Mitosis and Meiosis

BRING YOUR TEXT TO LAB!

Objectives:
1. To begin to understand the mechanics of cellular Reproduction/Life Cycles and how the process underlies inheritance.
2. To simulate the movement of chromosomes during mitosis.
3. To make direct observations of mitosis.
4. To simulate the movement of chromosomes during meiosis.

Introduction
As we discussed in class, biological growth is a multi-dimensional phenomena. In essence, the basic unit of life, the cell, must duplicate itself. One "mother" cell must become two "daughter" cells, through the process of Cell Division, mostly MITOSIS. This is a completely asexual event and involves replication of the nucleus and its constituents and dividing up of the cytoplasm, to make two identical cells.

MEIOSIS, on the other hand, is involved only in sexual reproduction, and is the main source of genetic variation. In meiosis, cells with a full complement of chromosomes divide to yield cells with one-half as many chromosomes. These cells become the "Egg" and the "Sperm" that unite later in a process known as FERTILIZATION.

The steps of mitosis and meiosis were worked out from direct observation of living and preserved materials. Cells in actively growing regions of plants (meristematic regions), such as roots and shoots tips, have cells that are easily stained to show the process of mitosis. In these tissues we can see cells actively dividing and at different steps in the process. The process of mitosis is ubiquitous in all diploid organisms. Often times, original, detailed descriptions of observations of processes have been the basis of future, experimental work. The observational descriptions we make today about mitosis and meiosis will allow us to develop hypothesis about genetic relationships in the next few labs.
A Context for the Exercise

*Here we will be concerned primarily with early steps in the Scientific Method:*

We will recapitulate some of the original **observations** of the early “cytologists” and attempt to put these observations and simulations in a biological context.

**Observations:** We will examine the onion root tip in order to observe various stages in the cell cycle – growth and division. Then we will observe and compare the stages seen in an insect testis.

**Question:** How do the static stages observed relate to one another? We will examine this question by simulating possible orders for the stages using models.

**Hypotheses:** Using your background knowledge of chromosomes and the observations we make on their separation during the division process you can make assumptions, as did the early cytologists, as to how traits are passed from generation to generation. This will provide you with a foundation for the analysis of upcoming material in lecture and lab – i.e. genetics.

The Cell Cycle and Mitosis

As a plant or animal grows, new cells and nuclei arise from preexisting cells during **Mitosis** and **Cytokinesis.** In plants mitosis occurs predominantly in meristematic regions: the root and stem tips, vascular and cork cambium, and in organs in their early stages of growth. The two stages of cell division are generally recognized as:

- **a. Mitosis** - division of the nucleus into two nuclei;
- **b. Cytokinesis** - division of the cytoplasm and building of a new cell wall, which is laid down between the new cells.

In actively dividing cells, the term **Cell Cycle** is used to describe the life history of the cell. This includes the cell origin from mitosis, it then enlarges and develops during interphase, and finally it divides into two new cells. In other words, repeated cellular divisions are separated by periods of time during which growth and preparation for the next division occur.

Interphase itself is also divided into segments; two G (growth or gap) phases during which protein and organelle content doubles and an intervening S phase. During the “S” or synthesis phase of the Cell Cycle **DNA** is replicated. Since DNA is the “stuff” of which **CHROMOSOMES** are made, this replication results in the **doubling of all chromosomes.** This is necessary because during mitosis the chromosomes of one nucleus are divided between the two new nuclei.
EXERCISE 1 – Mitosis in Onion (Allium cepa) root tips

A. Now you are going to make slides of tissue squashes of onion roots to find your own mitotic stages. Get a fresh onion root from the container and observe it under the dissecting 'scope. Next, using a new razor blade, cut away and save the first 1 millimeter of the root tip containing the apical meristem. Place the tip on a glass slide and add a few drops of acid-alcohol. The acid-alcohol dissolves the pectin that holds the root cells together. Use the dissecting probes to soften up the tissue by poking and working it apart. Add more acid-alcohol, if necessary, to keep the mass of tissue moist.

After 5 - 10 minutes, blot away the excess liquid and add one single drop of aceto-carmine stain and work this into the tissue using two probes. This takes time and patience, but soon the tissue will be a mass of bits. Finally, place a cover slip over the cells and squash with a medium downward pressure, as directed by your TA.

Observe your squash under the microscope and search carefully for a region of mitotic activity. Again, this takes much patience, work and luck. Draw what you see in your lab books.

B. Now that you have tried to make your own slide, look at a professionally made slide. Obtain a prepared slide of onion root tips in longitudinal section. Under low power (10x objective), note the location of the apical meristem (region of actively dividing cells) in relationship to cap and more mature regions. Under higher power (40x objective), try to find cells in various stages of mitosis.

Once you have found a region with actively dividing cells, count and record the number of cells you can see in each phase. Count as many cells in mitotic stages as you can.

What do the relative numbers of cells found in each mitotic stage mean for mitosis and the cell cycle?

EXERCISE 2 – Simulating mitosis with pop beads

Working in groups, obtain a chromosome simulation kit consisting of red pop beads, yellow pop beads, red centromeres, yellow centromeres, and clear centrioles.

Here, you will attempt to put the static stages you observed on the slides into a reasonable mechanical order. As you complete this exercise, diagram each stage in the space provided.

A. INTERPHASE

Interphase, the longest stage of the cell cycle, is the preparation phase for the next mitosis and cytokinesis. During this phase, DNA exists as chromatin, with a spaghetti-like granular appearance, not as distinct chromosomes.

The G1 phase begins. What happens during this time?
For the chromosome simulations, the two colors represent homologous chromosomes, and the length of the strands indicate different chromosomes. Before the S phase, a chromosome can be simulated by one strand of plastic beads, which is made of one double-stranded DNA molecule. If you attach two strands of the same color beads at their centromeres, you have a duplicated chromosome. This is how chromosomes appear after DNA Replication during the S phase of the Cell Cycle. Each half of the duplicated chromosome is called a sister chromatid. It is duplicated chromosomes that enter mitosis.

Make two strands of six red pop beads and attach each strand to a red centromere. Repeat with two strands of six yellow pop beads and a yellow centromere. These represent a homologous pair of chromosomes.

Next, make a homologous pair of chromosomes like above, but with eight beads. You now have two pairs of homologous chromosomes, differentiated by their size.

What is the diploid number for the cell you are simulating mitosis for? What is the haploid number?

What do you need to do with the pop bead chromosomes you made before you can begin simulating mitosis?

The G₂ phase begins. What happens during this time?

MITOSIS BEGINS:

B. PROPHASE

During prophase chromatin condenses and chromosomes appear. Centrioles migrate to opposite sides of the cell and spindle fibers begin to appear (use your imagination).

Arrange the four chromosomes randomly in an imaginary cell on your workspace. Place the centrioles in the correct positions.

C. METAPHASE

During metaphase chromosomes line up along the metaphase plate. The centromeres of each sister chromatid are attached by spindle fibers to the centrioles at opposite poles of the cell.

Arrange your four chromosomes along the metaphase plate, with the centrioles in the correct position.
D. ANAPHASE
During anaphase the chromatids of each chromosome separate at the centromeres and move to opposite sides of the cell. Each chromatid is now called a chromosome. Separate the chromatids of each chromosome and move them into the correct position.

E. TELOPHASE
During telophase the spindle apparatus disappears, and the nuclear membrane reappears and forms two separate nuclei, one for each daughter cell. The chromosomes uncoil and become chromatin.

F. CYTOKINESIS BEGINS
What happens during Cytokinesis? What happens now in your simulation? How do the two new cells compare to the original cell with which you began?

You have just simulated the process of the cell cycle, especially Mitosis and Cytokinesis, in an animal cell.
Is the process the same in Plants?
Keep your simulated chromosomes so you can use them in exercise 4 for Meiosis.

The process of Meiosis

Two critical events occur in the life history of all sexually reproducing organisms: meiosis and fertilization. During the interphase preceding meiosis, the chromosomal material (DNA) is replicated. Then, during meiosis, the nucleus undergoes two divisions, one of which is a reduction division. By a precise mechanism, meiosis produces four daughter nuclei, each with one-half the number of chromosomes, and thus one-half as much DNA, as the parent nucleus. Whereas the parent nucleus is diploid (2n), each of the daughter nuclei is haploid (n). In diploid cells, the chromosomes are present in matched pairs called homologous chromosomes. Each parent contributes one member of each pair during sexual reproduction. In the reduction division of meiosis, the pairs of homologous chromosomes are separated. Haploid cells, therefore, contain only one member of each homologous pair of chromosomes. Look at the series of pictures of the phases of meiosis in your text for details.

EXERCISE 3 – Observing the stages of meiosis

Obtain a prepared slide of insect testis. Scan this slide to observe cells with formed chromosomes. Take care to notice the particular arrangements of chromosomes with respect to each other. Compare that to the text diagrams.

Can you identify stages that match the text diagrams? List the stages you see below.

EXERCISE 4 – Simulating the stages of meiosis

Now, you will simulate the steps in the process using the Pop-bead set. The Meiosis simulation will use the two pairs of homologous chromosomes you made for the mitosis simulation.

Where and why does meiosis occur?

A cell entering meiosis has prepared almost exactly as a cell does before mitosis. There is an Interphase with G₁, S, and G₂ stages.

Why, what happens during Interphase that is needed before meiosis also?
In the following simulation of Meiosis, you will need to fill-in the information for each phase. Explain what happens to the chromosomes, and diagram the stages in the space provided. Then arrange/move the simulated chromosomes accordingly.

**MEIOSIS BEGINS:**

**MEIOSIS I:**

A. PROPHASE I *(Make sure to include Crossing Over)*

B. METAPHASE I *(How does Independent Assortment affect this?)*

C. ANAPHASE I

D. TELOPHASE I
MEIOSIS II:

A. PROPHASE II

B. METAPHASE II

C. ANAPHASE II

D. TELOPHASE II
The events of Prophase I and Metaphase I are especially significant for sexual reproduction.

*Why? How and why does Metaphase I differ from metaphase of mitosis?*

*How does meiosis fit into the sexual life cycle of an organism?*

*What happens to homologous chromosomes during meiosis?*

*What happens to sister chromatids during meiosis?*

*What do the terms haploid and diploid mean?*

*Write a description of meiosis using the following terms: homologous chromosomes, sister chromatids, haploid, diploid, crossing over, independent assortment.*

*Compare and contrast mitosis and meiosis.*