Lab 6. Mitosis and On-line Karyotyping

Prelab Assignment

Before coming to class ...

- Read carefully the introduction section, below, and pages 113 120 (i.e. sections 8.1 (The Basics o Cellular Reproduction, 8.2 (The Cell Cycle) and 8.3 Mitosis and Cytokinesis) in your textbook, *Essentials of Biology by Mader 2nd ed*.
- Answer the prelab questions on page 1 of the report sheet. Be prepared to hand in your responses to the prelab questions at the start of lab.

Goals of this Lab Exercise

- To understand the mechanisms of the cellular process called mitosis
- To understand the process and application of the technique called karyotyping
- To apply this knowledge to issues in today's society in relationship to karyotyping

Introduction

In the late 1800's new techniques to visualize cellular structure exploded with the discovery of vital stains and dyes. Most plant cells were fairly easy to visualize since most contain pigment molecules for photosynthesis, but animal cells were another matter. When viewed under a microscope lens sub-cellular structures, called organelles, were seen as simply the grainy consistency of the cytoplasm. When scientists would apply different pigments harvested from different plants the graininess took on a distinct form. Organelles could now be distinguished and studied as separate structures. Differences in cellular function could be attributed to the number and types of organelles found in various cells. By the end on the century scientist were using not only pigments from plants to stain cells, but were beginning to use heavy metals linked to pigments to further delineate structure. Dyes and stains became almost as an important discovery as the light microscope.

The new technology of stains allowed a number of scientists to peer into cells like they could not have done before their advent. One scientist, Walter Flemming, noted in salamander ovary cells that dark staining condensations appeared within the nucleus. These condensations were then separated toward opposite poles of the cell ("Dance of the Bodies") just prior to the cell splitting into two new cells. He eloquently described and named the process called **mitosis**.

Today's technology in the field of genetics and how genes affect the phenotype of an individual started over 140 years ago with the work of Gregor Mendel. His work with pea plants set the stage for how chromosomes are sorted and passed from generation to generation. In the early part of the 1900's another geneticist, Thomas Morgan, working with fruit flies (*Drosophila melangaster*) developed a technique that allowed him to visualize the structure of chromosomes. This technique harvested chromosomes from cells arrested in metaphase of mitosis, stained the chromosomes with a vital stain, and then matched the chromosomes based on staining patterns and size. This technique is called **karyotyping** and is used today to show the potential for genetic abnormalities within the genome of an individual. The specificity of the technique has been refined through the use of more specific stains (spectral analysis) which adhere to specific sites within the DNA molecules to further highlight the differences between chromosomes.

Part 1. Microscopic Observation of Mitosis

Instructions: Do part A or Part B below—do <u>not</u> do both parts!!

Part A. Observation of Mitosis in Onion Root Tips

Materials

- Compound light microscope (one per person)
- Slide of onion root tip (*Allium*)
- 1. Work individually, but share the task of locating the cells to sketch in step 5.
- 2. Obtain a prepared slide *Allium* (onion) root tip. This slide was prepared from the tip of an actively growing root. It was "fixed" by chemicals to preserve the cellular structure and stained with dyes help see the structures involved with mitosis.
- 3. Observe with the low and medium-power objectives. Note the root cap, and zone of cell division. The root cap protects the delicate root tip as it is pushed through the soil as the cells divide in the zone of cell division.
- 4. Focus on the zone of cell division just behind the root cap. Now observe with the *high power* <u>objective</u>, 40x.
- 5. Survey the zone of cell division at high power and locate the following stages of the cell cycle: *Interphase*, *prophase*, *metaphase*, and *telophase/cytokinesis*. In the appropriate spaces on the report sheet use a *sharp pencil* to make a sketch of a representative cell in each phase.
 - Label clearly: Cell wall, cytoplasm, nucleus, chromosomes and spindle fibers (collectively called mitotic spindle)

Part B. Observation of Mitosis in Whitefish Blastula

Blastulas are a convenient source of animal cells that are actively undergoing cell division. A *blastula* is a very young embryo and consists of solid ball of cells produced from the many divisions of a zygote (fertilized egg). You will observe a prepared slide containing cross sections of several different whitefish blastulas. By scanning these blastulas you should be able to locate cells at the various stages of mitosis and cytokinesis.

Materials

- Compound light microscope (one per person)
- Slide of white fish blastula
- 1. Work individually, but share the task of locating the cells to observe and sketch in steps 3 and 4.
- 2. Examine a slide of whitefish blastula with the low-power objective. The slide has several blastulas. Select one to observe at low and medium power, focus, and then switch to high power for detailed observation.
- 3. Survey one or more blastula at high power to locate cells at the following stages of the cell cycle: Interphase, prophase, metaphase, and telophase/cytokinesis.
- 4. In the appropriate spaces on the report sheet use a <u>sharp pencil</u> to make a sketch of a representative whitefish blastula cells in the following stages of the cell cycle: **Interphase**, **prophase**, **metaphase**, and **telophase/cytokinesis**.
 - Label clearly: Plasma membrane, cytoplasm, nucleus, chromosomes and spindle fibers (collectively called mitotic spindle)

Part 2. Web Karyotyping

In the following on-line activity you will play the role of a cytogenetic technician and complete the karyotype for three patients, then use these karyotypes to evaluate and diagnose each patient. Be careful! The emotional and physical well being of each patient is in your hands.....or almost in your hands!

- 1. Go to the Biology Project at: http://www.biology.arizona.edu/
- 2. Scroll down and click on "Human Biology."
- 3. Scroll down to "Activities" and then click on "Web Karyotyping."
- 4. Read the introduction and then complete the assignment as described. Record your responses on the attached report sheet.

Part 3. On-line Onion Root Tips: Phases of the Cell Cycle

This activity is a digital version of a classic microscope lab. You will classify cells from the tip of an onion root into the appropriate phases of the cell cycle, and then count up the cells found in each phase. You can use those numbers to predict how much time a dividing cell spends in each phase. In the process of doing this you will become familiar with the cell cycle and the process of mitosis and its stages, which are, oddly enough, the major goals of this activity!

- 1. Go back to the Biology Project at: http://www.biology.arizona.edu/
- 2. Scroll down and click on "Cell Biology."
- 3. Scroll down to "Activities" and then click on "On-line Onion Root Tips: Phases of the Cell Cycle."
- 4. Read the introductory page (about 3 total pages) and then complete the assignment as described. Record your responses on the attached report sheet.

Part 4. New Methods in Karyotyping: The Spectral Karyotype

In this activity you will learn about a new technique for diagnosing chromosomal abnormalities, spectral karyotyping". This technique is exciting because of its many applications, but also full of many controversial societal issues. On your report sheet are three questions pertaining to the old and new methods of karyotyping. Answer these questions on the report sheet as you do the following on-line reading assignment.

- 1. Go back to the Biology Project at: http://www.biology.arizona.edu/
- 2. Scroll down and click on "Human Biology."
- 3. Scroll down to "Activities" and then click on "New Methods in Karyotyping."
- 4. Read the introduction.
- 5. To learn about the methods involved click on "Methods" at the bottom of the "Introduction" page
- 6. To learn about some of the possible applications of this new method click on "Applications" at the bottom of the "Methods" page.
- 7. Don't forget to answer questions 1-3 located on the report sheet!