Rooting for Mitosis

Overview

Students will fix, stain, and make slides of onion root tips. These slides will be examined for the presence of cells in the four stages of mitosis.

Introduction

All new cells come from previously existing cells. New cells are formed by the process of cell division which involves both replication of the cell's nucleus (karyokinesis) and division of the cytoplasm (cytokinesis) to form two genetically identical daughter cells.

There are two types of nuclear division: mitosis and meiosis. Mitosis typically results in new somatic (body) cells. Formation of an adult organism from a fertilized egg, asexual reproduction, regeneration, and maintenance or repair of body parts is accomplished through mitotic cell division. Meiosis, on the other hand, results in the formation of either gametes or spores. These cells have half the chromosome number of the parent cell.

Where does one find cells in the process of mitosis? Plants and animals differ in this respect. In higher plants the process of forming new cells is restricted to special growth regions called meristems. These regions usually occur at the tips of stems or roots. In animals, cell division occurs almost anywhere as new cells are formed or as new cells replace old ones. Some tissues in both plants and animals, however, rarely divide once the organism is mature. To study the stages of mitosis in plants, you need to look for tissues where there are many cells in the process of mitosis. This restricts your search to the tips of growing plants such as the onion root tip.

When exploring plant cell mitosis, look for the following keys to help identify the stages of cell division: The non-dividing cell is in a stage called **interphase**. The nucleus may have one or more dark-stained nucleoli and is filled with a fine network of threads, the chromatin. Interphase is essential to cell division because the genetic material (DNA) is duplicated (replicated) during this stage.

The first sign of a division is **prophase**, in which a thickening of the chromatin threads occurs. Thickening continues until it is evident that the chromatin has condensed into chromosomes. With somewhat higher magnification you may be able to see that each chromosome is composed of two chromatids. As prophase continues, the chromatids continue to shorten and thicken. In late prophase the nuclear envelope and nucleoli are no longer visible, and the chromosomes are free in the cytoplasm. Just before this time the first sign of a spindle appears in the cytoplasm; the spindle apparatus is made up of microtubules, and it is thought that these microtubule may pull the chromosomes toward the poles of the cell where the two daughter nuclei will eventually form. It appears that centrioles are basal bodies that give rise to flagella and cilia in animals and lower plants such as mosses and ferns. Centrioles are not found in nonflagellated "higher" plants such as angiosperms.

At **metaphase**, the chromosomes have moved to the center of the spindle. One particular portion of each chromosome, the centromere, attached to the spindle. The centromeres of all the chromosomes lie at about the same level of the spindle, on an imaginary plane called the metaphase plate. At metaphase you should be able to observe the two chromatids of some chromosomes.

At the beginning of **anaphase**, the centromere regions of each pair of chromatids separate and are moved by the spindle fibers toward opposite poles of the spindle, dragging the rest of the chromatid behind them. Once the two chromatids separate, each is called a chromosome. The daughter chromosomes continue poleward movement until they form two compact clumps, one at each spindle pole.

Telophase, the last stage of division, is marked by a pronounced condensation of the chromosomes, followed by the formation of a new nuclear envelope around each group of chromosomes. The chromosomes gradually uncoil to form the fine chromatin network of interphase, and the nucleoli and nuclear envelope reappear. The cell develops into two new cells. In plants, a new cell wall is laid down between the daughter cells. In animal cells, the old cell will pinch off in the middle to form two new daughter cells. This division of the cytoplasm, in contrast to nuclear division (mitosis), is called cytokinesis.

Motivation

Cells are dividing all the time in living things. Where do you think the most rapidly dividing cells can be found? How might one induce an area of an organism to undergo more rapid cell division? Think of a plant. If one wanted to show cell division in action, where would be the best place(s) to look? How could one prove that cells were undergoing division? What if you were told you could make cells stop dividing, how could one prove it worked?

Objectives

At the completion of this laboratory you should be able to

- Detail the events of mitosis in plant cells
- Visually identify the stages of mitosis in plant cells
- Demonstrate a procedure to stain tissue for the identification of cells in the various stages of mitosis
- Explain and demonstrate the inhibitory effects of caffeine on cell division, and describe the significance

Materials

- Onions
- Microscope slides and cover slips
- Toluidine blue
- 1M hydrochloric acid
- Razor blades
- Latex gloves
- Safety goggles
- Kim wipes
- Pipette
- Tea or coffee

Associated California State Biology Standards

Interestingly, mitosis is not explicitly covered in the California High School Biology Standards. However, it is covered in the Seventh Grade Life Science Standards and is heavily tested at the high school level.

Procedure

1. Root and onion bulb in water by placing fresh onions in water so that only their bases remain water at a constant temperature of 25 ± 0.5° C. Move to a dark place. Roots should begin to appear in 48 hours.

2. Students should mark the root tip one or two days prior to staining the root tips. This will enable them to link rate of growth with mitotic index.
3. Obtain an onion bulb with roots about a half an inch long or more. Remove the plant material and use the thread and ruler to measure how much the root tips have grown since marked. Cut a root off. Then, cut off the bottom 1 or 2 mm of the root tip and place it on a slide
4. Wear proper safety equipment, including goggles and gloves, for the next steps.
5. Add a very small drop of 1M HCL acid to the root tip on the slide and let it sit for 4 min.
6. Using a Kim wipe draw the HCL away from the root tip. Throw the wipe away.
7. Cover the root tip with a drop of 1% toluidine blue and let stand for 2 minutes.
8. After two minutes, carefully blot around the root to remove excess stain with a new wipe. Rinse with water until the water runs clear. Add one drop of water and gently lay a coverslip over the root tip.
9. Place the slide, coverslip down on a paper towel. Using a pencil eraser, carefully apply pressure to the coverslip area, with pressure being applied from one edge to the other, in order to squash and spread the root tip tissue.
10. Mount the slide on your microscope.
11. Use the low power objective on your microscope to look for thin layers of cells and then use the 40X power objective to observe mitotic stages in individual cells.
12. Identify chromosomes at the various stages of mitosis. Make sketches of the stages observed.
13. Assuming mitosis (not counting interphase) in onion normally takes about 80 minutes at room temperature, calculate the time spent in each stage. Count 25 total cells on your slide that are undergoing mitosis and tally the number found in each phase. Calculate the percentage of cells in that phase. Then convert that number to the percentage of time a cell is in each phase, based on an 80 minute total for mitosis. Record your answers in the data table below.

	Prophase	Metaphase	Anaphase	Telophase	Total
# of cells					25
% of cells					100%
Time spent in:					80 min

Evaluation

The following questions are listed under the Analysis section of the student handout and may be used as part of a report, class discussion or assessment.

1. Why is an onion root tip a good place to find examples of mitotic cells? What would be other good sources? What would not?
2. Why do cells need to divide?
3. Do all our cells divide at the same time? What are some examples of your own cells that divide quickly? What about some of your cells that are really slow or do not divide?
4. What happens when cell division goes out of control?
5. When is DNA replicated in the cell division process?
6. Onion root tip cells in various stages of mitosis are pictured here: http://www.biology.arizona.edu/CELL_BIO/activities/cell_cycle/01.html . Students can test their identification with an online quiz.

Extension Activity
You may also take an onion that has been growing and has good roots and transfer it to a cup of cool tea or coffee and see what effect caffeine has on cell cycle. After placing the onion with roots in the tea or coffee, let it stand for 2-10 hours, then do a root tip squash as outlined above, same procedure. (Caffeine has been shown to interrupt and inhibit cytokinesis, therefore the cells visible in this activity should all stop after telophase and will be binucleate.)

Test Preparation

1. Which of the following does **not** occur by mitosis?
    (A) Growth
    (B) Production of gametes
    (C) Repair
    (D) Development in the embryo

2. In which stage of the life of a cell is the nucleolus always visible?
    (A) Prophase
    (B) Anaphase
    (C) Telophase
    (D) Cytokinesis
    (E) Interphase

3. If a cell has 24 chromosomes, how many will it have at the end of mitosis?
    (A) 12
    (B) 24
    (C) 48
    (D) The number varies with the species.

Student Sheet: Rooting for Mitosis Name:_____

Procedure

1. Mark the root tip one or two days prior to staining the root tips. This will enable you to link the rate of growth with mitotic activity. Use a permanent marker pen to mark a small dot about 2 mm from the end of some root tips. Replace the plant carefully in the water.
2. Obtain an onion bulb with roots about a half an inch long or more. Remove the plant material and use the thread and ruler to measure how much the root tips have grown since marked. Cut a root off. Then, cut off the bottom 1 or 2 mm of the root tip and place it on a slide
3. Wear proper safety equipment, including goggles and gloves, for the next steps.
4. Add a very small drop of 1M HCL acid to the root tip on the slide and let it sit for 4 min.
5. Using a Kim wipe draw the HCL away from the root tip. Throw the wipe away.
6. Cover the root tip with a drop of 1% toluidine blue and let stand for 2 minutes.
7. After two minutes, carefully blot around the root to remove excess stain with a new wipe. Rinse with water until the water runs clear. Add one drop of water and gently lay a coverslip over the root tip.
8. Place the slide, coverslip down on a paper towel. Using a pencil eraser, carefully apply pressure to the coverslip area, with pressure being applied from one edge to the other, in order to squash and spread the root tip tissue.
9. Mount the slide on your microscope.
10. Use the low power objective on your microscope to look for thin layers of cells and then use the 40X power objective to observe mitotic stages in individual cells.
11. Identify chromosomes at the various stages of mitosis. Make sketches of the stages observed on a separate sheet of paper or the back of this lab.
12. Assuming mitosis (not counting interphase) in onion normally takes about 80 minutes at room temperature, calculate the time spent in each stage. Count 25 total cells on your slide that are undergoing mitosis and tally the number found in each phase. Calculate the percentage of cells in that phase. Then convert that number to the percentage of time a cell is in each phase, based on an 80 minute total for mitosis. Record your answers in the data table below.

	Prophase	Metaphase	Anaphase	Telophase	Total
# of cells					25
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Analysis

On a separate sheet of paper please complete the following.

1. Why is an onion root tip a good place to find examples of mitotic cells? What would be other good sources? What would not?
2. Why do cells need to divide?
3. Do all our cells divide at the same time? What are some examples of your own cells that divide quickly? What about some of your cells that are really slow or do not divide?
4. What happens when cell division goes out of control?
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6. Onion root tip cells in various stages of mitosis are pictured here: http://www.biology.arizona.edu/CELL_BIO/activities/cell_cycle/01.html . Test your identification with an online quiz.