Mitosis in Eelgrass Root Tips (Lab)

Taken from: *http://www.huntington.org/Education/FIBR/download/2004/04PlantCell.pdf*

Objectives

You will fix, stain, and make slides of eelgrass root tips. These slides will be studied for the presence of cells in the four stages of mitosis.

At the completion of this laboratory you should be able to

- Stain tissue for the identification of cells in the various stages of mitosis
- Identify the stages of mitosis in plant cells

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 (in animals) or spores (in plants). 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. In both plants and animals, though, tissues rarely divide once the organism is mature.

To study the stages of mitosis, you need to look for tissues where there are many cells in the process of mitosis. In plants, this restricts your search to growing tips, such as the eelgrass root tip.

The phases of plant mitosis are:

The nondividing 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.

Preparing an eelgrass root tip squash

It is possible for you to make your own stained preparations of eelgrass root tips and observe mitotic figures. eelgrass bulbs have been rooted in water. Growth of new roots is due to the production and elongation of new cells. Mitotic divisions are usually confined to the cells near the tip of the root. Follow the procedure outlined below to make your own root tip preparation.

Procedure

1. Obtain an eelgrass bulb that has been rooted in water. Cut 2 or 3 roots off near the base of the bulb.

2. Then, cut off the bottom 1 or 2 mm of the root tip and place it in a petri plate. With a pasteur pipette, add a small puddle of 1M HCl and let root tip stand for 4 min.

3. Remove the root tip from the HCl with forceps and place on a slide.

4. Cover the root tip with 2 drops of 1% toluidine blue and let sit for 2 minutes.

5. Carefully blot around the root to remove excess stain. Rinse with water and blot until the water is clear. Add one drop of water and apply a coverslip.

6. Place the slide, coverslip down on a paper towel. Using a pencil eraser, carefully apply pressure to the coverslip area in order to squash and spread the root tip tissue. [This takes a little practice, so if your first squash does not yield good results, try another one or two.]

7. Mount the slide on your microscope.

8. 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.

9. Identify chromosomes at the various stages of mitosis.

10. Compare your slide to the prepared slides that contain stained sections of root tips.

11. Make sketches of the mitotic stages observed on the next page.

Use this side of page to draw sketches of what you see in the microscope.

Use this side of page to note stages and descriptions.

Answer the following questions.

A. Which stage(s) have the easiest chromosomes to see? Why is this so?

B. Why are the cells in mitosis located near the tip of the root?