[redacted]

01/27/09

Biology 215—Lab: Wed. 3

Chromosomes/Mitosis

15/15 - outstandings

BACKROUND/PURPOSE - Excellent!

Our bodies allow us to live everyday. They are very important to us. They are also very complex and significant because they undergo thousands of biological processes. One may wonder, "How can the body do this?" or "How does the body know what to do?" The answer is deoxyribonucleic acid (DNA). DNA is what you could call the genetic blueprint of cells, which our bodies (cells) are composed of. New cells are being created every second in our bodies by a process called mitosis. In this process, the cell goes through certain phases which ultimately lead to the division of chromosomes, condensed strands (molecules) of DNA. Before mitosis can occur, a different stage in the cell cycle takes place to prepare the cell for division. This stage is called interphase. During interphase, the cell goes through periods of growth and development. The DNA is duplicated during this time in synthesis, or the S-phase of interphase. It would be in its uncondensed form, known as chromatin. A nuclear membrane would be visible around the chromatin. Also, nucleoli would appear inside the nucleus. As we will find out later, the cell spends much of its time in interphase. When this stage of the cell is complete, it moves to mitosis.

Mitosis is composed of four main phases: prophase, metaphase, anaphase, and telophase. These phases could be broken down into further sub-phases, but we will just look at the main components. Prophase is the first stage in mitosis. It can be characterized as the preparation phase. Chromatin becomes visible as it coils into chromosomes and becomes denser. The nuclear membrane begins to vanquish (vanish?) as well as the nucleoli. Spindle fibers form from centrioles at opposite ends of the cell. They connect to the chromosomes on protein structures called kinetichores, which are located at the chromosome's centromere. Metaphase follows after the completion of prophase. During metaphase, chromosomes (continue to condense and) are moved to the center of the cell, also known as the metaphase plate. Their centromeres become aligned, forming a distinct line down the cell. Next, anaphase takes place. The spindle fibers begin to shorten. This causes the identical strands of each chromosome (sister chromatids) to be pulled apart towards opposite poles of the cell. Two different clusters of DNA should become visible at this stage. The last phase of mitosis is telophase. At this point, the chromosomes are located at the poles of the cell. The cell membrane begins to pinch in, forming two different cell

figures, each with identical chromosomes. The genetic material then starts to uncoil as the nuclear envelope and nucleoli gradually reappears. During cytokinesis, the cell actually splits into different cells called daughter cells.

In today's lab, we will use onion root tip to get a clear view and understanding of mitosis. This will allow us to physically examine chromosomes, using a microscope. The procedure will make it possible to look at hundreds of cells at the same time, providing real life images. The development of a cell and its mitotic stages will become tangible to the experimenter. A great understanding for cell division should be at grasp after completing the lab.

Materials

Fixed onion roots

6M Hydrochloric acid (careful!)

Plastic transfer pipettes

Carnoy fixative solution

Toluidine Blue stain

microscope

2 microscope slides with cover slips

plastic tray

forceps

razor blades

paper towels

squeeze water bottle

Procedure (Part A)

1. Taking care, use a transfer pipette to transfer a small amount of 6M HCl from the bottle to a plastic tray.
2. Using forceps obtain an onion root from the beaker. Use care to grab the roots at the opposite end from the tip, which is pointed and slightly yellow.
3. Place the root in the pool of 6M HCl in the plastic tray for 5 minutes. (do not skimp on the time!)
4. Carefully pour the HCl off into the sink (hold the root in place with gloved finger or forceps) and run water down the drain for the next 5 minutes to dilute the acid.
5. Using a clean transfer pipette, carefully transfer a small amount of Carnoy fixative to the root tip in the tray. Soak 5 minutes.
6. Pour off the Carnoy fixative and run water down the drain for 5 minutes to dilute.

7. Transfer the root to a glass slide and cut away all but 1-2 mM of the root tip. KEEP THE VERY TIP OF THE ROOT and discard the rest.
8. Place the slide on a paper towel and add 1 drop of toluidine blue dye to the root tip. Let stand for 1 minute.
9. Wick away the dye with the corner of a piece of paper towel, taking care not to touch the root tip. Add 13-14 drops of water with the water squirt bottle to the root tip, wick away extra water, and cover with the cover slip.
10. Using firm downward pressure, press (do not twist thumb) on the cover slip to squash the root tip into a single cell layer for viewing.
11. View the cells on low (100X) power to become familiar with the look of the cells. Look for mitotic phases. (Look for condensed chromosomes, rather than uniformly dark nuclei). Then view cells/ chromosomes on high power (400 X). **DO NOT USE OIL TO VIEW CELLS AT 1000X.**
12. **Sketch a cell in each of the 4 phases of mitosis. (If you are unable to find one or more of the phases, use a classmate's slide.**
13.

Part B.

In the second part of the lab we will use the commercially prepared onion root tip slides. These slides have a very thin (.007-.012 mM) longitudinal section of onion (*Allium*) root tips. The process for preparing these cells is much more complex (and expensive!) than the squashes you prepared. Briefly, onion roots are killed and fixed, dehydrated, and embedded in paraffin (wax). The sections were sliced on an instrument called a microtome, stained and mounted on a microscope slide. Briefly scan the slide and identify cells in interphase and in mitosis (prophase, metaphase, anaphase, or telophase).

Part C. Using the commercially made slide, observe at least 100 cells near the tip. Record the number of cells you observe in each of the phases of the cell cycle. Assume that *Allium* has a cell cycle of 5 hours. Use the data you collect to approximate the duration of interphase and each phase of mitosis. How long are these cells in mitosis? Sketch a cell cycle (circle) for this organism showing the relative duration (in minutes) of each phase. (Figure 2-6 in your text may help.)

Part D. Observe/sketch from the demo slide of human chromosomes, from cells arrested in mitosis. (Note the magnification.) How many chromosomes do you count? How many *should* you count? Can you recognize size or shape differences in the chromosomes? How do you think you could make a karyotype of a person's chromosomes? Why do we typically use another source of cells (not human) for general chromosome viewing?

DATA/OBSERVATIONS

Part A: 12. Next Page

Part B:

The cells from the commercially prepared onion root tip slides worked very well in our lab. They showed up quite clear and distinct under the microscope. It was easy to identify each phase of mitosis.

1. Explained in discussion.
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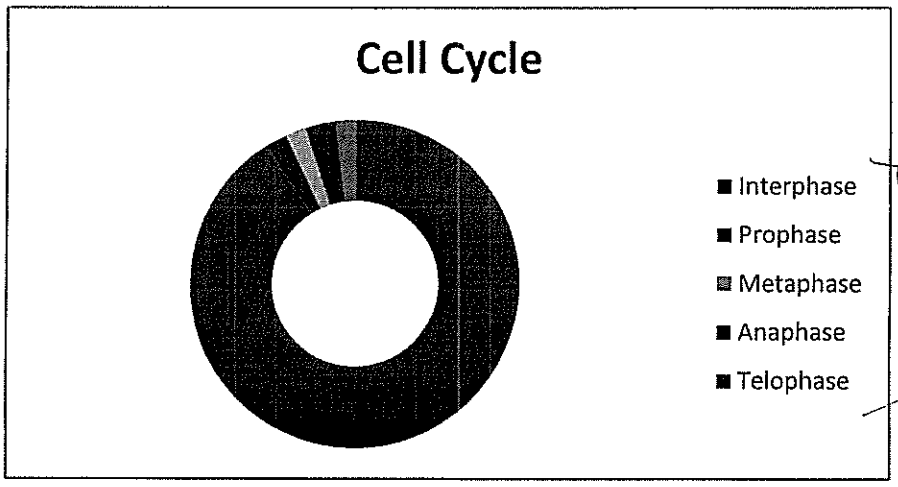
Mitotic Index of 40-50 cells in two different regions: average of regions: 12%

I wanted 50+ to compare 2 regions - nearer and farther from tip.

Mitotic Index appeared to increase towards tip of root.

Part C:

	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
Number of Cells	91	2	2	3	11	100
Percent of Cells	91%	2%	2%	3%	2%	100%
Time in Stage	4 hours 33 minutes	6 minutes	6 minutes	9 minutes	6 minutes	5 hours



really hard for those old eyes to distinguish those in black & white

Part D: Next Page

CONCLUSION/DISCUSSION

The lab was a great success, in my opinion, for my partner and me. Despite adding too much water to our slide, our squash turned out excellent. The cells were quite distinct and mitosis was clearly visible. Some cell regions were clearer than others but focusing cured these obstacles. It was possible that some of the cells were at different elevations on the slide. Nucleoli were quite visible in our squash. The chromosomes themselves had been stained well and were easily seen. They appeared as dense spots of DNA; sister chromatids were not distinctively visible, but arms could be seen during anaphase and telophase. We were able to utilize our slide to its maximal potential. We received all of the information we needed from it.

Although our slide turned out great, it is difficult to beat the clarity that was displayed from the commercially prepared cells. The cell walls, nucleoli, membranes, and chromosomes all appeared to be crisp and distinct. They were easier to count, and they could all be seen clearly on the same magnification and focus.

Overall, I thought the commercial slides were better in quality. The squashes, however, provided a hands-on experience. It took time to make the slides, but if I had to choose between the two in doing the experiment again, I would choose making the squash. I had fun making it. It gave me a sense of accomplishment when we got to see the results of our work. The slide turned out great, and we learned from the process. The commercial slides are expensive, and making them is a longer and more complicated process.

When counting the cells for the mitotic index, we got an index of 12%. This was the average index of two different regions. One region was located near the root tip, and the other had a higher location, further up the root. We later counted one hundred cells and reported how many of the cells were in each phase of mitosis or interphase (results in data/observations). It made sense that most of the cells were in interphase because it is a stage that includes many developmental processes of the cell. We found nine cells in mitosis; they all showed up in similar quantities. This did not give us a great idea about how long each phase lasts because we had very little mitotic cells to work with. We might have obtained better results if we would have counted more cells, maybe 200-500. This would have taken longer, but often in science, accuracy increases with quantity.

After executing the counting portions of the lab, it was easy to conclude that mitotic cells were more populous toward the tip of the onion root cell. After all, the root is growing most toward the tip. Therefore, the mitotic index was highest here. It is possible to increase the index by applying water to the root and by adding certain growth prohibitors such as fertilizer. Stopping cells while they are in mitosis is another alternative one can take in order to increase the mitotic index.

The slide that was prepared for us which displayed human chromosomes was quite interesting. It was nice to see the chromosomes as defined shapes. This was most likely possible because of the 1000X magnification we were using. It would have been great to see these chromosomes during mitosis. Other sources are most likely preferred because it is not easy to view these chromosomes during division. Focusing became difficult when viewing them, but the pairs of sister chromatids were a sight for sore eyes after looking at onion chromosomes for two hours.

Size!

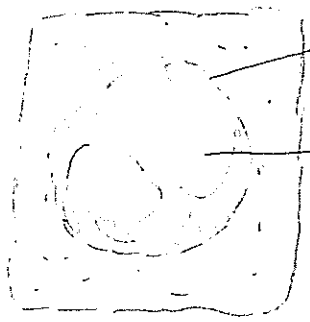
This experiment increased my knowledge of mitosis and gave me a better understanding of chromosomes. Lab is always enjoyable because of the hands-on experience. Making the slides and using the microscopes was an enjoyable process. In text books, cells and chromosomes almost look flawless. I learned in this lab that perfection is not easy to come by. This was a good thing. We had to search for cells; they were not handed to us on a sheet of paper. It was rewarding when we found these cells that we worked for. The lab was put together well; I enjoyed it, and more importantly, I learned from it.

Excellent job!

Prophase

400X

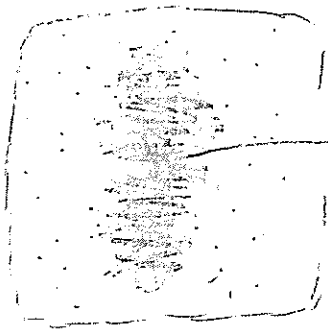
Interphase



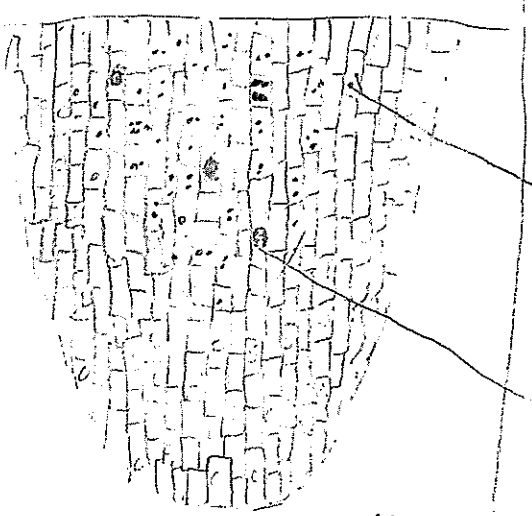
400X

AMAZING!!!

Metaphase

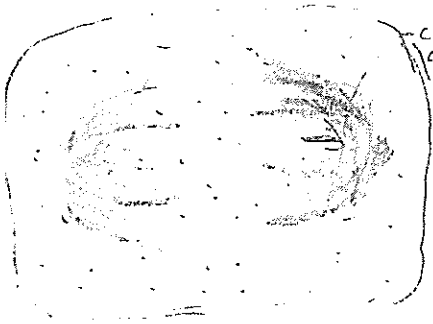


400X



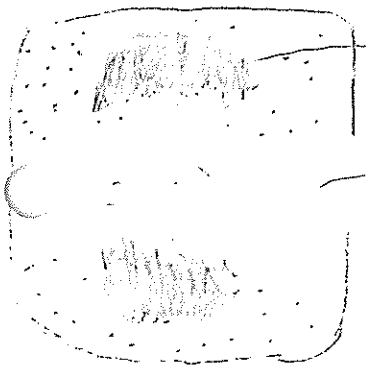
Commercial Onion Tip 400X

Anaphase



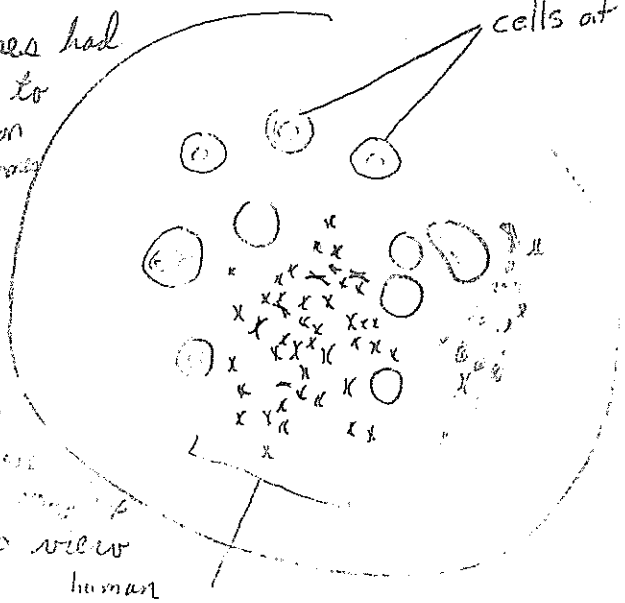
400X

Telophase



400X

D. We counted 46 chromosomes in the main cluster that we observed. We could see the sister chromatids much easier and more distinctly than those in the onion. The chromosomes had actual shape to them. The onion root chromosomes seemed more like a cluster or blob. It is easier to observe other species although [illegible] and easier to view [illegible] chromosomes which [illegible] smaller.



1000X