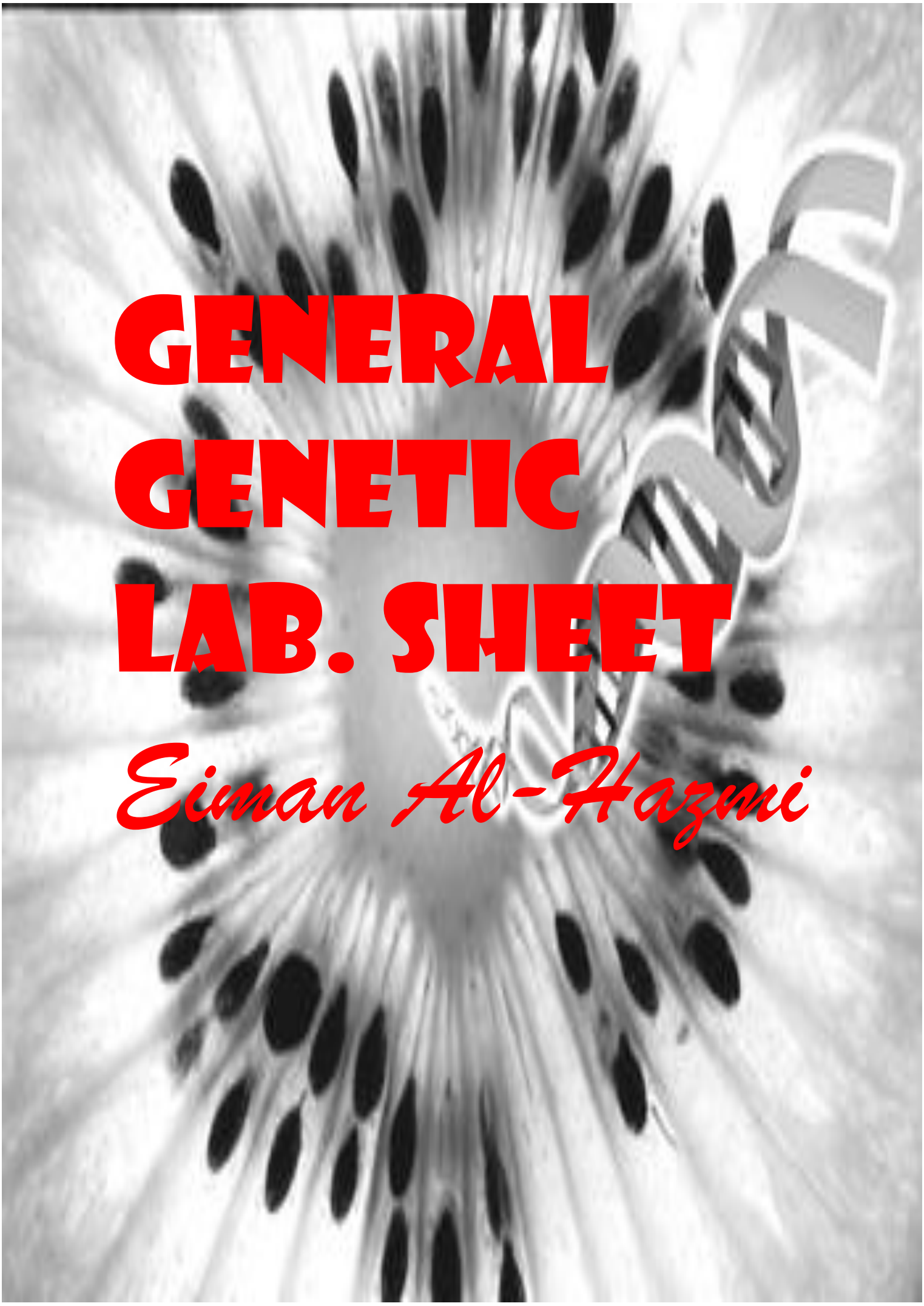

GENERAL
GENETIC
LAB. SHEET
Eiman Al-Hazmi

Lab (1)

GENERAL STRUCTURE AND CYTOLOGICAL PREPARATIONS

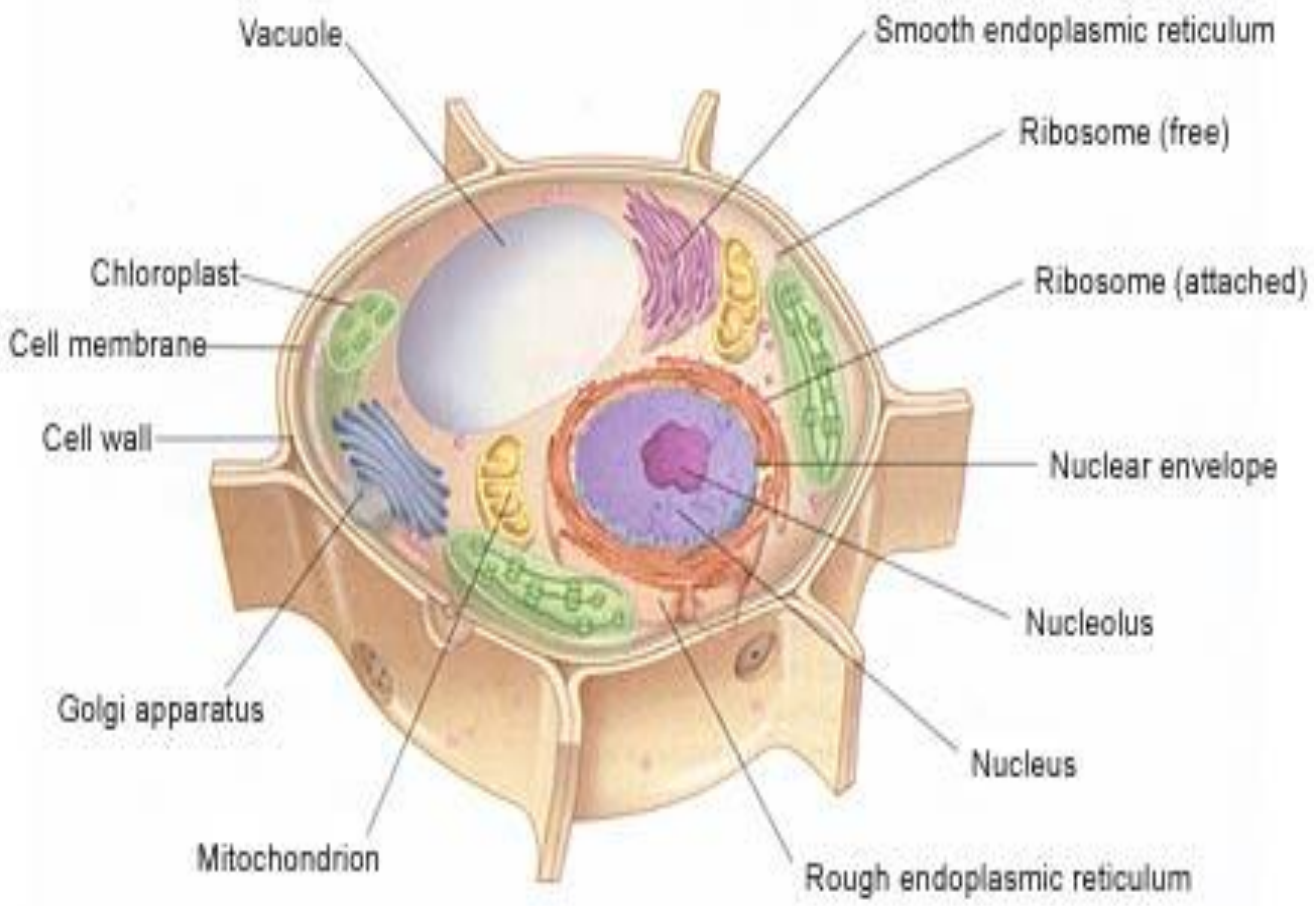
Cells are the fundamental units of all organisms. Some organisms made up of only one cell other of billions. A cell has several components that perform different functions which called "organelles." Among the most important are the nucleus, vacuoles, and mitochondria, all of which are enclosed within the cell membrane and immersed in cytoplasm.

Cell membrane: The cell membrane (or plasma membrane) surrounds all living cells, and is the cell's most important organelle. It function is to controls substances movement in and out of the cell and allow only certain fluids and chemicals, and is responsible for many other properties of the cell as well. Membranes are composed of phospholipids, proteins and carbohydrates arranged in a fluid mosaic structure. There are some carbohydrates that extend out from the proteins and are attached to it or sometimes to the phospholipids. Proteins with carbohydrates attached are called glycoproteins, while phospholipids with carbohydrates attached are called glycolipids. Plant cells also contain a rigid, protective cell wall provides and maintains the shape of these cells made up of polysaccharides usually cellulose.

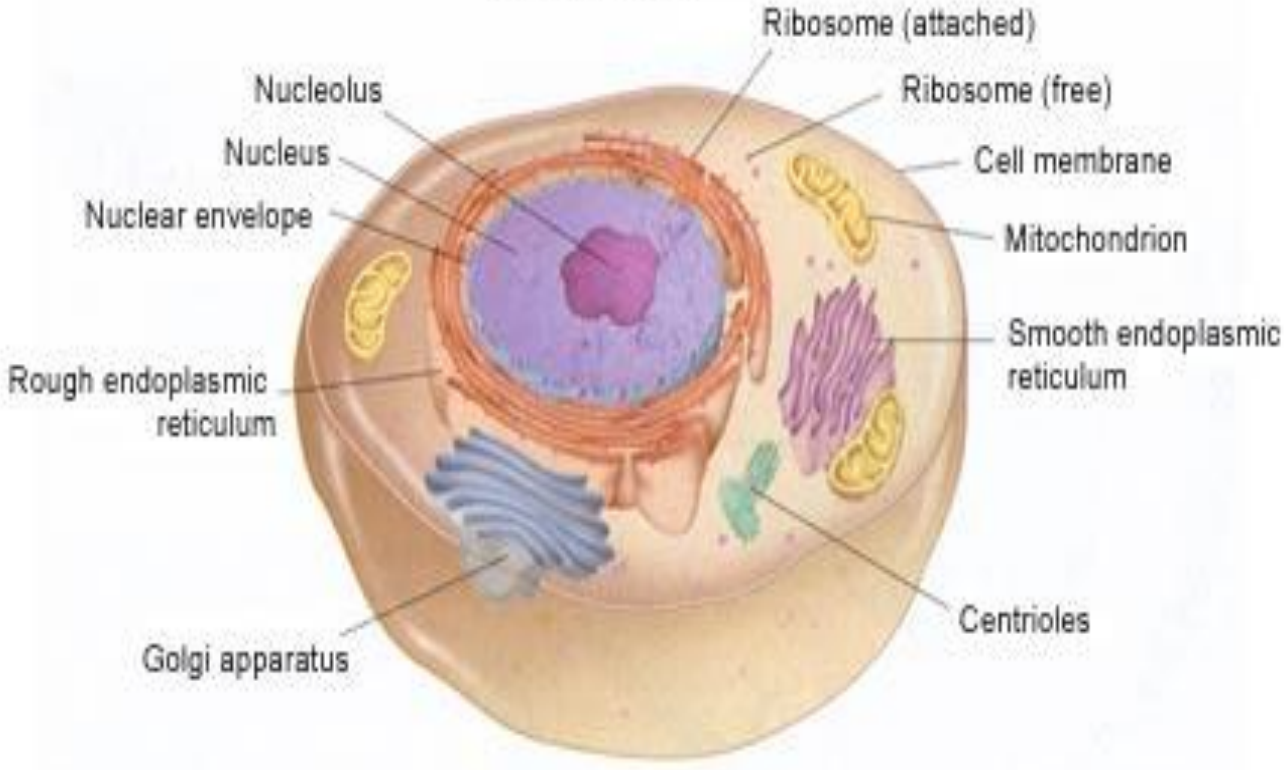
Cytoplasm: Cytoplasm is basically the substance that fills the cell. It is a jelly-like material that is 80٪ water and usually clears in color. Cytoplasm, which can also be referred to as cytosol, means cell substance. The cytoplasm is found inside the cell membrane, surrounding the nuclear envelope and the cytoplasmic organelles. It is made up of proteins, vitamins, ions, nucleic acids, amino acids, sugars, carbohydrates and fatty acids. All of the functions for cell expansion, growth and replication are carried out in the cytoplasm of a cell. The cytoplasm contains organelles, such as mitochondria, the endoplasmic reticulum, the Golgi apparatus, ribosomes, vacuoles, lysosomes, and in plant cells chloroplasts (green, colorless or colored). Cytoplasm is also the home of the cytoskeleton, a network of cytoplasmic filaments responsible for the movement of the cell and which gives the cell its shape. The animal cell has a centrosome composed of two centrioles, which separate during cell division and help in the formation of the mitotic spindle.

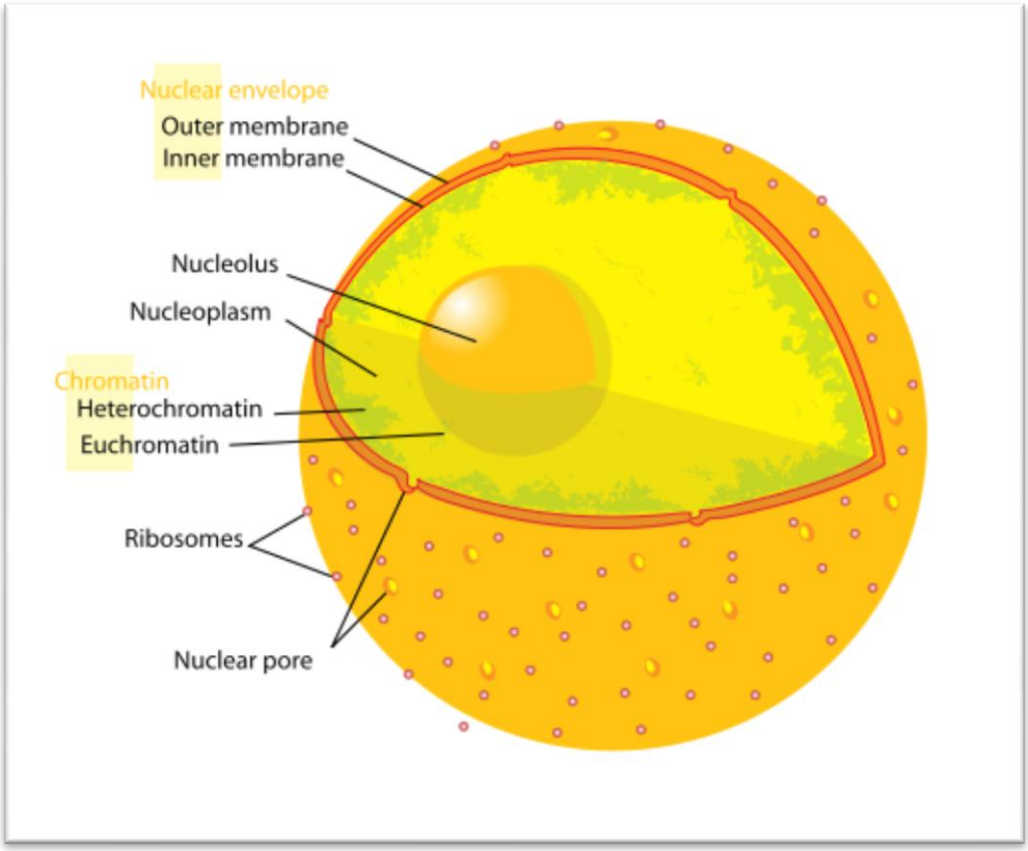
The nucleus:The nucleus is the most obvious organelle in any eukaryotic cell. It is the control center of cell activity and contains the genetic material (DNA) that is responsible for providing the cell with its unique characteristics and important for cell division. This genetic material is appears in a thread-like form called chromatin which is the complex of DNA and proteins but when cells replicate the chromatin condensed to form chromosomes which are thick, short and visible and easily seen by microscopy. The nucleus is enclosed in double membranes which have nuclear pores and it has a liquid in it called nucleoplasm. The prominent structure in the nucleus is the nucleolus (one, two or three) that produces ribosomes needed for protein synthesis.

Plant Cell



Animal Cell





Cytological preparations:

Many techniques are used for studying cells and its component and for studying the mitotic and meiotic division as well, but all these techniques depends on stable basis which are: collection of speciments, fixation and staining. Cell staining techniques and preparation depend on the type of stain and analysis used.

Collection of speciments:

In plant we can use meristemic zone in root, stem, and embryo for studying mitotic division. In animal cells from bone marrow, blood, amniotic fluid, cord blood, tumor can be cultured in order to increase their number. A mitotic inhibitor (colchicine, colcemid) is then added to the culture. This stops cell division at mitosis which allows an increased yield of mitotic cells for analysis. For studying meiosis division in plant we can use pollen mother cell (PMC) and using tissue obtained from testes and ovary in animal.

Fixation:

Fixation is to preserve cells and tissue constituents in as close a life-like state as possible and to allow them to undergo further preparative procedures without change. Fixation arrests autolysis and bacterial decomposition and stabilizes the cellular and tissue constituents so that they withstand the subsequent stages of tissue processing. It also arrests the division in required phases without any deformation of cells or Swollen or shrunken in chromosomes.

The most important and common fixative is Carnoy's fixative (1886), we use it in chromosomal studies because of it rapidly penetration and fixation and it widely used in cytological studies. Carnoy's fixative consist of glacial acetic acid which is a primary fixative that fixed nuclear proteins causes swollen of the cells and absolute ethanol alcohol which it is a primary fixative either that causes cells shrinkage. So in Carnoy's fixative we use both to avoid shrinking that result from using alcohol and swelling that result from glacial acetic acid.

Dehydration: This step usually necessary to achieve a good spreading for chromosomes between the slid and the cover slip. Tissue samples are gradually passed through 100% alcohol in order to remove and replace all the water with alcohol. Using HCL helps in soften the tissues because it effect may be associated with enzymes. Dehydration can dismantle the Baktinip salts of the Central plate and makes clear cytoplasm.

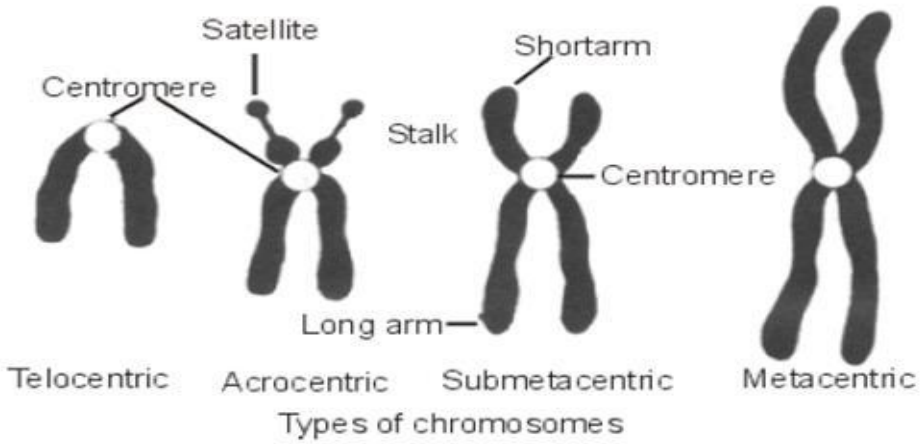
Staining of chromosomes: Chromosome identification has been traditionally dependent on morphological characteristics such as size, arm ratio and secondary constrictions at metaphase of mitosis and that can be observed by microscopy after the appropriate staining for it. A good stain gives clear cytoplasm In addition to stained nucleus. There is many kind of stains that are widely used in plant chromosomal staining but the most important one is Aceto-carmin dye, which is one of the most commonly used dyes in the chromosomal study due to its suitability and it speed in staining.

CHROMOSOMES AND MITOTIC DIVISION

Chromosome Number: The diploid chromosome number is the number of chromosomes in the somatic cell and is designated by the symbol 2N. The gametes, which have one-half the diploid number, have the haploid number N. In humans the diploid number is 46, with 23 inherited from each parent through the sperm or egg. The chromosomal number in living organisms differ from kind to another but it is stable in the same organism except in few kinds: such as honey bee it have a difference between the two sex that the female have 2N because it comes from fertilized egg otherwise the male have only 1N because it comes from non-fertilized egg. Same (homologous) chromosomes form a pair with one member from each parent. Thus, there are 23 pairs of chromosomes in human cells. Of these, 22 pairs are not directly involved in sex determination, and are known as autosomes. The remaining chromosome pair consists of the sex chromosomes, and is directly involved in sex determination. In females the two sex chromosomes are identical (XX), whereas in males the two sex chromosomes are not identical (XY). The Y chromosome is smaller than the X chromosome.

Chromosome Morphology: A typical metaphase chromosome consists of two arms separated by a primary constriction or centromere. Each of the two sister-chromatids contains a highly coiled double helix of DNA. A chromosome may be characterized by its total length and the position of its centromer.

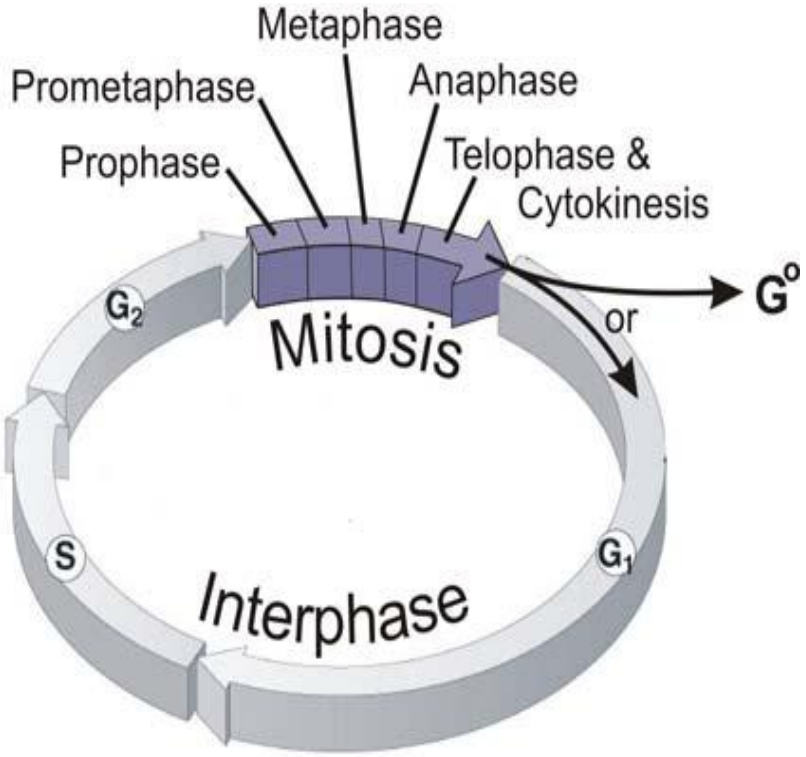
A chromosome with the centromere at or near the middle is called **metacentric**. A **submetacentric** chromosome has a centromere somewhat displaced from the middle point. If the centromere is obviously off center (e.g., halfway between the middle and the tip of the chromosome). **Acrocentric** chromosomes have their centromeres very near one end. **Telocentric** chromosomes, which are absent in human cells, have their centromeres at the very tip of one end. The short chromosome arm is designated p (petite) and the long arm q (one letter after p). Certain human chromosomes may also contain a secondary constriction called a satellite stalk near the tip.



Types of chromosomes

Homologous chromosomes: Are chromosome comes from the father and the mother which have the same length and the same position of centromere which we cane use them in karyotyping.

The cell cycle: Chromosomes are not visible under the light microscope in non-dividing (Interphase) cells. As the cell begins to divide, the threads of chromatin (DNA-protein complex) in the nucleus begin to condense into multiple levels of coiled structures recognizable as chromosomes. There are two modes of cell division: mitosis and meiosis. Mitosis is responsible for the proliferation of body (somatic) cells, whereas meiosis is responsible for the production of gametes. Because mitotic cells are easy to obtain, morphological studies are generally based on mitotic metaphase chromosomes. The transition of interphase to cell division (mitosis) and back to Interphase is called the cell cycle. Interphase of a dividing cell is divided into three stages: G1, S and G2, with "G" meaning gap. During the G1 stage, cytoplasmic components such as membranes, organelles and ribosomes begin to proliferate. G1 if followed by the S stage during which DNA synthesis occurs and the amount of DNA per chromosome is doubled, resulting in two sister-chromatids visible during prophase and metaphase. S phase is followed by a second period of growth, the G2 stage. At the end of G2, the cell starts to divide and enters the M (mitosis) stage. When the cell is not cycling, Interphase is said to be in G0. The cell cycle for human cells averages about 20 hours (8-10 hours for G1, 6-8 hours for S, 2-4 hours for G2 and 1 hour for M



MITOSIS

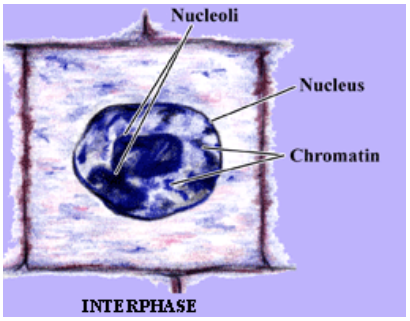
Mitosis is Asexual Reproduction Cell Cycle results in copying & equal duplication of parental cell's DNA and the equal division of chromosomes into two daughter cells so it is the process of cell division in which the nucleus divides to produce two nuclei with the same types and numbers of chromosomes. (rates = liver cells 1x/yr - epithelial cells 1x/day).

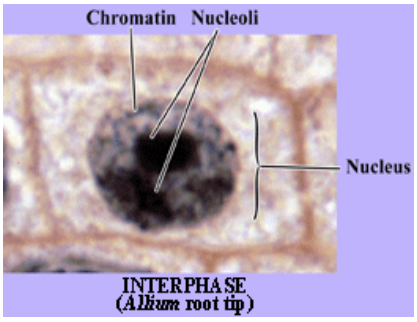
Interphase - period between successive divisions of a cell
3 parts = G1 - before, DNA synthesis (S), & G2 period after
MITOSIS* - nuclear division phase; separation & duplication of chromosomes: four stages: (1) prophase, (2) metaphase, (3) anaphase, and (4) telophase.

Cytokenesis* - physical division of cell into two parts: animals/plants

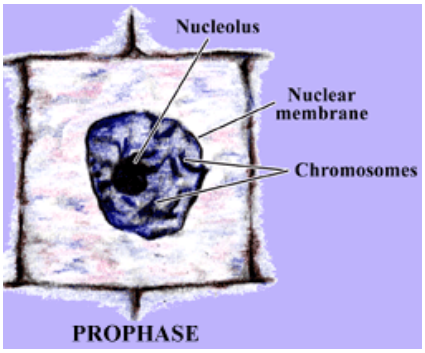
Mitotic stages:

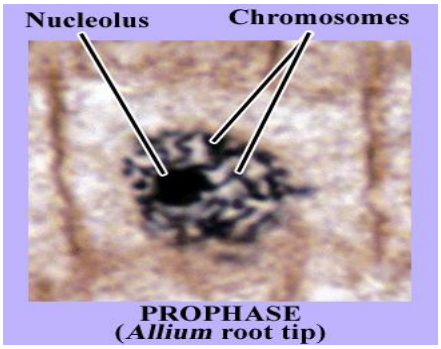
Interphase: Interphase is the stage in the life cycle of the cell when the cell is performing all its normal metabolic functions except division. In preparation for mitosis, the interphase cell begins preparations for division with the duplication of the chromosomes and the centrioles.



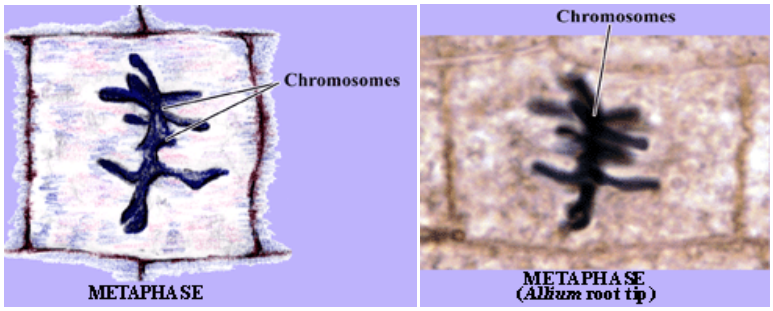


Prophase: Prophase is the first stage of mitosis and includes the following events: the nuclear membrane disappears, the nucleoli disappear, a pair of centrioles moves to form each pole and the mitotic spindle forms between the poles (centrioles), aster fibers extend from the poles (centrioles), and the replicated DNA begins to condense into distinctive chromosomes.

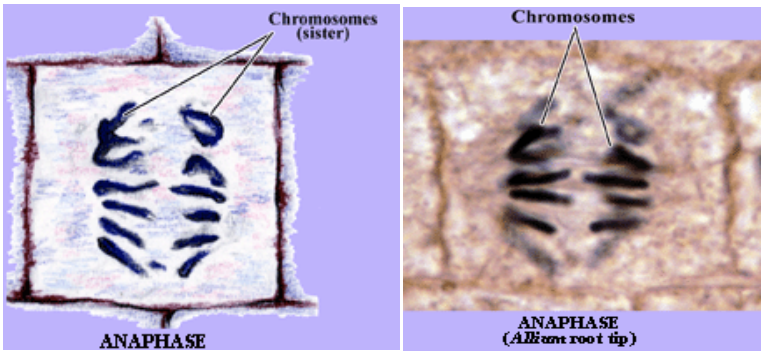




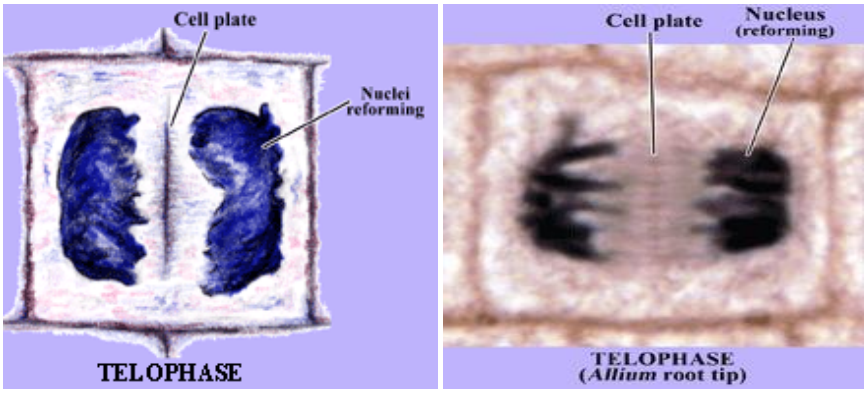
Metaphase: Metaphase is the second stage of mitosis and is characterized by the alignment of the chromosomes along the equator of the cell.



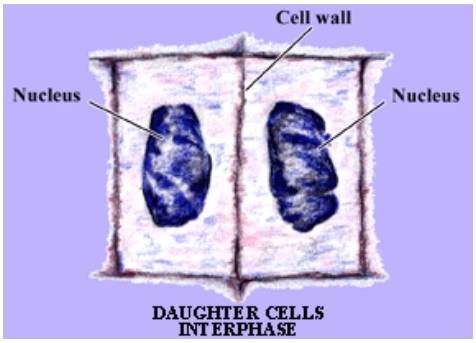
Anaphase: Anaphase is the third stage of mitosis and is characterized by the separation of the chromosomes and the movement of the each of the replicated chromosomes toward opposite poles.

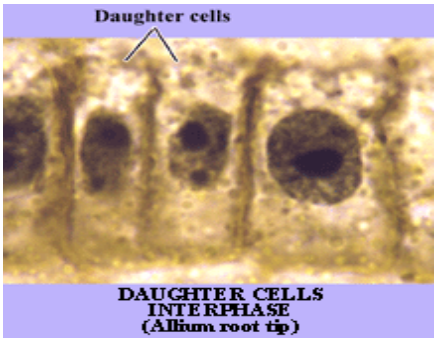


Telophase: Telophase is the fourth (last) stage of mitosis and begins when the chromosomes stop their movement at the poles. Telophase is characterized by the dispersal of the chromosomes into chromatin, the nuclear membrane reforms, the nucleoli reappear, and the mitotic spindle and aster fibers disappear. Mitosis is complete with the formation of two nuclei, each with the same number and types of chromosomes. The formation of a cell plate (new wall), part of cytokinesis (division of the cytoplasm) is evident during telophase.



Cytokinesis and Daughter Cells: At the completion of mitosis, the cell is divided into two cells, each with one of the newly formed nuclei, by the formation of a cell wall between the cells. Cytokinesis is defined as the division of the cytoplasm and in plant cells is characterized by the formation of a new wall between the daughter cells.





PROCEDURE FOR PREPARING ROOT TIP SQUASHES

While actively growing onions are present in the lab for you to observe, you will be provided with roots that have been previously harvested and treated with 2 mL Carnoy's fixative to stabilize the cells, ensure all tips are immersed. Cover the beaker with foil that it is air tight. Fix the root tips in Carnoy's fixative for at least 24 hours.

You will work in groups for this lab exercise.

The first step will be to 'soften' the roots so that they later can be spread on a microscope slide.

1.Using scissors, cut 2 roots tips about 1 cm long, and transfer them into a test-tube. (One of the rots will be an extra one.)

2. Fill the tube about 2/3 full with 1N HCl and cover the tube with gauze then tie it with rubber.***** Caution: Work with the HCl carefully, it is a strong acid. *****

3.Place the tube in a 60OC water bath, and allow the roots to incubate for 12 minutes.

4. After the 12 minute incubation period, remove the tube from the water bath.

5. Carefully remove the HCl from the test-tube and rinse the roots in H2O about 3 times.

6. After removing the water from the third rinse, cover the root with the aceto-carmine stain.***** Caution: Avoid staining your skin and cloth. *****

7. Incubate the roots in the stain for 12 minutes. During this time the very tip of the root will begin to turn red as the DNA stains the numerous small actively dividing cells at the tip.

8. Transfer a root to the center of a clean microscope slide and add a drop of water.

9. Using a razor blade cut off most of the unstained part of the root, and discard it.

10. Cover the root tip with a cover slip, and then carefully push down on the cover slide with the wooden end of a dissecting probe. Push hard, but donot twist or push the cover slide sideways. The root tip should spread out to a diameter about 0.5 – 1 cm.

11. Examine under the microscope at low power to ensure that the cells are adequately spread to a monolayer. If so, examine under higher power. Locate mitotic figures (near the tip end), and switch to oil immersion (1000x).