairs of sister chromatids that are tightly linked along their entire lengths by cohesin complexes.
- Unlike mitosis, however, two successive rounds of chromosome segregation then occur.
- The first of these divisions (meiosis I) solves the problem, unique to meiosis, of segregating the homologs.
- The duplicated paternal and maternal homologs pair up alongside each other and become physically linked by the process of genetic recombination.
- These pairs of homologs, each containing a pair of sister chromatids, then line up on the first meiotic spindle.
- In the first meiotic anaphase, duplicated homolog rather than sister chromatids is pulled apart and segregated into the two daughter nuclei.
- Only in the second division (meiosis II), which occurs without further DNA replication, are the sister chromatids pulled apart and segregated (as in mitosis) to produce haploid daughter nuclei.
- In this way, each diploid nucleus that enters meiosis produces four haploid nuclei, each of which contains either the maternal or paternal copy of each chromosome, but not both:

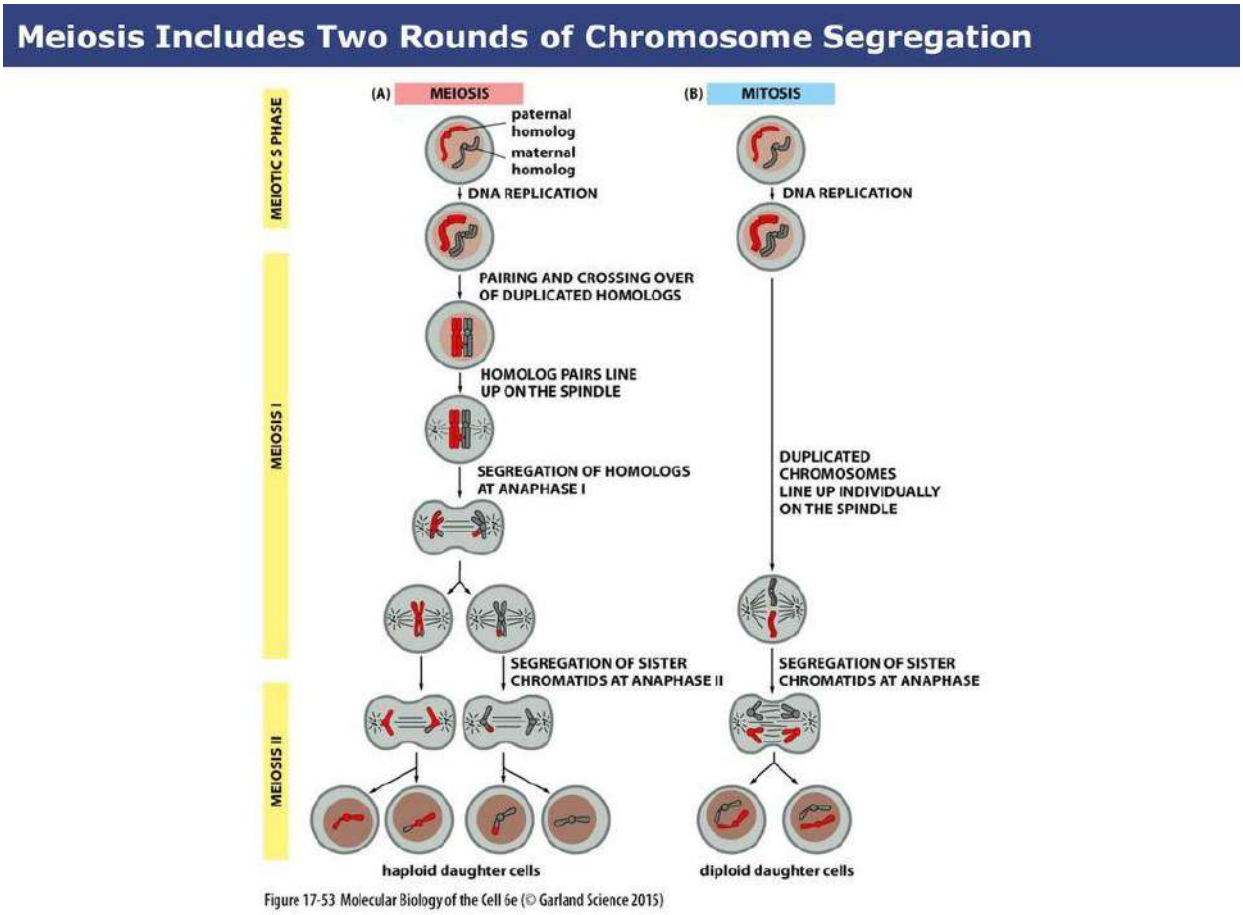
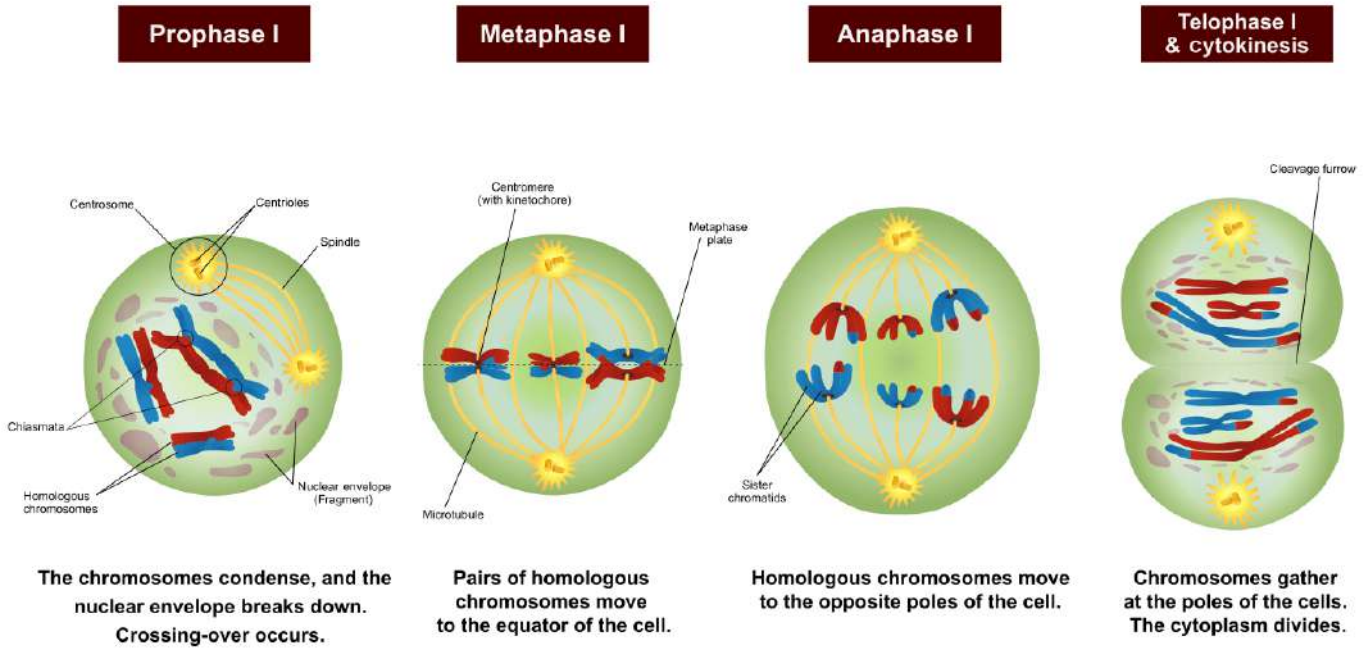


Figure 17-53 Molecular Biology of the Cell 6e (© Garland Science 2015)

Meiosis I

- Before entering meiosis, a cell must first go through interphase. As in mitosis, the cell grows during G_1 phase, copies all of its chromosomes during S phase, and prepares for division during G_2phase.
- During prophase I, differences from mitosis begin to appear. As in mitosis, the chromosomes begin to condense, but in meiosis I, they also pair up. Each chromosome carefully aligns with its homologue partner so that the two match up at corresponding positions along their full length.
- This process, in which homologous chromosomes trade parts, is called crossing over. It's helped along by a protein structure called the synaptonemal complex that holds the homologues together.
- The chromosomes would actually be positioned one on top of the other throughout crossing over; they're only shown side-by-side in the image above so that it's easier to see the exchange of genetic material.
- You can see crossovers under a microscope as chiasmata, cross-shaped structures where homologues are linked together. Chiasmata keep the homologues connected to
- each other after the synaptonemal complex breaks down, so each homologous pair needs at least one.
- It's common for multiple crossovers (up to 252525!) to take place for each homologue pair.
- The spots where crossovers happen are more or less random, leading to the formation of new, "remixed" chromosomes with unique combinations of alleles.
- After crossing over, the spindle begins to capture chromosomes and move them towards the center of the cell (metaphase plate). This may seem familiar from mitosis, but there is a twist. Each chromosome attaches to microtubules from just one pole of the spindle, and the two homologues of a pair bind to microtubules from opposite poles.

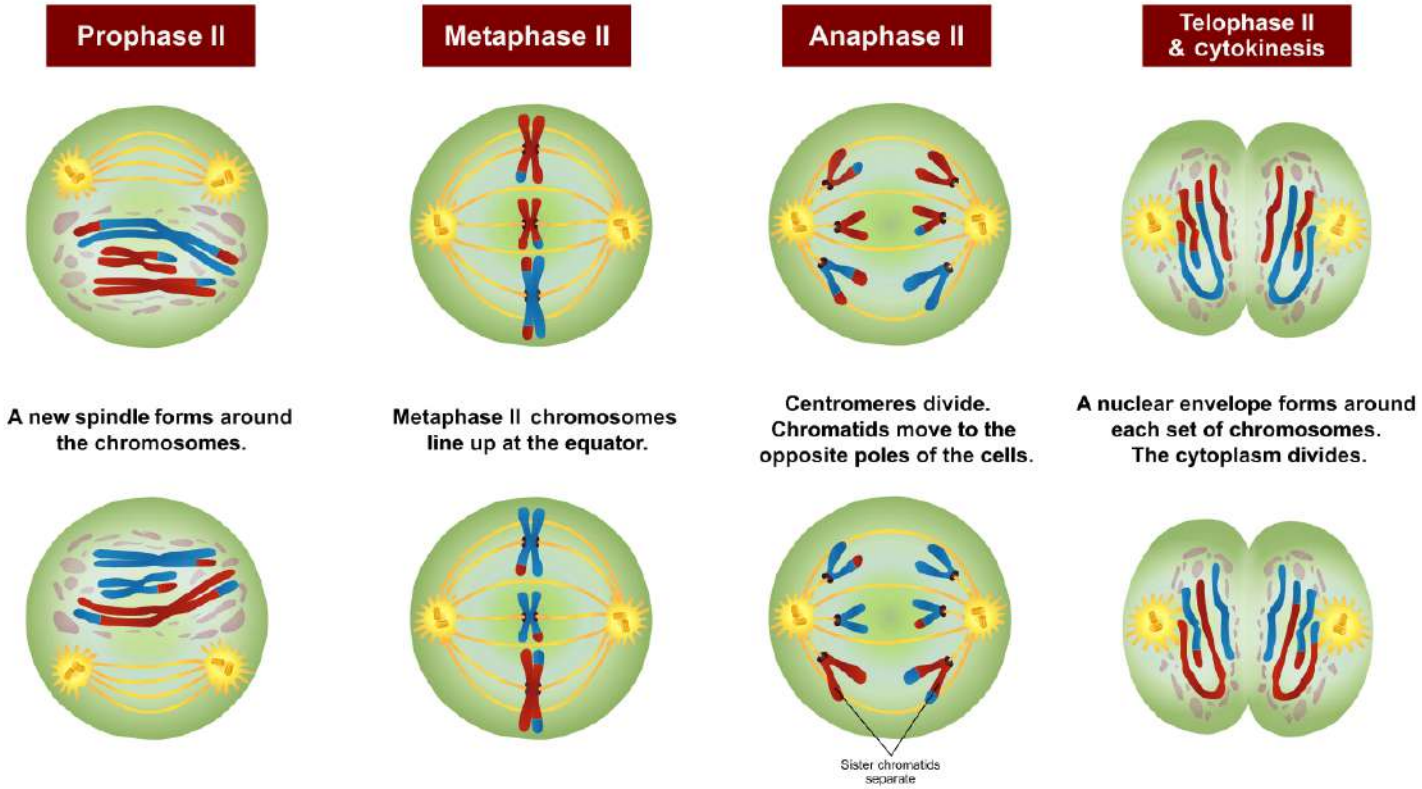
- So, during metaphase I, homologue pairs—not individual chromosomes—line up at the metaphase plate for separation.
- When the homologous pairs line up at the metaphase plate, the orientation of each pair is random.
- This allows for the formation of gametes with different sets of homologues.
- In anaphase I, the homologues are pulled apart and move apart to opposite ends of the cell.
- The sister chromatids of each chromosome, however, remain attached to one
- another and don't come apart.
- Finally, in telophase I, the chromosomes arrive at opposite poles of the cell. In some organisms, the nuclear membrane re-forms and the chromosomes decondense, although in others, this step is skipped—since cells will soon go through another round of division, meiosis II.
- Cytokinesis usually occurs at the same time as telophase I, forming two haploid daughter cells.

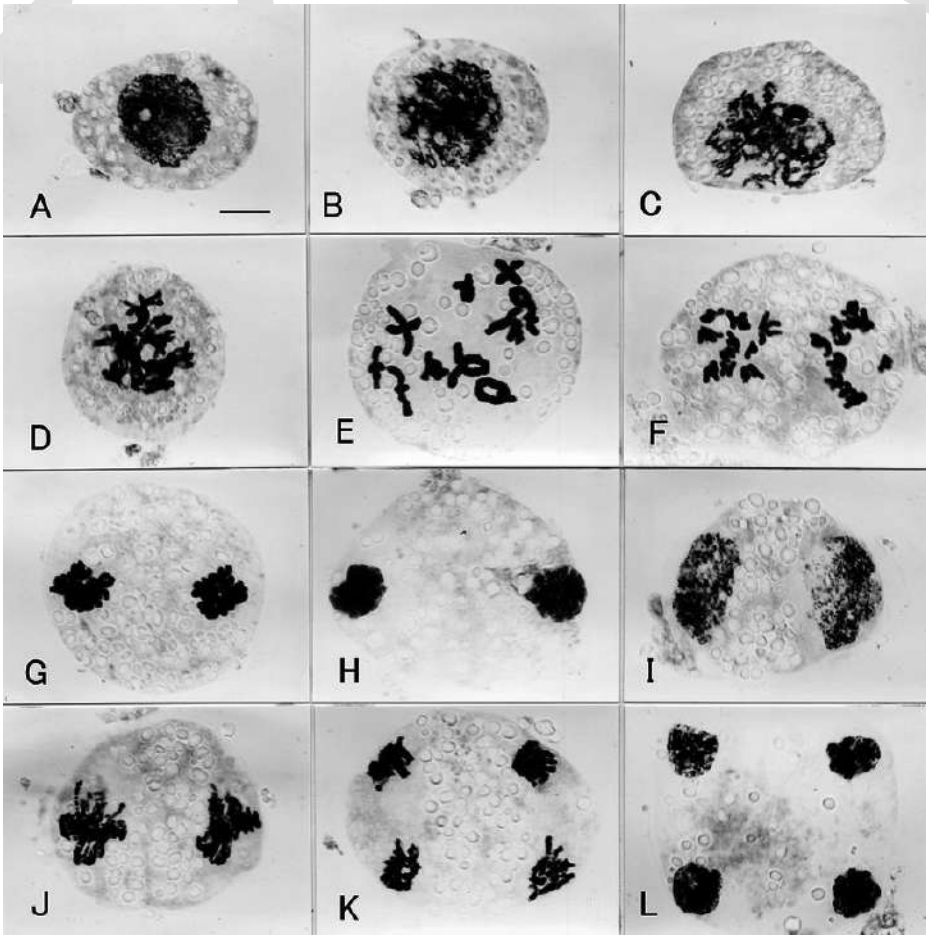


Meiosis II

- Cells move from meiosis I to meiosis II without copying their DNA. Meiosis II is a shorter and simpler process than meiosis I, and you may find it helpful to think of meiosis II as “mitosis for haploid cells."
- The cells that enter meiosis II are the ones made in meiosis I.
- These cells are haploid— have just one chromosome from each homologue pair—but their chromosomes still consist of two sister chromatids. In meiosis II, the sister chromatids separate, making haploid cells with non-duplicated chromosomes.
- During prophase II, chromosomes condense and the nuclear envelope breaks down, if needed.
- The centrosomes move apart, the spindle forms between them, and the spindle microtubules begin to capture chromosomes.
- The two sister chromatids of each chromosome are captured by microtubules from opposite spindle poles. In metaphase II, the chromosomes line up individually along the metaphase plate.
- In anaphase II, the sister chromatids separate and are pulled towards opposite poles of the cell.
- In telophase II, nuclear membranes form around each set of chromosomes, and the chromosomes decondense.

- Cytokinesis splits the chromosome sets into new cells, forming the final products of meiosis: four haploid cells in which each chromosome has just one chromatid.
- In humans, the products of meiosis are sperm or egg cells.





APOPTOSIS

- **Apoptosis** (from Ancient Greek ἀπόπτωσις, *apóptōsis*, "falling off") is a form of programmed cell death that occurs in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and globa mRNA decay.

- The average adult human loses between 50 and 70 billion cells each day due to apoptosis. For an average human child between the ages of 8 and 14, approximately 20–30 billion cells die per day.

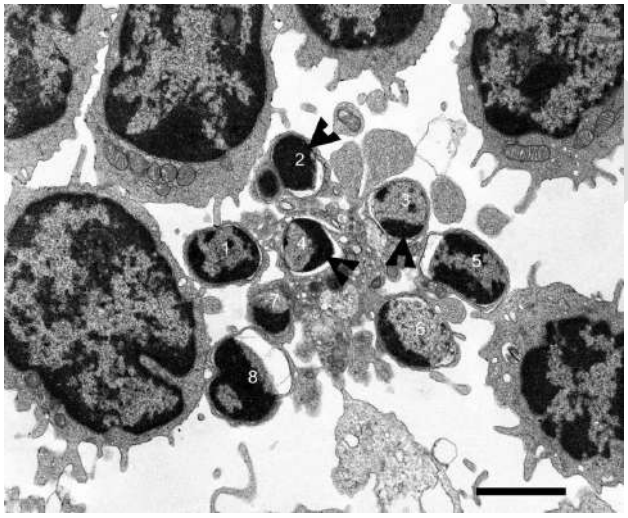
- In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis is a highly regulated and controlled process that confers advantages during an organism's life cycle. For example, the separation of fingers and toes in a developing human embryo occurs because cells between the digits undergo apoptosis.

- Unlike necrosis, apoptosis produces cell fragments called apoptotic bodies that phagocytic cells are able to engulf and remove before the contents of the cell can spill out onto surrounding cells and cause damage to them.

- Because apoptosis cannot stop once it has begun, it is a highly regulated process. Apoptosis can be initiated through one of two pathways. In the *intrinsic pathway* the cell kills itself because it senses cell stress, while in the *extrinsic pathway* the cell kills itself because of signals from other cells. Weak external signals may also activate the intrinsic pathway of apoptosis.

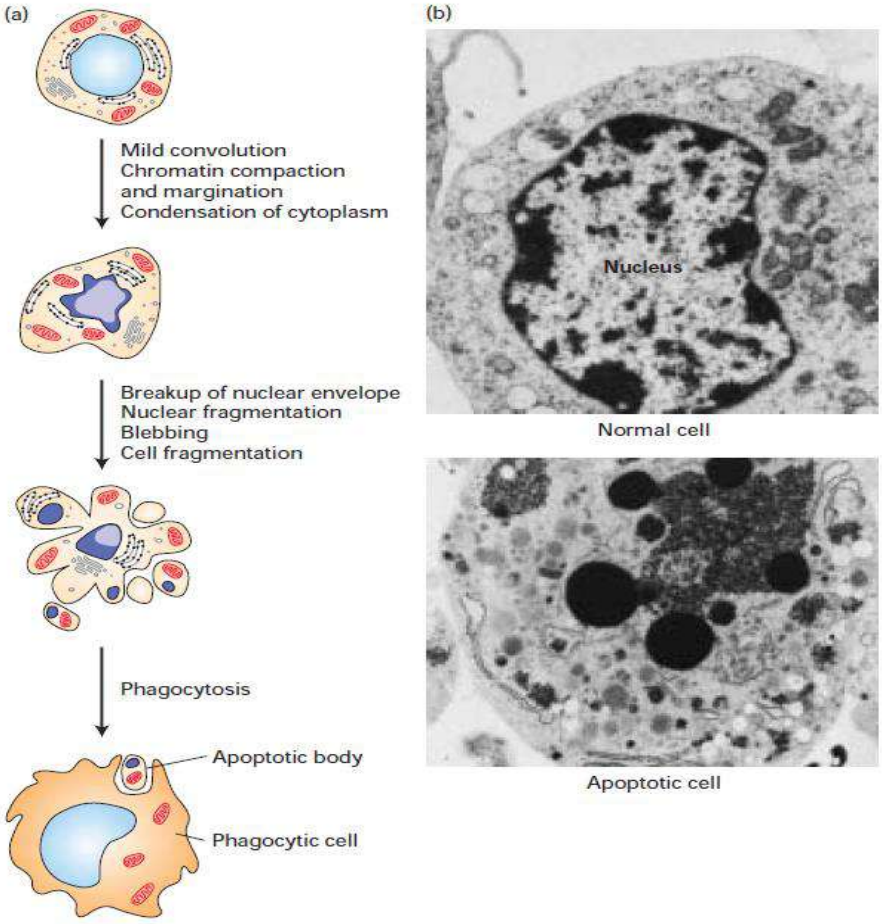
- Both pathways induce cell death by activating caspases, which are proteases, or enzymes that degrade proteins. The two pathways both activate initiator caspases, which then activate executioner caspases, which then kill the cell by degrading proteins indiscriminately.

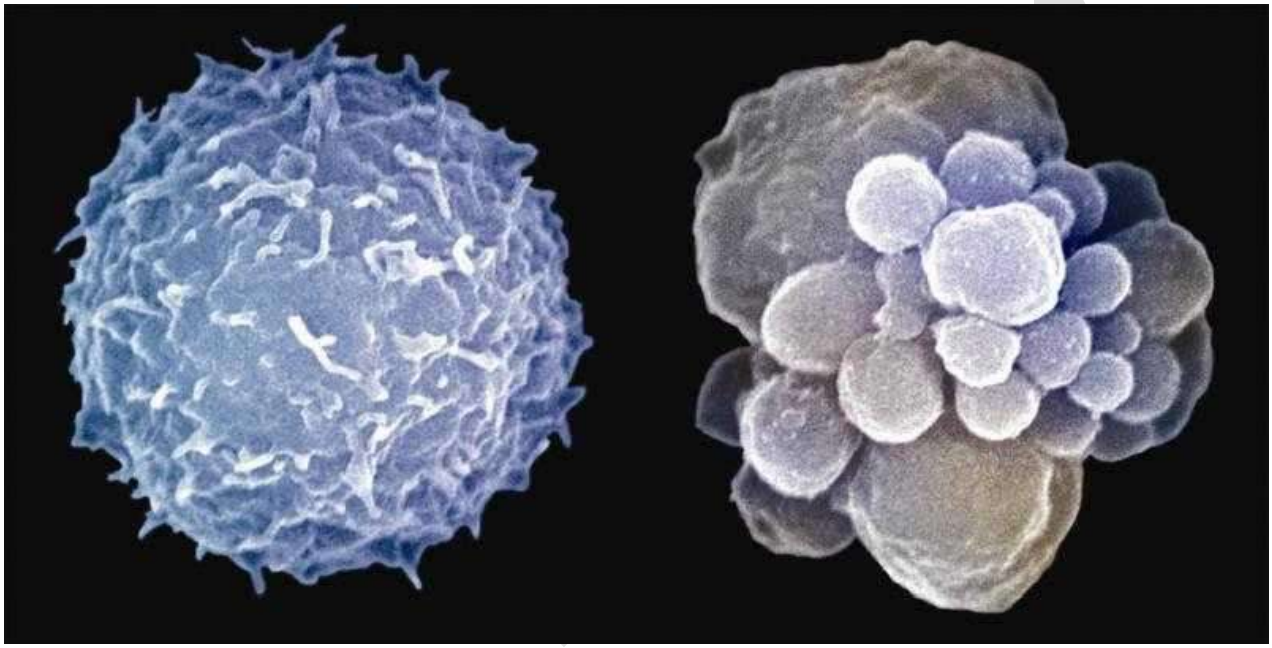
- In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in a wide variety of diseases. Excessive apoptosis causes atrophy, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer. Some factors like Fas receptors and caspases promote apoptosis, while some members of the Bcl-2 family of proteins inhibit apoptosis.



TEM image of apoptotic cell

SEM image of Brain cancer cell after apoptosis





NECROSIS

- Necrosis (from Ancient Greek νέκρωσις, *nékrōsis*, "death") is a form of cell injury which results in the premature death of cells in living tissue by autolysis. Necrosis is caused by factors external to the cell or tissue, such as infection, or trauma which result in the unregulated digestion of cell components.

- In contrast, apoptosis is a naturally occurring programmed and targeted cause of cellular death. While apoptosis often provides beneficial effects to the organism, necrosis is almost always detrimental and can be fatal.
- Cellular death due to necrosis does not follow the apoptotic signal transduction pathway, but rather various receptors are activated and result in the loss of cell membrane integrity and an uncontrolled release of products of cell death into the extracellular space.
- This initiates in the surrounding tissue an inflammatory response, which attracts leukocytes and nearby phagocytes which eliminate the dead cells by phagocytosis.
- However, microbial damaging substances released by leukocytes would create collateral damage to surrounding tissues.
- This excess collateral damage inhibits the healing process. Thus, untreated necrosis results in a build-up of decomposing dead tissue and cell debris at or near the site of the cell death. A classic example is gangrene.
- For this reason, it is often necessary to remove necrotic tissue surgically, a procedure known as debridement.