Making Mitosis Slides

Introduction

Discover the appearance and organization of plant cells in different phases of the cell cycle. All living cells are always in one of the phases of the cell cycle, whether they are performing their normal metabolic tasks or actively dividing to form new genetically identical daughter cells.

Concepts

- Mitosis
- Cell cycle
- Interphase

Background

The process of growth and division in a typical eukaryotic cell is called the cell cycle and is composed of two phases—interphase and the mitotic (M) phase. Each cell cycle begins with the formation of a new cell and continues until that cell divides into two offspring cells. Each offspring cell then begins the cycle again. Although the phases of the cell cycle are fixed, the amount of time spent in each phase, especially interphase, varies among different organisms and among different cells within an organism. In the apical meristem region of an onion root tip one complete cell cycle is typically completed in approximately 24 hours. Of that time, between 2–4 hours is spent in the mitotic phase of nuclear and cellular division.

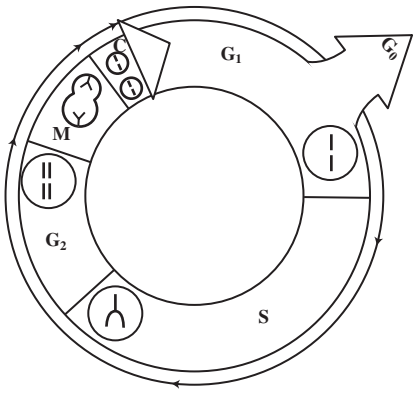

Figure 1. Cell Cycle

Interphase can be subdivided into three phases—G_1, S, and G_2. During interphase, normal metabolic activities such as the synthesis of RNA, proteins or other cellular macromolecules and the building of organelles cause the cell to grow in size as the cell prepares for its next division. In the apical meristem region of an onion, interphase lasts about 90 percent of the total cell cycle (20–22 hours). During the G_1 phase (gap 1) the cell is metabolically very active as it grows following the previous cell division. In an onion root tip, cells outside of the apical meristem go into an extended G_1 phase and rarely ever divide again—this is called a G_0 phase.

From the G_1 phase, meristem cells enter the S (synthesis) phase of interphase. In the S phase, an exact copy of DNA is made in the nucleus of the cell. Additional proteins, RNA, macromolecules, and organelles continue to be made during the S phase and also in the final phase of interphase, which is called the G_2 phase (gap 2). S phase is the longest phase of interphase, usually taking between 10–12 hours to complete.

The dramatic events of nuclear division take place as part of the mitotic phase or M phase. Chromosomes coil and become visible with a compound microscope during the mitotic phases. The M phase is subdivided into two parts—mitosis, or nuclear division, and *cytokinesis*, or cell division. Cytokinesis occurs simultaneously with the end phase of mitosis. After cytokinesis, each offspring cell enters G_1, and the cycle begins anew.

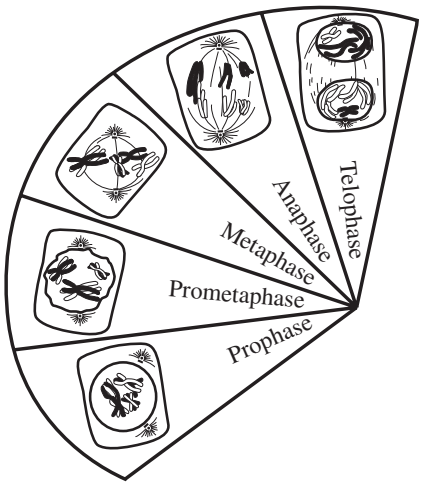

Figure 2.

Mitosis is subdivided into five phases as shown in Figure 2. During *prophase*, the nucleolus fades and chromatin condenses into chromosomes, and cytoskeleton microtubules create the spindle fibers necessary for chromosome separation. In *prometaphase*, the nuclear envelope breaks down, so there is no longer a recognizable nucleus, and spindle fibers elongate. 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 also separate, splitting the chromosome in half. The spindle fibers shorten and drag the attached half chromosomes, or chromatids, to opposite poles of the cell. In *telophase*, the chromatids arrive at the poles and the spindle fibers that have pulled them apart disappear. A nuclear envelope reforms around each cluster of chromosomes and these chromosomes return to their more extended form while cytokinesis begins.

Publication No. 10153
061616

Materials

Hydrochloric acid solution, HCl, 1 M
Methylene blue stain solution, 1%
Onions, bulbs or green
Water, deionized and tap
Compound microscope
Cover slips
Cup
Forceps
Microscope slides, glass
Paper towels
Pencil with eraser
Pipets, 3
Ruler
Scalpel

Safety Precautions

Hydrochloric acid solution is toxic by ingestion or inhalation and corrosive to skin and eyes. Methylene blue stain is a permanent stain on many objects. The scalpel is a sharp object—use care when cutting the root tips with the scalpel. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron throughout this lab. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all normal laboratory safety guidelines. Please review current Material Safety Data Sheets for additional safety, handling and disposal information.

Preparation

Actively growing onion root tips are required for this activity. Allow at least 2–4 days for new roots to grow. To grow root tips, obtain 5–6 onion bulbs or green onions. Remove any dried, old root growth from the bottom of the bulbs. Place each onion bulb into a plastic cup or jar of water so that only the root portion of the bulb is under water.

Procedure

1. Cut three roots from an actively growing plant using a scalpel. *Caution:* The scalpel is extremely sharp.
2. Trim the tip of each root to 1 cm; use only the tapered end of the root tip.
3. Use forceps to place 2–3 root tips (use only the 1-cm tips) on a glass microscope slide.
4. Using a clean, graduated pipet, add 2–3 drops of 1 M hydrochloric acid to cover the root tips on the microscope slide. *Note:* Hydrochloric acid is corrosive to skin and eyes.
5. Allow the root tips to soak in the acid for 5 minutes.
6. After 5 minutes, use a paper towel and carefully blot away excess hydrochloric acid from the slide. *Caution:* Avoid contact of the acid with skin.
7. Using a clean, graduated pipet, add 2–3 drops of deionized water to the root tips.
8. Use a paper towel to blot away excess water.
9. Repeat steps 7 and 8.
10. Using a clean, graduated pipet, add 2–3 drops of methylene blue stain to the root tip. *Note:* Methylene blue stain is a permanent stain.
11. Allow the root tips to soak in the stain for 3 minutes.
12. Use a paper towel to blot away excess methylene blue stain.
13. Add 1 drop of deionized water to the root tips.
14. Use forceps to move one root tip to a clean microscope slide.
15. Place a cover slip on the root tissue. Using the eraser end of a pencil, gently apply pressure on the cover slip to squash the root tissue. Apply an even downward pressure on the root tips and cover slip but not so hard as to break the cover slip. Do not twist or grind the cover slip.

16. Using low magnification on the microscope, focus on the root cells. Switch to medium power or high power as necessary to easily visualize the inside of the onion root cells.
17. Study all of the squashed tissue to locate cells in each stage of the cell cycle. *Note:* All stages of mitosis may not be present within a single field of view.
18. Repeat steps 14–17 using the remaining two root tips.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory wastes. Methylene blue stain can be disposed of according to Flinn Suggested Disposal Method #26b and hydrochloric acid according to method #24b. Microscope slides, cover slips and scalpels may be disposed of according to Flinn Suggested Biological Waste Disposal Method Type V. Plant material may be disposed of according to Flinn Suggested Biological Waste Disposal Method Type VI, common garbage wastes.

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
Form and function
Content Standards: Grades 5–8
Content Standard C: Life Science, structure and function in living systems, reproduction and heredity
Content Standards: Grades 9–12
Content Standard C: Life Science, the cell, molecular basis of heredity, matter, energy, and organization in living systems

Tips

- Prometaphase is included as a stage 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.
- Remove entire lengths of root from the onion before cutting the 1-cm root tips. Do not cut just partial roots. This may later cause you to remove a root that does not have a root tip for use in other classes. After the entire root has been removed, only the 1-cm tip should be cut off and used in the exercise. If using this as a student activity, several onions will be required to provide enough root tips for an entire class.
- 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 in the apical meristem region of the root.
- Students easily recognize cells in metaphase and anaphase but may need assistance with the remaining phases of the cell cycle.
- There are many excellent video sequences available showing cells dividing. These video clips can help students visualize the actual movement of chromosomes and are highly recommended for teaching the cell cycle.

References

Campbell, N. A.; Reece, J. B. *Biology*, 7th ed.; Pearson Education: San Francisco, 2005; Chapter 12.

Inside the Cell; U.S. Department of Health and Human Services. National Institutes of Health. National Institute of General Medical Sciences. NIH Publication No. 05–1051, September 2005 revision. 46–51.

Materials for *Making Mitosis Slides* are available from Flinn Scientific, Inc.

Catalog No.	Description
M0074	Methylene Blue Solution, 1%, 100 mL
H0013	Hydrochloric Acid, 1 M, 500 mL
ML1383	Cover Slips, Glass, No. 1, 22 × 22 mm, 1 oz
ML1398	Microscope Slides, Glass, Economy Choice

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