BIL 151 - Mechanisms of Mitosis

Part 1. Introduction and Literature Search

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The first person to ever observe and characterize cells was **Robert Hooke** in 1665, who observed the tiny holes that made up a slice of cork he was observing under one of the earliest microscopes. Those cell remnants were dead and hollow, so he had no idea what the internal structure of a living cell was like. It was not until 1674 that **Anton van Leeuwenhoek** first identified living cells (unicellular protists) under a microscope. Because they moved under their own power, he called them **animalcules** ("little animals") not realizing that they were no more than single cells.

Further observations and work by **Theodor Schwann**, **Matthias Jakob Schleiden**, and **Rudolf Virchow** led to the development of **cell theory**, which has evolved over the decades as new discoveries about cells have been made.

I. Cell Theory

The original cell theory stated that

- All living organisms are composed of one or more cells.
- The cell is the basic structural and functional unit of life.
- Cells arise from pre-existing cells by a process of cell division.

Modern discoveries have added that

- Energy flow occurs within cells via metabolism and biochemical reactions.
- Genetic/heritable information is passed from cell to cell upon division.
- All cells have the same basic chemical composition.

Recall that there are two basic cell types, **prokaryotic** and **eukaryotic**. Bacteria and archaeans are **prokaryotes** (from the Greek *pro*, meaning "before" and *karyon*, meaning "nut", which refers to the dark-staining nucleus prokaryotes don't have). All other organisms, including protists, animals, fungi, and plants, are **eukaryotes**.

Refer to your lecture notes and text readings to review the basic components and anatomy of prokaryotic and eukaryotic cells. Prokaryotic DNA is carried on a single, circular chromosome, whereas eukaryotic DNA is organized on multiple, linear chromosomes that undergo an organized duplication and segregation known as **mitosis**. Although prokaryotic divide in two (via **fission**) to reproduce, they do not undergo a mitotic cell cycle like that described here, which is unique to eukaryotes.

II. The Cell Cycle

One cannot truly understand biology without understanding the structure and function of a living cell. One of the most essential cell functions is reproduction, whether asexual (**mitosis**) or sexual (**meiosis**). The cell passes through (somewhat arbitrarily defined) stages as it divides. If you observe a population of growing cells, whether part of an organism's body or in a culture dish, you are likely to see cells in various phases of the cell cycle (Figure 1). The stages of active mitosis have been named for easy referral to events occurring in each phase.

A. Interphase

Cells spend the majority of their time in **interphase**. An interphase cell is busy undergoing the chemical reactions that facilitate energy transfer, growing, and preparing to divide. Interphase is not part of mitosis, which is defined as active cell division. Rather, it is the living stage of the cell. The chromosomes cannot be seen easily, as they are in the diffuse form

known as **chromatin**. The genes on the chromosomes are actively being transcribed and translated, with the particular genes being expressed depending on the identity and function of the particular cell. The stages known as **G1**, **S**, and **G2** occur during interphase, and are involved in normal cell growth regulation (G1), DNA synthesis (S), and protein and microtubule synthesis preceding mitosis (G2).

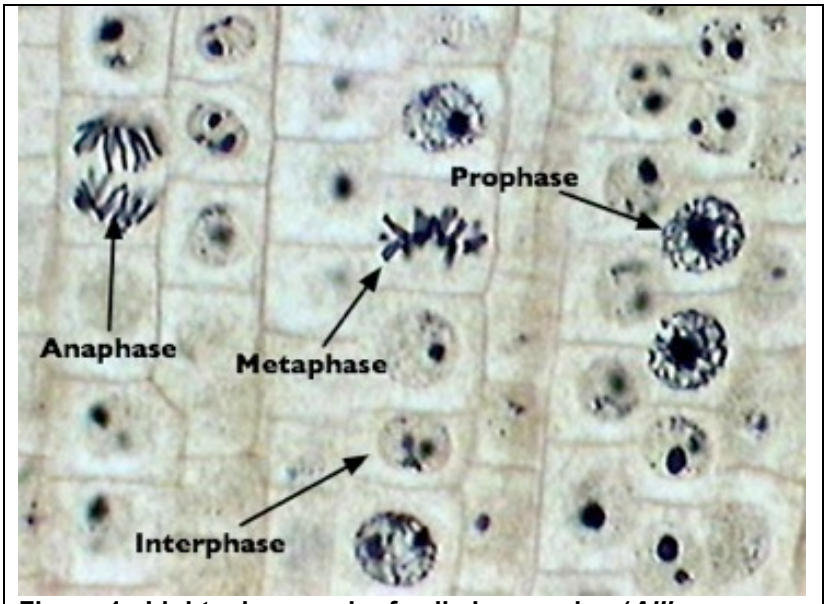

Figure 1. Light micrograph of cells in an onion (*Allium cepa*) root tip, where many cells are actively engaged in mitosis because the tissue is growing so rapidly.

B. Prophase
Prophase is the first stage of mitosis. The chromosomes condense and become visible. Although they have already duplicated, the chromosomes are not yet visible as separate sister chromatids. The nuclear membrane breaks down, and the spindle fibers begin to form at opposite poles of the cell.

C. Metaphase
In this second phase of mitosis, the duplicated chromosomes (now called **sister chromatids**) unwind from each other, and are attached only at a slightly constricted region, the **centromere**.

It is only now that the chromosomes take on the "X" shape so often seen in illustrations. This "X" actually represents not one, but two chromosomes: the identical sister chromatids produced during DNA replication.

Spindle fibers attach to the **kinetochore**, a protein structure located at the centromere, and the chromosomes are arranged along the equator of the cell.

D. Anaphase
Spindle fibers shorten, the kinetochores separate, and the chromatids (now **daughter chromosomes**) are pulled apart and begin moving to opposite poles.

E. Telophase
The daughter chromosomes arrive at the poles and the spindle fibers disappear. The nuclear envelope reorganizes around the chromosomes, which then resume their active, diffuse form, chromatin.

III. Literature Search and Review

By now you have enough experience to be able to do a thorough literature search in advance of designing a research project. Before beginning, it is very important to learn what is already known about your area of interest (as well as *why* it is important and relevant), so that you don't simply repeat work that has already been done (unless there is a good reason to replicate someone else's work). Previous research should give you ideas about what questions still need to be addressed.

The theme of this project is broad: **Understanding Mitosis**. The choices of available protocols, however, will be narrow: **Inhibition** or **Promotion of Mitosis**. One reason for this is that chemical substances that alter the processes of mitosis are often unbelievably deadly. To be honest, we're not sure we trust you not to lick the spoon.

But the main reason for narrowing your choices this time is to make sure we focus less on methods (though methods are, of course, very important) and more on the *question* we are

exploring. In this case, that question will be: **What are the cellular structures that facilitate the chromosomal movement we call mitosis?**

A. Basic Materials and Concepts

For this project, you and your teammates will predict the outcome of treating a rapidly dividing tissue (onion root tip) with a substance that either **promotes** or **inhibits** mitosis. The unknown in this system is exactly *how* each substance either promotes or inhibits mitosis. This will be for you and your teammates to discover, if possible.

Your team may choose to examine onion root tips that have been treated with either:

1. Green Light root stimulator and starter (active ingredient: **indole-3-butyric acid**)
2. Preen weed preventer (active ingredient: **trifluralin**)

It is known that **indole-3-butyric acid will *promote* mitosis**. But exactly how it does this, and at what phase of the cell cycle, is for your team to research. It's possible that its mechanism of action is not fully understood.

It is also known that **trifluralin will *inhibit* mitosis**. But exactly how it does this, and at what phase of the cell cycle, is for your team to research. As with indole-3-butyric acid, it's possible that its mechanism of action is not fully understood.

Now things can get interesting. Once you perform a literature search on each of these compounds, your team may be able to logically infer the exact cell structure or process that each of these two compounds affects. If you know this, you should be able to predict at which phase of mitosis the cells will show the effects of treatment with Compound X.

For example, if you have reason to suspect that Compound X kills mitotic cells by inhibiting cell wall formation, then you might predict that in an onion root tip treated with Compound X will have cells in all phases of mitosis except the final phase, when the cell wall is forming between the two daughter cells. You might treat onion root tips with Compound X for 24 hours (**the approximate time of the onion root tip cell cycle is 24 hours**), and then count the number of mitotic cells in each phase of mitosis. What would you expect to see, if Compound X kills mitotic cells by interfering with cell wall formation?

B. On Your Mark…Get Set…Search!

A **literature search** is an organized search for published material on a selected topic. You will use databases that retrieve academic sources of high quality and reliability. Literature databases allow you to search a wide array of journals and other sources, and enable you to collect scholarly references. Some of these may be in the form of an abstract, whereas others might be the full text of a journal article. You can save these to your own computer for future reference.

An effective literature search takes an organized approach:

1. Decide on a search topic

Before you begin, decide what keywords to use. The two chemical compounds listed above along with "mechanism of action" might be a good place to start. Hint hint.

2. Use the right keywords in your search

Identify important keywords. In the example above, you might include "mitosis, onion, spindle fiber, chromosome," or any number of terms you wish to include that might generate interesting findings. Also consider:

- When to use broad terms, and when to use narrower terms to refine your search
- Use synonyms for your keywords to find every possible variant of the vocabulary used in the research on this topic.
- Use dictionaries to check spelling and find keyword synonyms

- Using online encyclopedias (e.g., Wikipedia) to find initial background information that might help you refine your search or choose an area for your research topic. (But don't use these as a primary source of information.)

3. Choose a Database

GoogleScholar (**www.googlescholar.com)** is an excellent place to start, but there are other databases available through the UM library system that you may wish to use, as well.

4. Perform your Search

- Use **Boolean operators** (always use in upper case) to combine search keywords (http://www.csa.com/help/Search_Tools/boolean_operators.html)
- Truncate (shorten) your keywords to make your search broader.
- If you are not sure how to spell a keyword, use **wildcards** (http://www.oxfordscholarship.com/page/209/wildcards)
- To narrow your search, use phrases enclosed on quotation marks. For example, "trifluralin effect on spindle fibers".
- Use the database to search for keywords in different places, such as "title" or "abstract".
- If you find a useful article by a particular author, search that author's name to find papers on the same topic.
- If you find a useful article, search its Literature Cited section to find more sources.
- Make sure the literature you are citing is recent and current.
- Make sure the literature you use is from a peer-reviewed, scientific journal or academically reliable source.
- Make sure to identify whether your source is a journal article, a book, a thesis, etc.

5. Determine the availability of the material you wish to reference.

If the paper you wish to read is not available online, you may be able to get a copy by contacting the people at the Richter Library Help Desk. If our library does not have the paper you need, they may be able to get it via interlibrary loan. Since this requires turnaround time, this is one very good reason to start this assignment immediately, and not find yourself hamstrung by time constraints.

C. Putting the Literature Search to Work for You

Each team member should locate **at least three papers** relevant to the mitosis inhibitor (trifluralin) and/or promoter (indole-3-butyric acid) and how it affects mitotic cells. Remember that your sources should be from a peer-reviewed scientific journal or other reliable academic source (e.g., a textbook). Once you find a paper of interest, read it completely and analytically. Your assignment is to turn in (1) a copy of each of the three articles you found and (2) the completed literature search overview (use the template linked to the syllabus in this session's slot) to your instructor. **Also keep a copy of the entire assignment for yourself.**

Your literature search summary is due at your next laboratory session. **Use the template linked to the syllabus (it is in MS Word format) to complete this assignment.**

Next week, you and your teammates will meet to share what you have discovered in your literature searches and decide which chemical to use. In the third week, when you will be observing experimentally manipulated onions, your team will have available:

- onions that have been treated for 24 hours with indole-3-butyric acid (aqueous)
- onions that have been treated for 24 hours with trifluralin (aqueous)
- onions that have been growing for 24 hours in plain water

Given what you have learned about these chemicals in your literature search, you should be able to predict differences in mitotic stages between treated and untreated (control) roots.