

Biology 3A Laboratory

MITOSIS – Asexual Reproduction

OBJECTIVE

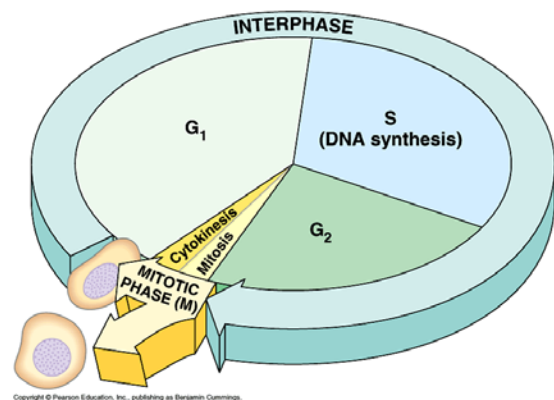
- To study the cell cycle and understand how, when and why cells divide.
- To study and identify the major stages of cell division.
- To relate the mitotic process to living organisms.
- To understand the differences between plant and animal cell division.
- To graphically illustrate the roles of cell division and cell elongation in root growth.
- To learn how chromosomes are stained.

INTRODUCTION

Mitosis is a form of cell division that eukaryotic cells employ for growth, repair or regeneration of **somatic (body) cells**. Some eukaryotic cells can even use mitosis as a form of asexual reproduction to increase their numbers. Certain bacteria, cyanobacteria and many single-celled organisms reproduce by another form of mitosis called **binary fission**. The ability of cells to reproduce more of its own kind is a basic principle of the cell theory.

Every cell in our body has a **cell cycle** (Figure 1) which is divided into two main phases: **Interphase** (non-dividing phase) and **Mitotic phase** (dividing phase). Interphase is the longest phase of the cell cycle. It comprises about 90% of the cell cycle and is subdivided into three phases: G₁, S and G₂. Most cells are arrested in the G₁ phase of Interphase. During this G₁ phase, cellular processes such as protein synthesis and metabolic activities occur. The **S phase** of interphase is when DNA replication takes place which results in the copying of the genetic information. The **G₂ (Gap) phase** is another cellular growth phase in which the cell with its copied genetic information is preparing for the mitotic phases.

Figure 1: A typical cell cycle.



The **Mitotic Phase** is the division of the duplicated genetic material (**karyokinesis**) and can be subdivided into four phases: prophase, metaphase, anaphase and telophase. **Prophase** is longest of the mitotic phases. During this phase, the nucleoli disappears, the nuclear envelope fragments and begins to disappear. The chromosomal material (**chromatin**) condenses becoming more distinct and visible as chromosomes. Each duplicated chromosome consist of **two sister chromatids** that are held together at the **centromere**. During **metaphase**, the chromosomes move to the **metaphase plate** and line up single file at the center of the cell. In **Anaphase**, the centromeres break and the sister chromatids separate and move toward opposite poles of the cell. After separation, each **chromatid** is considered a **chromosome**. In **Telophase**, as the cell continues to elongate, the nuclear envelope and nucleoli reappear. **Cytokinesis**, the division of the cytoplasm, occurs in conjunction with

telophase to create two separate daughter cells. In animal cells, a **cleavage furrow** develops to pinch the cell into while in plants, the **cell plate** begins to form between the new daughter cells. At the end of karyokinesis and cytokinesis, each daughter cell has a single nucleus with the same number of chromosomes as the parent cell.

A. PLANT AND ANIMAL MITOSIS

Although the process of mitosis is the same for both plants and animals, there are differences in presence or absences of certain cellular structures. Telophase and cytokinesis occur in both plant and animal cells. However, plant cells form a **cell plate** during telophase, which will eventually separate the two daughter cells. Animal cells form a **cleavage furrow** when the chromosomes are near the poles. Animal cells also have **centrioles** (asters) which assist in the shortening of the microtubules during anaphase.

When you are trying to locate and identify the various phases, not all of the cells will show the “typical” stages of mitosis. During the tissue preparation procedure, thin sections are taken through the whitefish blastula (early embryonic developmental stage) and the onion root tip in such a way that not all of the nuclear material may be present. If you encounter such a cell that does not contain any nuclear material, skip that cell and move on to one with distinct nuclear material present.

Procedure A:

1. Obtain a prepared slide of an onion root tip (*Allium sp.*). Note: there will be several root tips on this slide.
2. Place a piece of white paper in front of the slide in order to notice the three lightly stained root tips.
3. Place the slide on the microscope stage and focus on the root tip under scanning power (4X).
4. Find the rounded root cap and move to the area just above the root cap. This is the region in the root where mitosis is occurring.
5. Identify and draw the 5 mitotic stages on your worksheet. Do not forget to label the appropriate components.
6. Obtain a prepared slide of a whitefish blastula. Note: there will be several thin sections through the blastula on this slide.
7. Place a piece of white paper in front of the slide in order to notice the three lightly pinkish stained blastula sections.
8. Repeat the process as before with the onion root tip.
9. Make a note of the differences between plant and animal mitosis.

B. MITOTIC STAGE FREQUENCY

During the cell cycle, a cell will spend only a portion of its time in a particular phase. Interphase is the longest of all the phases, while prophase is the longest of the mitotic phases. To understand the relative time a cell spends in a particular phase, you will determine the frequency for each of the five major phase of mitosis for your group, your cumulative lab section's data and finally the cumulative data for all lab sections. By increasing the sample size (large population), this should increase the accuracy of the results.

The onion root tip is divided into three or more (or less) distinct regions: the region of cell division, the region of elongation and the region of maturation. However, there is no clear distinction between these regions. Keep in mind that the frequency of cell division will vary at different levels and in different tissues of the root tip. Some cells may even have elongation during interphase between successive divisions.

Procedure B:

1. Work in pairs.
2. Obtain a prepared slide of an onion root tip (*Allium spp.*). Note: there will be several root tips on this slide.
3. Place the slide on the microscope stage and focus on the root tip under scanning power.
4. Find the rounded root cap and move to the area just above the root cap as before.
5. Focus on low power and scan for a section in which the chromosomes appear distinct.
6. Change to high power and focus.
7. Randomly count 100 cells and note the stage of mitosis that each cell is in. If you cannot distinguish the stage or nothing is present, skip that cell.
NOTE: DO NOT count 100 cells for each stage. Count 100 cells **altogether**.
8. Make a running tally on Data Table 1 on your worksheet.
9. Calculate the frequency each stage is in by dividing the total for each stage by 100.
- 10. Record your groups data on Excel spreadsheet before you leave lab today.**
11. Using Excel, construct a graph your data, the mean lab section data and the mean entire Biology 3A laboratory sections' data on the same graph.
12. Replace the slide and the microscope correctly to their proper areas.

C. Chromosome Staining Technique

In the first two portions of the lab, you identified and labeled the five stages of mitosis and determined the relative time spent in each phase. In this section, you will learn a staining technique used to observe chromosomes in a fresh onion root tip.

Procedure C:

1. Remove two 2 mm tips from an onion with a razor blade (be careful).
2. Add the two root tips to a small beaker and cover with 1 N HCl to hydrolyze and macerate the tissue.
3. Place the beaker into 60C water bath for 5 – 10 minutes. Times could be longer or short than what is given.
4. Rinse out HCl with water.
5. Place the material in a small quantity of the stain in a tightly stoppered vial. Staining may take from a few minutes to several hours. Your instructor will divide the class into various staining time durations (5, 10, 15, 20, and 30 minutes).
6. Rinse in several changes of tap water.
7. Squash stained materials with 45% acetic acid.
8. Cut the meristematic region of the stained root tips at 0.1 mm thick and squeeze out the tissue with the tip of a dissecting needle.
9. Place the squeezed out material on a slide.
10. Apply a drop of Feulgen stain and a drop of 1 N HCl (1:1 ratio) for 1 – 3 seconds and remove with a piece of blotting paper.
11. Put on a coverslip.

12. Making the "squash":

- a. Tap the coverslip gently with the eraser end of a pencil after fixing a corner of the coverslip with a finger to avoid cover glass slipping.
- b. Or place your thumbs on top of each other and then on top of the coverslip. Using a rocking motion as you bear down with considerable force, squash the root tip.

13. Observe the preparation.

NOTE: the chromosomes should be bright magenta red in color whereas the cytoplasm is colorless.

14. Show your instructor your stained slide after you have finished observing them.

A. PLANT AND ANIMAL MITOSIS

1. Make representative drawings of the various distinct mitotic stages from both prepared slides of the onion root tip and whitefish blastula. **Label** (at least once) in both plant and animal cells, if present: nucleus, chromosomes, chromatid, centrioles or asters, spindle fibers, cleavage furrow, cell plate, cytokinesis, karyokinesis.

PLANT

ANIMAL

Interphase

Prophase

Metaphase

Anaphase

Telophase

- List several major differences you have observed between plant and animal mitosis.

B. MITOTIC STAGE FREQUENCY

- Data Table 1: Individual mitotic stage frequency for onion root tip.

Stage	# Observed - Tally	Total # Observed	Frequency
Interphase			
Prophase			
Metaphase			
Anaphase			
Telophase			

- Data Table 2: Duration of mitotic stages.

Phase	Student Pair		Lab Section		All Sections	
	# of cells/stage	Duration (min)	# of cells/stage	Duration (min)	# of cells/stage	Duration (min)
Total # of nuclei						
# in prophase						
# in metaphase						
# in anaphase						
# in telophase						
# in interphase						

- Estimate the duration of each stage based on your raw data, the mean lab section and the mean data for all lab sections. The duration of each mitotic stage may be estimated using the following equation:

$$\text{Time/mitotic stage} = \frac{\text{number of cells/stage}}{\text{total number of cells}} \times \frac{24 \text{ hr} \times 60 \text{ min}}{\text{mitotic 1 hr}}$$

- Using Excel, graph number of cells observed in each mitotic phase for your group's data, your cumulative lab section's data and the entire cumulative data for all lab sections on the same graph.
- Based upon the data in Table 2 on the number of cells undergoing mitosis, what phase or stage of active mitosis is the **longest** for: student pair, lab section and all sections?
- Based upon the class data on the number of cells undergoing mitosis, what phase or stage of active mitosis is the **shortest** for: student pair, lab section and all sections?

9. The nuclear envelope/membrane disappears during what stage of mitosis? **Explain why.**

10. When does the nucleolus reappear?

11. Are there any types of human cells that do not undergo mitosis after maturation? If so, which are they?

C. Chromosome Staining Technique

12. Were you able to determine the different mitotic stages in your slide? If you could not see any chromosomes, what should you do?

13. Data Table 3: Mitotic stage frequency for the fresh onion root tip.

Stage	Total # Observed	Frequency
Interphase		
Prophase		
Metaphase		
Anaphase		
Telophase		

14. How does your data compare with section A?

15. List several other types of stains that are used to stain chromosomes?