

Mitosis in Onion Root Tip Cells

A quick overview of cell division

The genetic information of plants, animals and other eukaryotic organisms resides in several (or many) individual DNA molecules, or **chromosomes**. For example, each human cell possesses 46 chromosomes, while each cell of an onion possesses 8 chromosomes. All cells must replicate their DNA when dividing. During **DNA replication**, the two strands of the DNA double helix separate, and for each original strand a new complementary strand is produced, yielding two identical DNA molecules. DNA replication yields an identical pair of DNA molecules (called sister **chromatids**) attached at a region called the **centromere**.

DNA replication in eukaryotes is followed by the process called **mitosis** which assures that each daughter cell receives one copy of each of the replicated chromosomes. During the process of mitosis, the chromosomes pass through several stages known as **prophase, metaphase, anaphase and telophase**. The actual division of the cytoplasm is called **cytokinesis** and occurs during telophase. During each of the preceding stages, particular events occur that contribute to the orderly distribution of the replicated chromosomes prior to cytokinesis.

The stages of mitosis

Prophase. During prophase, the chromosomes supercoil and the fibers of the spindle apparatus begin to form between **centrosomes** located at the pole of the cells. The nuclear membrane also disintegrates at this time, freeing the chromosomes into the surrounding cytoplasm.

Prometaphase. During prometaphase, some of the fibers attach to the **centromere** of each pair of sister chromatids and they begin to move toward the center of the cell.

Metaphase. At metaphase the chromosomes have come to rest along the center plane of the cell.

Anaphase. During anaphase, the **centromeres split** and the sister chromatids begin to **migrate** toward the opposite poles of the cell.

Telophase. During telophase, the chromosomes at either end of the cell cluster begin to cluster together, which facilitates the formation of a new nuclear membrane. This also is when **cytokinesis** occurs, leading to two separate cells. One way to identify that telophase has begun is by looking for the formation of the **cell plate**, the new cell wall forming between the two cells.

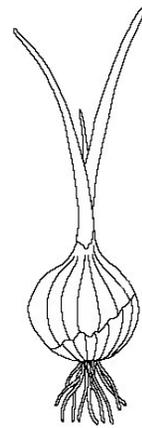
The objectives of this lab exercise are for you to:

- Better understand the process and stages of mitosis.
- Prepare your own specimens of onion root in which you can visualize all of the stages of mitosis.
- Apply an analytical technique by which the relative length of each stage of mitosis can be estimated.

I. Viewing mitosis in onion root tips.

Why use onion roots for viewing mitosis?

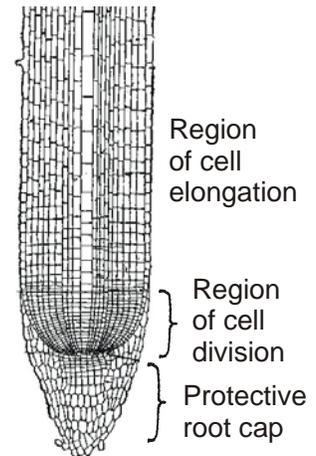
- The roots are easy to grow in large numbers.
- The cells at the tip of the roots are actively dividing, and thus many cells will be in stages of mitosis.
- The tips can be prepared in a way that allows them to be flattened on microscopes slide (“squashed”) so that the chromosomes of individual cells can be observed.
- The chromosomes can be stained to make them more easily observable.



Regions of Onion Root tips

There are three cellular regions near the tip of an onion root.

1. The **root cap** contains cells that cover and protect the underlying growth region as the root pushed through the soil.
2. The **region of cell division** (or **meristem**) is where cells are actively dividing but not increasing significantly in size.
3. In the **region of cell elongation**, cell are increasing in size, but not dividing.



Viewing Chromosomes

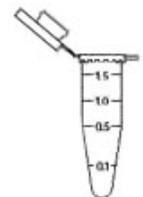
Chromosomes generally are not visible as distinct entities in nondividing cells, since the DNA is uncoiled, but the process of mitosis is facilitated by **supercoiling** of the chromosomes into a highly compacted form. Supercoiled chromosomes can be visualized in cells, particularly if they are treated with a DNA-specific stain, such as the **Feulgen stain**.

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 a fixative to stabilize the cells. You will work in groups of two 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 plastic micro-tube. (One of the roots will be an extra one.)
2. Fill the tube about 2/3 full with 1N HCl from a dropper bottle.
*** **Caution: Work with the HCl carefully, it is a strong acid.** ***
3. Place the tube in a 60°C 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.



Rinse the roots in H₂O.

1. Using forceps, carefully transfer the root tips to a small petri plate.
2. Using a plastic 'squeeze' pipet, carefully remove the HCl from the micro-tube and transfer it to the "discard flask".
3. Rinse the root tips 3 times with water from the dropper bottle, disposing of the rinses in the discard flask.

Staining the chromosomes.

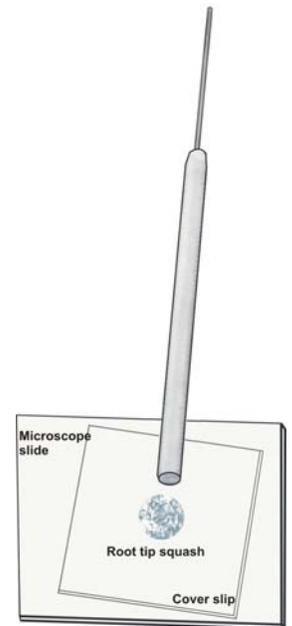
1. After removing the water from the third rinse, cover the root with the Feulgen stain.
*** **Caution: Although the Feulgen stain does not appear colored, it will strongly stain skin and clothing.** ***
2. Incubate the roots in the stain for 12 minutes. During this time the very tip of the root will begin to turn red as the DNA stains the numerous small actively dividing cells at the tip.

Remove the stain and again rinse the roots.

1. Using a plastic 'squeeze' pipet, carefully remove the Feulgen stain and discard it in the discard flask.
2. Again, rinse the root tips 3 times with water.

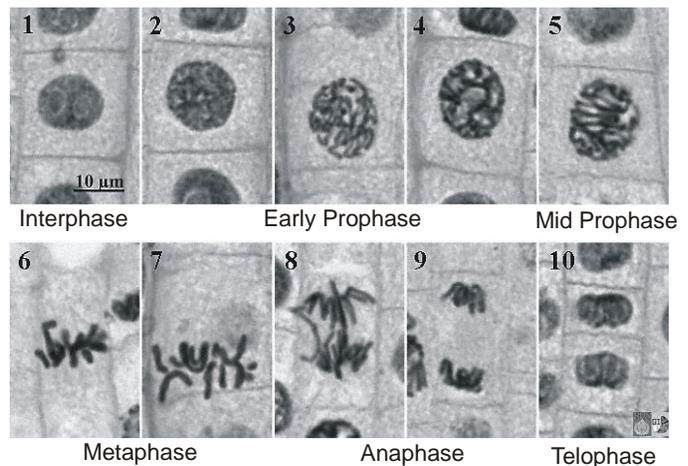
Preparing the root tip squash.

1. Transfer a root to the center of a clean microscope slide and add a drop of water.
2. Using a razor blade cut off most of the unstained part of the root, and discard it.
3. 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 do not twist or push the cover slide sideways. The root tip should spread out to a diameter about 0.5 – 1 cm.



Observations of onion root tip squash.

Scan the microscope under the 10x objective. Look for the region that has large nuclei relative to the size of the cell; among these cells will be found cells displaying stages of mitosis. Examples are shown in the figure to the right. Switch to the 40X objective to make closer observations. Since prophase and prometaphase are difficult to distinguish, classify all these cells as prophase. Record your observations in the table provided.

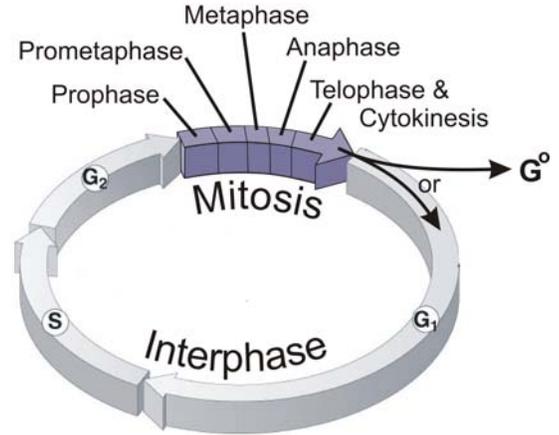


II. Estimating the relative length of each stage of mitosis.

For this procedure, we will use permanently mounted slides of onion roots. These slides are prepared by slicing the roots into thin sections, mounting them on microscope slides, staining, and then mounting under cover slip.

Mitosis and the cell cycle

While making your observations, consider the relative number of cells actually involved in mitosis. Some of these cells are still involved in the **cell cycle**, which encompasses all of the processes involved in cell replication. Cells that are actively dividing but not yet in mitosis are said to be in **interphase**, during which time the DNA is copied and the cell is otherwise preparing for replication. Some root cells have ceased dividing and are only increasing in size, whereas others have reached their final, mature size and function, and are said to be in the **G₀** stage.



Procedure for determining the length of the stages of mitosis

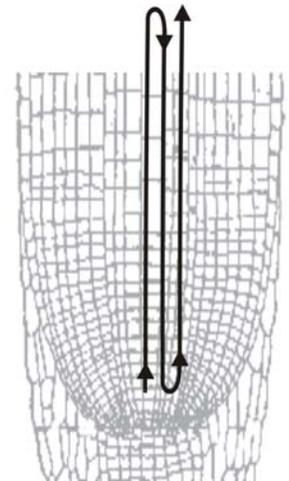
Locate the meristem region of the root tip.

1. Starting under the 10x objective, find the region of active cell division.
2. Switch to the 40x objective and begin observations at the lower end of this region.

Recording data

Students should take turns as **observer** and **recorder**. The observer should call out the stage of mitosis of each cell to be tallied by the recorder in the results table. Roles should be switched for the second slide. Since prophase and prometaphase are difficult to distinguish, classify these cells as prophase. **Only count as prophase cells that contain distinctly visible chromosomes.**

1. Systematically scan the root tip moving upward and downward through a column of cells.
2. Tally each cell in a stage of mitosis that you observe, being careful not to record the same cell twice. Tally the stages of 20 cells mitotic cells.
3. Tally numbers in the table below. Each group member should tally cells from a different slide.



Calculations

1. Pool your data with that of the class, and then record the class totals in the table provided below.
2. Calculate the percentage of cells in each stage.
3. The relative time span of each stage is equivalent to the percentage of cells found in that stage.

Name: _____

III. Results

Onion root tip squash

1. Find and draw a cell showing each stage of mitosis.

Prophase	Metaphase	Anaphase	Telophase

2. What is a distinguishing **visible** feature of each stage of mitosis?

Prophase:

Metaphase:

Anaphase:

Telophase:

Relative length of stages of mitosis

3. Tally the results of your cell counts and then calculate percentages.

	Your tally	Class totals	Percent of total (class)
Prophase			
Metaphase			
Anaphase			
Telophase			
Totals			

4. Based upon the class results, order the stages of mitosis from shortest (1) to longest (4). After the longest and shortest stage, give a brief explanation of why that stage may have that time period.

Prophase ____

Metaphase ____

Anaphase ____

Telophase ____

5. Many of the cells of the root meristem are not undergoing mitosis, rather they are in a stage called _____. Based upon the interpretations made above, interphase appears to be much _____ (shorter / longer) than mitosis. What processes occur in interphase cell prior to the onset of mitosis?

6. Once cell division ends, the cells will exit the cell cycle and enter the _____ stage. Why is it incorrect to say that these cells are “resting”?