

Lab 6. Cellular Reproduction: Mitosis and Meiosis

Cell Division - Mitosis

Sexually-reproducing, multicellular organisms begin life as a single cell, the fertilized egg. This cell, the **zygote**, through the process of **mitosis** (nuclear division) and **cytokinesis** (cytoplasmic division), becomes two daughter cells, which in turn become four cells, eight, sixteen, thirty-two, etc. as each cell continues to divide. At a particular point in this process of development (or embryogenesis), cells begin to **differentiate** into different types of cells that will perform specialized roles in the functioning of the whole organism. The information that controls the behavior and differentiation of each cell is carried in the **chromosomes**. In previous labs, you saw dark-staining nuclei in cells. These nuclei stain darkly because the chromosomes in the cells were 'unwound', forming diffuse chromatin in the nucleus. This is the way that the chromosomes are when they are actively functioning to direct the cell's function. When cells reproduce, this diffuse chromatin becomes reduced into discrete bodies (chromosomes) through a complex coiling process. Chromosomes are the 'packages' in which genetic information is passed during cell division. Chromosomes typically occur in homologous pairs (in diploid organisms). The number of pairs of chromosomes and the size and shape of each pair are characteristic and constant for a given kind of organism. Humans, for example, have 23 pairs of homologous chromosomes in each of their cells (except for egg and sperm cells), while the fruit fly, *Drosophila melanogaster*, has 4 pairs.

Prior to cell division, a cell synthesizes a duplicate of each chromosome. Duplicate chromosomes remain attached to one another in a region called the **centromere** until the actual division process occurs. While attached to one another, each duplicate is called a **chromatid**, and the two together - **sister chromatids**. It is important to remember that, although each chromosome is at this point doubled, consisting of two sister chromatids connected at the centromere, they still represent only ONE CHROMOSOME. They will not become separate chromosomes until they have separated from each other.

Mitosis (nuclear division) consists of the separation of the sister chromatids. As they separate, the cytoplasm with its constituent organelles is divided approximately equally between the daughter cells, a process called cytokinesis. The outcome of mitotic cell division is two identical cells with identical sets of chromosomes. Mitosis and cytokinesis are key processes in the growth and development of an organism. They are also the means by which wounds are repaired and dead cells are replaced.

Details of Mitotic Cell Division

Mitosis is part of the normal cell cycle. There are three general portions of the cell cycle: Interphase, Mitosis, and Cytokinesis.

Interphase

In interphase, the cell is actively growing, respiring, and synthesizing DNA, RNA, and proteins. Nuclear material is surrounded by a membrane. The dark-staining nucleoli are visible within the nucleus. Collectively, the chromosome mass is called chromatin. The chromosomes are not individually distinguishable because they are

uncoiled into long thin strands and appear only as dark granules within the nucleus. Chromosomes and organelles are duplicated during interphase.

Mitosis

The phases of mitosis are: Prophase, Metaphase, Anaphase, and Telophase.

- **Prophase** - The nuclear membrane and nucleoli break down. Chromosomes appear first as thin threads, and later as thicker, distinct bodies as they coil up. At this point, each chromosome contains two DNA molecules organized as sister chromatids held together by a centromere. The spindle, a structure composed of microtubules forms, although it may not be easily visible until the next stage. In animal cells, the formation of the spindle appears to be associated in some way with the centrioles, which move in opposite directions. The centrioles may have something to do with organizing the spindle. However, a spindle forms in a dividing plant cell, yet plant cells lack centrioles. In animal cells, additional microtubules radiate from the centrioles and form a star-shaped aster. Centrioles and asters have not been found in the cells of higher plants.
- **Metaphase** - The chromosomes move to the equatorial plane of the cell, and line up along the equator. It is useful to think of the chromosomes lining up end-to-end in metaphase of mitosis, although it doesn't actually happen this way.
- **Anaphase** - The centromeres holding sister chromatids together split apart, and sister chromatids move away from each other towards opposite ends of the cell.
- **Telophase** - Nuclear division is completed in telophase. The chromosomes are now at each end of the cell. New nuclear membranes begin to form around each group of chromosomes, and the chromosomes begin to uncoil. It is often difficult to distinguish late anaphase from early telophase. It should be remembered that mitosis is a continuous process (not stopping between each phase) and as such, each phase gradually grades into the next.

Cytokinesis

Cytokinesis usually begins during telophase. Cytokinesis is the division of the cytoplasm into two separate cells. In animal cells, the first sign of this splitting of cytoplasm is the formation of a crease around the cell, a **cleavage furrow**. As cytokinesis continues, the furrow becomes deeper and deeper, finally pinching the parent cell into two daughter cells.







In plant cells, division of the cytoplasm results from the formation of a **cell plate** across the center of the cell. This cell plate forms internally in the cell as the result of Golgi vesicles lining up and fusing.


Once mitosis and cytokinesis are complete, the daughter cells may again enter interphase of the cell cycle.


Observing mitosis and cytokinesis in plant cells: Onion root tip.

Obtain a slide of an onion root tip. Examine it with the scanning objective lens, and locate the region of active cell division just behind the dead cells at the tip of the root. Focus on this region, and switch to the medium-power objective. Focus again and switch to the high-power objective lens. Find cells in each of the following stages: Interphase, Prophase, Metaphase, Anaphase, Telophase, Early Cytokinesis. **Draw representative cells on the paper provided, labeling all structures.**

Because all the cells on your slide were stopped at once, the relative numbers of cells in each phase of the cell cycle provide a rough estimate of the relative duration of each phase. After you have drawn cells in each phase and are familiar with their appearance, count the number of cells in each of the six stages above that appear in ONE field of view under the high-power objective (40X). Record your data below.

-  How many cells are in interphase? _____
-  How many cells are in prophase? _____
-  How many cells are in metaphase? _____
-  How many cells are in anaphase? _____
-  How many cells are in telophase? _____
-  How many cells are in early cytokinesis? _____

 Which is the longest portion of the cell cycle?

 Which portions of the cell cycle are very brief?

Observing mitosis and cytokinesis in animal cells: Whitefish Blastula

The most convenient source of actively dividing cells in animals is the early embryo, where cells are large and divide rapidly with a short interphase. In the blastula (an early embryonic stage), a large percentage of cells will be dividing at any given time. By examining a cross section of a whitefish blastula, you should be able to locate many dividing cells in various stages of mitosis and cytokinesis.

Obtain a slide of a whitefish blastula. Find a cross section using the scanning objective, switch to the 10X objective, focus, and switch to the 40X objective. Find the six stages of the cell cycle above, and **draw and label what you see in each stage on the paper provided.**

Although the behavior of the chromosomes is the same as in plant cells, several distinctive animal structures should be visible:

- **Centrioles** - small dots at the poles around which the microtubules of the spindle seem to organize.
- **Asters** - a star-like array of microtubules surrounding each centriole pair.
- **Cleavage Furrow** - a constriction near the center of the dividing cell

Meiosis

Meiosis takes place in all organisms that reproduce sexually. Meiosis is necessary in sexually reproducing organisms because it prevents the chromosome number from doubling with every generation when fertilization occurs. Meiosis is the process by which the gametes (sperm and eggs) are produced. In animals, it only occurs in the gonads, while in plants it only occurs in structures called sporangia.

Meiosis consists of two nuclear divisions, meiosis I and II, with an atypical interphase between the divisions during which cells do not grow or synthesize DNA. This means that meiosis I and II result in a sum total of four cells from each parent cell. Each new cell contains half the number of diploid chromosomes, one chromosome from each homologous pair. Recall that cells with only one of each homologous pair of chromosomes are called 'haploid' cells.

Meiosis I

- **Prophase I** - Chromosomes coil and condense as they do in mitosis. The nuclear envelope and nucleoli break down. However, in meiosis, there is another essential event in prophase I. Homologous chromosomes, each composed of two sister chromatids, come together and coil around each other. This is called synapsis. While synapsed, the pair of chromosomes are called a tetrad. During synapsis, chromatids of homologous chromosomes exchange or 'trade' pieces of DNA. This exchange of genetic material is called crossing over or recombination.
- **Metaphase I** - Chromosomes move to the equatorial region of the cell as tetrads. In other words, the homologous pair goes to the center of the cell together. It is useful to think of the pair of them lying there side-by-side (as opposed to end-to-end like in mitosis).
- **Anaphase I** - Each chromosome (consisting of two sister chromatids held together at the centromere) separates from its homologue and one homologue of each pair moves toward each pole.
- **Telophase I** - The homologous chromosomes have aggregated at opposite poles. Cytokinesis begins and divides the original cell into two haploid daughter cells.

It is by the end of Meiosis I that the resulting two cells have reduced their chromosome number by half. Although each chromosome in each cell still consists of two sister chromatids connected by a centromere, there are half as many chromosomes as in the original parent cell.

Meiosis II

- **Interphase** - no DNA synthesis occurs
- **Prophase II** - No synapsis or crossing over occurs because the cell contains no homologous pairs to cross over with. Chromosomes condense.


- **Metaphase II** - The chromosomes move to the midline of the cell again. This time, they line up just like in mitosis. You can imagine them as being end-to-end.
- **Anaphase II** - Each centromere splits, and the single-chromatid chromosomes thus formed move away from each other toward opposite ends of the spindle
- **Telophase II** - the chromosomes begin to unwind, cytokinesis begins, and the nuclear membrane re-forms.

Differences between the sexes in the products of meiosis

Although the process is identical, the products of meiosis differ between males and females because of differences in the allocation of cytoplasm during cytokinesis. In males, the cytoplasm is divided equally between the daughter cells both during the first meiotic division and during the second. This results in four equal-sized cells being produced by one initial cell after meiosis. These four cells will become the sperm.

In females, the allocation of the cytoplasm is unequal during both meiotic divisions. This results in one larger daughter cell, and one smaller cell (the polar body) produced each time. During cytokinesis after meiosis I, the smaller daughter cell becomes the first polar body, and the larger cell will eventually give rise to the ovum after meiosis II. During meiosis II, this larger cell also divides unequally with respect to its cytoplasm, and the smaller cell becomes the second polar body. The larger cell, with most of the cytoplasm, is now the ovum. The polar bodies do not function.

Thus, in females, only one functional cell (the ovum) is produced by meiosis from one initial cell. The other two or three products (the polar bodies) degenerate.

-  On the paper provided, draw a complete meiotic cycle for both males and females. Assume that this organism has a diploid number of 4, two long chromosomes and two short chromosomes. The dark chromosomes came from the father, and the light chromosomes from the mother. You will begin in prophase I, with the chromosomes already duplicated. Be sure to show synapsis and crossing over, the alignment of the chromosomes at both metaphase I and II, and the cytoplasmic allocation during cytokinesis in each sex.

Mitosis in onion root tip

All drawings are done at total magnification = 400X

Label (where present): cytoplasm, nucleus, nucleolus (nucleoli), chromatin, spindle, chromosomes, cell plate, daughter cells.

Interphase	Prophase	Metaphase
Anaphase	Telophase	Recent Cytokinesis

Mitosis in whitefish blastula

All drawings are done at total magnification = 400X

Label (where present): cytoplasm, nucleus, nucleolus (nucleoli), chromatin, spindle, chromosomes, cell plate, daughter cells.

Interphase	Prophase	Metaphase
Anaphase	Telophase	Recent Cytokinesis

Meiosis in Males

Prophase I	Metaphase I	Anaphase I	Telophase I (and Cytokinesis)
Prophase II	Metaphase II	Anaphase II	Telophase II (and Cytokinesis)

Meiosis in Females

Prophase I	Metaphase I	Anaphase I	Telophase I (and Cytokinesis)
Prophase II	Metaphase II	Anaphase II	Telophase II (and Cytokinesis)