

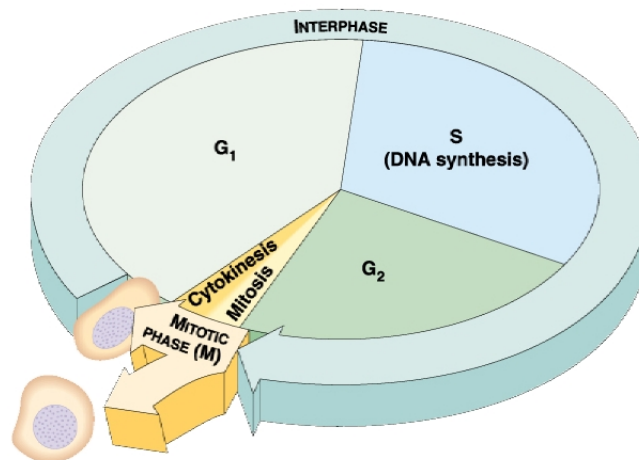
Mitosis and the Cell Cycle

The cell theory states that "all cells come from preexisting cells" by the process of cell reproduction, or cell division. Cell division is the process by which all the cells of a multicellular organism are formed during growth and development, and how many single celled organisms reproduce themselves. In multicellular organisms, cell division is responsible for repair and replacement of cells and tissues during one's lifetime. Asexual reproduction, a means of making more individuals, is also accomplished by cell division.

We know that all cells of an individual have exactly the same DNA in their chromosomes, and that each species has a fixed chromosome number, a number that does not change from generation to generation. To ensure that chromosomes and DNA remain the same in new cells when cells divide, it is crucial to have a mechanism that exactly **replicates (duplicates)** the DNA of the original cell and **distributes** the copied DNA equally to the new cells. **Mitosis** is the process by which the replicated chromosomes are equally distributed to new nuclei. To form new cells, we must also separate the **cytoplasm** and critical organelles, such as mitochondria and chloroplasts, of the original cell into the new cells formed. The distribution of the cytoplasm of the original cell into new cells is called **cytokinesis**.

Mitosis and cytokinesis are a part of the cell cycle that starts when a cell is formed and continues until it divides. Some cells never divide, others are specialized to divide (especially in plants, where virtually all cell division occurs in specialized tissue called **meristem**). Cell division is a brief part of the life cycle; most of the life of a cell is spent in normal activities of growth and maintenance.

The cell cycle involves the following:



Interphase

The period of time when a cell undergoes its normal activities, including

- Growth** (called G_1 or First Gap in the cell cycle)
- DNA Replication** (called S for synthesis in the cell cycle)
- Preparation for Division** (called G_2)

Cell Division (or cell reproduction), which includes

- Mitosis**, with
 - Prophase**
 - Prometaphase**
 - Metaphase**
 - Anaphase**
 - Telophase**
- Cytokinesis**

Cell reproduction in eukaryotes involves three events:

1. DNA Replication
Process of replicating the genetic material of the nucleus
This occurs during **Interphase**, when growth and/or normal metabolic activities take place.
2. Mitosis
Process of distributing the replicated DNA equally to the two new nuclei.
3. Cytokinesis
Process of separating the cytoplasm contents

In this laboratory you will have the opportunity to look for mitosis in fresh preparations of onion or *Narcissus* root tips as well as observing prepared slides of mitosis in the onion (*Allium cepa*) root tip meristem. You may also simulate the process of mitosis using pop-bead models to be sure that you understand how chromosomes "behave" during mitosis.

You will be using the oil immersion lens for your observations. Review the oil immersion procedure from the microscope lab prior to using the oil immersion lens. Use lens paper to clean your slides and the microscope surfaces (lenses, stage, etc.) after you have used the oil immersion lens. You may need to use the solvent, xylene, to remove all traces of the oil.

Exercise I Mitosis in Living Tissue

It is sometimes possible to observe mitosis in living tissues. (In fact the technique kills the cells so one should probably say "fresh-dead" tissues.) You will observe mitosis in the tissues of the onion or *Narcissus* root by doing what is known as a "squash" preparation.

Materials Needed

Onion or <i>Narcissus</i> bulb with living roots	Dissecting needles
Sharp razor blade	Paper toweling
Microscope slide	Dropper bottles of:
Coverslip	Acetocarmine solution
Alcohol lamp	5% Iron alum
Matches	Prepared slide of <i>Allium</i> root tip squash preparation
Lens paper	

Procedure

1. Remove an entire root from the onion or *Narcissus* bulb. On a piece of paper toweling at your lab table, cut the tip 5 to 8 mm from the tip of the root. Discard the rest of the root.
2. Place the cut tip on a clean microscope slide in a drop of acetocarmine. Note: Acetocarmine stains!
3. Use a pair of dissecting needles to tease apart the meristematic tip as much as possible. When you have the root in tiny bits add a drop of 5% iron alum, and place a coverslip on top of your preparation.
4. Warm the slide gently over the alcohol lamp for about one minute. Do not allow the slide to get hot to the touch; you don't want to cook either your fingers or the root. Do not let the root dry out. This takes a bit of practice. You may need to repeat steps 1 - 3 if your first attempt results in an "overdone" preparation.
5. Cover the slide with 2 - 3 layers of lens paper. Squash the slide with your thumb using a firm and even pressure. Avoid squashing with such force that the coverslip breaks or slides. This step also requires some skill, luck and/or practice. Don't hesitate to make several slides if your early attempts meet with less success than anticipated.
6. Observe your slide looking for the phases of mitosis described in Exercise II. Remember that a cell spends the majority of its cell cycle in interphase. The cells of the onion root tip undergo mitosis about once in 24 hours.

Exercise II Mitosis in *Allium* (onion) Root Tip Meristem

Cell division in plants occurs in regions called meristems. Primary meristems are found in the growing tips of shoots and roots. Many plant cells have the ability to become meristems. For example, when you do a stem cutting, certain cells of the stem dedifferentiate (become unspecialized) to become root meristem for the formation of new roots.

Materials Needed

Prepared slides of *Allium* root tip mitosis, Is (Do not look at slides labeled "squash".)
Lens paper
Immersion oil
Xylene

Although mitosis is separated into phases for the convenience of discussion, you should remember that the process is a continuous one and the separations are arbitrary. You may see many cells in some point intermediate between the phases that you expect to see.

Procedure

1. Obtain a slide of *Allium* root tip. The root tip has been cut longitudinally. Each slide should have about 3 root tip slices.
2. Locate the meristem region with the scanning objective. Continue to scan the meristem region, consisting of about 200 cells with the 10x objective. The mitotic figures will be too small to distinguish detail at this magnification, although each slide should have several cells in each of the mitotic phases. Which phase of the cell cycle is most abundant in the root tip? Why is this so?
3. When you find a promising cell for the mitotic phase you are planning to observe, center it in the field of view and rotate your 45x objective into position to observe the detail of the mitotic figures. You may want to use the oil immersion lens for this exercise to see superb detail.
4. Locate cells that are good representatives of each of the phases of mitosis described below. Compare the phases of mitosis that you find in the microscope with those illustrated below.

The Phases of Mitosis and the Cell Cycle

Interphase

Most of the cells of the meristem will be in interphase. The granular chromatin material in the nucleus is distinctive although no individual chromosomes are visible. You may also see nucleoli. DNA replication occurs during interphase. What other cellular events are associated with interphase?

Prophase and Prometaphase

Replicated chromosomes condense from the diffuse chromatin and become visible as threadlike structures. Each replicated chromosome is composed of its two identical replicas (called chromatids) held together at their centromere. Replicated chromosomes continue to condense and become thicker as prophase progresses. The **nucleolus** region (an aggregation of chromosome bits and concentrated RNA and protein) of the nucleus will start to disappear. The replicated chromosomes are firmly attached at their centromeres throughout this condensation and coiling.

Microtubules and associated proteins initiate **spindle** formation during prophase. The spindle apparatus originates from a **microtubule organizing center**, also called the **centrosome**. The centrosome is self-replicating and replicates during interphase. In animal cells, **centrioles** are found within the centrosome, but not in cells of higher plants, including the onion. Microtubules radiating from the centrosomes are called **asters**.

By the end of prophase (often separated into a phase called **prometaphase**), the spindle apparatus will extend from the poles of the cell through the center of the cell to the opposite pole of the cell. Some microtubules from each pole of the cell attach to a protein structure, called the **kinetochore**, located in the centromere region of each replicated chromosome.

The nuclear membrane degrades in **prometaphase** (or late prophase) into small vesicles that will be used to synthesize new nuclear membrane material in the new cells.

Metaphase

The spindle apparatus has moved the chromosomes to the equator of the cell, aligning the centromeres of each replicated chromosome along the equator. Centromeres of each sister chromatid are aligned with each other and each sister chromatid is connected at its kinetochore to a microtubule. This alignment of chromosomes along the equatorial plane of the cell is often called the **metaphase plate**, and is the distinctive feature of metaphase.

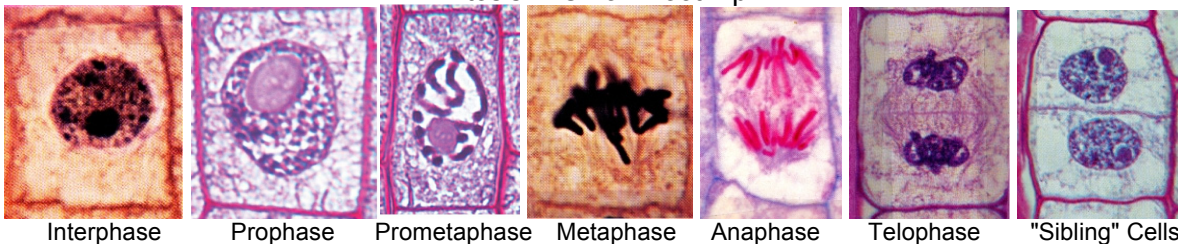
Anaphase

Centromeres of each replicated chromosome separate to start anaphase. (This also signals that metaphase has been completed.) By definition, each sister chromatid is now a single unreplicated chromosome. Microtubules from each pole pull the chromosomes away from each other and toward the respective poles of the cell. Each of the two clusters of chromosomes being pulled to the two poles of the cell has one copy of each original chromosome.

Telophase

Membrane vesicles and membrane fragments form new nuclear membranes around each group of separated chromosomes at the poles of the cell. Chromosomes stretch back out and become indistinct as chromatin. The spindle microtubules disperse and the spindle apparatus disappears. New nucleoli form. At the completion of telophase, there will be two new nuclei, each identical to the original nucleus.

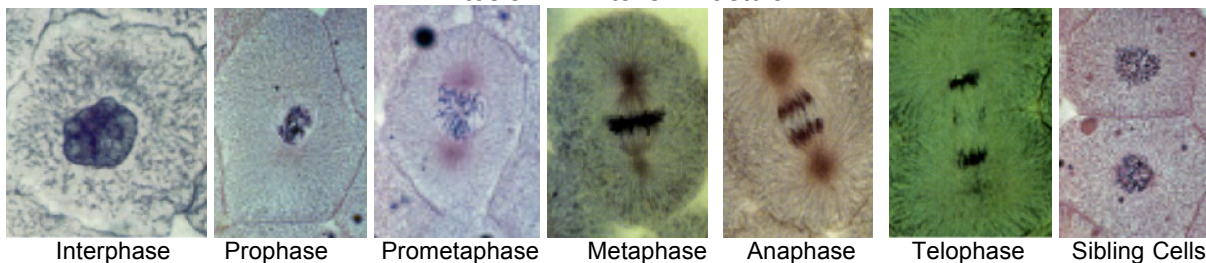
Mitosis in Onion Root Tip



Comparing Mitosis in Plant and Animal

Compare and contrast the illustrations of mitosis in the Whitefish blastula with the onion meristem previously observed. How are they different? In what ways are they similar?

Mitosis in Whitefish Blastula



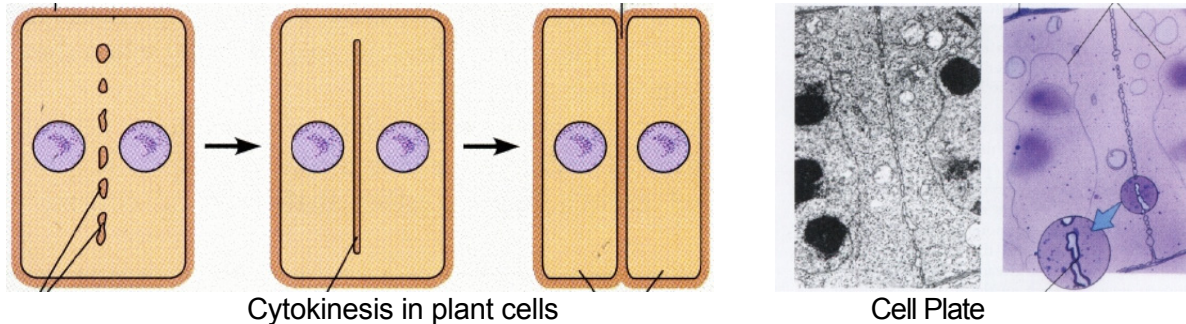
Cytokinesis: Separation of the Cytoplasmic Contents

Mitosis describes events of chromosomes and nuclei. Most cells accompany mitosis with cytokinesis, the separation of the cytoplasm of the original cell into two new cells. Cytokinesis coincides with the events of telophase or occurs immediately after, so that at the completion of mitosis, the original cell is separated into two cells, each with a nucleus and DNA identical to that of the original cell.

Cytokinesis in Plant Cells

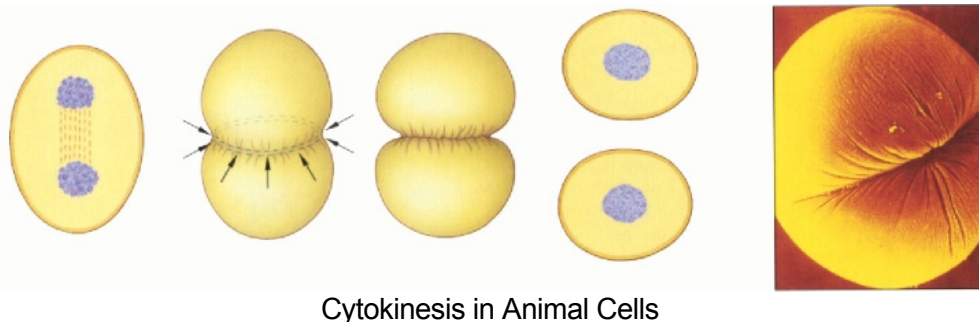
Each cell of a plant is surrounded by a rigid cell wall. Cytokinesis in plant cells requires synthesis of new wall material. This process is called **cell plate formation**. Cell plate formation involves making a cross wall at the equator of the original cell. Golgi vesicles containing wall material fuse along microtubules forming a disk-like structure that is called the **phragmoplast** or **cell plate**. As cellulose and other fibers are deposited, the cell plate is formed creating a boundary and new cell wall between the two new cells. Membrane material from the original cell fuses to each side of the cell plate forming new cell membranes on the dividing sides of the original cell into the two new cells.

Look for the formation of the "cell plate" in the telophase cells of your onion root tip that signals the start of the new cell wall separating the daughter cells.



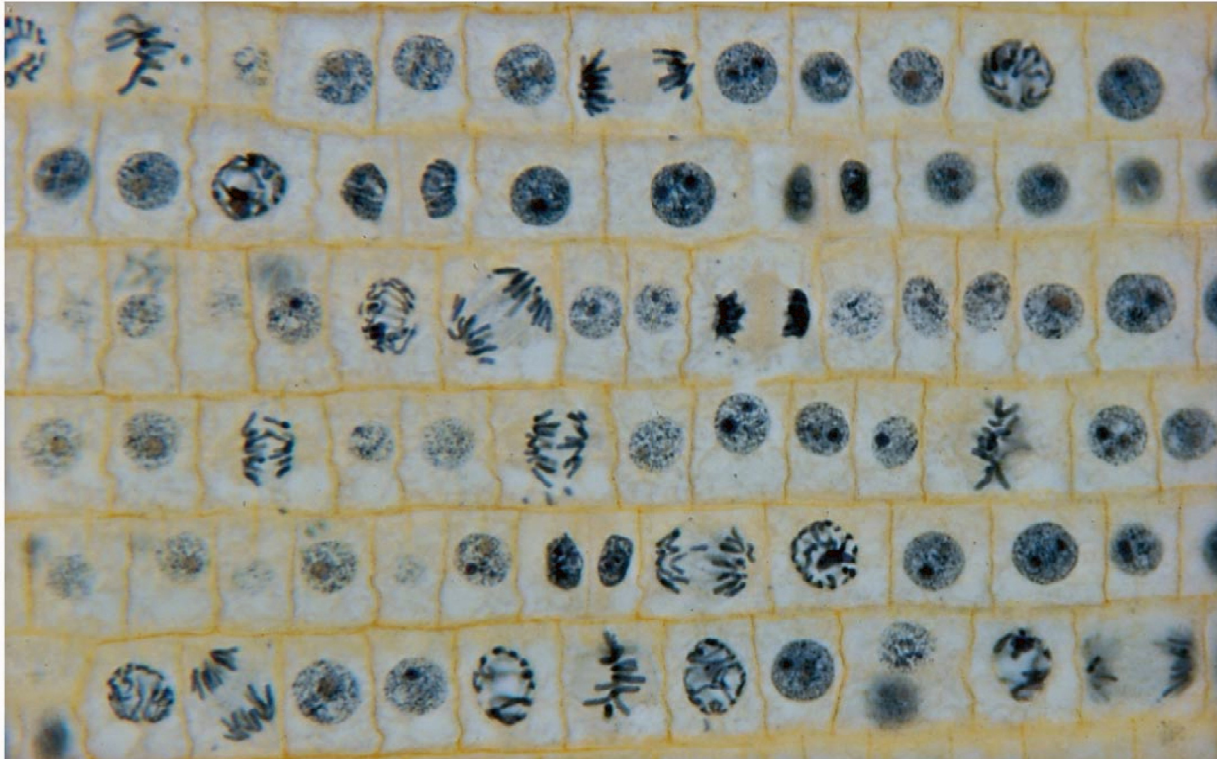
Cytokinesis in Animal Cells

Cytokinesis in animal cells starts with the formation of a **cleavage furrow**, a depression or pinching in of the plasma membrane, caused by a ring of microfilaments (the **contractile ring**), which forms across the middle of the cell after the chromatids are separated in anaphase. This ring contracts, pinching the membrane toward the center of the cell. This eventually pinches the cell in two.



Exercise III Relative Time Spent in Cell Cycle Phases

You can estimate the relative length of time each phase of the cell cycle takes by recording the frequency with which you find each phase in meristem regions where cell division is occurring. There are more than 60 cells in the micrograph of onion mitosis shown below. Record how many cells of each cell cycle phase you see in Table 1. Now go back and observe your slide of root tip mitosis. Using high power, count how many cells you find of each phase of mitosis in your field of view. Repeat this for two or three different fields of view.



Mitosis in Allium root tip meristem

Cell cycle Phase	Micrograph	View 1	View 2	View 3	Total	Percent of Total
Interphase						
Prophase						
Metaphase						
Anaphase						
Telophase						
Grand Total:						

Clean Up

If you have used the oil immersion lens for your observations be sure that the oil is removed from all lens surfaces, microscope surfaces and all microscope slides.

Exercise IV Mitosis Simulation – Optional

Although this exercise is optional, it is recommended to test your understanding of how mitosis works by simulating the process using pop-bead "chromosomes". A clear understanding of the behavior of chromosomes during mitosis is essential to understanding the process of **meiosis**, a reduction division, which you will be observing later. For your mitosis model you will create a diploid cell with a chromosome number of 4.

Materials Needed

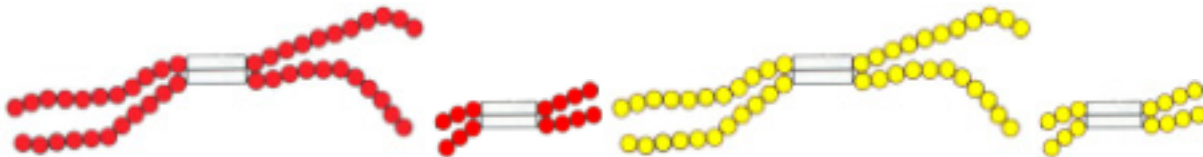
- Red pop-beads
- Yellow pop-beads
- 8 Magnetic centromeres

Procedure

1. Using your pop-beads, construct two long chromosomes (one red and one yellow) and two short chromosomes (one of each color). Be sure that the two long chromosomes have the same number of pop-beads and that the two short ones have the same number of pop-beads. Each chromosome should have a magnetic centromere. The centromeres of the long and short chromosomes of each color should be located in corresponding places.



2. Replicate each of your chromosomes by making an identical set of pop-bead chromosomes. Attach the **identical** replicas by their magnetic centromeres. This replication occurs in which phase of the cell cycle?



3. Imagine that a "square" of your lab table is a cell. Place your chromosomes in the nucleus. With your replicated pop-bead chromosomes you are now ready to go through the entire process of mitosis. Review the process of mitosis as outlined in Exercise II above as you take your simulated chromosomes through each phase.