BIL 151 - Mechanisms of Mitosis
A Sample Protocol

The Role of Spindle Fibers in Mitosis

NOTE: YOU WILL NOT BE DOING THIS EXPERIMENT. THIS IS A *SAMPLE PROTOCOL ONLY, MEANT TO HELP GUIDE YOU IN THINKING THROUGH YOUR OWN PROJECT.*

1. Introduction: Structure and Function of Spindle Fibers

Spindle fibers play an important role throughout the mitotic process. For example, late prophase is characterized by movement of the mitotic spindle to opposite ends of the cell. In metaphase, sister chromatids become visibly associated with spindle fibers attached at each pole of the cell. Spindle fibers pull sister chromatids apart during anaphase, and then disassemble at telophase.

The spindle fibers contain a highly organized array of microtubules which are long, hollow, unbranched tubes composed of globular proteins. Microtubules are also components of other cellular structures, including the cytoskeleton, and they form the core of both cilia and flagella.

Microtubules are not only involved in separating chromosomes during mitosis and meiosis, but also function as structural supports and organizers for cells. In plant cells, for example, microtubules maintain cell shape through their influence on the formation of the cell wall during interphase. Studies also showed that microtubules play a role in maintaining the internal organization of cells and are responsible for intracellular motility of macromolecules and organelles. Treatment of cells with microtubule-disrupting drugs, such as nocodazole or colchicine, can seriously affect the location of membranous organelles, including the endoplasmic reticulum (ER) and Golgi complex. It is also known that the transport of materials from one membrane compartment to another depends on the presence of microtubules because specific disruption of these cytoskeletal elements brings the movements to a halt.

Even though microtubules of the mitotic spindle or the cytoskeleton are stiff enough to resist forces that might compress or bend the fiber, they are extremely sensitive to disassembly. Living cells can be subjected to a variety of treatments that lead to the disassembly of liable cytoskeletal microtubules without disrupting other cellular structures. Disassembly can be induced by cold temperature, hydrostatic pressure, elevated calcium ions (Ca^{2+}) concentration, and a variety of chemicals including colchicine, vinblastine, vincristine, nocodazole, and podophyllotoxin.

2. Methods: Disrupting of Microtubule Function

In order to demonstrate that microtubules are, indeed, essential for a particular process, one can disrupt their function and observe the results. A useful model organism is the common onion (*Allium cepa*), the roots of which grow rapidly, and so are a good place to find many dividing cells.

Two groups of onion root tips will be used, and a chromosome squash will be performed in order to visualize the state of the cell, in terms of mitosis. The chromosome squash allows the investigator to observe cells and various stages of mitosis under a light microscope.

Colchicine is an alkaloid compound derived from the corm and other parts of the Autumn Crocus, *Colchicum autumnale*. Colchicine induces the disassembly of microtubule fibers and thus stops the mitotic process. It is also known as a mitotic poison. If used properly, it can be employed to stop mitosis “in its tracks” so that chromosome morphology can be studied, chromosome counts can be made, or induction of polyploidy can be performed.

One group of onion root cells will be grown in a beaker with plain water under normal conditions (12-hour light/dark cycle, 75^{O}F, etc.). The second group of root cells will be grown for a short period of time in a dilute colchicine solution.

3. Hypothesis and Predictions

The working hypothesis for this investigation is:

The spindle apparatus, composed of microtubules, is essential to the mitotic process.

When designing experimental procedures to test this hypothesis, we must remember that microtubules not only play an important part in separating chromosomes, but also are involved in other cellular activities. Disassembly of microtubules by colchicine does not only terminate cell division, but also affects the structure, intracellular organization, and macromolecule transport of the cells. We must determine what features in a dividing cell population might tell us that colchicine treatment has successfully inhibited spindle formation ***selectively***, and what features might have simply stopped mitosis because it killed the cell.

For example, if we see many empty cells, or cells with a densely stained nuclear zone but no evidence of mitosis in treated onion root tips, it would probably mean that the colchicine has simply killed the cells.

However, an observation that most cells are in prophase and metaphase, but very few are in anaphase or telophase in treated onion root tips would more clearly indicate that colchicine has arrested cell division of most cells by inhibiting the formation of a spindle apparatus. If the guidance and mechanical force of the apparatus are not impaired (as in the control onion root tips), we should see cells in all stages of mitosis represented equally (statistically speaking).

Considering these factors, we can make the following predictions to test the hypothesis.

I. If the formation of the spindle apparatus in living cells is inhibited, these cells will stop dividing.

II. If the inhibitor of the spindle apparatus formation is eliminated, spindle apparatus will be rebuilt and cell division will resume for these cells.

To test these predictions, we will apply the following strategies:

1. First, we will test a series of concentrations of colchinie solution for a fixed duration of exposure to identify the highest tolerable concentration of colchicine solution treatment for onion root tip tissue to be alive, and the lowest concentration for the treatment to be effective over the selected time period. From this experiment, we can select the optimum colchicine concentration treatment that produces cells only at prophase and metaphase.

2. Second, we will test a series of different time durations of treatment with the optimum concentration of colchicine to determine the minimum time period required to allow the treatment to be effective.

3. Finally, we will transfer onion bulbs treated with the optimum concentration of colchicine solution to a water bath for an extended length of time to wash away the colchicine. If the onion bulbs are free of colchicine, cells should resume normal division and new roots will sprout.

When you complete your literature search on the mitosis inhibitor and promoter we will be using, consider the way this protocol has been outlined, and how it focuses less on methods than on what is being tested. This is how you should be thinking about your own mitosis project.