Mitosis and Meiosis Simulation Lab

**Background: Mitosis**

With the exception of the original cells on Earth, all new cells come from previously existing cells. New cells are formed by the process of cell division which involves both replication of the cell's nucleus (*karyokinesis*) and division of the cytoplasm (*cytokinesis*). It was discovered in 1858, by Rudolf Virchow, that new cells can only arise from previously existing cells. This is done in two ways: mitosis and meiosis. Somatic (body) cells divide exclusively through mitosis and cytokinesis, while germ cells produce gametes through meiosis. Mitosis typically results in new body cells with the same complement of chromosomes as the parent cell (2n). Plant cells simply enlarge, essentially by absorbing water. When they reach a certain size, they divide, forming two identical daughter cells. The various parts of the cell are divided in such a way that the new daughter cell is identical to the parent cell.

Strictly speaking, mitosis implies only the division of the nucleus, and is therefore distinct from cell division, in which the cytoplasm is divided. In most organisms, cells divide by the ingrowth of the cell wall, if present, and the contraction of the plasma membrane, which cuts through the spindle fibers. In land plants (bryophytes and vascular plants) and a few algae, cell division takes place when a cell wall forms. Small droplets appear across the equatorial plate of the cell and gradually fuse, forming a disc that grows outward until it reaches the wall of the dividing cell, completing the separation of the two daughter cells.

The circular DNA of prokaryotes is simply replicated before division. In eukaryotes, however, the hereditary material is part of their complex chromosomes. Equal division of this material requires a more complicated method in order for the chromosomes to be replicated, separated, and apportioned precisely between the daughter cells.

Mitosis, or nuclear division, is this complicated process that ensures the equal division of the nuclear material between the daughter cells in eukaryotic organisms. During mitosis the chromosomes appear as long, slender threads; they become shorter and shorter as their coiling tightens, and move to the center of the cell where they fully contract. They then split longitudinally into two identical halves that appear to be pulled to opposite poles of the cell by a series of microtubules. In these two genetically identical groups, the coiling of the chromosomes relaxes again, and they are reconstituted into the nuclei of the two daughter cells. The cell cycle is a continuous process that can be divided into two major phases: interphase, and mitosis. Within mitosis are four distinct smaller phases, known as: prophase, metaphase, anaphase, and telophase.

Where does one find cells undergoing mitosis? Plants and animals differ in this respect. In higher plants the process of forming new cells is restricted to special growing regions called meristems. These regions usually occur at the tips of stems or roots. In animals, cell division occurs anywhere new cells are formed or as new cells replace old ones. However, some tissues in both plant and animals rarely divide once the organism is mature.

**Interphase**

Interphase is generally considered to be a resting phase. Interphase is made up of three phases. G1, S, and G2. As you will see, the cell is hardly at rest during interphase, as a great deal is going on.

- **G1**: is usually the longest phase of the cell cycle. The cell is recovering from the cell division; it will usually double the number of organelles and in overall size, while it performs its assigned functions.
- The S phase is also called the synthesis phase. During this phase each chromosome is duplicated, resulting in a doubled chromosome consisting of two chromatids attached at a centromere.
- G2: is the final stage of interphase, and the cell is preparing for nuclear division. The cell synthesizes proteins and enzymes needed for cell division.

Mitosis:
- **Prophase:** Just prior to mitosis, the pair of centrioles duplicates. During prophase, the two pairs of centrioles migrate to opposite poles. Centrioles form spindle fibers, which become microtubules and eventually attach to the centromere. Additional fibers known as asters also radiate outward from the centrioles. The chromosomes first become visible, starting out as long threads in the nucleus and condense, becoming shorter and thicker. Each chromosome is composed of two longitudinal halves, called chromatids, joined in a narrow area known as the centromere, where the chromatids are not coiled. The centromere divides the chromosomes into two arms of varying lengths.

- **Metaphase:** The spindle fibers enter the nuclear region, extend from the centrioles to the centromere, and attach at a point known as the kinetochore. Once the spindle fibers are attached, they align the centromeres along the equatorial region of the cell known as the metaphase plate, so that the arms of the chromosomes point towards the poles of the cell.

- **Anaphase:** The centromere divides and the two chromatids separate from each other, forming two identical daughter chromosomes. The spindle fibers attach to the centromere and pull the newly-divided chromosomes towards the poles and away from the metaphase plate. The spindle fibers appear to move, but in fact, the microtubules are continuously formed at one end of the spindle fiber and then disassembled at the other.

- **Telophase:** After the chromosomes reach the poles, a nuclear membrane forms around each set of daughter nuclei and the chromosomes uncoil and elongate, once again becoming invisible. The spindle fibers break down and disappear. In animal cells, a cleavage furrow, an indentation in the cell membrane between the daughter nuclei begins to develop. This marks the end of mitosis. In plant cells, a cell plate forms dividing the two daughter cells.
Cytokinesis:
- As mitosis ends, cytokinesis begins, resulting in the formation of two daughter cells. The cleaved membrane mentioned above in telophase slowly draws together, forming a narrow bridge, then separates the cell into two daughter cells. The cells now enter interphase of the next cycle.

Mitosis ends when the processes are complete and the chromosomes have once more disappeared from view. The two daughter cells enter interphase. The daughter nuclei produced are identical to one another and to the nucleus that divided to produce them.

In order to investigate the process of mitosis, plant and animal tissues where cells are dividing rapidly must be examined. In animals, the most rapidly growing and dividing tissues are found in the embryonic stages of development. Although most animal tissues continue to undergo mitosis throughout the life cycle of the organism, they do so very slowly when compared to their embryos. Some animal cells, like most plant tissues, rarely replicate after the organism reaches maturity.

In plants, these tissues are primarily found in the tips of stems and roots. The root tips of plants are exceptionally good places to look for cells undergoing mitosis. Plant root tips consist of several different zones where various developmental and functional processes of the root are performed. The primary region for the formation of new cells is the apical meristem. The root cap offers protection for the rest of the root, the region of elongation is the area where the bulk of cell growth occurs, and the region of maturation is where tissue differentiation occurs.

Meiosis

There are two types of nuclear division: mitosis and meiosis. Mitosis typically results in new somatic (body) cells, with the same complement of chromosomes as the parent cell (2n). Formation of an adult organism from a fertilized egg, asexual reproduction, regeneration, and maintenance or repair of body parts are accomplished through mitotic cell division. Meiosis results in the formation of either gametes (in animals) or spores (in plants). These cells have half the chromosome number of the parent cell (n).

Sexual reproduction provides a mechanism to produce genetic variation, since the genes of two different individuals can be arranged in various ways. This requires that the chromosome number of the parent cell, normally diploid, be reduced to half that, to create a haploid cell. The type of cell division resulting in a haploid parent cell is called meiosis.

In meiosis, a germ cell divides into four haploid gametes. When two gametes—an egg and a sperm for most animals—combine during fertilization, forming a zygote, the diploid chromosome number is restored. Meiosis consists of one DNA replication and two nuclear divisions, meiosis I and II. This results in the formation of four daughter cells, each with only half the number of chromosomes as the parent.

Genetic variability is further increased by a process called crossing over. In the early stages of meiosis, the homologous pairs of chromosomes move close together in such a way that all four chromatids are entwined, forming a tetrad. This process, known as synapsis, allows for the exchange of chromosome sections between the homologous pairs.
Lab Objectives:
Before doing this lab you should at least have a basic understanding of
- The different stages of cell cycles
- The significance of cell cycles to living organisms
- How to use a compound light microscope, and determine magnification
After doing this lab you should
- Have a deeper understanding of the processes of the cell cycle
- Be able to calculate exactly how long each phase of the cell cycle takes in onion meristematic tissue

Materials for Mitosis Simulation Lab
Each group should have:
- About 60-100 beads of each color (red and yellow)
- 4 magnetic centromeres
- 2 centrioles
- 2 plastic zipper-lock baggies
- 4 pieces of string about 30” long (75 cm)
- Onion root tip slides
- Compound light microscope

Part A: Mitotic Cell Cycle
Throughout this activity, you will be completing a simulation of the process of the Mitotic Cell Cycle. Do each successive task in order, and answer the questions in your as you go. The questions are italicized, and highlighted in bold font.

1. Gather all materials needed
2. Assemble two units (one of each color), such as the illustration here shows. Make sure they are different not only in color, but in size as well. Don’t make them HUGE!
3. Join the identical units together in the middle with the magnetic centromeres, as shown. These will, of course simulate the two chromosomes (four sister chromatids) that you’ll be using to illustrate mitosis. The cells of the organism you are simulating mitosis in, contain 2 chromosomes.
4. Place them in a zipper-seal bag, which will represent the nuclear membrane, containing the chromosomes.

To simulate Interphase:
1. Create duplicates of each of your two chromosomes. They should be identical to each of the ones you previously created in every way.
2. Place them inside the baggie with the other ones.
   **Question A: What stage of interphase does #2 above represent?**
3. Place the baggie near the center of your work space, and position the two centrioles near the nucleus at right angles to each other (see illustration) Remember, at this point the chromosomes are not condensed, so look like a jumbled mess.
   At this point, we will NOT simulate the plasma membrane of this organism.
To simulate Mitosis/Prophase:
1. Separate the two centrioles, and position them to each side of the baggie/nucleus as illustrated to the right, pointing toward the nucleus.
2. Dump the four chromosomes out of the baggie/nucleus and set the bag aside.
3. Tie a string to EACH individual centromere, (magnets connecting sister chromatids) and allow the other ends of the string to be positioned at the centrioles.

*Question B: What do the strings from #3 above represent?*

To simulate Mitosis/Metaphase:
1. Pull gently on the strings to align the chromosomes centrally between the centrioles, end to end. They can be randomly positioned, with no worries about red/yellow combinations. See illustration to the right. The other ends of the strings should still be positioned by their associated centriole. (also illustrated at right) Remember, the illustration only shows TWO chromosomes. Your simulation will have FOUR, because they chromosomes duplicated!

To simulate Mitosis/Anaphase:
1. Pull gently on the strings until the centromeres are separated. When the centromeres separate, the chromatids become daughter chromosomes. Continue pulling the chromosomes toward the centrioles. See figure below and to the right:

To simulate Mitosis/Telophase:
1. Remove the strings. Pile the two chromosomes together near each centriole. A nuclear membrane would now begin to form and division of the cytoplasm would occur, completing cell division. (see below)

Repeat as necessary several times, until you are familiar with the major events of mitosis.

**Part B: Determining the time for Mitosis**

For this procedure, we will use permanently mounted slides of onion roots. These slides are prepared by slicing the roots into thin sections, mounting them on microscope slides, staining, and then mounting under a cover slip.

**Procedure:**

Locate the meristem region of the root tip (this would be the area actively dividing as it is growing down into the soil) under the compound light microscope.
1. Starting with the 10x objective, find the region of active cell division (meristem region)
2. Switch to the 40X objective and begin making observations at the lower end of this region.
Recording data
Take turns being the observer and the recorder. The observer should call out the stage of mitosis of each cell to be tallied by the recorder in the results table. Roles should be switched for the second slide. Since prophase and prometaphase are difficult to distinguish, classify these cells as prophase. **Only count as prophase those cells that contain distinctly visible chromosomes.**

1. Systematically scan the root tip upward and downward through a column of cells.
2. Tally each cell in a stage of the cell cycle that you observe, being careful not to record the same cell twice. Tally the stages of 100 cells.
3. Tally the numbers in the table below. Each group member should tally cells from a different slide or a totally different part of the slide.

Calculations
1. Pool your data with that of the class, and record the class totals in the table provided below.
2. Calculate the percentage of cells in each stage
3. The relative time span of each stage is equivalent to the percentage of cells found in that stage.

Results
Onion root tip squash

1. Find and draw a cell showing each stage of the cell cycle. Below your diagram, indicate the distinguishing features of each stage of the cell cycle.

<table>
<thead>
<tr>
<th>Interphase</th>
<th>Prophase</th>
<th>Metaphase</th>
<th>Anaphase</th>
<th>Telophase</th>
</tr>
</thead>
</table>

Relative length of stages of cell cycle
2. Tally the results of your cell counts and then calculate percentages.

<table>
<thead>
<tr>
<th></th>
<th>Your Tally</th>
<th>Class Totals</th>
<th>Percent of Total (class)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prophase</td>
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<tr>
<td>Telophase</td>
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<td></td>
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<tr>
<td>Totals</td>
<td></td>
<td></td>
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</tbody>
</table>
3. Based upon the class results, order the stages of mitosis from shortest (1) to longest (4). After the longest and shortest stage, give a brief explanation of why that stage may have that time period.

   Prophase
   Metaphase
   Anaphase
   Telophase

4. Many of the cells of the root meristem region are not undergoing mitosis, rather they are in a stage called ________________. Based upon the interpretations made above, this stage appears to be much _____________ (shorter/longer) than mitosis. What processes occur in this stage prior to the onset of mitosis?

5. What stage were the majority of the cells in?

6. What evidence in this lab shows that mitosis is a continuous process, not a series of separate events?

7. The onion plant began as a single cell. The cell had X number of chromosomes (the exact number does not matter, we will just call that number “X”). How many chromosomes are in each of the cells that you observed? (give the answer in terms of X). How do you know?
Part C: Meiotic Cell Cycle
Throughout this activity, you will be completing a simulation of the process of meiosis. Do each successive task in order, and answer the questions as you go. The questions are italicized, and highlighted in bold font.

**Question A:** What important step happens during the “S” phase of interphase?

Meiosis I
To simulate Interphase:
1. Construct two strands of seven red pop beads and attach each strand to a red centromere. Repeat with two strands of seven yellow pop beads and a yellow centromere.
2. Cut a long piece of yarn to place around your chromosomes.

**Question B:** What structure does this yarn represent?

3. Place the chromosomes in the center of the yarn circle
4. DNA replication occurs, producing a duplicate of each chromosome. Construct two strands identical to the ones you made previously. Each half of the duplicated chromosome is called a chromatid. Join both red chromatids at the centromere to form a pair of sister chromatids. Repeat for the yellow chromosome.
5. Place a pair of clear plastic centrioles at ninety degree angles, just outside of your nuclear membrane. The centrioles also replicate during interphase so place another pair next to them in your cell. It may be helpful to tape your centrioles together during the exercise

Prophase I
1. A process called synapsis occurs in which homologous chromosomes move close together and pair up along their entire length. A tetrad (group of four chromatids) is formed. Centrioles move to opposite poles of the cell and the nuclear membrane begins to break down. (Simulate by removing yarn barrier)
2. Align your homologous chromosomes and entwine them in the center of the nucleus.
3. Simulate crossing over and chiasmata by removing yellow pop beads from area beyond the chiasmata of one homologous chromatid, and replace with the non-sister chromatid’s corresponding red pop beads. Replace the missing red beads at the end of the red chromosome with the yellow beads.

**Question C:** What happened here that wouldn’t happen in prophase of mitosis?

Metaphase I
Chromosomes disentangle and become aligned in the center of the cell in homologous pairs.
1. Disentangle your chromosomes, and align them side by side in the center of the cell in homologous pairs.

**Question D:** How would metaphase of mitosis look different?

Anaphase I
The homologous chromosomes separate and are drawn to opposite sides of the cell by spindle fibers connected from the centriole at the pole, to the centromere joining the sister chromatids.
1. Move each homologous pair toward its respective centriole. Move the chromosome pairs by the centromere, noting how the chromosome arms trail the centromere as movement occurs.

**Question E:** How would this look different in anaphase of mitosis?
Telophase I
During meiosis, cell division occurs and centrioles replicate, resulting in two daughter cells still containing paired chromatids.

*Question F: The chromatid sisters may be different from the chromatids in the parent cell. Why?*

1. Keeping each paired strand near its respective centrioles, create two cell membranes around your paired strands using the string (not the nuclear membrane, but the plasma membrane) representing the two separate daughter cells.
2. Position paired centrioles together near the outside of the nuclear membrane

*Question G: During what stage of meiosis I would this duplication of centrioles have occurred?*

Meiosis II

Prophase II
The duplicated centrioles move to opposite poles of both daughter cells. The chromosomes move toward the center of the daughter cells.
1. Move the centrioles to opposite poles of each daughter cell. Place the centromeres of the paired strands in the center of each daughter cell.

*Question H: How is this different than what happens in prophase I of meiosis?*

Metaphase II
All of the chromosomes line up, single file, in the center of the cell.
1. Center the paired strands along an imaginary line across the center of the cell. Line up the strands so they are centered in each daughter cell opposite the corresponding centrioles (which have migrated to the poles)

*Question I: How is this different from Metaphase I in meiosis?*

Anaphase II
The chromatids of each paired strand separate and are drawn to opposite poles of the cell. Each chromatid, with a well-defined centromere, is now a chromosome.
1. Separate each chromatid from its pair at its centromere. Move each strand toward its respective centrioles, noting how the chromosome arms trail the centromere as it moves towards each pole. Repeat this procedure for both daughter cells from meiosis I.

Telophase II and Cytokinesis
Cell division is completed and four daughter cells are formed. Each contains half of the chromosome number of the original parent cell. A nuclear membrane forms around each cell’s chromosomes and the daughter cells from meiosis I finish dividing completely. Centrioles remain outside the nuclear membrane of each of the four daughter cells.
1. Place each chromosome strand near its respective centrioles. Cut yarn to create a nuclear membrane around each chromosome and put some cut string around each nucleus (modeling the cell’s plasma membrane) to show complete division in the daughter cells from meiosis I, resulting in the four daughter cells.
Analysis Questions for Meiosis:
Make sure you have answered questions A & B from Mitosis portion, and questions A-I from Meiosis portion above.

1. Describe which stage during meiosis many chromosomal abnormalities may occur. Why would this be true?

2. If an onion were to reproduce sexually, it would need to produce sperm and/or eggs by the process of meiosis. After meiosis, how many chromosomes would be in each sex cell (in terms of X)?

3. Design a chart comparing and contrasting mitosis and meiosis. You must have four similarities, and four differences (for each).