Mitosis and the Cell Cycle

Introduction:

The cell theory states that "all cells come from preexisting cells" by the process of cell reproduction, or cell division. Cell division is the process by which all the cells of a multicellular organism are formed during growth and development, and how many single celled organisms reproduce themselves. In multicellular organisms, cell division is responsible for repair and replacement of cells and tissues during one's lifetime. Asexual reproduction, a means of making more individuals, is also accomplished by cell division.

We know that all cells of an individual have exactly the same DNA in their chromosomes, and that each species has a fixed chromosome number, a number that does not change from generation to generation.

To ensure that chromosomes and DNA remain the same in new cells when cells divide, it is crucial to have a mechanism that exactly replicates (duplicates) the DNA of the original cell and distributes the copied DNA equally to the new cells. Mitosis is the process by which the replicated chromosomes are equally distributed to new nuclei. To form new cells, we must also separate the cytoplasm and critical organelles, such as mitochondria and chloroplasts, of the original cell into the new cells formed. The distribution of the cytoplasm of the original cell into new cells is called cytokinesis.

Mitosis and cytokinesis are a part of the cell cycle that starts when a cell is formed and continues until it divides. Some cells never divide, others are specialized to divide (especially in plants, where virtually all cell division occurs in specialized tissue called meristem). Cell division is a brief part of the life cycle; most of the life of a cell is spent in normal activities of growth and maintenance.

The cell cycle involves the following:

Interphase
The period of time when a cell undergoes its normal activities, including
- Growth (called G1 or First Gap in the cell cycle)
- DNA Replication (called S for synthesis in the cell cycle)
- Preparation for Division (called G2)

Cell Division (or cell reproduction), which includes
- Mitosis, with
  - Prophase
  - Metaphase
  - Anaphase
  - Telophase

Cytokinesis
Cell reproduction in eukaryotes involves three events:

1. **DNA Replication**
   - Process of replicating the genetic material of the nucleus.
   - This occurs during **Interphase**, when growth and/or normal metabolic activities take place.

2. **Mitosis**
   - Process of distributing the replicated DNA equally to the two new nuclei.

3. **Cytokinesis**
   - Process of separating the cytoplasm contents

In this laboratory you will have the opportunity to look for mitosis in prepared slides of mitosis in the onion (*Allium cepa*) root tip.

**Exercise I Mitosis in Allium (onion) Root Tip Meristem**

Cell division in plants occurs in regions called meristems. Primary meristems are found in the growing tips of shoots and roots. Many plant cells have the ability to become meristems. For example, when you do a stem cutting, certain cells of the stem dedifferentiate (become unspecialized) to become root meristem for the formation of new roots.

**Materials Needed**
- Compound Microscope
- Prepared slides of *Allium* root tip mitosis
- Blank paper for drawings and a sharp pencil

Although mitosis is separated into phases for the convenience of discussion, you should remember that the process is a continuous one and the separations are arbitrary. You may see many cells in some point intermediate between the phases that you expect to see.

**Procedure**

1. Obtain a slide of *Allium* root tip. The root tip has been cut longitudinally. Each slide should have about 3 root tip slices.
2. Locate the meristem region with the scanning (4x) objective to locate the correct location of the meristem (near the tip). Continue to scan the meristem region, with the 10x objective. The mitotic figures will be too small to distinguish detail at this magnification, although each slide should have several cells in each of the mitotic phases. Which phase of the cell cycle is most abundant in the root tip? Why is this so?
3. When you find a promising cell for the mitotic phase you are planning to observe, center it in the field of view and rotate your 40x objective into position to observe the detail of the mitotic figures.
4. Locate cells that are good representatives of each of the phases of mitosis described below. Compare the phases of mitosis that you find in the microscope with those illustrated below.

The Phases of Mitosis and the Cell Cycle

Interphase
Most of the cells of the meristem will be in interphase. The granular chromatin material in the nucleus is distinctive although no individual chromosomes are visible. You may also see nucleoli. DNA replication occurs during interphase. What other cellular events are associated with interphase?

Prophase
Replicated chromosomes start to condense from the diffuse chromatin and become visible as threadlike structures. Each replicated chromosome is composed of its two identical replicas (called chromatids) held together at their centromere. Replicated chromosomes continue to condense and become thicker as prophase progresses. The nucleolus region (an aggregation of chromosome bits and concentrated RNA and protein) of the nucleus will start to disassemble. The replicated chromosomes are firmly attached at their centromeres throughout this condensation and coiling.

Microtubules and associated proteins initiate spindle formation during prophase. The spindle apparatus originates from a microtubule organizing center, also called the centrosome. The centrosome is self-replicating and replicates during interphase. In animal cells, centrioles are found within the centrosome, but not in cells of higher plants, including the onion. Microtubules radiating from the centrosomes are called asters. By the end of prophase, the spindle apparatus will extend from the poles of the cell through the center of the cell to the opposite pole of the cell. Some microtubules from each pole of the cell attach to a protein structure, called the kinetochore, located in the centromere region of each replicated chromosome.

The nuclear membrane degrades in late prophase) into small vesicles, used to synthesize new nuclear membrane material in the new cells.

Metaphase
The spindle apparatus has moved the chromosomes to the equator of the cell, aligning the centromeres of each replicated chromosome along the equator. Centromeres of each sister chromatid are aligned with each other and each sister chromatid is connected at its kinetochore to a microtubule. This alignment of chromosomes along the equatorial plane of the cell is often called the metaphase plate, and is the distinctive feature of metaphase.

Anaphase
Centromeres of each replicated chromosome separate to start anaphase. (This also signals that metaphase has been completed.) You can't actually see this; the separating chromosomes are the first visual sign of anaphase. By definition, each sister chromatid is now a single unreplicated chromosome. Since sister chromatids are identical, each of the two clusters of chromosomes being pulled to the two poles of the cell has one copy of each original chromosome.

Microtubules from each pole pull the chromosomes away from each other and toward the respective poles of the cell. The microtubules attach at the centromere region of the chromosomes so that chromosomes are pulled centromere first towards the poles of the cell. As the chromosomes are pulled to the poles, they begin to lengthen out.

Telophase
Membrane vesicles and membrane fragments form new nuclear membranes around each group of separated chromosomes at the poles of the cell. Chromosomes stretch back out and become indistinct as chromatin. The spindle microtubules disperse and the spindle apparatus disappears. New nucleoli form. At the completion of telophase, there will be two new nuclei, each identical to the original nucleus.
Cytokinesis: Separation of the Cytoplasmic Contents
Mitosis describes events of chromosomes and nuclei. Most cells accompany mitosis with cytokinesis, the separation of the cytoplasm of the original cell into two new cells. Cytokinesis coincides with the events of telophase or occurs immediately after, so that at the completion of mitosis, the original cell is separated into two cells, each with a nucleus and DNA identical to that of the original cell.

Cytokinesis in Plant Cells
Each cell of a plant is surrounded by a rigid cell wall. Cytokinesis in plant cells requires synthesis of new wall material. This process is called cell plate formation. Cell plate formation involves making a cross wall at the equator of the original cell. Golgi vesicles containing wall material fuse along microtubules forming a disk-like structure that is called the cell plate. As cellulose and other fibers are deposited, the cell plate is formed creating a boundary and new cell wall between the two new cells. Membrane material from the original cell fuses to each side of the cell plate forming new cell membranes on the dividing sides of the original cell into the two new cells.

Look for the formation of the "cell plate" in the telophase cells of your onion root tip that signals the start of the new cell wall separating the daughter cells.

Before proceeding to the next exercise be sure that you can recognize each of the phases of mitosis in the onion root tip.
Cytokinesis in Animal Cells

Cytokinesis in animal cells starts with the formation of a **cleavage furrow**, a depression or pinching in of the plasma membrane, caused by a ring of microfilaments (the **contractile ring**), which forms across the middle of the cell after the chromatids are separated in anaphase. This ring contracts, pinching the membrane toward the center of the cell. This eventually pinches the cell in two.

Exercise II - Relative Time Spent in Cell Cycle Phases

You can estimate the relative length of time each phase of the cell cycle takes by recording the frequency with which you find each phase in meristem regions where cell division is occurring. Using high power, find an area with 10 x 10 cells – 100 cells in total. Record how many cells of each cell cycle phase you see in Table 1. Repeat this with two more different slides (views) of Allium root tip.
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<thead>
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<th>Cell Cycle Phase</th>
<th>View 1</th>
<th>View 2</th>
<th>View 3</th>
<th>Total</th>
<th>Percent of Total</th>
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<tr>
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<td>Anaphase</td>
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<td>Telophase</td>
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<td><strong>Grand Total</strong></td>
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Questions:

1: Which phase appears to take the longest time to complete? Why might this be so?

2: Why were root tips and not some other part of the onion selected for this activity?

3: Normal cells from a chicken’s stomach complete a full mitotic division in about 625 minutes. Cancerous stomach cells require only 450 minutes (the time spent in interphase and prophase is greatly reduced). What is the significance of these findings.