

Observing Mitosis in Plant Cells

Introduction

One of the basic tenets of cell theory is that "all cells only arise from pre-existing cells." In fact, new cells are formed by the process of cell division, which gives two genetically identical daughter cells.

Concepts

- Cell cycle
- Cytokinesis
- Meristem
- Mitosis

Background

In higher plants, cell division occurs in areas called meristems. *Meristems* are usually found at the tips of stems or roots and are responsible for plant lengthening and enlarging as well as leaf, flower, stem, and fruit production. Instead of repairing or replacing damaged cells, plant cells create new organs, such as a leaf, at the meristem locations. The root tip of a plant contains an *apical meristem* that facilitates the growth in length of the root. The apical meristem is just a small area at the end of the root tip where mitosis occurs (see Figure 1). A root tip can be broken down into zones. Each zone can overlap into the adjacent zones; there are no distinct lines of separation. The first zone is the *root cap*. The root cap protects the apical meristem when the root pushes through the soil as the root grows. The apical meristem is also called the *zone of cell division*. All phases of the cell cycle occur in this zone. The *zone of elongation* is the region in which each cell elongates. Roots grow when the cells in the zone of elongation push the root tip farther into the soil. In the *zone of maturation*, the cells complete their differentiation and develop into dermal, ground, or vascular tissue cells.

In the zone of cell division, the cells complete a full cell cycle in about 24 hours. The *cell cycle* is composed of five stages— G_1 , S, G_2 , M and C (see Figure 2). The M stage, or mitosis, only comprises the stages in which the nucleus divides. Mitosis represents only a brief segment, typically two to four hours, in the overall life cycle of the cell. Most of the life of a cell is spent performing normal metabolic activities, growing, and preparing the cell for its next division. These stages are collectively termed interphase and include the G_1 , S, and G_2 stages. During interphase the cell is metabolically active. Throughout most of interphase, the cell is producing (synthesizing) RNA, proteins, and cytoplasm as it respire and metabolizes. Cells in the root cap and the zone of elongation enter interphase and rarely divide again. Cells in the zone of cell division prepare for mitosis during interphase by replicating DNA and organelles. During the end of mitosis the cell begins to divide in two. In the C stage, or cytokinesis, the parent cell's cytoplasm and organelles are divided to produce two offspring cells.

Mitosis is further subdivided into five phases as shown in Figure 3 on page 2. During *prophase*, the nucleolus fades and chromatin condenses. Microtubules of the cytoskeleton begin to create the *spindle fibers* necessary for chromosome separation. In *prometaphase*, the nuclear envelope breaks down so there is no longer a recognizable nucleus. During *metaphase*, the chromosomes reach a position called the *metaphase plate*, which is midway between the poles. At the onset of *anaphase*, the sister chromatids separate, splitting each chromosome in half. The spindle fibers shorten and drag the attached chromatids to opposite poles of the cell. In *telophase*, the daughter chromosomes arrive at the poles and the spindle fibers that have pulled them apart disappear. A nuclear envelope reforms around

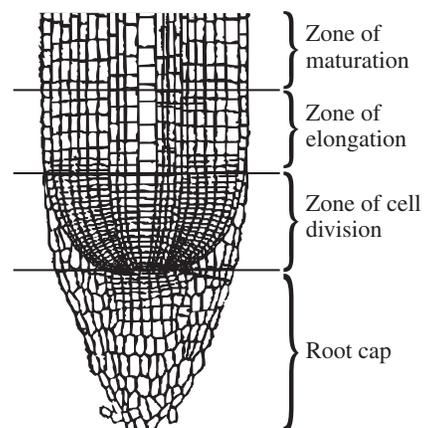


Figure 1. Apical meristem

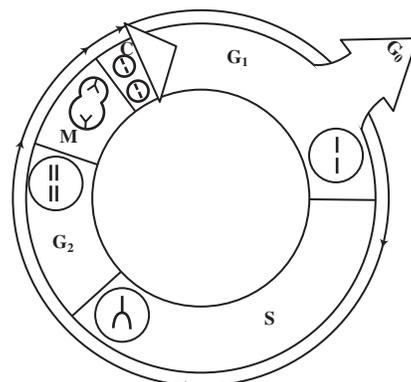


Figure 1. Cell Cycle

each cluster of chromosomes and these chromosomes return to their more extended form while cytokinesis begins.

During plant cell cytokinesis, a cell plate is synthesized between the two daughter cells. To do this, plant cells send vesicles derived from the Golgi apparatus to the middle of the cell. The vesicles fuse to form the cell plate which expands until it forms a complete cell wall.

A prepared longitudinal cross section of an onion root tip provides a snapshot of the cell cycle. The portion of cells in each phase of the cell cycle should correspond closely with the amount of time spent in each phase. In onions, the time to complete one cell cycle is typically 24 hours or 1440 minutes. By counting the number of cells in each phase of the cell cycle, the relative duration of each phase can be calculated.

Materials

Allium, microscope slide, l.s.

Microscope, compound

Safety Precautions

Although this lab is considered nonhazardous, please follow all normal laboratory safety guidelines.

Procedure

- Using the 4X (low power) objective on the microscope, focus on the root tip.
- Switch to the 10X (medium power) objective and focus on the root tip. Observe the root cap, zone of cell division, and zone of elongation.
- Center the zone of cell division in the field of view and switch to the 40X (high power) objective. Use the fine focus to focus. Observe the cells in the zone of cell division. Locate cells in the following phases of the cell cycle: interphase, prophase, prometaphase, metaphase, anaphase, and telophase. *Note:* All phases of mitosis may not be present within a single field of view.
- While observing a single field of view in the zone of cell division, count the number of cells in each phase of the cell cycle. Record the number for each phase in a table. Count *all* of the cells in the field of view.
- Count at least two full fields of view or a minimum of 200 cells, whichever is greater.
- Total the number of cells counted in each phase and record the total.
- Calculate the percent of cells in each phase of the cell cycle using Equation 1.

$$\text{Percent of cells in phase} = \frac{\text{Total number of cells in phase}}{\text{Total number of cells counted}} \times 100 \quad \text{Equation 1}$$

- It takes an average of 24 hours (or 1,440 minutes) for root tip cells to complete the cell cycle. Calculate the amount of time spent in each phase of the cell cycle from the percent of cells in that phase using Equation 2.

$$\text{Time in phase (minutes)} = \frac{\text{Percent of cells in phase} \times 1440 \text{ minutes}}{100} \quad \text{Equation 2}$$

Tips

- Repeat the activity using prepared slides of Whitefish blastula.
- Prometaphase is included as a phase in mitosis since it is included in literature published by the National Institutes of Health. Include prometaphase events with metaphase events if prometaphase is not included in your textbook.

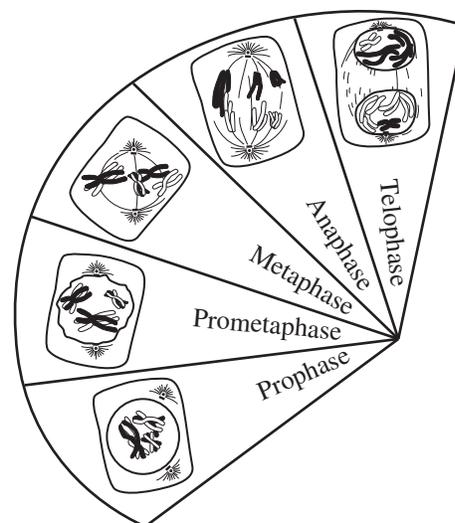


Figure 3. Mitosis "Wedge"

- We can see mitosis in action in the root tips of sprouting onion (*Allium sp.*) because the chromosomes are particularly large, and the mitotic rate is high.
- A 100X oil immersion lens is a useful tool to visualize spindle fibers and cell plates.
- Quadruple stain *Allium* microscope slides provide excellent contrast between the cell wall, nuclear membrane, and the chromosomes.
- Each phase of mitosis may not appear in every thin section of *Allium*. If students experience difficulty locating a particular phase, remind them that these prepared slides are the thin sections of a living plant. They may find a beautiful example of the phase on another thin section on the same or on another slide.
- On average, 10–40 cells will be undergoing mitosis in each zone of cell division. The exact number of cells will vary in each thin section on the *Allium* slide. *Allium* is a living tissue so variation is expected to occur.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12

Systems, order, and organization

Constancy, change, and measurement

Content Standards: Grades 5–8

Content Standard A: Science as Inquiry

Content Standard C: Life Science, structure and function in living systems, reproduction and heredity, regulation and behavior

Content Standards: Grades 9–12

Content Standard A: Science as Inquiry

Content Standard C: Life Science, the cell, molecular basis of heredity, organization in living systems

Materials for *Observing Mitosis in Plant Cells* are available from Flinn Scientific, Inc.

| Catalog No. | Description |
|-------------|--|
| ML1163 | Allium Microscope Slide |
| ML1164 | Allium Microscope Slide, quadruple stain |
| ML1261 | Whitefish Mitosis Microscope Slide |
| MS1048 | Flinn Advanced Student Compound Microscope |

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.