LABORATORY INQUIRY
Mechanisms of Mitosis

The first person to ever observe and characterize cells was Robert Hooke in 1665, who observed the tiny holes that made up a slice of cork he was observing under one of the earliest microscopes. Those cell remnants were dead and hollow, so he had no idea what the internal structure of a living cell was like. It was not until 1674 that Anton van Leeuwenhoek first identified living cells (unicellular protists) under a microscope. Because they moved under their own power, he called them animalcules ("little animals") not realizing that they were no more than single cells.

Further observations and work by Theodor Schwann, Matthias Jakob Schleiden, and Rudolf Virchow led to the development of cell theory, which has evolved over the decades as new discoveries about cells have been made.

I. Cell Theory
The original cell theory stated that

- All living organisms are composed of one or more cells.
- The cell is the basic structural and functional unit of life.
- Cells arise from pre-existing cells by a process of cell division.

Modern discoveries have added that

- Energy flow occurs within cells via metabolism and biochemical reactions.
- Genetic/heritable information is passed from cell to cell upon division.
- All cells have the same basic chemical composition.

Recall that there are two basic cell types, prokaryotic and eukaryotic. Bacteria and archaeans are prokaryotic (from the Greek pro, meaning “before” and karyon, meaning “nut”, which refers to the dark-staining nucleus prokaryotes don’t have). All other organisms, including protists, animals, fungi, and plants, are eukaryotes.

Be sure to refer to your lecture notes and text readings to review the basic components and anatomy of prokaryotic and eukaryotic cells. Note that prokaryotic cells, though they do divide in two to reproduce, do not undergo a cell cycle like that described here, which is unique to eukaryotes.

II. The Cell Cycle
One cannot truly understand biology without understanding the basic structure and function of a living cell. One of the most basic functions of cells is reproduction, whether asexual (mitosis) or sexual (meiosis). Scientists have characterized the life cycle of a cell, and we now know that the cell passes through defined stages as it lives. If you observe a population of living cells, whether part of an organism’s body or in a culture dish, you are likely to see cells in various phases of the cell cycle (Figure 1). The stages of active mitosis have been named for easy discussion and referral to the events occurring in each phase.

A. Interphase
Interphase is the cell cycle stage in which cells spend the majority of their time. An interphase cell is busy undergoing the chemical reactions that facilitate energy transfer,
growing, and preparing to divide. Interphase is not part of mitosis, which is defined as active cell division. Rather, it is the living stage of the cell. The chromosomes cannot be clearly seen, as they are in the diffuse form known as chromatin. The genes on the chromosomes are actively being transcribed and translated, though which genes are active depends on the identity and function of the individual cell. The stages known as G1, S, and G2 occur during interphase, and are involved in normal cell growth (G1), DNA synthesis (S), and protein and microtubule synthesis preceding mitosis (G2).

**B. Prophase**
Prophase is the first stage of mitosis. The chromosomes condense and become visible. Although they have already duplicated, the chromosomes are not yet visible as separate sister chromatids. The nuclear membrane breaks down, and the spindle fibers begin to form at opposite poles of the cell.

**C. Metaphase**
In this second phase of mitosis, the duplicated chromosomes unwind from each other, becoming visible as two identical sister chromatids attached only at a slightly constricted region, the centromere. It is only now that the chromosomes take on the “X” shape so often seen in illustrations. But this “X” actually represents not one, but two chromosomes: the identical sister chromatids produced during DNA replication.

Spindle fibers attach to the kinetochore, a protein structure located at the centromere, and the chromosomes are arranged along the equator of the cell.

**D. Anaphase**
Spindle fibers shorten, the kinetochores separate, and the chromatids (daughter chromosomes) are pulled apart and begin moving to opposite poles.

**E. Telophase**
The daughter chromosomes arrive at the poles and the spindle fibers disappear.

**III. Designing a Project About Cells/Mitosis**
For this lab project, your team should choose some aspect of mitosis, and attempt to determine the function of a particular cellular structure or molecule in the mitotic process. The following section outlines a sample project in which the role of the spindle fibers is investigated. Your team should use this type of approach to analyze another structure or phenomenon that occurs during asexual cell division.

The description of the function of microtubules at the start of this next section may give you some ideas for a project of your own. But note that this is the type of
information you should include in your team’s presentation when your project is complete.

A. Sample Project: The Role of Spindle Fibers in Mitosis

Introduction: Structure and Function of Spindle Fibers

Spindle fibers play an important role throughout the mitotic process. For example, late prophase is characterized by movement of the mitotic spindle to opposite ends of the cell. In metaphase, sister chromatids become visibly associated with spindle fibers attached at each pole of the cell. Spindle fibers pull sister chromatids apart during anaphase, and then disassemble at telophase.

The spindle fibers contain a highly organized array of microtubules which are long, hollow, unbranched tubes composed of globular proteins. Microtubules are also components of other cellular structures, including the cytoskeleton, and they form the core of both cilia and flagella.

Microtubules are not only involved in separating chromosomes during mitosis and meiosis, but also function as structural supports and organizers for cells. In plant cells, for example, microtubules maintain cell shape through their influence on the formation of the cell wall during interphase. Studies also showed that microtubules play a role in maintaining the internal organization of cells and are responsible for intracellular motility of macromolecules and organelles. Treatment of cells with microtubule-disrupting drugs, such as nocodazole or colchicine, can seriously affect the location of membranous organelles, including the endoplasmic reticulum (ER) and Golgi complex. It is also known that the transport of materials from one membrane compartment to another depends on the presence of microtubules because specific disruption of these cytoskeletal elements brings the movements to a halt.

Even though microtubules of the mitotic spindle or the cytoskeleton are stiff enough to resist forces that might compress or bend the fiber, they are extremely sensitive to disassembly. Living cells can be subjected to a variety of treatments that lead to the disassembly of liable cytoskeletal microtubules without disrupting other cellular structures. Disassembly can be induced by cold temperature, hydrostatic pressure, elevated calcium ions (Ca\(^{2+}\)) concentration, and a variety of chemicals including colchicine, vinblastine, vincristine, nocodazole, and podophyllotoxin.

Methods: Disrupting of Microtubule Function

In order to demonstrate that microtubules are, indeed, essential for a particular process, one can disrupt their function and observe the results. A useful model organism is the common onion \((\text{Allium cepa})\), the roots of which grow rapidly, and so are a good place to find many dividing cells.

Two groups of onion root tips will be used, and a chromosome squash will be performed in order to visualize the state of the cell, in terms of mitosis. The chromosome squash allows the investigator to observe cells and various stages of mitosis under a light microscope.

Colchicine is an alkaloid compound derived from the corm and other parts of the Autumn Crocus, \(\text{Colchicum autumnale}\). Colchicine induces the disassembly of microtubule fibers and thus stops the mitotic process. It is also known as a mitotic poison. If used properly, it can be employed to stop mitosis “in its tracks” so that
chromosome morphology can be studied, chromosome counts can be made, or induction of polyploidy can be performed.

One group of onion root cells will be grown in a beaker with plain water under normal conditions (12-hour light/dark cycle, 75°F, etc.). The second group of root cells will be grown for a short period of time in a dilute colchicine solution.

Hypothesis and Predictions

The working hypothesis for this investigation is:

**The spindle apparatus, composed of microtubules, is essential to the mitotic process.**

When designing experimental procedures to test this hypothesis, we must remember that microtubules not only play an important part in separating chromosomes, but also are involved in other cellular activities. Disassembly of microtubules by colchicine does not only terminate cell division, but also affects the structure, intracellular organization, and macromolecule transport of the cells. We must determine what features in a dividing cell population might tell us that colchicine treatment has successfully inhibited spindle formation selectively, and what features might have simply stopped mitosis because it killed the cell.

For example, if we see many empty cells, or cells with a densely stained nuclear zone but no evidence of mitosis in treated onion root tips, it would probably mean that the colchicine has simply killed the cells.

However, an observation that most cells are in prophase and metaphase, but very few are in anaphase or telophase in treated onion root tips would more clearly indicate that colchicine has arrested cell division of most cells by inhibiting the formation of a spindle apparatus. If the guidance and mechanical force of the apparatus are not impaired (as in the control onion root tips), we should see cells in all stages of mitosis represented equally (statistically speaking).

Considering these factors, we can make the following predictions to test the hypothesis.

I. **If the formation of the spindle apparatus in living cells is inhibited, these cells will stop dividing.**

II. **If the inhibitor of the spindle apparatus formation is eliminated, spindle apparatus will be rebuilt and cell division will resume for these cells.**

To test these predictions, we will apply the following strategies:

1. First, we will test a series of concentrations of colchicine solution for a fixed duration of exposure to identify the highest tolerable concentration of colchicine solution treatment for onion root tip tissue to be alive, and the lowest concentration for the treatment to be effective over the selected time period. From this experiment, we can select the optimum colchicine concentration treatment that produces cells only at prophase and metaphase.

2. Second, we will test a series of different time durations of treatment with the optimum concentration of colchicine to determine the minimum time period required to allow the treatment to be effective.
3. Finally, we will transfer onion bulbs treated with the optimum concentration of colchicine solution to a water bath with circulatory water supply for an extended length of time to wash away the colchicine. If the onion bulbs are free of colchicine, cells should resume normal division and new roots will sprout.

**Materials**
Microscope, microscope slide, cover slips, prepared slides of onion root tip mitosis

- Acetocarmine stain in dropping bottle
- 1 M hydrochloric acid (HCL) in dropping
- Watch glasses
- Dissecting needles
- Scalpel or razor blades
- Forceps
- Alcohol lamp or slide warmer

Supply of onion bulbs
Beakers in which to let onions sprout
Colchicine solution
Absolute ethyl alcohol
70% ethyl alcohol
Glacial acetic acid
Chloroform

**Procedure**
Onion bulbs will sprout roots if they are placed in water for several days (Figure 2).

The bulbs should be placed in water about four days before the cell observations are to be done. Note that individual batches of onion bulbs may respond quite differently to conditions suitable for root growth. Many onions obtained commercially have been treated to prevent sprouting, and will produce few roots. For this reason, it is important to do a “test sprouting” before your experiment, to be sure the variety of onion you have chosen is viable. Green onions (scallions) are not treated to prevent sprouting, and can be used instead of bulb onions (Figure 3).
Results and Analysis
If you were to perform this colchicine experiment, you would prepare a report and presentation in which you would consider the following questions. Use these ideas and apply them to your team’s research project.

1. Can you locate the various stages of mitosis – prophase, metaphase, anaphase, and telophase?
2. In what stage are most of the cells?
3. How many cells from untreated onion root tip can you see in the field of view?
4. How many cells from treated onion root tip can you see in the field of view?
5. Do you observe more cells in mitotic stages than you observed in the untreated root tip? Identify all stages in both samples, and be sure to report your numbers as a proportion of the total observable cells.
6. Do you observe any polyploid cells (i.e., those with multiple chromosome sets)?
7. What mitotic stages are most prevalent? Does this differ between treatment and control?
8. Are any mitotic stages completely lacking? Again, explain this, and any difference between treatment and control.
9. Do your data indicate rejection or failure to reject each of your competing hypotheses?
10. Since colchicine stops spindle formation and mitosis, might there be any potential application value of this chemical to future areas research? Explain.

B. How to Do a Chromosome Squash
To examine the mitotic process in the cells of the onion root tip, you must soften the root so the cells can be separated and flattened, thus making it possible to see the chromosomes, nuclei, spindles, and other cell parts. Use the following procedure.

NOTE: If you wish to preserve samples for later analysis, you will need to preserve them in Caroy’s solution: 6 parts absolute ethyl alcohol: 3 parts chloroform: 1 part glacial acetic acid. Place the root tip in Caroy’s solution for 24 hours and then store in 70% ethyl alcohol until the time of use.

1. Clip the terminal 1 cm of the root tip from a growing onion bulb and use it immediately.
2. Place a few ml of 1 M HCL in a watch glass or Syracuse dish, enough to cover your root tip. (Caution! HCl is caustic and corrosive! Handle with care, and flush well with clean water if you get any on your skin or clothing.)
3. Into this acid place the terminal 3 or 4 mm of the 1-cm-long onion root.
4. In a short time (a few minutes) the root tip will feel soft when touched with a dissecting needle.
5. Now, using forceps or a needle, pick up the softened root tip and transfer it to a drop of acetocarmine stain on a clean slide.
6. Using a razor blade or a sharp scalpel, chop the root tip into tiny pieces. Note: Iron in the scalpel or dissecting needle reacts with the acetocarmine stain (Caution!
Acetocarmine is caustic and corrosive! Handle with care, and flush well with clean water if you get any on your skin or clothing. Acetocarmine may stain skin and permanently stain clothing., to give a better staining reaction.

6. Once this procedure is complete, apply a clean cover glass to the slide and heat it gently over an alcohol lamp or slide warmer. **Do not boil!** Then inverted the slide on a paper towel and push downward firmly, applying pressure with your thumb over the cover glass. This should flatten the cells and disperse them so they can be observed under the microscope.

7. Examine under lower power (100x) and then under high power (430x).

8. Use the same procedure to examine your treatment and control roots.

9. Count the number of cells you can identify in each stage of mitosis. Use these data for statistical analysis, to determine whether there is a significant difference between your treatment and control samples.

10. For comparison, you may wish to examine a commercially prepared slide of an onion root tip (available from your instructor). Observe the slide using low and high power.

**C. Your Team Research Project**

Of course, the colchicine project described above is only a sample of something your team might do. The idea is for you to explore the mechanisms of mitosis and cellular function, ask a question about how something in this area works, and then create cunning hypotheses that will allow you to clearly reject (or fail to reject) one of two competing hypotheses. It’s all in how you word your hypotheses. Be careful and clever, and remember the advice of John R. Platt in his seminal article, *Strong Inference*.

Your team should be ready to provide your instructor with a list of supplies by the end of your planning session, and then spend the next two weeks executing the experiment meticulously and with appropriate replications, treatments, controls and replications. By now you have enough experience to be able to do a thorough literature search to give you some ideas on an interesting, relevant project.

Go forth, read, and research!