Cell Division: Mitosis

How do eukaryotic cells divide to produce genetically identical cells or to produce gametes with half the normal DNA?

BACKGROUND

One of the characteristics of living things is the ability to replicate and pass on genetic information to the next generation. Cell division in individual bacteria and archaea usually occurs by binary fission. Mitochondria and chloroplasts also replicate by binary fission, which is evidence of the evolutionary relationship between these organelles and prokaryotes.

Cell division in eukaryotes is more complex. It requires the cell to manage a complicated process of duplicating the nucleus, other organelles, and multiple chromosomes. This process, called the cell cycle, is divided into three parts: interphase, mitosis, and cytokinesis (Figure 1). Interphase is separated into three functionally distinct stages. In the first growth phase (G1), the cell grows and prepares to duplicate its DNA. In synthesis (S), the chromosomes are replicated; this stage is between G1 and the second growth phase (G2). In G2, the cell prepares to divide. In mitosis, the duplicated chromosomes are separated into two nuclei. In most cases, mitosis is followed by cytokinesis, when the cytoplasm divides and organelles separate into daughter cells.

This type of cell division is asexual and important for growth, renewal, and repair of multicellular organisms.

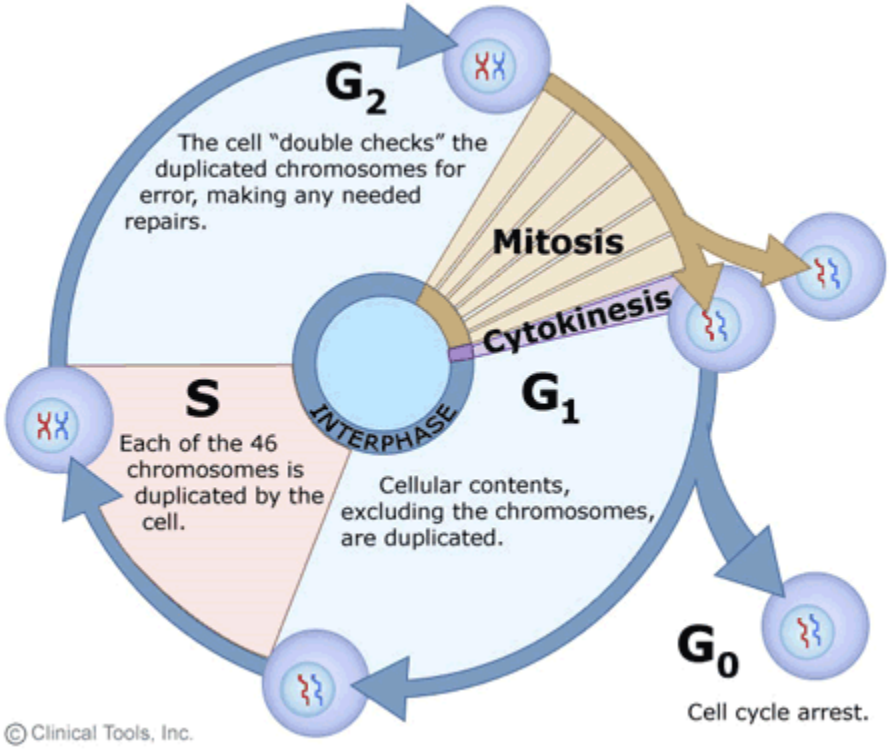
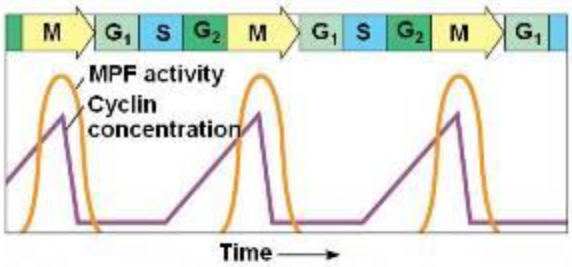


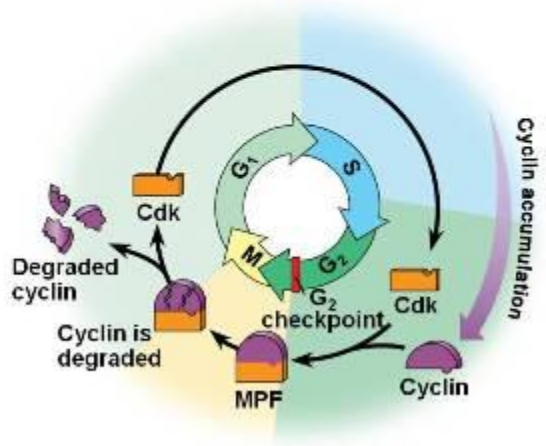
Figure 1. The Cell Cycle Showing G1, S, and G2,Phases, Mitosis, and Cytokinesis

Cell division is tightly controlled by complexes made of several specific proteins. These complexes contain enzymes called cyclin-dependent kinases (CDKs), which turn on or off the various processes that take place in cell division. CDK partners with a family

of proteins called cyclins. One such complex is mitosis-promoting factor (MPF), sometimes called maturation-promoting factor, which contains cyclin A or B and cyclin-dependent kinase (CDK). (See Figure 2a.) CDK is activated when it is bound to cyclin, interacting with various other proteins that, in this case, allow the cell to proceed from G_2 into mitosis. The levels of cyclin change during the cell cycle (Figure 2b). In most cases, cytokinesis follows mitosis.



(a) Fluctuation of MPF activity and cyclin concentration during the cell cycle



(b) Molecular mechanisms that help regulate the cell cycle

Figure 2. MPF Production During the Cell Cycle

As shown in Figure 3, different CDKs are produced during the phases. The cyclins determine which processes in cell division are turned on or off and in what order by CDK. As each cyclin is turned on or off, CDK causes the cell to move through the stages in the cell cycle.

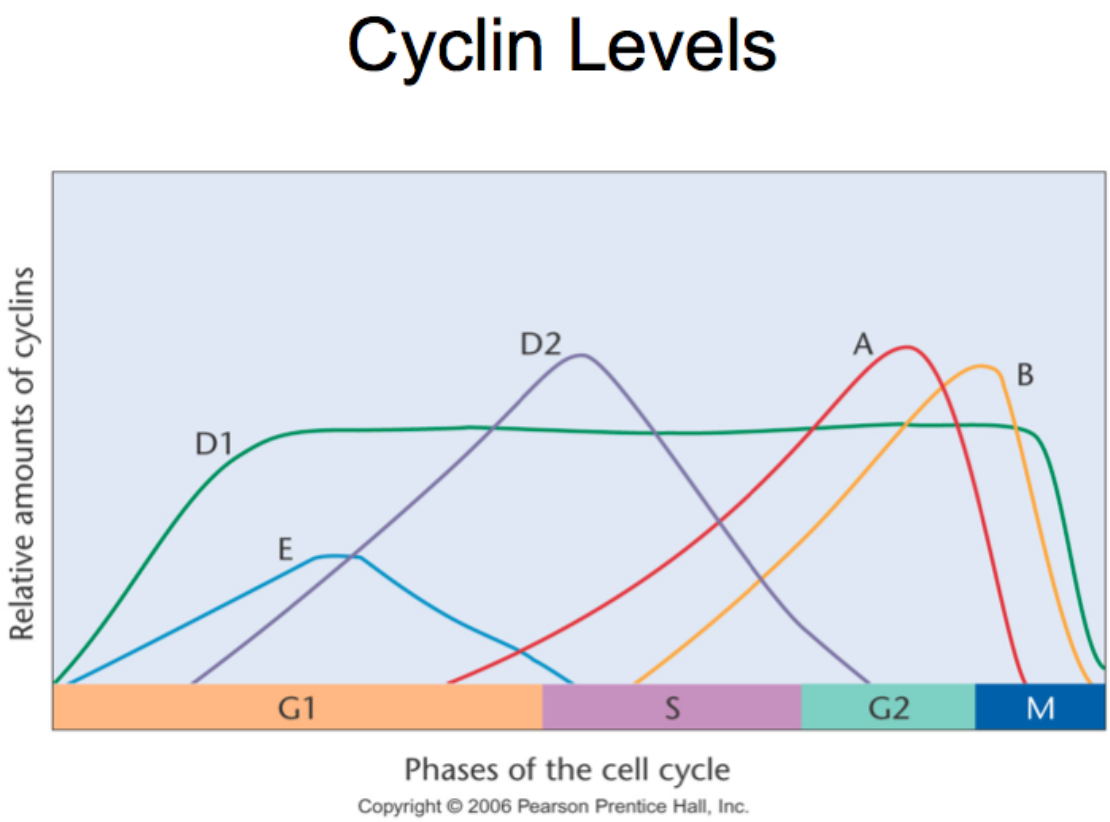


Figure 3. Levels of CDKs During the Cell Cycle

Cyclins and CDKs do not allow the cell to progress through its cycle automatically. There are three checkpoints a cell must pass through: the G1 checkpoint, G2 checkpoint, and the M-spindle checkpoint (Figure 4). At each of the checkpoints, the cell checks that it has completed all of the tasks needed and is ready to proceed to the next step in its cycle. Cells pass the G1 checkpoint when they are stimulated by appropriate external growth factors; for example, platelet-derived growth factor (PDGF) stimulates cells near a wound to divide so that they can repair the injury. The G2 checkpoint checks for damage after DNA is replicated, and if there is damage, it prevents the cell from going into mitosis. The M-spindle (metaphase) checkpoint assures that the mitotic spindles or microtubules are properly attached to the kinetochores (anchor sites on the chromosomes). If the spindles are not anchored properly, the cell does not continue on through mitosis. The cell cycle is regulated very precisely. Mutations in cell cycle genes that interfere with proper cell cycle control are found very often in cancer cells.

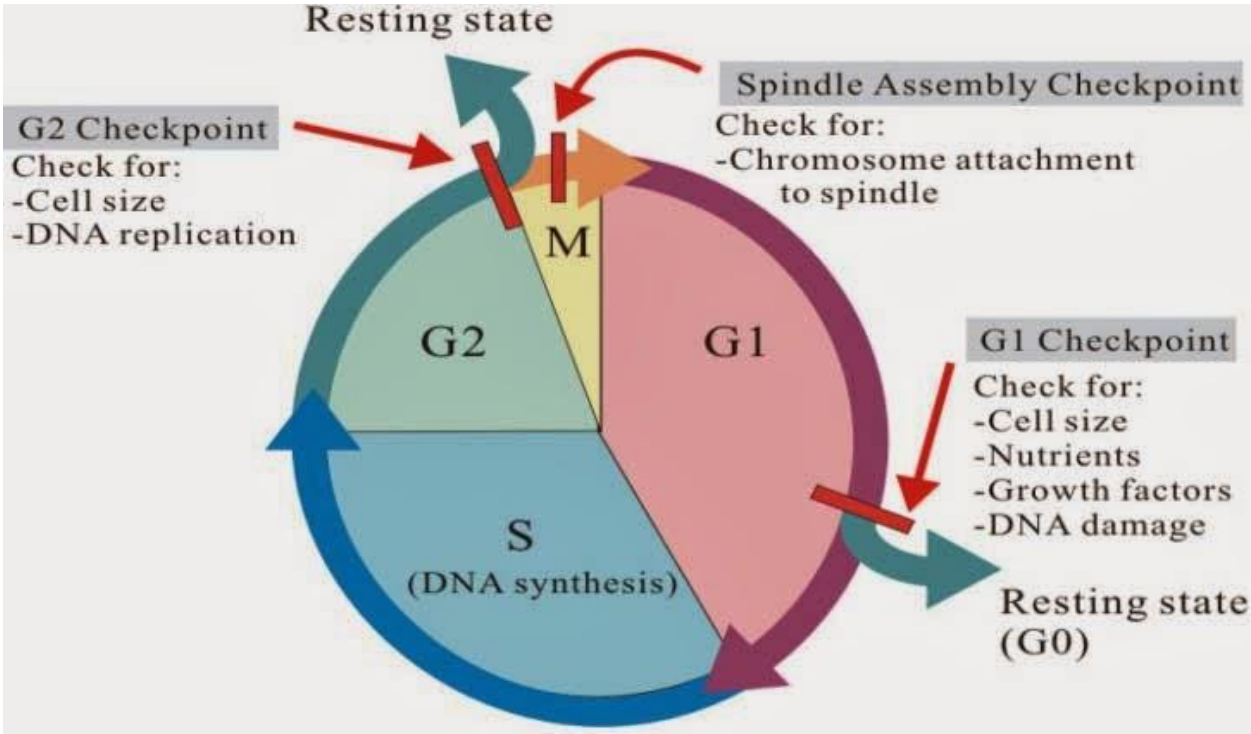


Figure 4. Diagram of the Cell Cycle Indicating the Checkpoints

Part 1: Effects of Environment on Mitosis

Introduction:

All new cells come from previously existing cells. New cells are formed by the process of cell division, which involves the replication of the cell's internal structures and the division of the cytoplasm (cytokinesis).

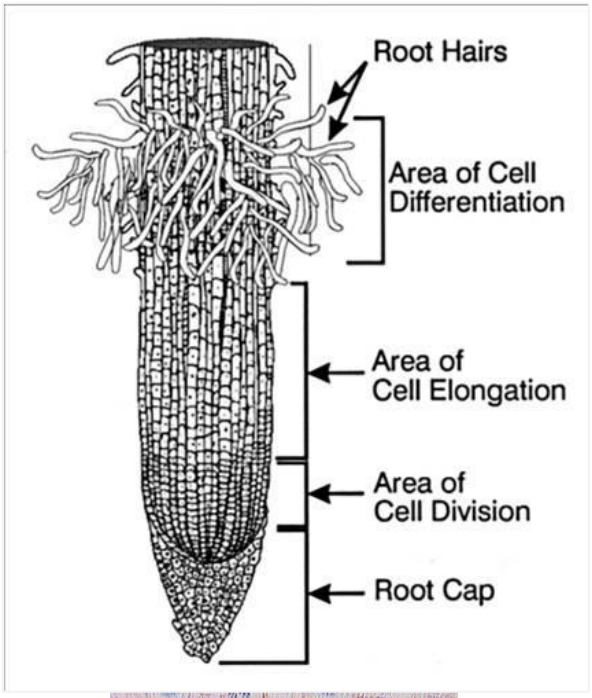
Background:

The health of a plant or animal depends upon both biotic and abiotic factors. Imagine the parking lot of your school. A few plants may be growing in cracks and crevices of the pavement. In these cracks there is at least a subsistence level of nutrients and water for a plant to survive. A few meters away an unpaved area with soil and little foot traffic may have more plants. The plants compete for space, water, nutrients, and light but, more than one plant is in the soil area. If you were to compare plants from the paved and soil areas you would likely see differences in the height of the stems, the number of leaves, and the number and length of the roots. This is a simple example of abiotic factors in the environment affecting plant growth.

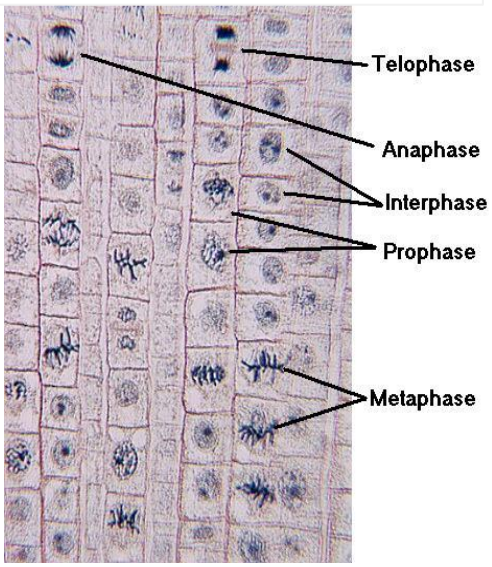
Many biotic factors also affect plant growth. A classic example of a beneficial biotic effect is the mutualistic relationship between legumes (beans, peas , clover, and alfalfa) and the nitrogen-fixing bacterium, rhizobia. Rhizobia (singular=rhyzobium), live in nodules on the roots of beans and other plants. Chemicals released by the plant cause the bacterium to migrate toward the plant roots. Entry of the bacterium into the root causes a cascade of cell signals.

That area of the root enters a phase of rapid cell division producing a nodule where the bacteria flourish.

Not all biotic interactions benefit a plant. Parasitic interactions may harm a plant by increasing mitosis. For example, the plant pathogen Agrobacterium tumefaciens (now called Rhizobium radiobactor) causes plant cancer or galls. By triggering a plant to undergo rapid cell division, the pathogen forces the plant to expend more energy in that location and not in the other roots, stems, and leaves. This weakens the plant and may cause death.



A. tumefaciens enters the plant through a wound and infects cells by inserting a plasmid into the cell. The plasmid inserts into the DNA and causes several important genes to be transcribed. One gene codes for the plant hormone indole-3-acetic acid, IAA. IAA is a plant hormone that triggers cell division—it has been used commercially as a rooting compound for many years.



The plant of choice when studying mitosis is the common onion. Onions germinate easily without soil so the chemicals provided to the plant can be easily controlled. Onion root tips also grow quickly and are only a few cells thick. A stain is used to dye condensed chromosomes like those undergoing mitosis a very dark color. By viewing the onion root tip using a light microscope it is easy to determine if a particular cell is in interphase or mitosis. Note that cell division occurs only in the meristem region, not in the other regions of the root tip. Recall also that 90% of the time a cell in this region will be in interphase, since mitosis typically makes up only 10% of a full cell cycle. Onions are alive and therefore the onion slide preparation will have more than one layer of cells present in each preparation. In order to reduce the total depth of the slide preparation the onion root tip needs to be treated and then squashed between the cover slip and the microscope slide.

Pre Lab Questions

How did you develop from a single-celled zygote to an organism with trillions of cells?

How many mitotic cell divisions would it take for one zygote to grow into an organism with 100 trillion cells?

How is cell division important to a single-celled organism?

What must happen to ensure successful cell division?

How does the genetic information in one of your body cells compare to that found in other body cells?

What are some advantages of asexual reproduction in plants?

Why is it important for DNA to be replicated prior to cell division?

How do chromosomes move inside a cell during cell division?

How is the cell cycle controlled? What would happen if the control were defective?

Experimental Overview

Onion root tips germinated in a solution containing IAA (or plant food solution) will be compared to onion root tips germinated in water only. A minimum number of cells will be tallied to determine the percentage of cells in interphase and mitosis for each treatment. The data from this baseline activity will be analyzed using a Chi-square statistical analysis test to determine if any observed variation in percent mitosis is statistically significant. The results of this baseline activity will provide a procedure and model for open inquiry and student-designed experiments.

Materials

Onion Bulbs (4 per group)
Plastic Cups (2 per group)
Sand
IAA Solution (or plant food solution)
Spring Water (or drinking water)
Dissection scissors
Forceps

Day 1: Germinate Root Tips

1. Fill 2 plastic cups about 1/3 full with sand.
2. Label one cup "Control" and the other "IAA treatment (Experimental)".
3. Get 4 onions and remove any dried roots from the bottom of each onion.
4. Insert 2 onions in each cup. Make sure they are about 1/3 submerged in the sand (bottom down).
5. Add enough spring water or drinking water to the "Control" cup to completely wet the sand.
6. Add enough of the IAA solution or Experimental Solution to the "IAA treatment (Experimental)" cup to completely wet the sand.
7. Loosely place plastic wrap on top of each cup to prevent excess evaporation.
8. Allow plants to grow for 3-5 days under a light source.
9. Water plants each day with the appropriate solution.
10. Take pictures of any shoot growth throughout the experiment.

Baseline Activity

1. Gently remove the control bulbs and rinse away the sand.
2. Take pictures of your specimens.
3. Cut off the roots of your control onion and measure each root to get an average growth rate. Record your data.
4. Measure the shoot growth of each bulb and record the data.
5. Repeat procedures 1-4 with your experimental bulbs.

Opportunities for Inquiry

1. Consider the following questions while reflecting upon your knowledge of biotic and abiotic factors that may influence root growth and mitosis in plants.
    a. In areas where there are very few plants growing, what biotic and abiotic factors may be affecting the rate of mitosis and the ability of plants to thrive?
    b. What chemicals may be expected to increase or decrease the rate of mitosis in plants?
    c. Of the factors identified in the above questions, which can be replicated as an experiment in the laboratory?
2. Plan, discuss, execute, evaluate, and justify an experiment to test a question regarding the rate of mitosis in plants.
    a. Decide upon one question that your group would like to explore.
    b. Develop a testable hypothesis.
    c. Discuss and design a controlled experiment to test the hypothesis.
    d. List any safety concerns and the precautions that will be implemented to keep yourself, your classmates, and your instructor safe during the experimental phase of this laboratory.
    e. Determine what and how you will collect and record the raw data.
    f. How will you analyze the raw data to test your hypothesis?
    g. Review your hypothesis, safety precautions, procedure, data tables, and proposed analysis with your instructor prior to beginning the experiment.
    h. Once the experiment and analysis are complete, evaluate your hypothesis and justify why or why not the hypothesis was supported by your data.
    i. Present and defend your findings to the class.
    j. Make suggestions for a new or revised experiment to modify or retest your hypothesis.

Part 2 : Counting Cells and Analyzing Data

1. Get a prepared control onion root slide.
2. Observe the cells at high magnification (400 X).
3. Look for well-stained, distinct cells.
4. Within the field of view, count the cells in each phase. Record your data in Table 1.
5. Repeat the counts in two other root tips.
6. Repeat steps 1-5 with the IAA (experimental) slide. Record your data in Table 2.
7. Total the number of cells from each group in interphase and in mitosis.

Table 1. Onion Root Tip Cell Phase Data; Control Group

Tip	Number of Cells		
	Interphase	Mitotic	Total
1			
2			
3			
Total			

Table 2. Onion Root Tip Cell Phase Data; IAA (Experimental) Group

Tip	Number of Cells		
	Interphase	Mitotic	Total
1			
2			
3			
Total			

8. Collect the class data for control and experimental groups and enter the values into Table 3; these are the observed values for the four groups.
9. Enter the observed values (o) from Table 3 into Table 6.
10. Use the totals from Table 3 to and the formulas from Table 5 to calculate the expected values (e).
11. Enter the expected values (e) into Table 6.
12. Calculate the remaining values for Table 6.
13. Calculate the chi-square ($\chi 2$) value for the data by adding together the numbers in the right column.
14. Compare this value to the critical value in Table 7.

Table 3. Onion Root Tip Cell Phase Class Data; Control Group and Experimental Group

Class Data	Number of Cells		
	Interphase	Mitotic	Total
Control Totals			
Experimental Totals			
Total			

Table 4. Formula of Observed Values (o)

	Interphase	Mitosis	Total
Control Totals	A	B	A+B
Experimental Totals	C	D	C+D
Total	A+C	B+D	A+B+C+D=N

Table 5. Formula of Expected Values (e)

	Interphase	Mitosis
Control Totals	$\frac{(A+B)(A+C)}{N}$	$\frac{(A+B)(B+D)}{N}$
Experimental Totals	$\frac{(C+D)(A+C)}{N}$	$\frac{(C+D)(B+D)}{N}$

Table 6. Calculation of Chi-Square Value

Group	Observed (o)	Expected (e)	(o-e)	$(o-e)^2$	$(o-e)^2/e$
Control Interphase					
Control Mitosis					
Experimental Interphase					
Experimental Mitosis					

Total of $(o - e)^2/e$ = chi-square ($\chi 2$) =_____

Statistical Analysis

The observed distribution of onion root tip cells in mitosis versus interphase for the treated samples will likely not coincide exactly with the percentages observed for the control group. The question, however, is whether the difference is statistically significant, that is, whether the observed difference in the percent mitosis for the two sets of samples may be due to chance. A chi-square ($\chi 2$) "goodness of fit" test is commonly used to determine whether a frequency distribution of results in various categories (in this case percent mitosis versus interphase) fits a predicted or expected distribution. Applying this statistical test to experimental results is done by formulating the so-called *null hypothesis* in which the observed distribution for the treated group can be described by the expected or control distribution.

The calculated chi-square value is then compared with a critical value (χ^2_c) that depends on two factors, the degrees of freedom (DF) for the distribution and the selected probability (*p*) for statistical significance (see Table 1). The degrees of freedom is equal to the number of categories for the results (*k*) minus one (DF = *k* – 1). The results in this experiment fall into two categories (percent mitosis and percent interphase), so DF = 1. The probability is usually selected at a 95% confidence level, p = 0.05, corresponding to a 5% probability that the observed difference is due to chance.

Table 7. Critical Values of the Chi-Square Distribution

	Degrees of Freedom (DF)				
Probability	**1**	**2**	**3**	**4**	**5**
0.1	2.71	4.61	6.25	7.78	9.24
0.05	3.84	5.99	7.82	9.49	11.1
0.01	6.64	9.21	11.3	13.2	15.1
0.001	10.8	13.8	16.3	18.5	20.5

If the calculated chi-square value is greater than the critical value obtained from the table for the degrees of freedom and the selected probability value, then the **null hypothesis is rejected** and the observed difference in percent mitosis or rate of mitosis between the treated sample and the control sample is considered statistically significant.

1. In terms of this part of the investigation, what does it mean if your null hypothesis is rejected?

2. Is your null hypothesis rejected? Explain why or why not.

Postlab Review

What was the importance of collecting the class data?

Was there a significant difference between the groups?

Did the IAA (experimental solution) increase the number of root tip cells in mitosis?

What other experiments should you perform to verify your findings?

Does an increased number of cells in mitosis mean that these cells are dividing faster than the cells in the roots with a lower number of cells in mitosis?

What other way could you determine how fast the rate of mitosis is occurring in root tips?

Create a graph that analyzes the data of the control root growth and the experimental root growth. Attach pgs. 6, and 9-13 on top of your graph and turn in when due.