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.

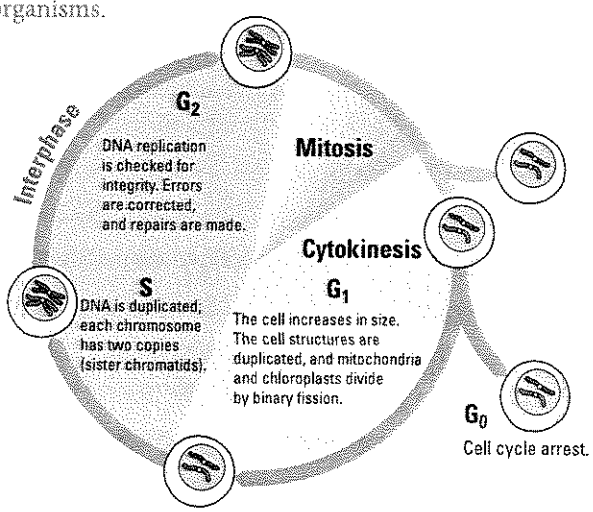

Figure 1. The Cell Cycle Showing G_1, S, and G_2 Phases, Mitosis, and Cytokinesis

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.

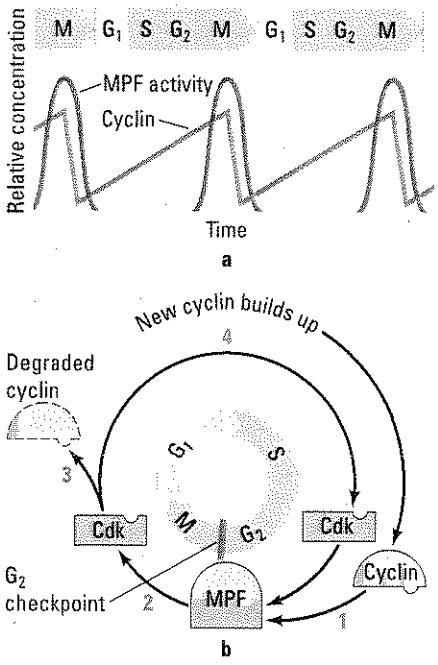

Figure 2. MPF Production During the Cell Cycle

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.

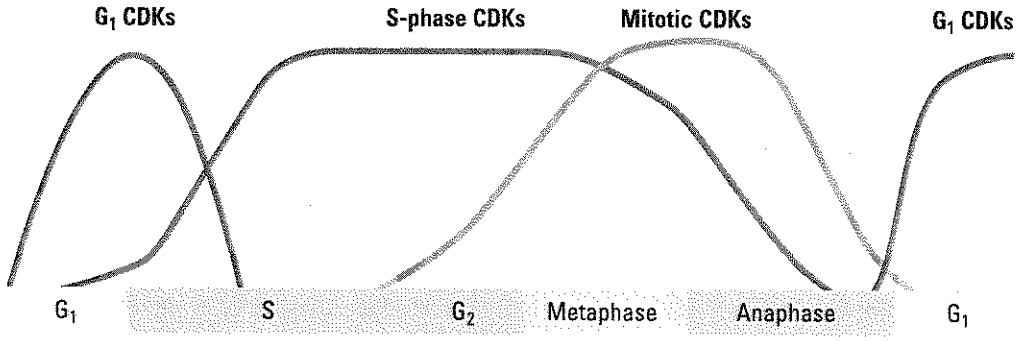

Figure 3. Levels of CDKs During the Cell Cycle

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.

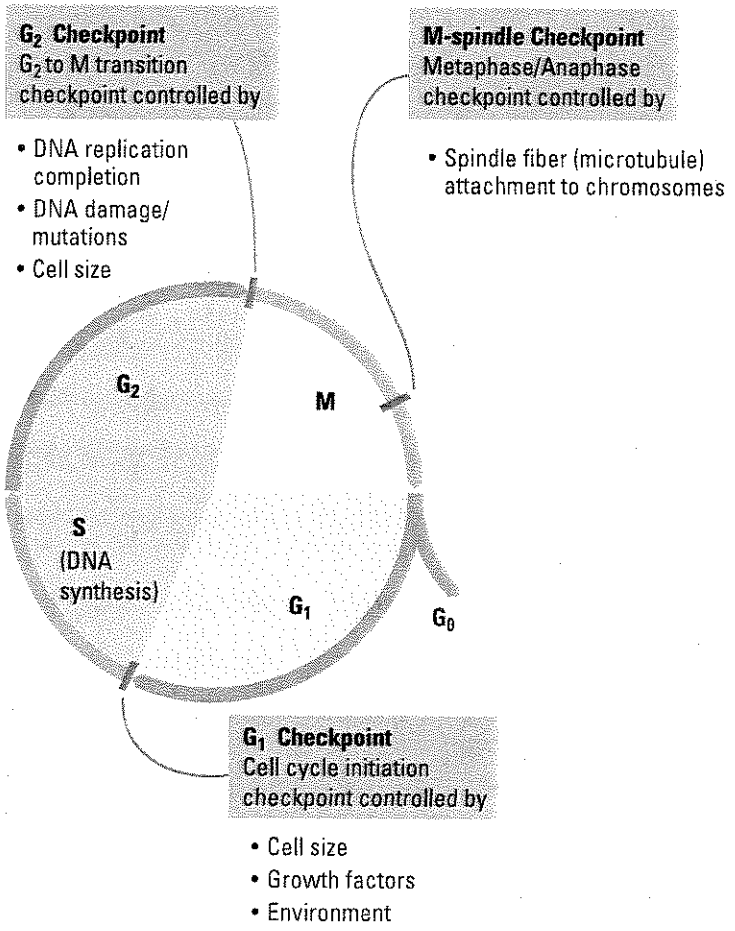

Figure 4. Diagram of the Cell Cycle Indicating the Checkpoints

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?

In 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. Therefore, we will examine the tips of growing plants such as the onion root tip.

Procedure

Examine prepared slides of longitudinal sections of onion root tips using the 10X objective. Locate the meristematic region near the root cap. Using the 40X objective, examine individual cells in this region to study the phases of plant mitosis. Use the pictures in your textbook for assistance in locating the stages described below:

1. **Interphase** - These are cells that are not dividing. The nucleus will be distinct and may have one or more dark-stained nucleoli.

2. **Prophase** - The first sign of division is the thickening of the chromatin threads inside the nucleus. The thickening continues until the chromatin has condensed into the chromosomes. The appearance of the nucleus is variable during the chromatin thickening process.

3. **Prometaphase** the nuclear envelope and nucleoli are no longer visible and the chromosomes are free in the cytoplasm. At this time, the spindle apparatus may become visible, but stains are needed to make them apparent.

4. **Metaphase** - The chromosomes have moved to the center of the spindle, usually in the middle of the cell. One portion of each chromosome, the centromere, attaches 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 may be able to observe the two chromatids of some of the chromosomes.

5. **Anaphase** - The centromere regions of each pair of chromatids separate and are moved by the spindle fibers toward opposite poles of the spindle. The ends of the chromosomes are dragged behind, creating a series of "v-shaped" structures. Once the duplicate chromatids separate, each is called a chromosome. The daughter chromosomes continue poleward movement until they form two compact clumps, one at each spindle body.

6. **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 uncoil back to their chromatin form. In plants, cytokinesis is accomplished when a new cell wall (cell plate) is laid down between the daughter cells, forming two new cells.

Analysis of Results – for the Lab Report

Make a drawing of a representative cell at each phase of mitosis and combine the drawings into a "foldable". Label the parts (nucleus, cell plate, spindle etc.) and the give the magnification used to make the drawing. Make the drawing large enough so that sufficient detail can be included. Use colored pencils, if desired, to give additional detail to the drawings.

Relative Lengths of Mitotic Stages

Procedure

To estimate the relative length of the time that a cell spends in the various stages of mitosis, you will examine the meristematic region of a prepared slide of the onion root tip. Since these cells

represent a "one point in time" sample, the number of cells in a particular phase of mitosis will approximate the amount of time an individual cell might spend in that mitotic phase.
Students should work individually or in pairs on the following steps:

1. Using the low-power objective (10X), locate the meristematic region. Shift to the higher-power objective (40X) and tabulate the number of cells that are in each stage of mitosis. Record the data in Table 1.

2. Repeat this count in at least two more non-overlapping fields of view.

3. Calculate the percentage of total cells represented by each stage of mitosis.

Table 1. Mitosis Phase Counts.

	Field 1	Field 2	Field 3	Total	% of total cells counted
Prophase					
Metaphase					
Anaphase					
Telophase					

Total Cells Counted = _____

For the lab report

1. Based on the data in Table 1, what can you infer about the relative length of time an onion root tip cell spends in each stage of mitosis? Explain.

2. Compare the number of cells in the various phases of mitosis to the number of cells observed in interphase. What can you infer about the relative length of time an onion root tip cell spends in interphase? Explain.

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?

INQUIRY BASED/ DESIGNED *

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?