II M.SC. BOTANY

CELL AND MOLECULAR BIOLOGY

UNIT: II

Cell cycle

The cell cycle, or cell-division cycle, is the series of events that take place in a cell that cause it to divide into two daughter cells. These events include the duplication of its DNA (DNA replication) and some of its organelles, and subsequently the partitioning of its cytoplasm and other components into two daughter cells in a process called cell division.

In cells with nuclei (eukaryotes), (i.e., animal, plant, fungal, and protist cells), the cell cycle is divided into two main stages: interphase and the mitotic (M) phase (including mitosis and cytokinesis). During interphase, the cell grows, accumulating nutrients needed for mitosis, and replicates its DNA and some of its organelles. During the mitotic phase, the replicated chromosomes, organelles, and cytoplasm separate into two new daughter cells. To ensure the proper replication of cellular components and division, there are control mechanisms known as cell cycle checkpoints after each of the key steps of the cycle that determine if the cell can progress to the next phase.

In cells without nuclei (prokaryotes), (i.e., bacteria and archaea), the cell cycle is divided into the B, C, and D periods. The B period extends from the end of cell division to the beginning of DNA replication. DNA replication occurs during the C period. The D period refers to the stage between the end of DNA replication and the splitting of the bacterial cell into two daughter cells

The cell-division cycle is a vital process by which a single-celled fertilized egg develops into a mature organism, as well as the process by which hair, skin, blood cells, and some internal organs are renewed. After cell division, each of the daughter cells begin the interphase of a new cycle. Although the various stages of interphase are not usually morphologically distinguishable, each phase of the cell cycle has a distinct set of specialized biochemical processes that prepare the cell for initiation of the cell division.

The eukaryotic cell cycle consists of four distinct phases: G_1 phase, S phase (synthesis), G_2 phase (collectively known as interphase) and M phase (mitosis and cytokinesis). M phase is itself composed of two tightly coupled processes: mitosis, in which the cell's nucleus divides, and cytokinesis, in which the cell's cytoplasm divides forming two daughter cells. Activation of each phase is dependent on the proper progression and completion of the previous one. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase.

Schematic of the cell cycle. Outer ring: I = Interphase, M = Mitosis; inner ring: M = Mitosis, G_1 = Gap 1, G_2 = Gap 2, S = Synthesis; not in ring: G_0 = Gap 0/Resting[2]

State	Phase	Abbreviation	Description
Resting	Gap 0	**G_0**	A phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	**G_1**	Cells increase in size in Gap 1. The *G_1 checkpoint* control mechanism ensures that

			everything is ready for DNA synthesis.
	Synthesis	**S**	DNA replication occurs during this phase.
	Gap 2	**G_2**	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The *G_2 checkpoint* control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Cell division	Mitosis	**M**	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (*Metaphase Checkpoint*) ensures that the cell is ready to complete cell division.

After cell division, each of the daughter cells begin the interphase of a new cycle. Although the various stages of interphase are not usually morphologically distinguishable, each phase of the cell cycle has a distinct set of specialized biochemical processes that prepare the cell for initiation of cell division.

G_0 phase (quiescence)

G_0 is a resting phase where the cell has left the cycle and has stopped dividing. The cell cycle starts with this phase. Non-proliferative (non-dividing) cells in multicellular eukaryotes generally enter the quiescent G_0 state from G_1 and may remain quiescent for long periods of time, possibly indefinitely (as is often the case for neurons). This is very common for cells that are fully differentiated. Some cells enter the G_0 phase semi-permanently and are considered post-mitotic, e.g., some liver, kidney, and stomach cells. Many cells do not enter G_0 and continue to divide throughout an organism's life, e.g., epithelial cells.

The word "post-mitotic" is sometimes used to refer to both quiescent and senescent cells. Cellular senescence occurs in response to DNA damage and external stress and usually constitutes an arrest in G_1. Cellular senescence may make a cell's progeny nonviable; it is often a biochemical alternative to the self-destruction of such a damaged cell by apoptosis.

Interphase

Interphase is a series of changes that takes place in a newly formed cell and its nucleus before it becomes capable of division again. It is also called preparatory phase or intermitosis. Typically interphase lasts for at least 91% of the total time required for the cell cycle.

Interphase proceeds in three stages, G_1, S, and G_2, followed by the cycle of mitosis and cytokinesis. The cell's nuclear DNA contents are duplicated during S phase.

G_1 phase (First growth phase or Post mitotic gap phase)

The first phase within interphase, from the end of the previous M phase until the beginning of DNA synthesis, is called G_1 (G indicating *gap*). It is also called the growth phase. During this phase, the biosynthetic activities of the cell, which are considerably slowed down during M phase, resume at a high rate. The duration of G_1 is highly variable, even among different cells of the same species. In this phase, the cell increases its supply of proteins, increases the number of organelles (such as mitochondria, ribosomes), and grows in size. In G_1 phase, a cell has three options.

- To continue cell cycle and enter S phase
- Stop cell cycle and enter G_0 phase for undergoing differentiation.
- Become arrested in G_1 phase hence it may enter G_0 phase or re-enter cell cycle.
-

The deciding point is called check point (Restriction point). This check point is called the restriction point or START and is regulated by G_1/S cyclins, which cause transition from G_1 to S phase. Passage through the G_1 check point commits the cell to division.

S phase (DNA replication)

The ensuing S phase starts when DNA synthesis commences; when it is complete, all of the chromosomes have been replicated, i.e., each chromosome consists of two sister chromatids. Thus, during this phase, the amount of DNA in the cell has doubled, though the ploidy and number of chromosomes are unchanged. Rates of RNA transcription and protein synthesis are very low during this phase. An exception to this is histone production, most of which occurs during the S phase.

G_2 phase (growth)

G_2 phase occurs after DNA replication and is a period of protein synthesis and rapid cell growth to prepare the cell for mitosis. During this phase microtubules begin to reorganize to form a spindle (preprophase). Before proceeding to mitotic phase, cells must be checked at the G_2checkpoint for any DNA damage within the chromosomes. The G_2 checkpoint is mainly regulated by the tumor protein p53. If the DNA is damaged, p53 will either repair the DNA or trigger the apoptosis of the cell. If p53 is dysfunctional or mutated, cells with damaged DNA may continue through the cell cycle, leading to the development of cancer.

Mitosis:

Mitosis describes the division of one cell into two identical **daughter cells**. It occurs in several stages, each stage describing a stereotyped set of changes in cell contents and structure. In this article, we will look at the stages of mitosis and a clinical application of mitosis.

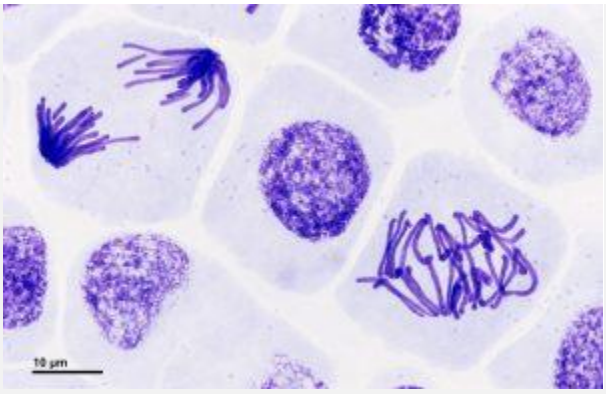


Fig 1 – Microscope image of cells in various stages of mitosis

Stages of Mitosis

Prophase

Each chromosome is made of two genetically identical chromatids, joined by a centromere. During DNA replication, the genetic material is loosely packed as chromatin. For mitosis however, the DNA needs to be more tightly packed to allow for easier separation in anaphase. At the start of prophase, **chromatin begins condensing into chromosomes**.

In addition, mitotic spindles begin to form**.** Mitotic spindles are structures made from **microtubules** that aid in the organisation and arrangement of chromosomes. The spindles originate from an organelle known as the centrosome. Each cell in mitosis has two centrosomes. During prophase, the centrosomes begin to move in opposite directions**.**

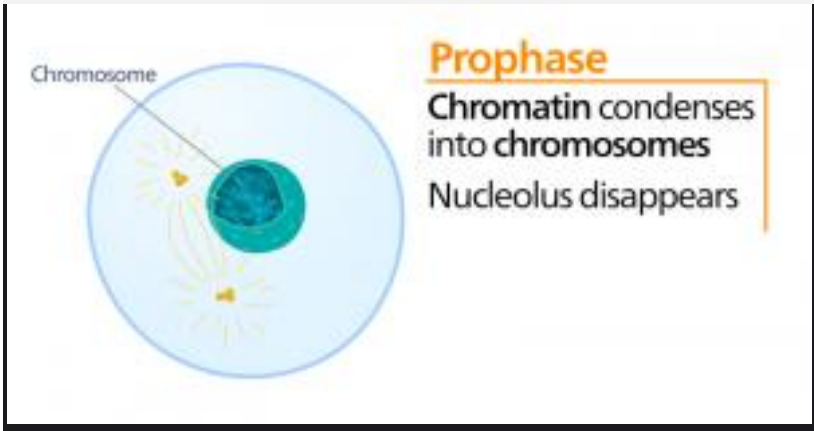


Fig 2 – Prophase

Prometaphase

In this stage the chromosomes finish condensing into their compact state. The nuclear envelope begins to breakdown, allowing **spindle fibres to attach** to the chromosomes. The mitotic spindles attach at a site called the **kinetochore**. The kinetochore is an area of the centromere on each

sister chromatid. The sister chromatids are attached to spindles that originate from the opposite centrosome.

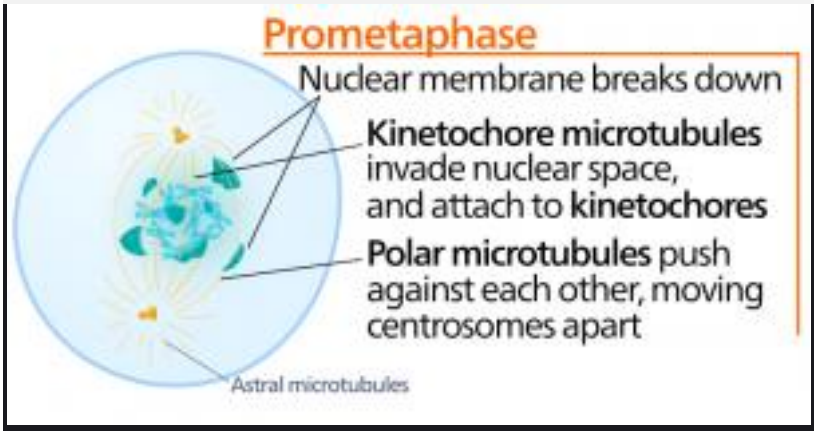


Fig 3 – Prometaphase

Metaphase

At this stage, the chromosomes align upon a theoretical line known as the **metaphase plate**. Furthermore, the centrosomes have orientated themselves to opposite ends of the cell. At this stage, the cell will check that all the chromosomes are aligned along the metaphase plate, with their kinetochores correctly attached. This helps to ensure sister chromatids are split evenly between the two daughter cells. An error in alignment or in a spindle attachment will result in the cell halting further progress until the problem is fixed.

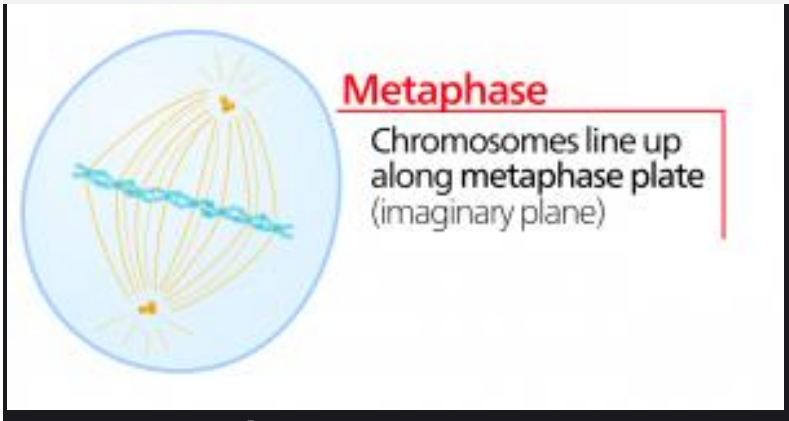


Fig 4 – Metaphase

Anaphase

During this stage the **sister chromatids** are pulled to opposite ends of the cell. The **spindle fibres contract**, breaking the chromatids at the centromere and moving them to opposite poles of the cell. Spindle fibres not attached to chromatids will elongate the cell to prepare the cell for division.

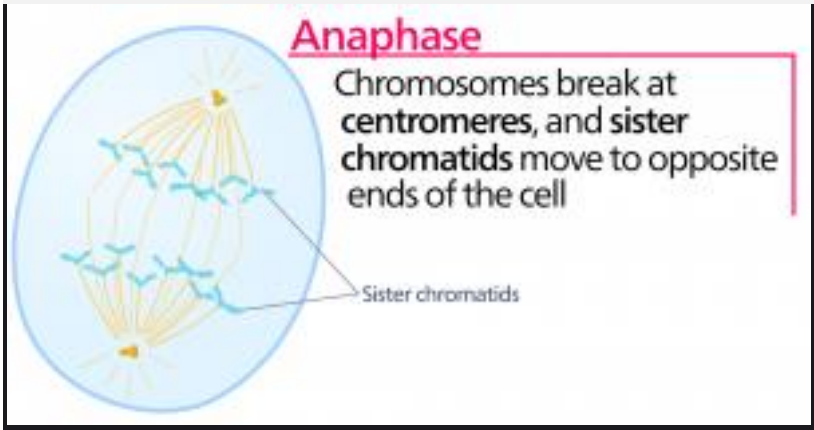


Fig 5 – Anaphase

Telophase

In this phase the cell has elongated and is nearly finished dividing. Cell-like features begin to reappear such as **reformation** of two nuclei (one for each cell). The chromosomes decondense and the mitotic spindles fibres are broken down.

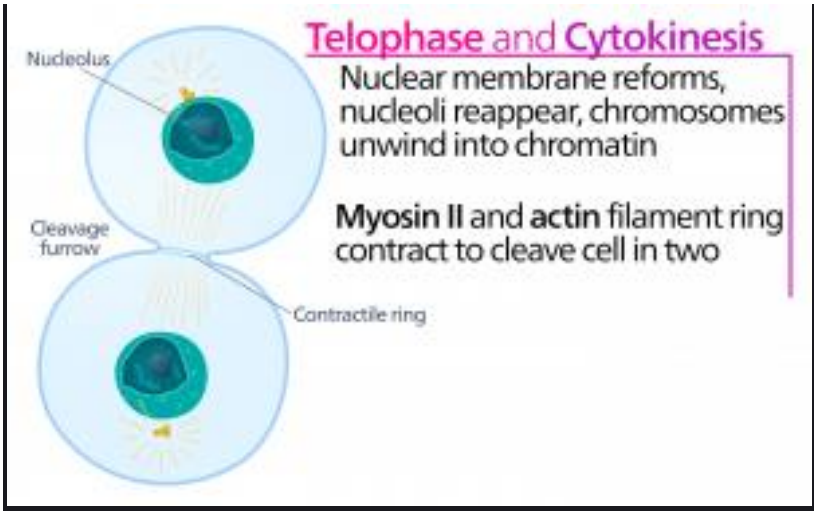


Fig 6 – Telophase & Cytokinesis

Cytokinesis

This is the division of the cytoplasm to form two new cells. This stage actually begins in either anaphase or telophase however it doesn't finish until after telophase. To separate the two cells, a ring of protein (actin ring) pinches the

cytoplasm along a crease known as a cleavage furrow. This splits the cytoplasm equally between the two cells.

Meiosis:

Meiosis describes a specific process of cell division by which gametes are made. In this process, we begin with a cell with double the normal amount of DNA, and we will end up with 4 non-identical haploid daughter gametes, after two divisions.

There are six stages within each of the divisions, namely prophase, prometaphase, metaphase, anaphase, telophase and cytokinesis. In this article, we will look at the stages of meiosis and consider its significance in disease.

Meiosis I

In meiosis I, homologous chromosomes are separated into two cells such that there is one chromosome (consisting of two chromatids) per chromosome pair in each daughter cell.

Prophase I

Prior to prophase, chromosomes replicate to form **sister chromatids**. There are initially four chromatids (c) and two chromosomes (n) for each of the 23 chromosome pairs (4c, 2n). The nuclear envelope disintegrates and the chromosomes begin to condense. Spindle fibres appear which will be important for successful division of the chromosomes.

To further increase the genetic diversity, homologous chromosomes exchange parts of themselves such that one chromosome contains both maternal and paternal DNA. This process is known as crossing over, and the points at which this occurs on a chromosome are referred to as **chiasmata.**

Prometaphase I

Now the spindle fibres attach to the chromosomes at a points along the chromosomes called centromeres. While this is happening the chromosomes continue to condense.

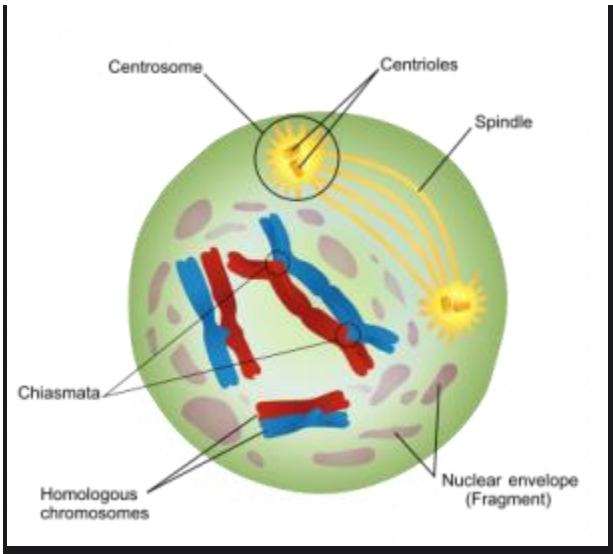


Fig 2 – Image of prometaphase I.

Metaphase I

Next, maternal and paternal versions of the same chromosome align along the equator of the cell. These are the **homologous chromosomes.** A process called **independent assortment** occurs – this is when maternal and paternal chromosomes line up randomly align themselves on either side of the equator. This is turn determines to which gamete chromosomes are allocated to, which leads to genetic diversity among offspring.

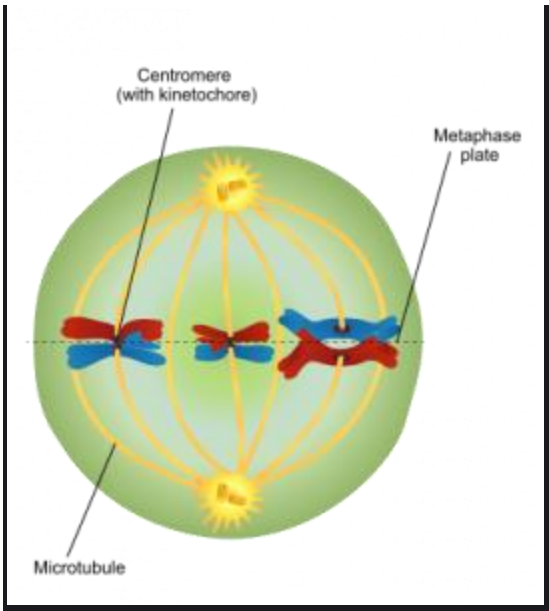


Fig 3 – Image of Metaphase I

Anaphase I

Here each of the homologous chromosomes get pulled towards opposite poles of the cell as the spindle fibres retract to divide the DNA between the two cells which will be formed.

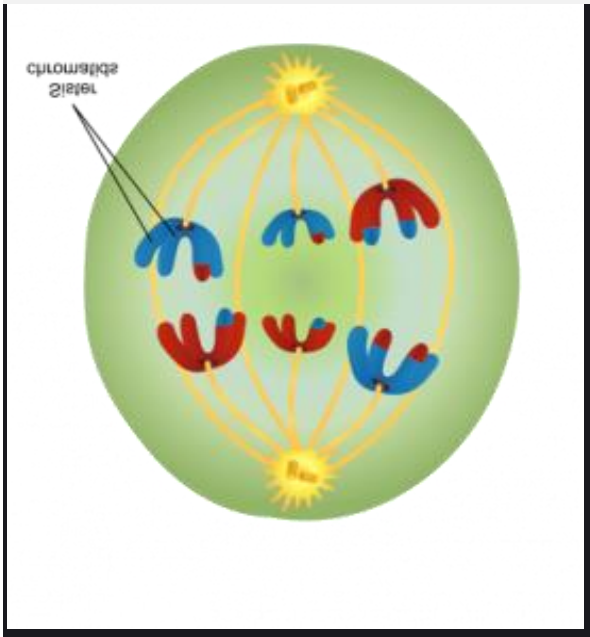


Fig 4 – Image of Anaphase I.

Telophase I and Cytokinesis I

During telophase I, the nuclear envelope reforms and spindle fibres disappear. In Cytokinesis I, the cytoplasm and cell divides resulting in two cells that are technically haploid – there is one chromosome and two chromatids for each chromosome (2c, n).

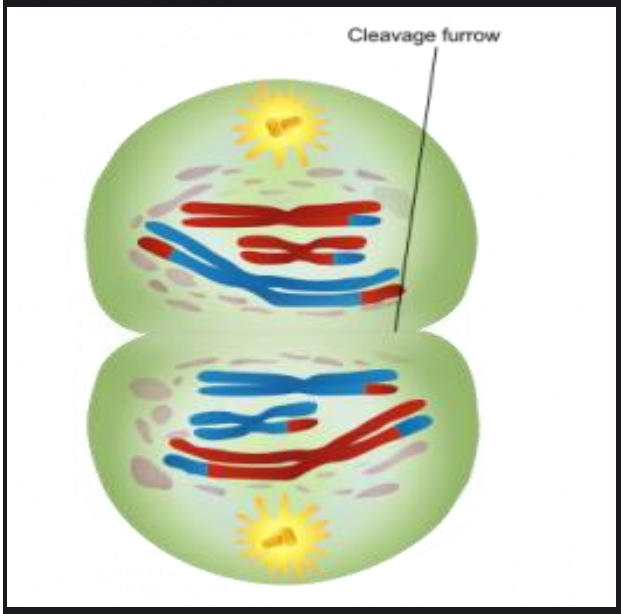


Fig 5 – Image of Telophase I and Cytokinesis I

Meiosis II

Prophase II and Prometaphase II

These stages are identical to their counterparts in meiosis I.

Metaphase II

Now chromosomes line up in single file along the equator of the cell. This is in contrast to Metaphase I where chromosomes lined up in homologous pairs.

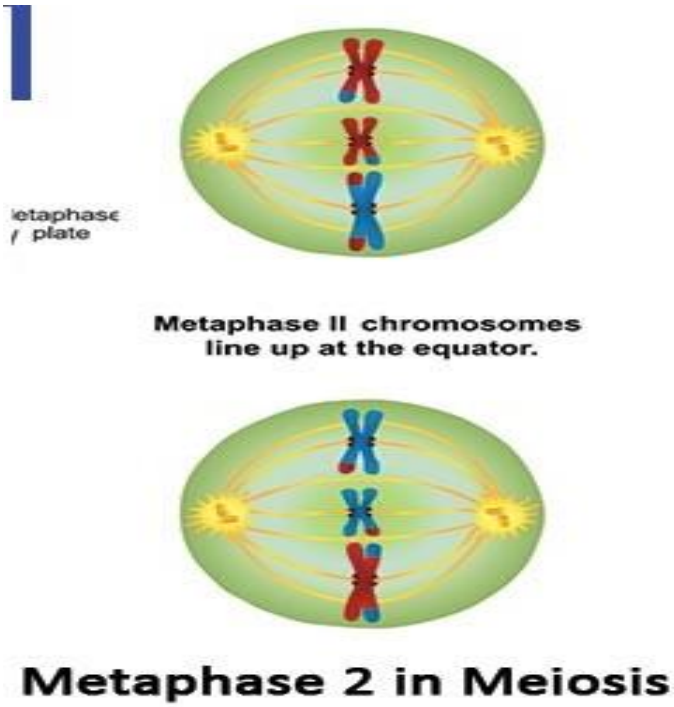


Fig 6 – Image of metaphase II

Anaphase II

Next, **sister chromatids** are pulled to opposite poles of the equator.

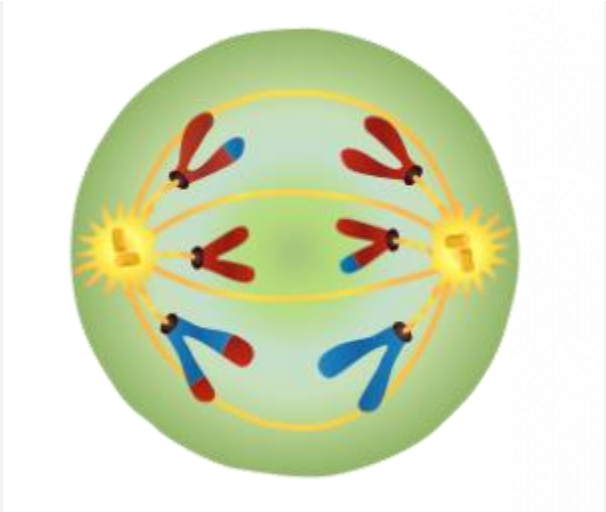


Fig 7 – Image of Anaphase II.

Telophase II

Cytokinesis II

Again, the cytoplasm and cell divides producing 2 non-identical haploid daughter cells, but as this is happening in both cells produced by meiosis I, the net product is 4 non-identical **haploid** daughter cells, each comprising one chromosome consisting of one chromatid . These are gametes.

Control of the Cell Cycle

By the end of this section, you will be able to:

- Understand how the cell cycle is controlled by mechanisms both internal and external to the cell
- Explain how the three internal control checkpoints occur at the end of G_1, at the G_2/M transition, and during metaphase
- Describe the molecules that control the cell cycle through positive and negative regulation

The length of the cell cycle is highly variable, even within the cells of a single organism. In humans, the frequency of cell turnover ranges from a few hours in early embryonic development, to an average of two to five days for epithelial cells, and to an entire human lifetime spent in G_0 by specialized cells, such as cortical neurons or cardiac muscle cells. There is also variation in the time that a cell spends in each phase of the cell cycle.

When fast-dividing mammalian cells are grown in culture (outside the body under optimal growing conditions), the length of the cycle is about 24 hours. In rapidly dividing human cells with a 24-hour cell cycle, the G_1 phase lasts approximately nine hours, the S phase lasts 10 hours, the

G_2 phase lasts about four and one-half hours, and the M phase lasts approximately one-half hour. In early embryos of fruit flies, the cell cycle is completed in about eight minutes. The timing of events in the cell cycle is controlled by mechanisms that are both internal and external to the cell.

Regulation of the Cell Cycle by External Events

Both the initiation and inhibition of cell division are triggered by events external to the cell when it is about to begin the replication process. An event may be as simple as the death of a nearby cell or as sweeping as the release of growth-promoting hormones, such as human growth hormone (HGH). A lack of HGH can inhibit cell division, resulting in dwarfism, whereas too much HGH can result in gigantism. Crowding of cells can also inhibit cell division. Another factor that can initiate cell division is the size of the cell; as a cell grows, it becomes inefficient due to its decreasing surface-to-volume ratio. The solution to this problem is to divide.

Whatever the source of the message, the cell receives the signal, and a series of events within the cell allows it to proceed into interphase. Moving forward from this initiation point, every parameter required during each cell cycle phase must be met or the cycle cannot progress.

Regulation of cell cycle:

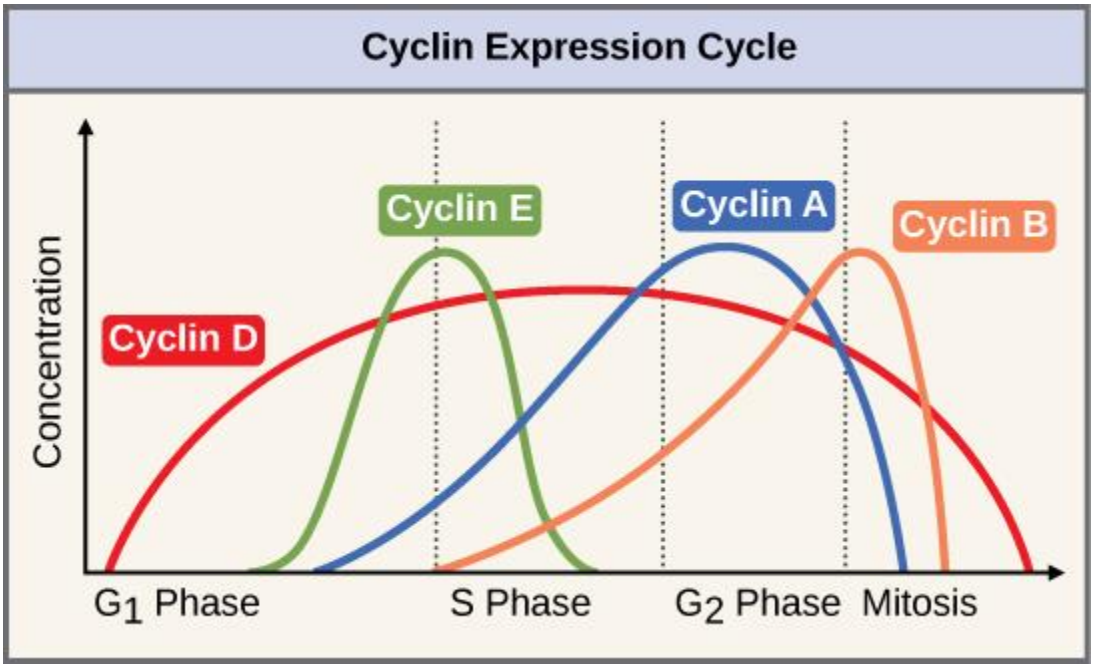
It is essential that the daughter cells produced be exact duplicates of the parent cell. Mistakes in the duplication or distribution of the chromosomes lead to mutations that may be passed forward to every new cell produced from an abnormal cell. To prevent a compromised cell from continuing to divide, there are internal control mechanisms that operate at three main cell cycle checkpoints. A checkpoint is one of several points in the eukaryotic cell cycle at which the progression of a cell to the next stage in the cycle can be halted until conditions are favorable. These checkpoints occur near the end of G_1, at the G_2/M transition, and during metaphase

Regulator Molecules of the Cell Cycle

In addition to the internally controlled checkpoints, there are two groups of intracellular molecules that regulate the cell cycle. These regulatory molecules either promote progress of the cell to the next phase (positive regulation) or halt the cycle (negative regulation). Regulator molecules may act individually, or they can influence the activity or production of other regulatory proteins. Therefore, the failure of a single regulator may have almost no effect on the cell cycle, especially if more than one mechanism controls the same event. Conversely, the effect of a deficient or non-functioning regulator can be wide-ranging and possibly fatal to the cell if multiple processes are affected.

Positive Regulation of the Cell Cycle

Two groups of proteins, called cyclins and cyclin-dependent kinases (Cdks), are responsible for the progress of the cell through the various checkpoints. The levels of the four cyclin proteins fluctuate throughout the cell cycle in a predictable pattern (Figure 2). Increases in the concentration of cyclin proteins are triggered by both external and internal signals. After the cell moves to the next stage of the cell cycle, the cyclins that were active in the previous stage are degraded.



Chromosome morphology:

Chromosomes were first seen by Hofmeister (1848) in the pollen mother cells of Tradescantia in the form of darkly stained bodies. The term chromosome (Gr: chrom=colour; soma=body) was used by Waldeyer (1888) to designate their great affinity to basic dyes.

Their functional significance was described by IV.S. Sutton (1900) when he traced parallelism between segregation of chromosomes during meiosis and transmission of hereditary factors during gametoeenesis.

General reviews on the morphology of chromosomes have been published by Heitz (1935), Kuwada (1939), Geitler (1940) and Kaufmann (1948).

Chromosomes are the most significant components of the cell, particularly they are apparent during mitosis and meiosis. Their presence was demonstrated long before they were named “chromosomes” by Waldeyer in 1888.

A chromosome can be considered as a nuclear component having special organization, individuality and function. It is capable of self-reproduction while maintaining its morphologic and physiologic properties through successive cell divisions.

Morphology:

The morphology of chromosome can be best studies at the metaphase or anaphase of mitosis when they are present as definite organelles, being most condensed or coiled.

Number:

The number of chromosomes in a given species is usually constant containing diploid number (2n) of chromosomes in their somatic cells and haploid (gametic or reduced) number (n) of chromosomes in their sex cells (sperms and ova). The number of chromosomes is variable from one to several hundred among different species

For example, in Ascaris megalocephala it is 2, while in certain protozoans (Aggreta), there are more than 300 chromosomes, in Paramecium 30 to 40, in radiolarians as many as 1600, in Hydra vulgaris 32, Musca

domestica 12, Rana esculenta 26, Columba livia 80, Oryctolagus cuniculus 44, Gorilla gorilla 48 and Homo sapiens (man) 46.

The chromosome numbers are also helpful for taxonomy. In the angiosperms the most frequent haploid number is 12 and members of this group have a range from 3 to 16. Similarly, in fungi, haploid number ranges from 3 to 8.

In primates this haploid number is from 16 to 30. This haploid set of chromosomes present in the nucleus of gametes in while in a diploid cell there will be two genomes. The diploid cells are the somatic cells in body. The diploid cells get the diploid set of chromosomes by the union of the haploid male and female gametes in the sexual reproduction.

Telomere:

Telomeres are repetitive stretches of DNA located at the ends of linear chromosomes. They protect the ends of chromosomes in a manner similar to the way the tips of shoelaces keep them from unraveling.

In many types of cells, telomeres lose a bit of their DNA every time a cell divides. Eventually, when all of the telomere DNA is gone, the cell cannot replicate and dies.

White blood cells and other cell types with the capacity to divide very frequently have a special enzyme that prevents their chromosomes from losing their telomeres. Because they retain their telomeres, such cells generally live longer than other cells.

Telomeres also play a role in cancer. The chromosomes of malignant cells usually do not lose their telomeres, helping to fuel the uncontrolled growth that makes cancer so devastating.

Lampbrush Chromosome:

Lampbrush chromosome (immature eggs) of most animals, except mammals. They were first described by Walther Flemming in 1882.[1] Lampbrush chromosomes of tailed and tailless amphibians, birds and insects are described best of all. Chromosomes transform into the lampbrush form during the diplotene stage of meiotic prophase I due to an active transcription of many genes. They are highly extended meiotic half-bivalents, each consisting of 2 sister chromatids. Lampbrush chromosomes are clearly visible even in the light microscope, where they are seen to be organized into a series of chromomeres with large chromatin loops extended laterally. Amphibian and avian lampbrush chromosomes can be microsurgically isolated from oocyte nucleus (germinal vesicle) with either forceps or needles.

Each lateral loop contains one or several transcription units with polarized RNP-matrix coating the DNA axis of the loop.

Giant chromosomes in the lampbrush form are useful model for studying chromosome organization, genome function
and gene expression during meiotic prophase, since they allow the individual transcription units to be visualized

Moreover, lampbrush chromosomes are widely used for high-resolution mapping of DNA sequences and construction of detail cytological maps of individual chromosomes.[11]

Polytene chromosome:

Polytene chromosomes are large chromosomes which have thousands of DNA strands. They provide a high level of function in certain tissues such as salivary glands of insects

Polytene chromosomes were first reported by E.G.Balbiani in 1881. Polytene chromosomes are found in dipteran flies: the best understood are those of *Drosophila*, *Chironomus* and *Rhynchosciara*. They are present in another group of arthropods of the class Collembola, a protozoan group Ciliophora, mammalian trophoblasts and antipodal, and suspensor cells in plants.[2] In insects, they are commonly found in the salivary glands when the cells are not dividing.

They are produced when repeated rounds of DNA replication without cell division forms a giant chromosome. Thus polytene chromosomes form when multiple rounds of replication produce many sister chromatids which stay fused together.

Polytene chromosomes, at interphase, are seen to have distinct thick and thin banding patterns. These patterns were originally used to help map chromosomes, identify small chromosome mutations, and in taxonomic identification. They are now used to study the function of genes in transcription.[3]

Function:

In addition to increasing the volume of the cells' nuclei and causing cell expansion, polytene cells may also have a metabolic advantage as multiple copies of genes permits a high level of gene expression. In *Drosophila melanogaster*, for example, the chromosomes of the larval salivary glands undergo many rounds of endoreduplication to produce large quantities of adhesive mucoprotein ("glue") before pupation. Another example within the fly itself is the tandem duplication of various polytene bands located near the centromere of the X chromosome which results in the Bar phenotype of kidney-shaped eyes.

The interbands are involved in the interaction with the active chromatin proteins, nucleosome remodeling, and origin recognition complexes. Their primary functions are: to act as binding sites for RNA pol II, to initiate replication and, to start nucleosome remodeling of short fragments of DNA.

Structure:

In insects, polytene chromosomes are commonly found in the salivary glands; they are also referred to as "salivary gland chromosomes". The large size of the chromosome is due to the presence of many longitudinal strands called chromonemata; hence the name polytene (many stranded). They are about 0.5 mm in length and 20 μm in diameter. The chromosomal strands are formed after repeated division of the chromosome in the absence of cytoplasmic division. This type of division is called endomitosis. The polytene chromosome contains two types of bands, dark bands and interbands. The dark bands are darkly stained and the inter bands are lightly stained with nuclear stains. The dark bands contain more DNA and less RNA. The interbands contain more RNA and less DNA. The amount of DNA in interbands ranges from 0.8 - 25%.

The bands of polytene chromosomes become enlarged at certain times to form swellings called puffs. The formation of puffs is called puffing. In the regions of puffs, the chromonemata uncoil and open out to form many loops. The puffing is caused by the uncoiling of individual chromomeres in a band. The puffs indicate the site of active genes where mRNA synthesis takes place. The chromonemata of puffs give out a series of many loops laterally. As these loops appear as rings, they are called Balbiani rings after the name of the researcher who discovered them. They are formed of DNA, RNA and a few proteins. As they are the site of transcription, transcription mechanisms such as RNA polymerase and ribonucleoproteins are present.

Polytene chromosomes were originally observed in the larval salivary glands of *Chironomus* midges by Édouard-Gérard Balbiani in 1881.[8] Balbiani described the chromosomal puffs among the tangled thread inside the nucleus, and named it "permanent spireme". In 1890, he observed similar spireme in a ciliated protozoan *Loxophyllum meleagris*.[1] The existence of such spireme in *Drosophila melanogaster* was reported by Bulgarian geneticist Dontcho Kostoff in 1930. Kostoff predicted that the discs (bands) which he observed were "the actual packets in which inherited characters are passed from generation to generation."

Occurrence:

Polytene chromosomes are present in secretory tissues of dipteran insects such as the Malpighian tubules of *Sciara* and also in protists, plants, mammals, or in cells from other insects. Some of the largest polytene chromosomes described thus far occur in larval salivary gland cells of the chironomid genus *Axarus*.

In plants, they are found in only a few species, and are restricted to ovary and immature seed tissues such as in *Phaseolus coccineus* and *P. vulgaris* (Nagl, 1981), and the anther tapetum of *Vigna unguiculata* and of some *Phaseolus* species.

Polytene chromosomes are also used to identify the species of chironomid larvae that are notoriously difficult to identify. Each morphologically distinct group of larvae consists of a number of morphologically identical (sibling) species that can only be identified by rearing adult males or by

cytogenetic analysis of the polytene chromosomes of the larvae. Karyotypes are used to confirm the presence of specific species and to study genetic diversity in species with a wide range of genetic variation.

Isochromosome:

Isochromosomes are chromosomes composed of mirror images of one of the arms of the chromosome. As a result, the opposite chromosome arm may be deleted and the cells only have a single copy of the genetic material in the arm present in the normal member of the homologous pair. The other homolog has both the normal short arm and normal long arm (Fig. 5.2). As a result, the cells with an isochromosome have trisomy for the arm that is duplicated in the isochromosome and monosomy for the arm that is not present in the isochromosome. Several mechanisms have been postulated for the formation of isochromosomes, including misdivision of the centromere or sister chromatid breakage and reunion in an area adjacent to the centromere. Many isochromosomes have two centromeres and are dicentric.

An isochromosome of the long arm, the X chromosome, is found in 15%–18% of Turner syndrome cases, and the phenotype is due to having only a single copy of Xp (on the other, normal X). Isochromosomes of Yp are usually dicentric and loss of this structurally abnormal Y may result in mosaicism for a 45,X cell line.

Unlike the isochromosome of the long arm of the X and the isodicentric Y, other isochromosomes encountered in prenatal diagnosis are often small supernumerary derivatives that are mosaic. Several of these were initially categorized as marker chromosomes until the source of the genetic material was identified and a syndrome described. Isochromosomes that may be encountered in prenatal diagnosis include

(1) cat eye syndrome (CES) due to an isochromosome consisting of a fusion of the short-arm centromeres and proximal long arm of chromosome 22;

(2) tetrasomy 15q due to an isochromosome consisting of the short arms, centromeres, and proximal long arm of 15q;

(3) Pallister-Killian syndrome that is typically due to mosaicism for supernumerary isochromosome of 12p (tetrasomy 12p). Associated diaphragmatic hernia often leads to diagnosis by amniocentesis when the fetus has Pallister-Killian syndrome. An interesting feature of Pallister-Killian is tissue-limited mosaicism in which peripheral blood often does not demonstrate the finding by chromosome analysis.

Heterochromatin and Euchromatin:

Heterochromatin is a tightly packed or condensed DNA that is characterized by intense stains when stained with nuclear stains, containing transcriptionally inactive sequences.

- It exists in multiple variations, up to four to five state, each of which is marked with combinations of epigenetic markers.
- The staining of heterochromatin might result in heteropycnosis; heteropycnosis is the differential staining of parts of chromosomes.
- This chromosome is different from euchromatin in that the genes in these chromosomes are usually inactivated and are not expressed.

- Heterochromatin is present in the nucleus towards the periphery. It is also not present in prokaryotic cells, indicating this form appeared later during evolution.

- However, the two most common heterochromatin include; constitutive heterochromatin and facultative heterochromatin.

- Constitutive heterochromatin usually packages the same sequences of DNA in all cells of the same species. It is usually repetitive and is present in structural forms like telomeres and centromeres.

- The genes in constitutive heterochromatin might affect the genes present near the tightly packed chromosomes.
- In humans, genes 1, 9, 16, and the Y chromosomes in men contain larger quantities of this heterochromatin.

- Facultative heterochromatin packages genes that are usually silenced through various mechanisms; however, unlike constitutive heterochromatin, facultative chromatin packages different genes in different organisms within the same species.

- The facultative chromosome is not repetitive but has the same structural components as the constitutive heterochromatin.

- The formation of facultative heterochromatin is regulated by the process of morphogenesis or differentiation.

- In humans, one of the two X chromosomes in women is inactivated as facultative heterochromatin while the other is expressed as euchromatin.

- Heterochromatin has multiple functions. Some of which include gene regulation and chromosomes integrity

- The tightly packaged DNA in heterochromatin prevents the chromosomes from various protein factors that might lead to the binding of DNA or the inaccurate destruction of chromosomes by endonucleases.

- Besides, heterochromatin also allows gene regulation and the inheritance of epigenetic markers.

Euchromatin:

Euchromatin is a more lightly packed DNA that is characterized by less intense staining and DNA sequences that are transcriptionally active or might become transcriptionally-active at some point during growth.

- Euchromatin is present towards the center of the nucleus and accounts for about 90% of the genome in an organism.

- Under an optical microscope, it appears as light-colored bands after staining. All parts of euchromatin are uniformly stained, which doesn’t result in heteropycnosis

- Under an electron microscope, however, it appears as an elongated 10 nm microfibril.

- The structure of euchromatin can be represented as an unfolded set of beads in a string where the beads are the nucleosomes. The

nucleosomes contain histone proteins that coat a particular number of DNA around.

- In euchromatin, the wrapping around by histone proteins is loose, and thus the individual DNA sequences might be accessible.

- The conformation of euchromatin is said to be controlled by a methylated part in the chromosome called histone tail.

- Euchromatin is the only confirmation of chromosomes in the case of the prokaryotic genome, which suggests that this form evolved earlier than heterochromatin

- Unlike heterochromatin, euchromatin doesn’t exist in two forms. It only exists as constitutive euchromatin.

- Euchromatin is extremely important as it contains genes that are transcripted into RNA, which are then translated into proteins.

- The unfolded structure of DNA in euchromatin allows regulatory proteins and RNA polymerase to bind to the sequences so that the process of transcription can initiate.

- It is possible for some genes in the euchromatin to be converted into heterochromatin when they are not to be transcribed and are no longer active.

- The transformation of euchromatin to heterochromatin acts as a method for regulating gene expression and replication.

- For this purpose, some genes like housekeeping genes are always arranged in euchromatin conformation as they have to be continuously replicated and transcribed.

Chromosome identification*:*

Chromosomes in metaphase can be identified using certain staining techniques, so called **banding**. Cells are cultured and then stopped in metaphase to maximize the number of suitable cells. They are then spread on a slide, stained with a suitable dye and visualized in the microscope.

Chromosome banding techniques produce a series of consistent landmarks along the length of metaphase chromosomes that allow for both recognition of individual chromosomes within a genome and identification of specific segments of individual chromosomes. These landmarks facilitate assessment of chromosome normalcy, identification of sites of chromosome breaks and alterations, and location of specific genes. This unit covers these basic banding techniques (Q-banding, G-banding, and R-banding), which produce virtually identical patterns of bands along the length of human chromosomes, although the bands and polymorphic regions highlighted may differ with each technique.

These techniques highlight reproducible landmarks along the length of the chromosome and specialized staining techniques can be used to highlight particular regions of chromosomes, such as heterochromatic and repeated-sequence segments. These specialized techniques, nucleolar organizer region (NOR) staining, centromeric heterochromatin staining (C-banding), methylated satellite DNA staining (distamycin-DAPI banding), and replication banding are also presented in this unit.

Structure of gene:

Genes are actually DNA strands thus are made up of the nucleotide chain. The chemical structure of a gene comprises nucleotides.

A part of DNA- genes are made up of A, T, G and C nucleotides. With the nucleotides of the opposite strand, it binds with hydrogen bonds and with the adjacent nucleotide, it binds with phosphodiester bonds. The nucleotides are the combination of nitrogenous bases (A, T, G and C), phosphate and pentose sugar.

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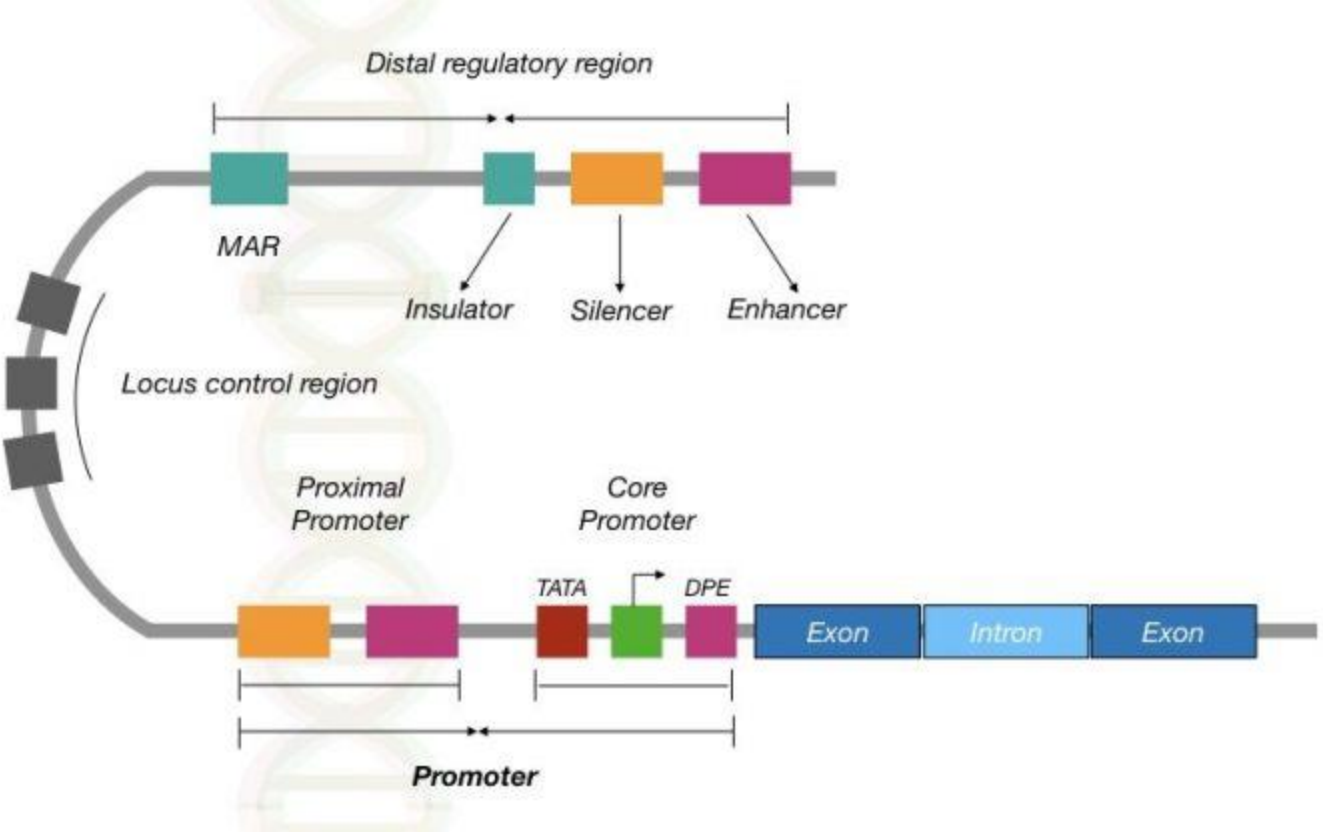
The core elements or sequences actually take parts in protein formation. While the regulatory elements maintain gene expression. Exons are core elements. Sequences on the other side like promoters, enhancers and silencers are regulatory elements of a gene.

The third type of element called maintenance elements possesses information for DNA repair, modification and replication. The functional or physical structure of a gene comprises introns, exons, promotes, enhancer and UTRs.

Introns are intervening non-coding sequences removed from the final transcript.

Exons are coding part of a gene which are joined after splicing and constructs the final transcript.

Regulatory elements are located on the extreme ends of a gene.



The molecular structure of a gene.

Promotes are non-coding sequences but facilitates binding sites for enzymes and transcriptional factors to work. The promoter consists of TATA box and CCAAT sequences for enzyme binding.

The entire promoter region is located on the 5’ end and made up of core promoter and proximal promoter sequences (see the above image).

The core promoter facilitates RNA polymerase bindings (and other proteins) to start transcription. While the proximal promoter provides bindings for transcriptional factors.

The enhancer induces transcription while the silencer represses it. Collectively, enhancers and silencers located far away from exon, regulate gene expression.

The 3’ untranslated regions are non-coding regions of gene helps in aborting the process of transcription and to form the final transcript.

Once the RNA polymerase reaches the untranslated region it stops synthesizing RNA and detached from the strand.

The eukaryotic gene structure consists of more regulatory sequences than prokaryotic genes. In addition to this, the entire machinery of transcription and translation is different in both.

The operon concept of prokaryotic genes consists of a gene cluster of similar function. Introns are not a part of an operon.

Promoters, enhancers, silencers, activators, insulators, locus control regions and MARs- matrix attachment regions are categorized into *cis*-elements.
While other transcriptional proteins which are formed from some genes are categorized into *trans*-elements. The in-depth structure of a gene with all elements are shown in the figure above.

Functions of gene:

The main function of a gene is to form or manufacture a protein, however, it’s not the only function. Indeed It’s partially true.
Some genes can’t form protein, although they transcribe into mRNA. For instance, the microRNAs are the type of tiny ribonucleic acid formed from some genes but it doesn’t undergo protein formation. It helps in gene regulation instead.

Transposons:

Transposon**,** class of genetic elements that can “jump” to different locations within a genome. Although these elements are frequently called

"jumping genes," they are always maintained in an integrated site in the genome. In addition, most transposons eventually become inactive and no longer move.

Class II Transposons

Class II elements are simply segments of DNA that move from one place to another via a "cut and paste" mechanism. Most, if not all, of these elements encode an enzyme called transposase, which acts to cleave the ends of the transposon, freeing it from its initial location in the genome. Transposase also cleaves target sites where the element is to be inserted. Once the transposon is ligated (bound) into its new position, gaps that are left in the DNA sequence are filled in through the synthesis of nucleotides. Class II transposons range in length from 1,000 to as many as 40,000 base pairs.

Retrotransposons

Retrotransposons represent a highly unique group of transposable elements and form large portions of the genomes of many eukaryotes (organisms with cells containing a clearly defined nucleus). Retrotransposons function by a "copy and paste" mechanism. Thus, they leave behind the original copy and generate a second copy that is inserted elsewhere in the genome. This process results in the insertion of repetitive sequences of DNA throughout the genome and is the mechanism responsible for the vast spread of transposable elements in many higher organisms.

The first step in retrotransposition occurs when the transposable DNA is copied into RNA. The RNA segment then jumps to another location in the genome. However, in order to be inserted into the genome at the new site,

the RNA must be copied back into DNA by an enzyme called reverse transcriptase. There are several different types of retrotransposons, including long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). About 20 percent of the human genome is made up of LINEs.

Transposons and Disease

The functions of transposons remain unclear. They have long been referred to as “junk” DNA because they appear to serve little or no purpose or as “selfish” DNA because they serve only to copy and amplify themselves within genomes. In rare cases, however, transposons are associated with genetic mutations or chromosomal rearrangements that cause disease in humans. Disease typically arises from the insertion of transposons into particular regions of genes that are involved in regulating gene activity. For example, insertions near promoter regions, which are short segments of DNA that are used to initiate gene transcription (the synthesis of RNA from DNA), can lead to over activity of genes. In some cases this can give rise to cancer. In other cases the site where a class II element is cut out of the genome is not repaired correctly, resulting in mutations that interfere with gene regulation and thereby cause cell dysfunction.