Cell division and Cell cycle, Cell cycle regulation

After studying all about cell lets study how cells give rise to a new cell. During the current lecture we will be discussing types of cell division and its various phases.

Cell division and its significance:

Continuity of life depends on cell division. All cells are produced by divisions of pre-existing cell (Please recall our discussion about the cell theory in our first lecture). A cell born after a division, proceeds to grow by macromolecular synthesis, and divides after reaching a species-determined division size. Growth of a cell is an increase in size or mass which is an irreversible process that occurs at all organizational levels.

Cell cycle:

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 division. Cells have the property of division and multiplication and consist of three major phases namely mitosis (M phase) or the nuclear division, cytokinesis or the division of the cell and interphase where replication of genetic material occurs. The M phase lasts only for an hour in a period of 24 hour required for a eukaryotic cell to divide. The interphase can be further divided into G1 (gap phase 1), S (synthesis) and G2 (gap phase 2) phases (Figure 1). This division of interphase into three separate phases based on the timing of DNA synthesis was first proposed in 1953 by Alma Howard and Stephen Pelc of Hammersmith Hospital, London, based on their experiments on plant meristem cells. Cell cycles can range in length from as short as 30 minutes in a cleaving frog embryo, whose cell cycles lack both G1 and G2 phases, to several months in slowly growing tissues, such as the mammalian liver. Cells that are no longer capable of division, whether temporarily or permanently, remain in G0 phase. A cell must receive a growth-promoting signal to proceed from the quiescent stage or G0 into G1 phase and thus reenter the cell cycle.

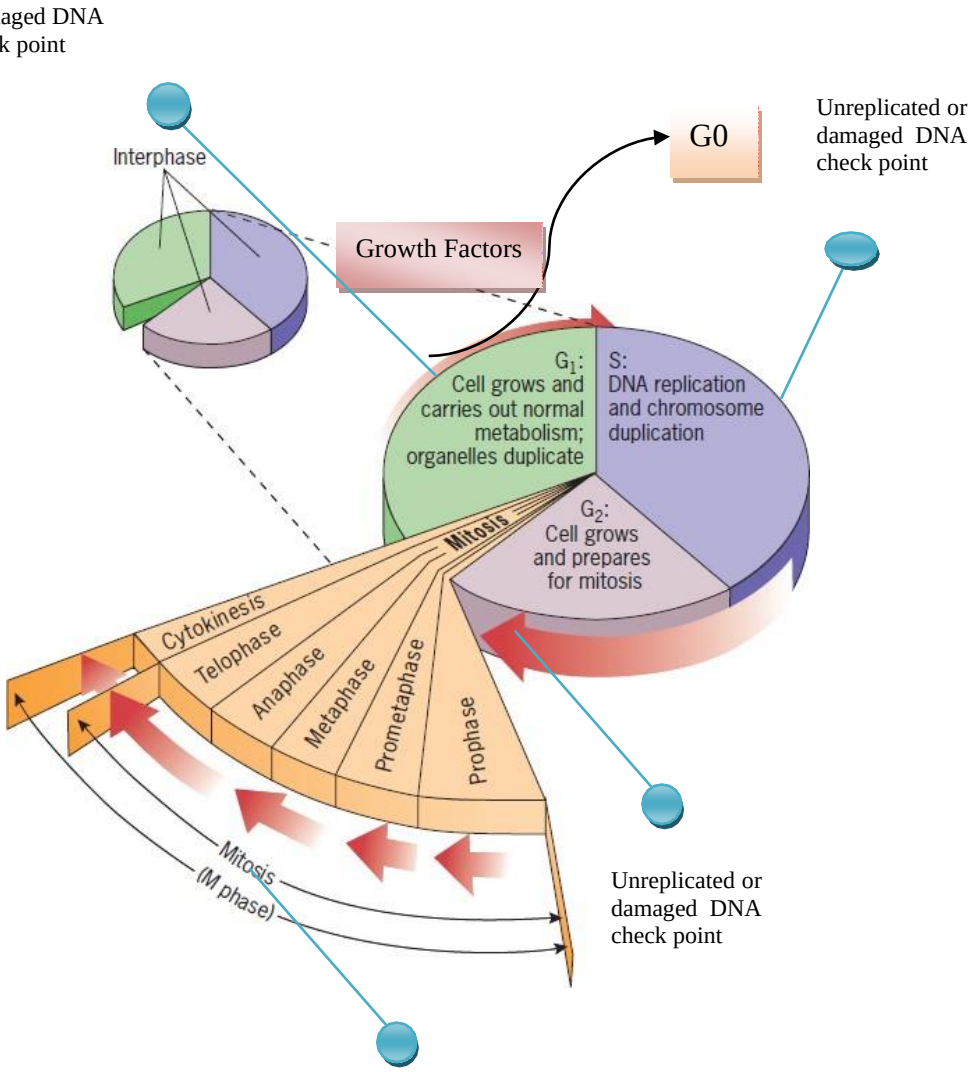


Figure 1: An overview of the cell cycle.

This figure has been adapted with permission from Cell and Molecular Biology Concepts and Experiments by Karp, 2010.

Interphase: During interphase the chromosomes are not visible with a light microscope when the cell is not undergoing mitosis. The genetic material (DNA) in the chromosomes is replicated during the period of interphase to carry out mitosis and is called S phase (S stands for *synthesis* of DNA). DNA replication is accompanied by chromosome duplication. Before and after S, there are two periods, called G1 and G2, respectively, in which DNA replication does not take place. The order of cell cycle events is G1 → S → G2 → M and then followed by cytokinesis. The G1 phase, S phase and G2 phase together form the interphase.

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 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 is stored as blebs on the surface of the cells about to divide. Events in interphase takes place in three distinct phases.

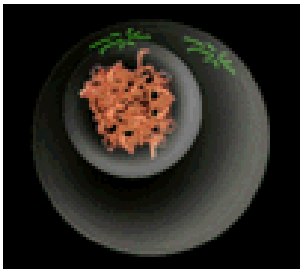


Fig.2: Interphase

G1 Phase: 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 also called first gap phase, as no DNA synthesis takes place during this stage. It is also known as the 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 enhancing size. During G1 phase, chromatin is fully extended and not distinguishable as discrete chromosomes with the light microscope. Thus, it 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 (a) regulatory proteins which control various events of mitosis (b) enzymes (DNA polymerase) necessary for DNA synthesis of the next stage and (c) tubulin and other mitotic apparatus proteins. G1 phase is most variable as to duration it either occupies 30 to 50 per cent of the total time of the cell cycle. *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.*

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. At the end of S phase, each chromosome has two DNA molecules and a duplicate set of genes. S phase occupies roughly 35 to 45 per cent time of the cell cycle.

G2 phase: 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.

Dividing phase: There are two types of cell division possible. Mitosis and meosis. 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 growth of organism, replacement of cells lost to natural friction or attrition, wear and tear and for wound healing. Hence, mitosis is remarkably similar in all animals and plants. It is a smooth continuous process and is divided into different stages or phases.

Mitosis

Mitosis is a process of cell division in which each of two identical daughter cells receives a diploid complements of chromosomes same as the diploid complement of the parent cell. It is usually followed by cytokinesis in which the cell itself divides to yield two identical daughter cells.

The basics in mitosis include:

1. Each chromosome is present as a duplicated structure at the beginning of nuclear division (2n).
2. Each chromosome divides longitudinally into identical halves and become separated from each other.
3. The separated chromosome halves move in opposite directions, and each becomes included in one of the two daughter nuclei that are formed.

Mitosis is divided into four stages: prophase, metaphase, anaphase and telophase. The stages have the following characteristics:

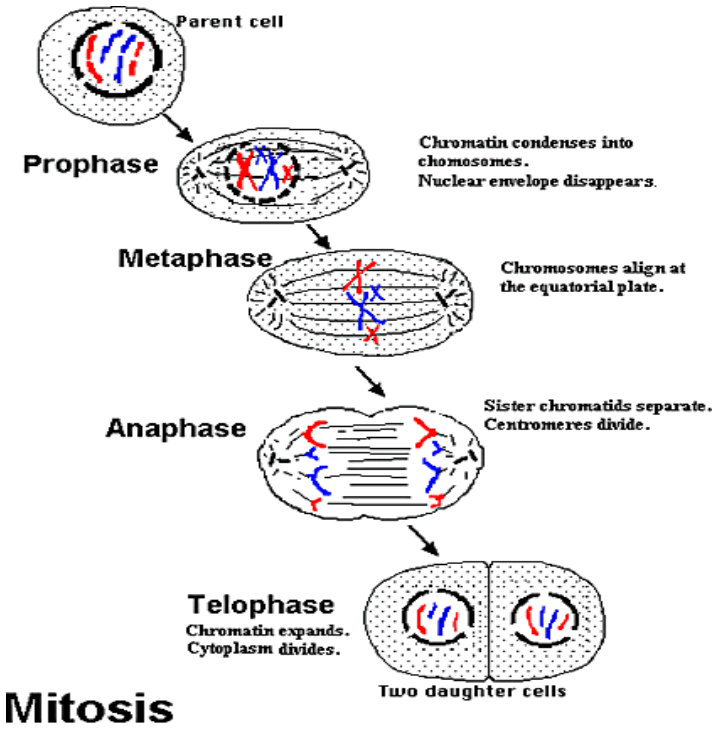


Fig.3: Mitosis cell cycle

1. **Prophase:**

The chromosomes are in the form of extended filaments and cannot be seen with a light microscope as discrete bodies except for the presence of one or more dark bodies (i.e. nucleoli) in the interphase stage. The beginning of prophase is marked by the condensation of chromosomes to form visibly distinct, thin threads within the nucleus. Each chromosome is already longitudinally double, consisting of two closely associated subunits called chromatids which are held together by centromere. Each pair of chromatids is the product of the duplication of one chromosome in the S period of interphase. As prophase progresses, the chromosomes become shorter and thicker as a result of intricate coiling. At the end of prophase, the nucleoli disappear and the nuclear envelope, a membrane surrounding the nucleus, abruptly disintegrates.

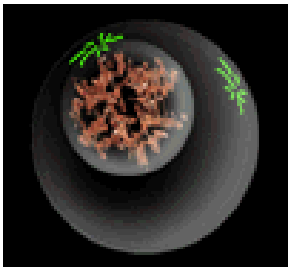


Fig.4: Prophase

2. **Metaphase:**

At the beginning of metaphase, the mitotic spindle forms which are a bipolar structure and consist of fiber-like bundles of microtubules that extend through the cell between the poles of the spindle. Each chromosome attached to several spindle fibers in the region of the centromere. The structure associated with the centromere to which the spindle fibers attach is known as the kinetochore. After the chromosomes are attached to spindle fibers, they move towards the center of the cell until all the kinetochores lie on an imaginary plane equidistant from the spindle poles. This imaginary plane is called the metaphase plate. Hence the chromosomes reach their maximum contraction and are easiest to count and examine for differences in morphology. The signal for chromosome alignment comes from the kinetochore, and the chemical nature of the signal seems to be the dephosphorylation of certain kinetochore-associated proteins. The role of the kinetochore is demonstrated by the finding that metaphase is not delayed by an unattached chromosome whose kinetochore has been destroyed by a focused laser beam. The role of

dephosphorylation is demonstrated through the use of an antibody that reacts specifically with some kinetochore proteins only when they are phosphorylated. Unattached kinetochores combine strongly with the antibody, but attachment to the spindle weakens the reaction. In chromosomes that have been surgically detached from the spindle, the antibody reaction with the kinetochore reappears. Through the signaling mechanism, when all of the kinetochores are under tension and aligned on the metaphase plate, the metaphase checkpoint is passed and the cell continues the process of division.

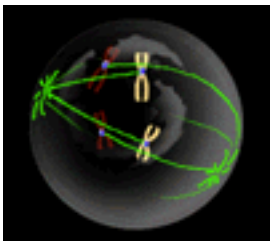


Fig.5: Prometaphase

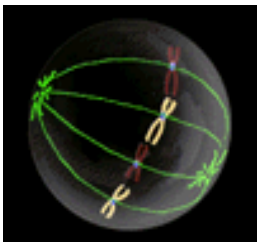


Fig. 6: Metaphase

3. Anaphase:

In anaphase, the centromeres divide longitudinally, and the two sister chromatids of each chromosome move toward opposite poles of the spindle. Once the centromere divide, each sister chromatid is treated as a separate chromosome. Chromosome movement results from progressive shortening of the spindle fibers attached to the centromeres, which pulls the chromosomes in opposite directions toward the poles. At the completion of anaphase, the chromosomes lie in two groups near opposite poles of the spindle. Each group contains the same number of chromosomes that was present in the original interphase nucleus.

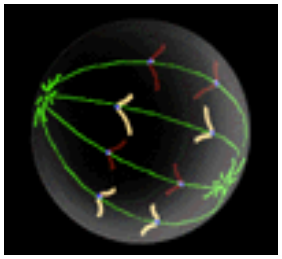


Fig.7: Anaphase

4. Telophase:

In telophase, a nuclear envelope forms around each group of chromosomes, nucleoli are formed, and the spindle disappears. The chromosomes undergo a reversal of condensation until and unless they are no longer visible as discrete entities. The two daughter nuclei slowly goes to interphase stage the cytoplasm of the cell divides into two by means of a gradually deepening furrow around the periphery.

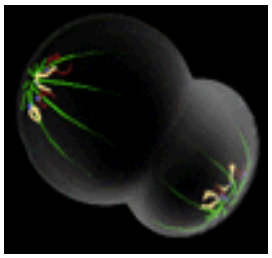


Fig.8: Telophase

5. Cytokinesis:

The chromosomes moved close to the spindle pole regions, and the spindle mid-zone begins to clear. In this middle region of the spindle, a thin line of vesicles begins to accumulate. This vesicle aggregation is an indication to the formation of a new cell wall that will be situated midway along the length of the original cell and hence form boundary between the newly separating daughter cells.

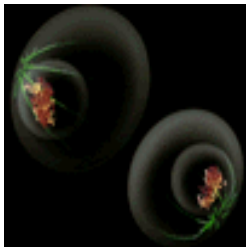


Fig.9: Cytokinesis

Interesting Facts:

- The drug Colchicine arrests cell cycle proression.
- A disregulation of the cell cycle components may lead to tumor formation.
- Several methods can be used to synchronise cell cultures by halting the cell cycle at a particular phase. For example, serum starvation and treatment with thymidine or aphidicolin halt the cell in the G1 phase.
- Some organisms can regenerate body parts by mitosis. For example, starfish regenerate lost arms through mitosis.
- Some organisms produce genetically similar offspring through asexual reproduction. For example, the hydra.
- Although errors in mitosis are rare, the process may go wrong, especially during early cellular divisions in the zygote.
- Endomitosis is a variant of mitosis without nuclear or cellular division, resulting in cells with many copies of the same chromosome occupying a single nucleus.

Meiosis

Meiosis

In the last chapter you studied about mitosis as cell division. Meiosis is the second type of cell division occurring in the gametic cells. Meiosis was first described by the German biologist Oscar Hertwig in 1876 in the sea urchin egg. Meiosis is the process of cell division that occurs only in the germ cells of eukaryotes unlike mitosis which takes place in the somatic cells. Unlike mitosis meiosis is only initiated once in the life cycle of eukaryotes **(John 1990)**. The cells produced by meiosis are known as gametes or spores. Meiosis leads to reduction of chromosome number, of a diploid cell (2n) to half (n). Meiosis begins with one diploid cell containing two copies of each chromosome and ultimately produces four haploid cells containing one copy of each chromosome which have undergone recombination, giving rise to genetic diversity in the offspring. High order transcriptional and translational control of genes known as "meiome" controls the events of meiosis **(Snustad 2008)**.

Cell cycle and Meiosis

The preparatory steps that lead up to meiosis are identical in pattern to mitosis and occurs in the interphase of the mitotic cell cycle. Interphase is followed by meiosis I and then meiosis II.

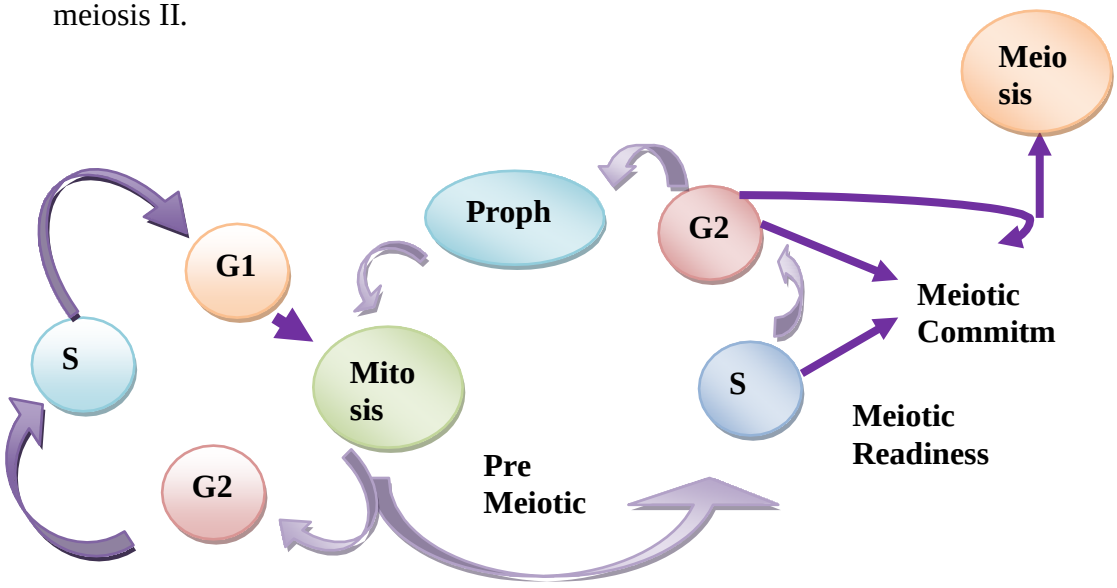


Fig 1: Position of meiosis in the Cell cycle.

Stages of meiosis

Meiosis can be separated into two phases which are meiosis I and meiosis II and they can be further subdivided into numerous phases which have particular identifiable features. They have been broadly described in the following sections.

Meiosis I

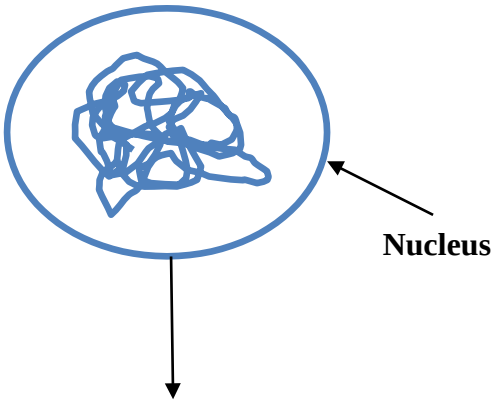
In meiosis I, chromosomes in a diploid cell segregate, producing four haploid cells generating genetic diversity. The stages of meiosis I are:

A. Prophase I

During this phase DNA is exchanged between homologous chromosomes or sister chromatids in a process called homologous recombination. The replicated chromosomes are called bivalents and have two chromosomes and four chromatids, with one chromosome coming from each parent. This phase can be further subdivided into Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis. The different stages have been pictorially presented in the following section.

1. Leptotene

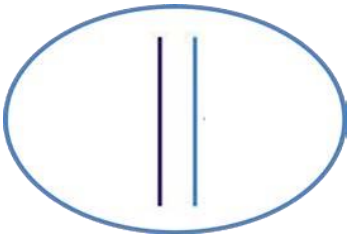
It is a very short duration stage and progressive condensation of chromosomes takes place. In this stage the chromosomes are first observed as thin threads and are said to be in a diffused state. The sister chromatids are tightly packed and indistinguishable from one another.



2. Zygotene

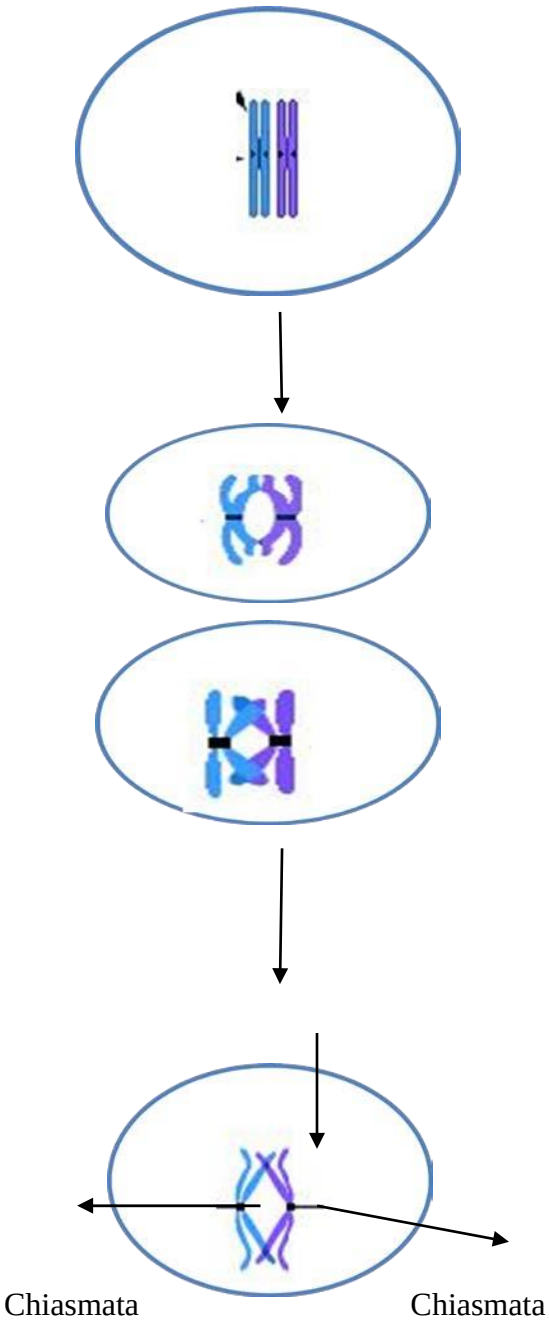
Chromosome duplication occurs and the homologous chromosomes pair up with each other.

Purple and blue represent homologous duplicated chromosomes.



3. Pachytene

Chromosomal crossover (crossing over) occurs by chiasma formation between homologous chromosomes. Nonsister chromatids of homologous chromosomes may exchange segments over regions of homology by a process called recombination. The region where crossing over occurs is known as chiasmata.



4. Diplotene

Homologous chromosomes separate from one another a little but remain attached at the chiasmata.

5. Diakinesis

Chromosomes condense further during the diakinesis stage. This is the first point in meiosis where the four parts of the tetrads are actually visible. Sites of crossing over

entangle together, effectively overlapping, making chiasmata clearly visible. The rest of the stage closely resembles prometaphase of mitosis; the nucleoli disappear, the nuclear membrane disintegrates into vesicles, and the meiotic spindle begins to form.

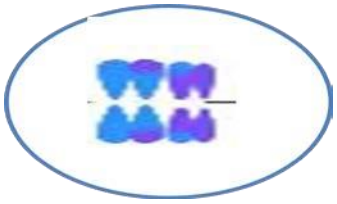


Figure 2: Stages of Meiosis I

Metaphase I

Homologous pairs move together along the metaphase plate: As kinetochore microtubules from both centrioles attach to their respective kinetochores, the homologous chromosomes align along an equatorial plane that bisects the spindle, due to continuous counterbalancing forces exerted on the bivalents by the microtubules emanating from the two kinetochores of homologous chromosomes. The physical basis of the independent assortment of chromosomes is the random orientation of each bivalent along the metaphase plate, with respect to the orientation of the other bivalents along the same equatorial line (see Fig 3).

Anaphase I

Homologous chromosomes are pulled apart by shortening of spindle fibres, each chromosome still containing a pair of sister chromatids. The cell then elongates in preparation for division down the center (see Fig 3).

Anaphase I

Chromosomes are at two different poles in the cell and the nuclear envelopes may reform, or the cell may quickly start meiosis II. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids (see Fig 3).

Telophase I

The two daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. The spindle networks disappear, and a new nuclear membrane forms. The chromosomes decondensation occurs and finally cytokinesis pinches the cell membrane in animal cells or the formation of the cell wall in plant cells, occurs, completing the creation of two daughter cells.

Meiosis II

Meiosis II is the second stage of the meiotic process. The overall process is similar to mitosis. The end result is production of four haploid cells. The four main steps of Meiosis II are: Prophase II, Metaphase II, Anaphase II, and Telophase II (see Fig 3).

Prophase II

In prophase II the nucleoli and nuclear envelope disappear. Centrioles move to opposite poles and arrange spindle fibers for the second meiotic division (see Fig 3).

Metaphase II

In metaphase II, the centromeres contain two kinetochores that attach to spindle fibers from the centrosomes (centrioles) at each pole. The new equatorial metaphase plate is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plate (see Fig 3).

Anaphase II

This is followed by anaphase II, where the centromeres are cleaved, allowing microtubules attached to the kinetochores to pull the sister chromatids apart. The sister chromatids by convention are now called sister chromosomes as they move toward opposing poles (see Fig 3).

Telophase II

The process ends with telophase II, which is similar to telophase I, and is marked by uncoiling and lengthening of the chromosomes and the disappearance of the spindle. Nuclear envelopes reform and cleavage or cell wall formation eventually produces a total of four daughter cells, each with a haploid set of chromosomes. Meiosis is now complete and ends up with four new daughter cells (see Fig 3).

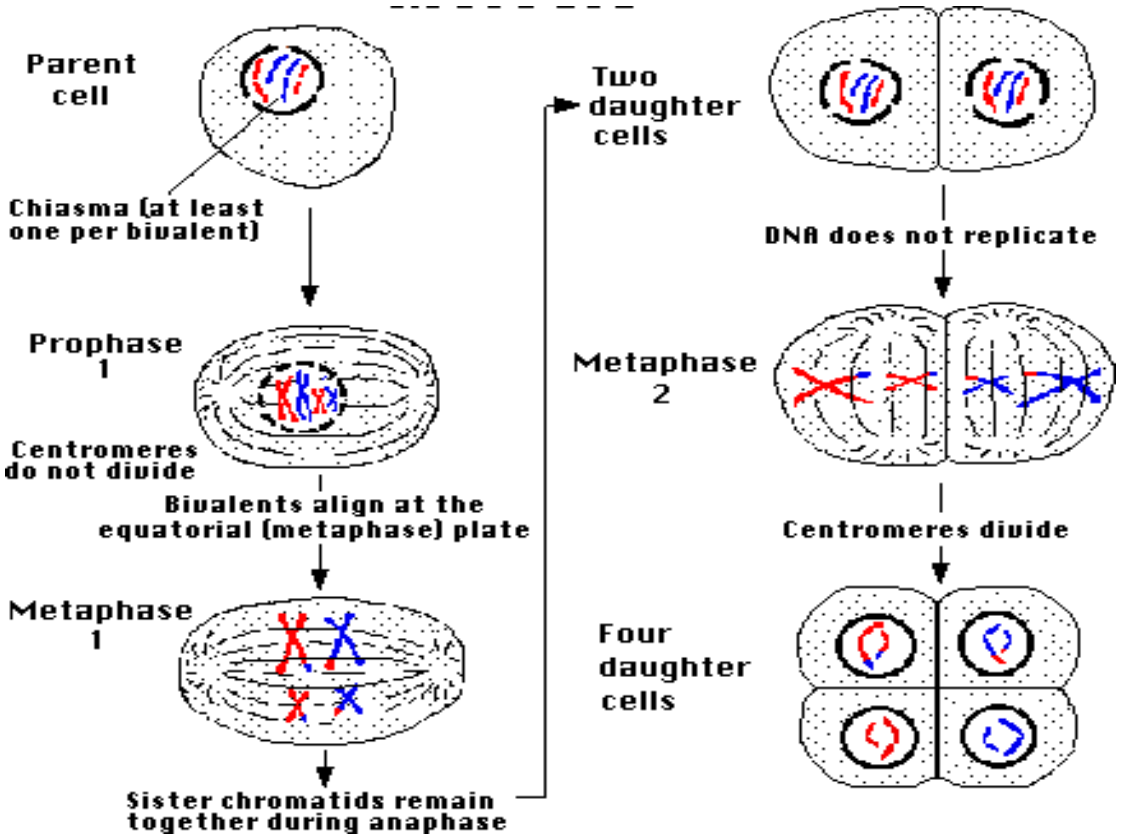


Figure 3: Events in meiosis I and II

The difference between male and female meiosis

There are mainly three differences between male and female meiosis

1. Male meiosis creates sperm, while female meiosis creates eggs.

2. Male meiosis takes place in the testicles, while female meiosis takes place in the ovaries.

3. A male will generally have one X and one Y sex chromosome, while a female have two X chromosomes, however only one of the two is active and the other is known as a barr body . During meiosis I, the sex chromosomes separate and enter different sperm or egg cells (gametes). Males will end up with one half X sperm and the other half Y sperm, while females will all have X eggs because they had no Y chromosome in the first place. There are more subtle differences though. At the end of meiosis I females have two daughter cells and meiosis II only occurs if and when fertilization occurs by a sperm cell. At that time both daughter cells divide to form 4 cells and of the 4 cells formed, 3 are discarded as polar bodies and the 4th cell having an enhanced cytoplasmic component combines its nuclear component with the sperm cell's nuclear component and crossing

over occurs to form the embryo which then begins to divide via mitosis to become two cells, then four and so on.

Interesting Facts:

- Meiosis was discovered and described for the first time in sea urchin eggs in 1876 by the German biologist Oscar Hertwig.

- Saccharomyces cerevisiae reproduces mitotically (asexually) as diploid cells when nutrients are abundant, but switches to meiosis (sexual reproduction) under starvation condition.

- Abnormalities in meiosis in human causes the following diseases.
    - Down Syndrome - trisomy of chromosome 21.
    - Patau Syndrome - trisomy of chromosome 13.
    - Edward Syndrome - trisomy of chromosome 18.
    - Klinefelter Syndrome - extra X chromosomes in males - i.e. XXY, XXXY, XXXXY, etc.
    - Turner Syndrome - lacking of one X chromosome in females - i.e. X0.
    - Triple X syndrome - an extra X chromosome in females.
    - XYY Syndrome - an extra Y chromosome in males.

Cell cycle regulation

After studying mitosis and meiosis it is important to know how are cell cycles regulated. The present chapter talks about the cell cycle regulatory methods.

Cell cycle regulation:

Cell cycle is a highly regulated and coordinated process mediated by extracellular signals from the environment, as well as by internal signals. In most cells, this coordination between different phases of the cell cycle is dependent on a series of cell cycle checkpoints that prevent entry into the next phase of the cell cycle until the events of the preceding phase have been completed. The major cell cycle regulatory check point occurs late in G1 and controls progression from G1 to S. Other check points function to ensure complete genome transmittance to daughter cells. DNA damage checkpoints in G1, S, and G2 lead to cell cycle arrest in response to damaged or unreplicated DNA. Another checkpoint, called the spindle assembly checkpoint, arrests mitosis if the chromosomes are not properly aligned on the mitotic spindle (Figure 1).

To restrict DNA replication once per cell cycle the G2 checkpoint ensures that the genome is replicated only once per cell cycle and that incompletely replicated DNA is not distributed to daughter cells. The molecular mechanism underlying this involves the action of the MCM (minichromosome maintenance complex) helicase that bind to replication origins together with the origin recognition complex (ORC) proteins. The MCM proteins are allowed to bind to replication origins during G1, leading to DNA replication when the cell enters S phase. After initiation the MCM proteins are dissociated from the origin, so replication cannot initiate again until next cell cycle. The association of MCM proteins with DNA during the S, G2 and M phases of the cell cycle is blocked by activity of the protein kinases that regulate cell cycle progression.

The cell cycle itself is under genetic control and the mechanisms of control are identical in all eukaryotes. There are two critical transitions: from G1 into S and from G2 into M. The G1/S and G2/M transitions are called "checkpoints" because the transitions are delayed unless key processes have been completed. For example, at the G1/S checkpoint, either sufficient time must have elapsed since the preceding mitosis or the cells have attained sufficient size for DNA replication to be initiated. Similarly, the G2/M

checkpoint requires that DNA replication and repair of any DNA damage be completed for the M phase to commence.

Both control points are regulated in a similar fashion and use a specialized protein kinase called the p34 kinase subunit that regulates the activity of target proteins by phosphorylation and regulates cellular processes also. To become activated, this p34 polypeptide subunit combines with several other polypeptide chains called cyclins. At the G1/S control point, one set of cyclins combines with the p34 subunit to yield the active kinase which triggers DNA replication and other events of the S period. Similarly, at the G2/M control point, a second set of cyclins combines with the p34 subunit to yield the active kinase which initiates condensation of the chromosomes, breakdown of the nuclear envelope, and reorganization of the cytoskeleton in preparation for cytokinesis.

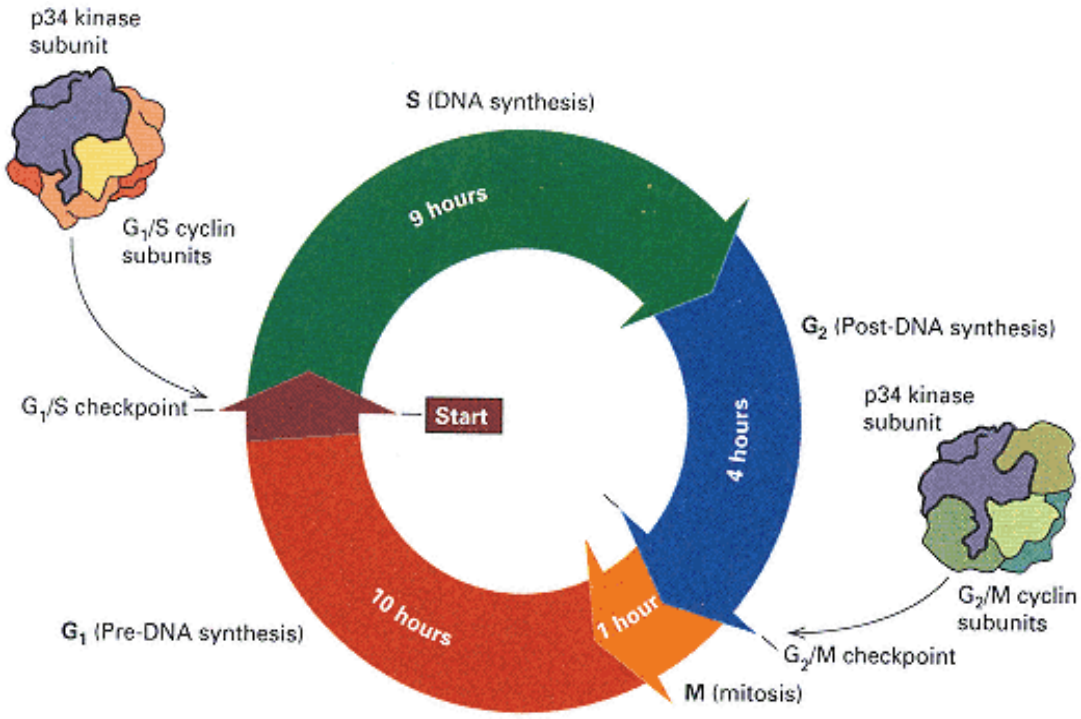


Figure 1: The cell cycle of a typical mammalian cell growing in tissue culture with a generation time of 24 hours. The critical control points for the G_1S and G_2M transitions are governed by a p34 kinase that is activated by stage-specific cyclins and that regulates the activity of its target proteins through phosphorylation.

Cell cycle regulatory elements

Cyclin dependent kinases (Cdks) are the central components that coordinate activities throughout the cell cycle whose activities in turn are regulated by cyclin binding. The cyclin-Cdk complex causes phosphorylation of proteins that control chromosome condensation, nuclear envelope breakdown and other events that occur at the onset of mitosis. Cyclins can be divided into four classes.

1. G1/S cyclin: They activate Cdks in late G1 and their level fall in S phase.
2. S cyclin: They stimulate DNA replication and their level remains high until mitosis.
3. M cyclin: Activate Cdks that stimulate entry into mitosis at the G2/M checkpoint.
4. G1 cyclins: Governs the activities of G1/S cyclins.

The cyclin protein not only activates Cdks but directs them to specific target proteins phosphorylating a different set of proteins. The different cyclin and Cdks of vertebrates has been presented in Table 1.

Table 1: The major cyclins and Cdks

Cyclin-Cdk complex	Vertebrates	
	Cyclin	Cdk partner
G1-Cdk	D	Cdk4, Cdk6
G1/S	E	Cdk2
S	A	Cdk2
M	B	Cdk1

Full activation of cyclin-Cdk complex occurs when Cdk-activating kinase phosphorylates an amino acid residue near the active site of Cdks. Furthermore Cdk activity peaks and falls during cell cycle and this process is controlled by Cdk-Inhibitory proteins (CKI) like p27 which inactivates cyclin A-Cdk2 complex. The structural basis of Cdk activation is illustrated in Figure 2. In inactive state without bound cyclin the active site is blocked by a protein region known as the T-loop. Cyclin binding causes T-loop to move out and its phosphorylation by CAK.

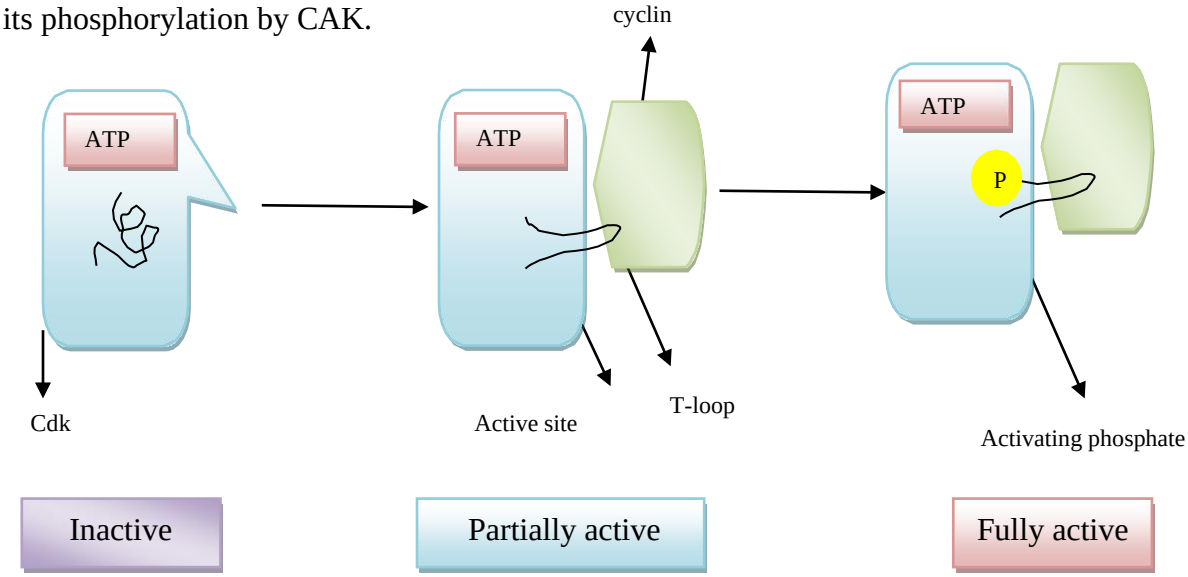


Figure 2: The structural basis of Cdk activation.

Other than phosphorylation/dephosphorylation, protein degradation also controls cell cycle progression. During the metaphase to anaphase transition the key regulator which is the anaphase promoting complex (APC) catalyses ubiquitinylation and proteosomal destruction of S and M cyclins. Destroying these cyclins inactivates most Cdks in the cell. Another ubiquitin ligase called SCF ubiquitinylates certain CKIs in late G1phase controlling activation of S-Cdks and thus DNA replication. APC activity is in turn regulated by subunits which are Cdc20 during anaphase or Cdh1 during early G. An overview of cell cycle control system is illustrated in **Figure 3.**

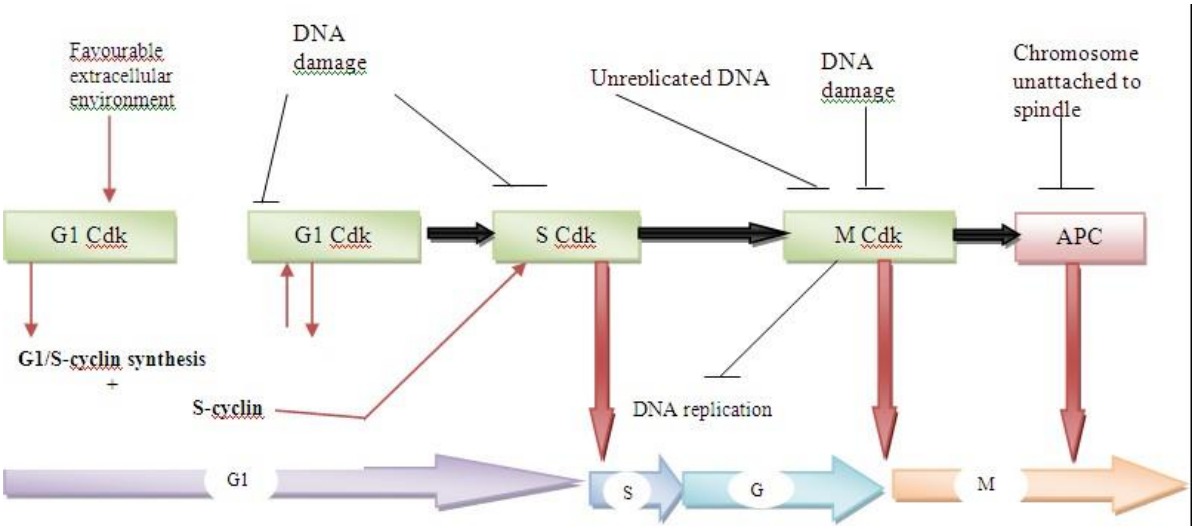


Figure 3: An overview of the cell cycle control system. Activation of G1-Cdk is stimulated through various external and internal signals. This in turn activates genes encoding G1/S and S cyclins. G1/S Cdk results in wave of S-Cdk activity which initiates chromosome replication in S-phase and contributes to some early events in mitosis. M-Cdk activity then triggers progression through G2/M checkpoint. APC with its activator Cdc20 triggers metaphase to anaphase transition. Further multiple mechanisms suppress Cdk activity after mitosis resulting in stable G1 period. This figure has been adapted from "Molecular Biology of the Cell" by Alberts B et al., 2008 Vth edition, Garland Science, USA.

Events of cell cycle in S-Phase

1. DNA replication starts at origins of replication and cell cycle ensures that replication occurs once per cell cycle.

2. In late mitosis and early G1 complex of proteins known as prereplicative complex (pre-RC) assemble at origin of replication. S-Cdk activity leads to the assembly of pre initiation complex.

3. After initiation pre-RC is dismantled and cannot be reassembled until the following G1. Assembly of pre-RC is stimulated by APC thus ensuring pre-RC assembly only at late mitosis and early G1 when Cdk activity is low and APC activity is high. The events of cell cycle during S-phase has been schematically represented in Figure 4.

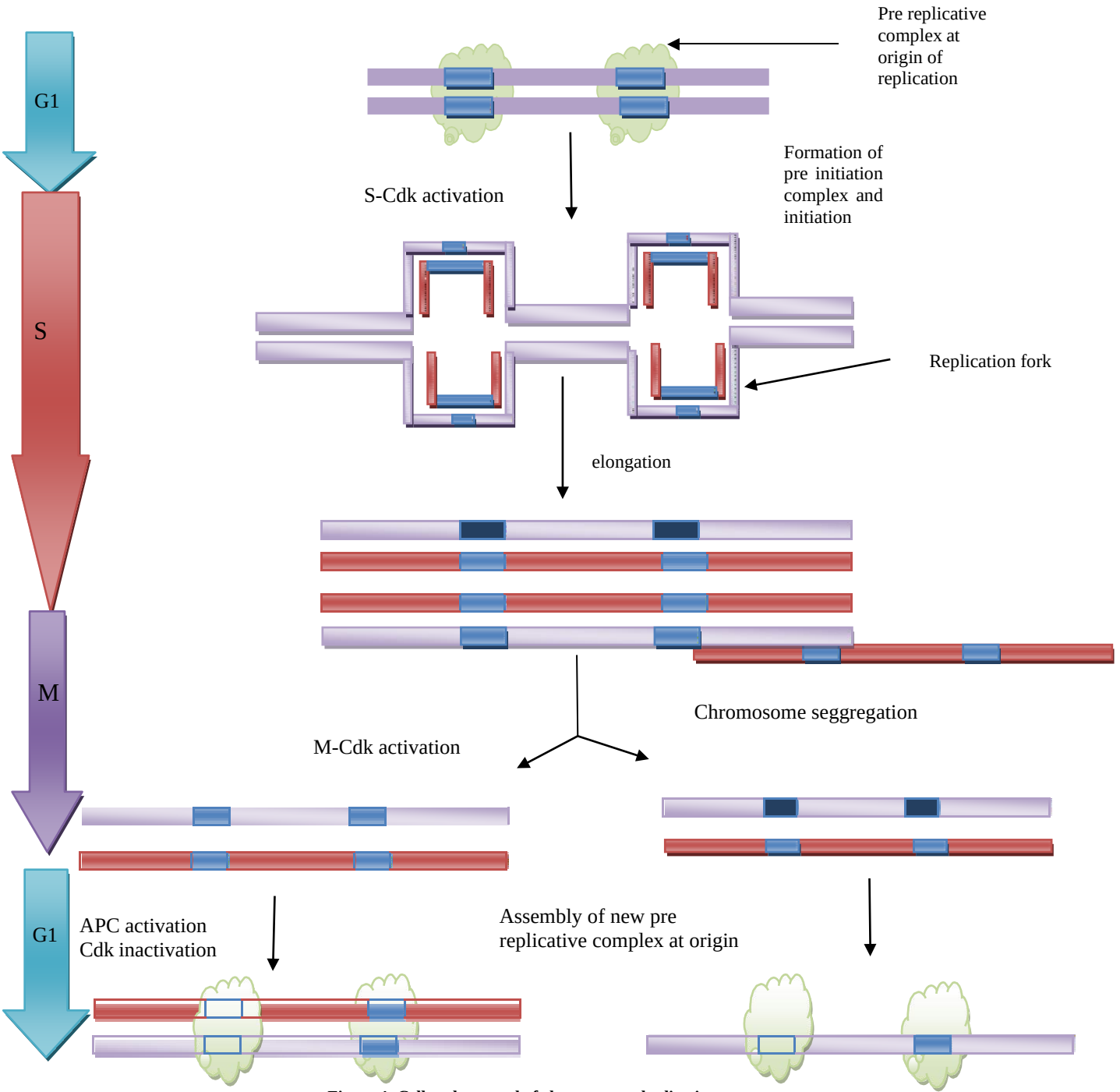


Figure 4: Cell cycle control of chromosome duplication.

Proteins involved in the initiation of DNA replication

Many proteins play part in initiation of DNA replication. The events are summarized in the following text and Figure 5.

1. A large multiprotein complex (origin recognition complex/ORC), binds to the replication origin throughout the cell cycle.

2. In late mitosis and early G1, proteins Cdc6 and Cdt1 bind to the ORC at origin and load a group of six related proteins called the Mcm proteins. This protein complexes leads to origin of replication.

3. The six Mcm proteins form a ring around the DNA and serves as the major DNA helicase causing unwinding of DNA when DNA synthesis begins and replication forks move out of the origin.

4. The activation of S-Cdk in late G1 causes assembly of several other protein complexes at the origin causing formation of large pre-initiation complex that unwinds the helix and begins DNA synthesis.

5. Parallel action of S-Cdk triggers the disassembly of some pre-RC components at the origin. Cdk's phosphorylates both the ORC and Cdc6.

6. Inactivation of APC in late G1 occurs and in turn turns off pre-RC assembly. In late mitosis and early G1 the APC triggers the destruction of a protein called geminin that binds and inhibits the Cdt1 protein.

7. S and M-Cdk activity along with low activity of APC block pre-RC formation at S-phase and thereafter.

8. After the end of mitosis APC activation leads to the inactivation of Cdks and destruction of geminin. Pre-RC components are dephosphorylated and Cdt1 is activated leading to pre-RC assembly to prepare the cell for the next S-phase.

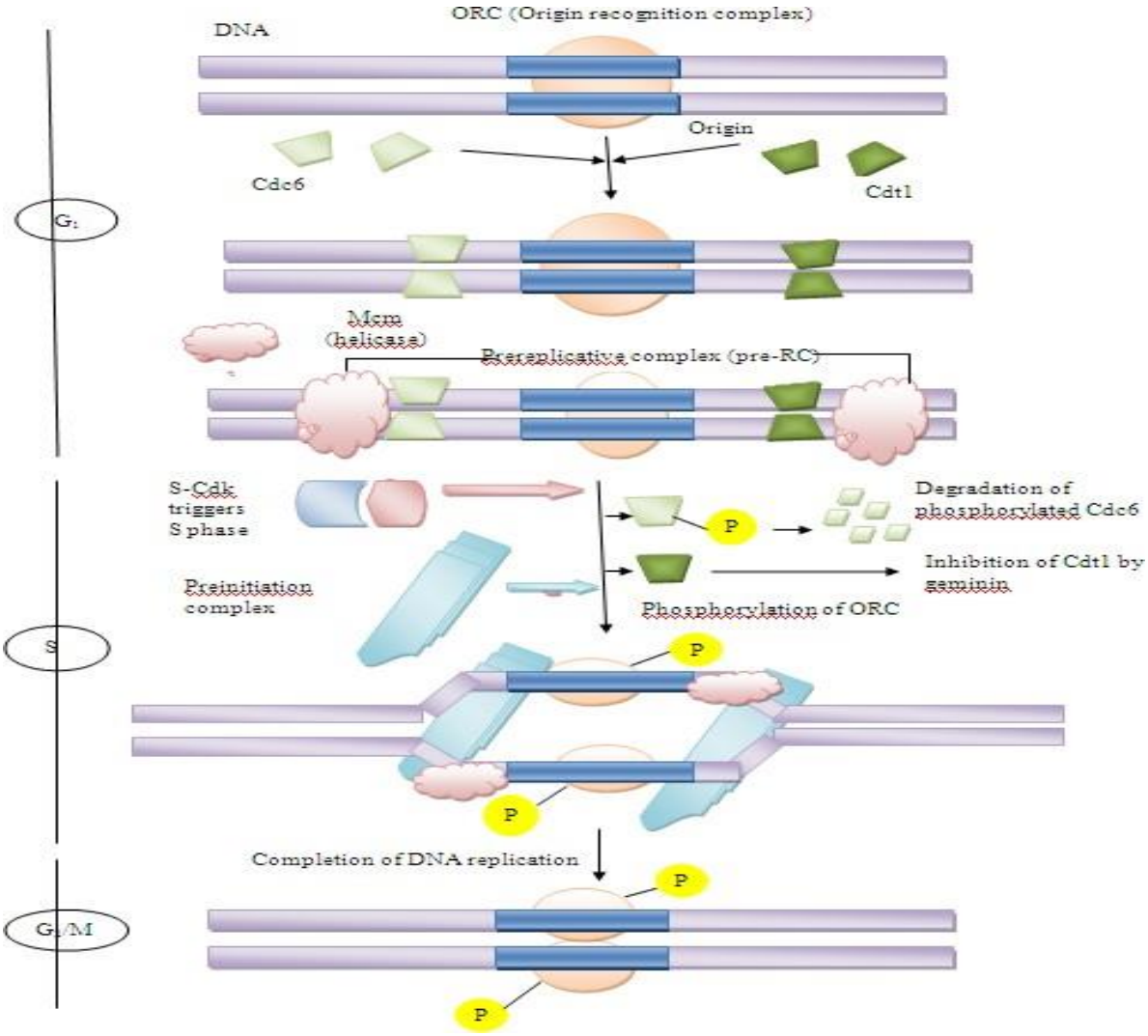


Figure 5: Control of the initiation of DNA replication.

How cell division is blocked by DNA damage?

When DNA is damaged for example by X-rays, protein kinases are activated and recruited to the site of damage. They in turn initiate a signaling cascade that causes arrest of the cell cycle. The first kinase at the site of damage is either ATM (Ataxia telangiectasia mutated) or ATR (Ataxia telangiectasia and Rad3 related) which recruits Chk1 and Chk 2 kinases at the same site. These kinases cause phosphorylation of the gene regulatory protein p53. Phosphorylation of p53 blocks Mdm2. Mdm2 is responsible for p53 ubiquitinylation and its proteosomal degradation. Thus blocking Mdm2 keeps p53 activity intact causing high level p53 accumulation. p53 then leads to transcription of

CKI protein p21. The p21 binds and inactivates G1/S-Cdk and S-Cdk arresting the cell cycle at G1.

Interesting facts:

- Two families of genes, the cip/kip family (CDK interacting protein/Kinase inhibitory protein) and the INK4a/ARF (Inhibitor of Kinase 4/Alternative Reading Frame) prevent the progression of the cell cycle. Because these genes are instrumental in prevention of tumor formation, they are known as tumor suppressors.
- Synthetic inhibitors of Cdc25 could also be useful for the arrest of cell cycle and therefore be useful as antineoplastic and anticancer agents.
- A semi-autonomous transcriptional network acts in concert with the CDK-cyclin machinery to regulate the cell cycle.