

# Lab 7

## Mitosis

### **Objectives**

1. Identify the various stages of mitosis in onion root tips.
2. Prepare root tip squashes to observe mitosis in bean roots
3. Observe how colchicine affects mitosis in bean roots.

### **Safety Precautions**

1. Colchicine is known to cause cancer in animals. Acetic acid is corrosive. Use gloves and goggles when handling bean root tips.
2. Dispose coverslips in sharps containers.

### **Materials**

Pop-bead Chromosome kit

Compound light microscope

Microscope slides and coverslips

Prepared onion root tip slides

Preserved broad bean root tips treated with colchicine and stained with Schiff's reagent

Preserved broad bean root tips treated with deionized water and stained with Schiff's reagent

45% acetic acid

Glass rods

Razor blades

Paper towels

### **Introduction**

Cancer is a leading cause of death in the United States; only heart disease claims more lives. New cells in our skin, blood, stomach lining, hair and other parts of our body are produced each day through a process of cellular division called mitosis. Cancer occurs when a cell accumulates mutations in its DNA that cause it to ignore the normal control systems governing cellular division. If the body's control systems cannot regulate it, uncontrolled proliferation of the mutated cell occurs.

During mitosis, the DNA contained in the chromosomes of the mother cell is replicated and distributed along with cytoplasm into two daughter cells. Unless a mutation occurs during the replication of the DNA, the daughter cells are identical to the mother cell. They are clones of the original cell. Like a dance, the process of mitosis is continuous but we can observe different stages of mitosis in actively dividing cells when we observe them under the microscope. In today's lab you will observe "snapshots" of the dance of mitosis captured by the slides we prepare and use.

Before mitosis, a cell is in a stage called interphase. (See Fig. 8-1.) The chromosomes in the nucleus are indistinguishable from one another using an ordinary light microscope, because they are stretched into long thin threads. During interphase, prior to mitosis, each chromosome is replicated to form two identical **chromatids**. The chromatids are attached to one another by at a region called the centromere. In the first stage of mitosis, **prophase**, the chromatids begin to coil

more tightly, condensing into discrete chromosomes visible with the light microscope. Two structures called **centrioles** move toward opposite ends of the cells. They will serve as starting points for the formation of long thin structures called **microtubules**. The membrane surrounding the nucleus begins to break up during prophase. In the next phase of mitosis, **metaphase**, the centromeres of each pair of chromatids begin to line up between the two centrioles across the center of the cell. They are moved into this position by the microtubules radiating from the centrioles. In the third phase of mitosis called **anaphase**, the centromeres of each chromatid separate. The chromatids are now once again called chromosomes. The centromeres of each chromosome are pulled by microtubules toward one of the poles of the cell. In the final phase of mitosis, **telophase**, identical sets of chromosomes have been gathered at opposite ends of the cell. They begin to uncoil, and return to the threadlike appearance they had during interphase. A nuclear membrane forms around each group of chromosomes and the cytoplasm is divided.

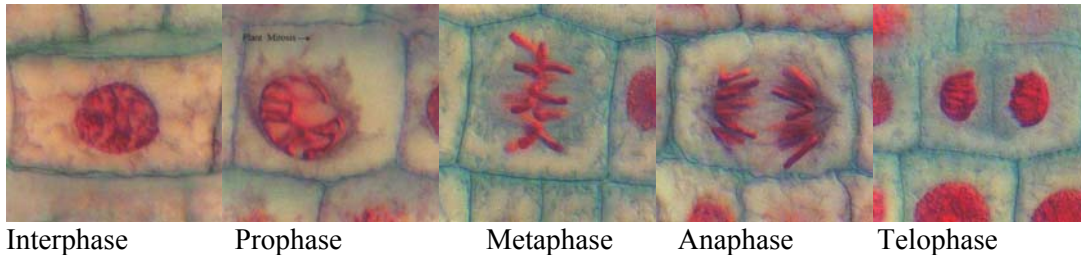


Fig. 8-1. Interphase and the stages of mitosis in an onion root tip slide.

A normal cell undergoes mitosis when it receives a growth-promoting signal, which activates the genes inside the nucleus responsible for initiating cell division. In some cancers these genes have undergone a mutation causing the cell to produce too much growth promoting signal or causing the cell to grow and divide regardless of growth-promoting signal.

A variety of options exist for the treatment of cancer. One option is the use of chemotherapy. Chemotherapy is particularly useful when the cancer has metastasized or spread throughout the body. Chemotherapeutic drugs interfere with cancer cells' ability to make DNA or to divide. In today's laboratory, you will observe bean root tip cells treated with colchicine, a mitotic inhibitor. Colchicine has limited therapeutic use, however, its action in the cell is similar to vinblastine and vincristine, which are used to treat cancers such as leukemia, lymphoma, and Karposi's sarcoma. Colchicine, vinblastine, and vincristine prevent cells from assembling microtubules needed to move the chromosomes.

## **Procedure**

### *Modeling Mitosis*

Modeling the sequence of activity in mitosis can be very helpful in understanding the process. Obtain a pop bead chromosome kit and kit instructions. Notice that in the "Assembly" section of the instructions, you are asked to prepare 'four units (two of each color).' By doing so, you will be preparing two different chromosomes (using different color beads to distinguish between the two chromosomes) and then replicating the DNA to create two chromatids of each chromosome. Two identical chromatids will be linked by the magnetic centromeres. Model each stage of mitosis and then answer assignment question 1.

### *Observing Mitosis in Onion Root Tip cells*

1. Obtain a prepared slide of onion root tips. Actively dividing cells are found at the end of roots. A root cap protects the region. Find the area where actively dividing cells are present.

Study the cells in this area and compare them to Fig. 8-1 until you can easily recognize cells in interphase, prophase, metaphase, anaphase and telophase.

2. Using high power, count the number of nuclei visible in a high power field and record your answer in assignment question 2.
3. In the same field, count the number of cells in each of these phases: prophase, metaphase, anaphase and telophase. Record your results in assignment question 2.
4. Like a snapshot, the prepared onion root tip slide catches cells in various stages of mitosis. The probability of finding a cell in a certain stage of mitosis is proportional to the length of the time a cell remains in that stage. If we know how frequently onion root tip cells divide, then we can calculate the duration of each stage of mitosis. Scientists have determined that the control system governing cellular reproduction dictate that onion root tip cells divide every 24 hours. In other words the cycle duration is 1440 minutes. Calculate the duration of each phase of mitosis using the following formula:

$$\text{Duration of a stage (min)} = \frac{\text{observed \#}}{\text{total \#}} \times \text{cycle duration (min)}$$

#### *Observing Mitosis in Colchicine-Treated and Untreated Broad Bean Root Tips*

Before this lab, *Vicia faba* seeds were germinated in vermiculite. The seedlings were transferred to either an aerated solution of 0.1% (w/v) colchicine or an aerated solution of deionized water for four hours. Your TA will stain the root tips with Schiff's reagent, staining the DNA of the broad bean tips. Your job is to prepare a "squash" of the colchicine-treated and water-treated broad bean root tips and observe the differences.

1. Place a water-treated root tip on a clean slide. Separate the intensely pink terminal end from the relatively unstained portion leaving 0.25-0.5 cm of intensely pink root tip on your slide.
2. Immediately add 1-2 drops of acetic acid and allow the root tip to remain in the acetic acid for two minutes. The acid softens the cell walls, allowing you to separate the cells more easily.
3. Carefully macerate the root tip by tapping up and down with the rounded end of a glass rod. When the tissue has been broken into small fragments, lower a cover slip onto the preparation.
4. Place a folded paper towel on top of the coverslip. Squeeze out the excess moisture by gently drawing the index finger of one hand across the paper towel, while holding onto the edge with your other hand.
5. Flatten the tissue on the slide by placing your thumb on the paper towel over the coverslip and gradually increasing pressure. Roll your thumb from side to side gently increasing the pressure with each roll. Avoid sliding the coverslip from side to side. Press down with the eraser end of your pencil to complete the squash.
6. Remove the paper towel and examine your root tip under the microscope. A well-made squash will have a monolayer of well-separated cells, with only a few larger chunks of tissue. Find dividing cells using the low power objective. If you did not succeed in preparing a slide in which cells undergoing mitosis can be seen, attempt the squash with a new root tip following steps 1-5.
7. Find a cell in metaphase, in which the chromosomes are clearly separated, either using your slide or another person's slide. Using high power, observe the cell. Make a drawing of the cell in your assignment.
8. Inspect 50-100 cells and count the number of cells in prophase, metaphase, anaphase, and telophase. Record your results and the pooled class results in your assignment.
9. Repeat steps 1-6 for a bean root treated with colchicine. Inspect 50-100 cells and count the number of cells in prophase, metaphase, anaphase, and telophase. Record your results and the pooled class results in your assignment.

## **References**

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Name \_\_\_\_\_

Lab Section Day & Time \_\_\_\_\_

### Lab 7 Assignment

#### *Modeling mitosis*

1. Answer the following questions:
  - a. What happens to the original cell undergoing mitosis, the mother cell?
  
  
  
  
  
  
  
  
  
  
  - b. How many chromatids are in the cell you modeled after DNA replication?
  
  
  
  
  
  
  
  
  
  
  - c. How many chromatids are in each daughter cell at anaphase?
  
  
  
  
  
  
  
  
  
  
  - d. Why is it important that the DNA is replicated?

#### *Onion root tip slide observations*

2. Record the results from your observation of the onion root tip slide and transcribe the pooled results from your lab section into the column labeled pooled results. Then calculate the duration of each phase of mitosis using the pooled results. Duration is calculated:

$$\text{Duration of a stage (min)} = \frac{\text{observed \#}}{\text{total \#}} \times \text{cycle duration (min)}$$

The control systems for cellular reproduction in onion root tip dictate that onion root tips divide once per day or once every 1440 minutes. Therefore the cycle duration for onion root tip is 1440 minutes.

	Number of cells/high power field		Duration (minute)
	My results	Pooled results	
Total number of nuclei			
Prophase			
Metaphase			
Anaphase			
Telophase			

3. Compare the duration of each phase of mitosis to that reported in scientific literature for onion root tip (prophase = 133 minutes, metaphase = 22.9 minutes; anaphase- 14.1 minutes, telophase =20.5 minutes). Are your results similar? What might explain differences you observed between your lab section's results and that reported in scientific literature? What reasons might exist for the varying length of each phase of mitosis?

*Colchicine and broad bean root tips*

4. Draw a broad bean root tip in metaphase with the chromosomes widely spread apart.

5. Inspect 50-100 broad bean root tip nuclei. Count the number of cells in each stage of mitosis for both a bean root aerated only with water, and a bean root aerated with colchicine. Transcribe your class' results into this table.

	Bean roots aerated in water		Bean roots aerated in colchicine	
	My results	Pooled class results	My results	Pooled class results
Total number of cells observed				
Prophase				
Metaphase				
Anaphase				
Telophase				

6. Describe the differences you observed in the bean root tips treated with colchicine. Explain how treatment with colchicine affects bean root tips.

*Thought questions*

7. To treat a patient with Kaposi's sarcoma (a cancer that develops in AIDS patients, especially men) a physician has prescribed vincristine. Vincristine is neurotoxic. In high doses, it causes peripheral nerve damage. To avoid neurotoxicity, the recommended dosage for adults is 10-30 micrograms per kilogram of body weight once a week. The patient's weight is 135 pounds. The physician has ordered 2 milligrams of vincristine to be given. Is this dosage within the recommended range? If not, what is the maximum amount of vincristine that should be administered? Show your calculations.
  
8. Examine the duration of each phase of mitosis in onion root tip. Like colchicine, vincristine prevents the formation of microtubules during prophase. Assume that human cells show a similar pattern in the duration of each phase of mitosis as onions, why might it be advantageous for a chemotherapeutic drug to affect activity in prophase rather than metaphase, anaphase or telophase?
  
9. Why do people undergoing chemotherapy often lose their hair and have problems with their digestive systems?