INVESTIGATION 7

CELL DIVISION: MITOSIS AND MEIOSIS

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 (G_1), the cell grows and prepares to duplicate its DNA. In synthesis (S), the chromosomes are replicated; this stage is between G_1 and the second growth phase (G_2). In G_2, 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.

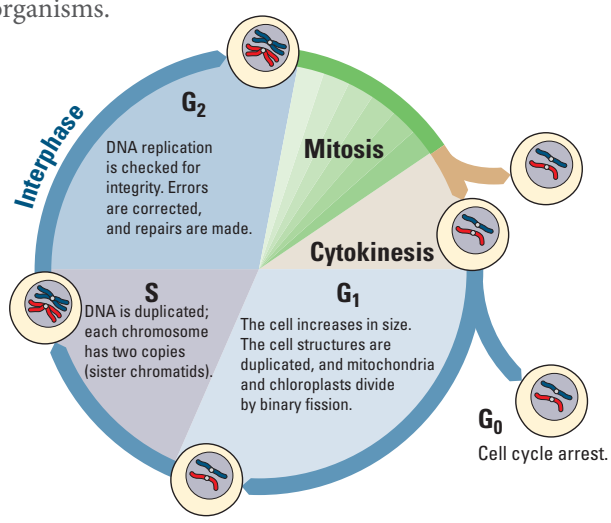

Figure 1. The Cell Cycle Showing G_1, S, and G_2 Phases, Mitosis, and Cytokinesis

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.

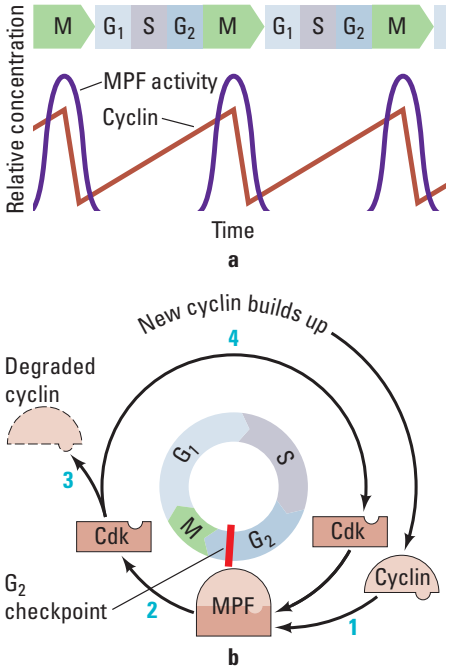

Figure 2. MPF Production During the Cell Cycle

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.

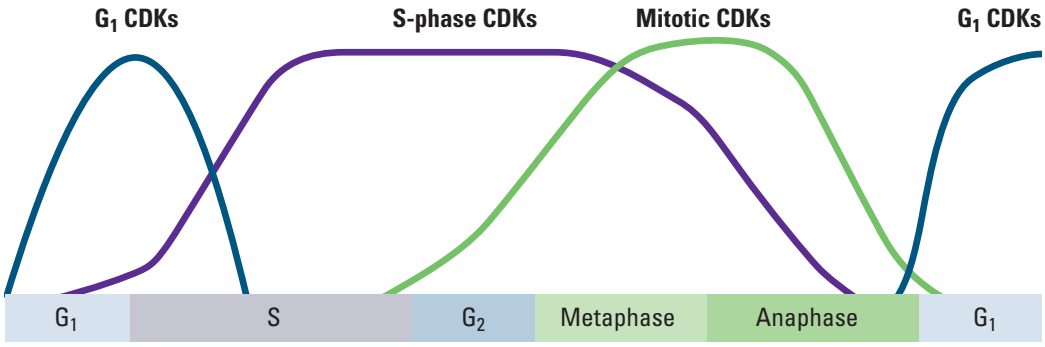

Figure 3. Levels of CDKs During the Cell Cycle

Cyclins and CDKs do not allow the cell to progress through its cycle automatically. There are three checkpoints a cell must pass through: the G_1 checkpoint, G_2 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 G_1 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 G_2 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.

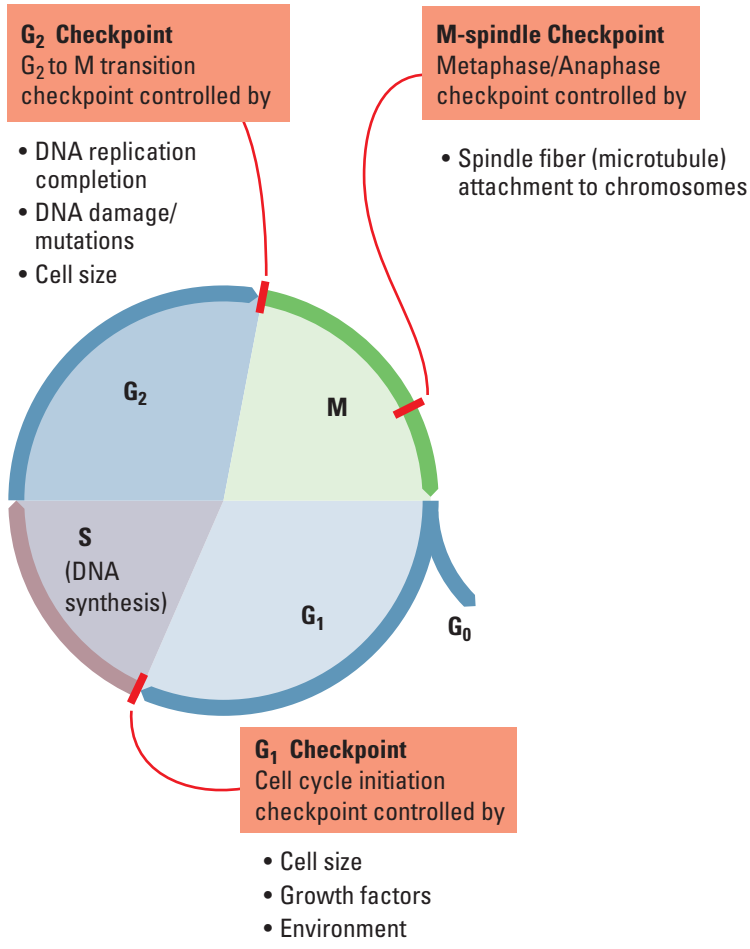

Figure 4. Diagram of the Cell Cycle Indicating the Checkpoints

Learning Objectives

- To describe the events in the cell cycle and how these events are controlled
- To explain how DNA is transmitted to the next generation via mitosis
- To explain how DNA is transmitted to the next generation via meiosis followed by fertilization
- To understand how meiosis and crossing over leads to increased genetic diversity, which is necessary for evolution

General Safety Precautions

You must be careful when preparing specimens for viewing under the compound microscope. Always cover the cover slip with a scientific cleaning wipe, such as a Kimwipe, and press down using a pencil eraser.

You should wear safety goggles or glasses and disposable gloves when handling the chemicals and razor blades in Parts 2 and 5. All materials should be disposed of properly as per your teacher's instructions.

THE INVESTIGATIONS

Getting Started

These questions are designed to see how well you understand and can explain the key concepts related to cell division before you begin your investigations.

1. 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?
2. How is cell division important to a single-celled organism?
3. What must happen to ensure successful cell division?
4. How does the genetic information in one of your body cells compare to that found in other body cells?
5. What are some advantages of asexual reproduction in plants?
6. Why is it important for DNA to be replicated prior to cell division?
7. How do chromosomes move inside a cell during cell division?
8. How is the cell cycle controlled? What would happen if the control were defective?

Procedures

Part 1: Modeling Mitosis

You will investigate mitosis using models. Your teacher will give you sockosomes, clay chromosomes, or pipe-cleaner chromosomes. or pop beads

Review chromosome duplication and movement using these model chromosomes. Think about these questions as you review the cell cycle and mitosis.

- If a cell contains a set of duplicated chromosomes, does it contain any more genetic information than the cell before the chromosomes were duplicated?
- What is the significance of the fact that chromosomes condense before they are moved?
- How are the chromosome copies, called sister chromatids, separated from each other?
- What would happen if the sister chromatids failed to separate?

Part 2: Effects of Environment on Mitosis

Scientists reported that a fungal pathogen, may negatively affect the growth of soybeans *(Glycine max)*. Soybean growth decreased during three years of high rainfall, and the soybean roots were poorly developed. Close relatives of *R. anaerobis* are plant pathogens and grow in the soil. A lectin-like protein was found in the soil around the soybean roots. This protein may have been secreted by the fungus. Lectins induce mitosis in some root apical meristem tissues. In many instances, rapid cell divisions weaken plant tissues.

You have been asked to investigate whether the fungal pathogen lectin affects the number of cells undergoing mitosis in a different plant, using root tips.

- What is your experimental hypothesis? Your null hypothesis? Are these the same?
- How would you design an experiment with onion bulbs to test whether lectins increase the number of cells in mitosis?
- What would you measure, and how would you measure it?
- What would be an appropriate control for your experiment?

Your teacher will provide you with untreated and lectin-exposed roots. You should be comfortable identifying cells in mitosis or in interphase before you begin examining the chromosome squashes.

Preparing Chromosome Squashes

1M HCl (correction 2013)

1. Place the onion root tip in 12 M HCl for 4 minutes.
2. Transfer the tip to Carnoy's fixative for 4 minutes.
3. Remove the slide from Coplin jar containing 70% ethanol, dry with a scientific cleaning wipe, and label it.
4. Place the tip on the slide and cut off the distal 2 mm portion of the tip; discard the remainder of the tip.
5. Cover the root tip piece with carbol-fuschin stain for 2 minutes.
6. Blot off excess stain and cover the tip with 1–2 drops of H_2O.
7. Place the cover slip over the tip and cover the cover slip with a scientific cleaning wipe.
8. Firmly press down on the cover slip with the eraser end of a pencil. Do not twist the slide, and be careful not to break the cover slip.

Counting Cells and Analyzing Data

1. Observe the cells at high magnification (400–500 X).
2. Look for well-stained, distinct cells.
3. Within the field of view, count the cells in each phase. Repeat the counts in two other root tips.
4. Collect the class data for each group, and calculate the mean and standard deviation for each group. You must make a table in your notebook for the class data.
5. Compare the number of cells from each group in interphase and in mitosis.

Table 1. Onion Root Tip Cell Phase Data; Treatment Group_____

Tip	Number of Cells		
	Interphase	Mitotic	Total
1			
2			
3			
Total			

There are 4 additional pages C & D that replace this procedure for Chi-square (pg 10-13 of this pdf)

1. For this experiment, the number of treated cells in interphase and mitosis will be the observed (o) values.
2. To find out what your expected values are, complete the following steps:
   a. Calculate the percentage of cells in interphase and mitosis in the *control* group from Table 1.
   b. Multiply the percentages by the total number of cells in the *treated* group; this will give the expected numbers (e).
3. Calculate the chi-square (χ^2) value for the test.
4. Compare this value to the critical value in Table 2.

Table 2. Critical Values of the Chi-Square Distribution

Probability	Degrees of Freedom (DF)				
	1	2	3	4	5
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

1. The degrees of freedom (df) equals the number of groups minus one. In this case, there are two groups, interphase and mitosis; therefore, df = 2-1, or 1.
2. The p value is 0.05, and the critical value is 3.84. If the calculated chi-square value is greater than or equal to this critical value, then the null hypothesis is rejected. If the calculated chi-square value is less than this critical value, the null hypothesis is not rejected. In terms of this part of the investigation, what does it mean if your null hypothesis is rejected?

Postlab Review

- What was the importance of collecting the class data?
- Was there a significant difference between the groups?
- Did the fungal pathogen lectin 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?

DESIGNING AND CONDUCTING YOUR INVESTIGATION

Now that you have worked with the root tip model system, design and conduct an investigation to determine what biotic or abiotic factors or substances in the environment might increase or decrease the rate of mitosis in roots. For instance, what factors in the soil might affect the rate of root growth and development? Consider, for example, abiotic soil factors such as salinity and pH or biotic factors, including roundworms, that might alter root growth.

Part 3: Loss of Cell Cycle Control in Cancer

Many of us have family members who have or have had cancer. Cancer can occur when cells lose control of their cell cycle and divide abnormally. This happens when tumor-suppressor genes, such as p53 or Rb (retinoblastoma), are mutated. There are many questions you should consider before beginning your investigation.

Review from Part 1

- How is the cell cycle controlled in normal cells?
- What are cyclins and cyclin-dependent kinases? What do these proteins do in a cell?

Prelab Questions for Part 3

- How are normal cells and cancer cells different from each other?
- What are the main causes of cancer?
- What goes wrong during the cell cycle in cancer cells?
- What makes some genes responsible for an increased risk of certain cancers?
- Do you think that the chromosomes might be different between normal and cancer cells?

The last question is the focus of this part of the lab. With your group, form a hypothesis as to how the chromosomes of a cancer cell might appear in comparison to a normal cell and how those differences are related to the behavior of the cancer cell.

For each of the following cases, look at pictures of the chromosomes (karyotype) from normal human cells. Compare them to pictures of the chromosomes from cancer cells. For each case, count the number of chromosomes in each type of cell, and discuss their appearance. Then answer the following questions.

- Do your observations support your hypothesis?
- If not, what type of information might you need to know in order to understand your observations?
- If yes, what type of information can you find that would validate your conclusions?

Case 1: HeLa cells

HeLa cells are cervical cancer cells isolated from a woman named Henrietta Lacks. Her cells have been cultured since 1951 and used in numerous scientific experiments. Henrietta Lacks died from her cancer not long after her cells were isolated. Lacks's cancer cells contain remnants of human papillomavirus (HPV), which we now know increases the risk of cervical cancer.

- From your observations, what went wrong in Henrietta Lacks's cervical cells that made them cancerous?
- How does infection with human papillomavirus virus (HPV) increase the risk of cervical cancer?

Your teacher may ask you to read *The Immortal Life of Henrietta Lacks* by Rebecca Skloot. As you read it, think about the following questions:

- Should tissue be removed from a patient without his or her consent for research?
- How was the HeLa cell line cultured?
- What virus infected Henrietta Lacks and may have caused her cervical cancer? What cellular process is affected by this virus?
- Was there bias in the way Henrietta Lacks was treated at Johns Hopkins?
- Put the use of HeLa cells on trial. Debate what is more important: an individual's rights to his/her own body tissues or the medical knowledge gained by studying a patient's tissues?
- Should Henrietta Lacks's family be compensated for the discoveries made using her cells?
- Do companies or universities have the right to patent discoveries made using a patient's tissues or genes without consulting the patient?
- What other legal and ethical questions are raised in this book?

Case 2: Philadelphia Chromosomes

In normal cells, mitosis usually is blocked if there is DNA damage. Sometimes, though, DNA damage makes cells divide more often. Certain forms of leukemia have a unique feature called a Philadelphia chromosome. Look at the karyotype of leukemia cells in Figure 5, and answer the following questions:

- What happens in a normal cell if the DNA has mutations?
- What would happen if cells with mutated DNA replicated?
- How do cells monitor DNA integrity?
- How are the chromosomes different in the cancer cells compared to normal cells?
- How could these differences lead to cancer?

Table 2. Table of Observed Values (o)

	Interphase	Mitosis	Total
Control	A	B	A + B
Treated	C	D	C + D
Total	A + C	B + D	A + B + C + D = N

1. Collect the class data and enter the values into Table 1; these are the observed values for the four groups.
2. Use the data from Table 1 to calculate the totals using the formulas found in Table 2. (For example, A equals the number of interphase cells in the control group.)
3. Use the totals from Table 2 to calculate the expected values (e) using the formulas from Table 3.
4. Enter the observed values (o) from Table 2 and expected values (e) from Table 3 for each group into Table 4. Calculate the chi-square (χ^2) value for the data by adding together the numbers in the right column.
5. Compare this value to the critical value in Table 5.

Table 3. Table of Expected Values (e)

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

Table 4. Calculation of Chi-Square Value

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

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

Table 5. Critical Values of the Chi-Square Distribution

Probability	Degrees of Freedom (DF)				
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0.01	6.64	9.21	11.3	13.2	15.1
0.001	10.8	13.8	16.3	18.5	20.5

1. The degrees of freedom (df) equals the number of treatment groups minus one multiplied by the number of phase groups minus one. In this case, there are two treatment groups (control, treated) and two phase groups (interphase, mitosis); therefore df = (2 - 1) (2 - 1) = 1.
2. The ρ value is 0.05, and the critical value is 3.84. If the calculated chi-square value is greater than or equal to this critical value, then the null hypothesis is rejected. If the calculated chi-square value is less than this critical value, the null hypothesis is not rejected.

SAMPLE DATA

Sample Table 2: Table of Observed Values (o)

	Interphase	Mitosis	Total
Control	148	25	173
Treated	161	88	249
Total	309	113	422

Sample Table 3: Table of Expected Values (e)

	Interphase	Mitosis
Control	127	46
Treated	179	67

Sample Table 4: Calculation of Chi-Square Value

Group	Observed (o)	Expected (e)	(o - e)	$(o - e)^2$	$(o - e)^2/e$
Control Interphase	148	127	21	441	3.47
Control Mitosis	25	46	-21	441	9.59
Treated Interphase	161	182	-21	441	2.42
Treated Mitosis	88	67	21	441	6.58

Total of $(o - e)^2/e$ = chi-square (χ^2) = 22.06

Since the calculated χ^2 is greater than the table value, the null hypothesis (treatment has no effect) is rejected.

Table 2. Table of Observed Values (o)

	Interphase	Mitosis	Total
Control	A	B	A + B
Treated	C	D	C + D
Total	A + C	B + D	A + B + C + D = N

1. Collect the class data and enter the values into Table 1; these are the observed values for the four groups.
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Table 3. Table of Expected Values (e)

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

Table 4. Calculation of Chi-Square Value

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

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

Table 5. Critical Values of the Chi-Square Distribution

Probability	Degrees of Freedom (DF)				
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