

Pollen Tube Formation and the Central Dogma of Biology

INTRODUCTION

In this laboratory activity, you will be studying the phenomenon of pollen tube growth and its relationship to a set of concepts generally known as the “central dogma of biology.” Pollen grains of various species function as important model systems for studies in plant biology, including studies in plant genetics. Pollen grains represent a remarkable model system because they are incredibly small and simple (composed of two or three cells at maturity), but they exhibit very complex developmental responses.

Pollen is also important from an applied perspective because of the significance of pollination to the agricultural industry. For example, studies on pollen/stigma compatibility have revealed a complex interplay between numerous genetic and physiological components that determine where and when a pollen grain will germinate and form a pollen tube.

The concept of the central dogma describes the functional relationships among DNA, RNA, and protein. This important relationship (described later) forms the foundation for our understanding of how genes function.

POLLEN TUBE GROWTH

Pollen grains are small structures (usually about 10–50 μm in diameter) that contain either two or three haploid nuclei when released from the anther (i.e., at anthesis). When a viable pollen grain lands on the stigma of a compatible flower, it produces a tube several hundred to several thousand micrometers long (up to several centimeters long, in some cases!). It is through these pollen tubes that pollen nuclei travel to the ovaries of compatible flowers. Pollen grains are morphologically simple, and the process of tube formation is a relatively uncomplicated example of growth and development. For these reasons, and because of the rapid rate of tube formation *in vitro* exhibited by some species, pollen tube formation has become a model system for studying growth and development in plants.

One area of research that has yielded valuable insights relates to the relative roles mRNA transcription and protein translation have in the process of pollen tube growth. In this lab activity, you will be studying the relationships between these phenomena by measuring the growth of pollen tubes under several conditions.

THE CENTRAL DOGMA OF BIOLOGY

Following the elucidation of the structure of DNA by Watson and Crick in 1953, a central focus of biology became the study of how messages encoded in DNA direct growth and function of cells and

organisms. During the 1950s and 1960s, many of the details of this process became known. The concepts describing how the information stored in DNA is used in the cell have become known collectively as the “central dogma of biology.” While these concepts are certainly “central” to the study of biology, the term *dogma* is a bit pretentious. In fact, one of the tenets of the central dogma is that RNA is copied from DNA and not the other way around. This assertion was shown to be less than universal when it was discovered that certain viruses make DNA by copying RNA.

The discoveries of the 1950s and 1960s provided the following general picture of information flow in cells (see Figure 13.1). It was demonstrated that DNA is reproduced when “new” DNA strands are copied from “old” DNA strands (i.e., DNA is copied from DNA; this is called *DNA replication*). This results in the faithful transmission of genetic instructions from one generation of cells to the next. To use these instructions, cells first make messenger RNA (mRNA) “copies” of specific genes found in the DNA (a process known as *transcription*). These mRNAs function as intermediate “message carriers.” In eukaryotic organisms, mRNAs are made in the nucleus and then transported into the cytoplasm where the messages are decoded. The decoding of the messages results in the production of specific proteins (a process called *translation*). The proteins, which are the final products of this sequence of events, then control how the cells grow and function.

Much additional information is also available regarding the details of these processes. For example, two other types of RNA, ribosomal RNA (rRNA) and transfer RNA (tRNA), are also produced by transcription and are necessary for translation. Furthermore, each of the processes requires numerous enzymes and other factors that help to “fine-tune” these processes.

The details of the central dogma have been elucidated through many careful studies utilizing numerous experimental approaches. One technique that has been useful in defining the relative roles of transcription and translation is the use of biochemical inhibitors. Various inhibitors are available that have relatively specific capabilities to block certain biochemical processes. For instance, *actinomycin D* is a substance that binds tightly to DNA double helices and prevents transcription. The substance can be used to assess the relative importance of mRNA production during specified stages of development. Several other inhibitors, including *cycloheximide*, block translation, inhibiting the production of new proteins. Other, more specific, inhibitors are also available that affect the function of specific proteins. An example of one such inhibitor is *cytochalasin B*, which binds to the growing ends of actin microfilaments (a major cytoskeletal component), preventing their elongation.

In this lab exercise, you will measure pollen tubes from pollen in control cultures and those from cultures that have been treated with actinomycin D, cycloheximide, and cytochalasin B. You will use your results to determine the relative roles of the processes that each inhibitor affects in the process of tube growth.

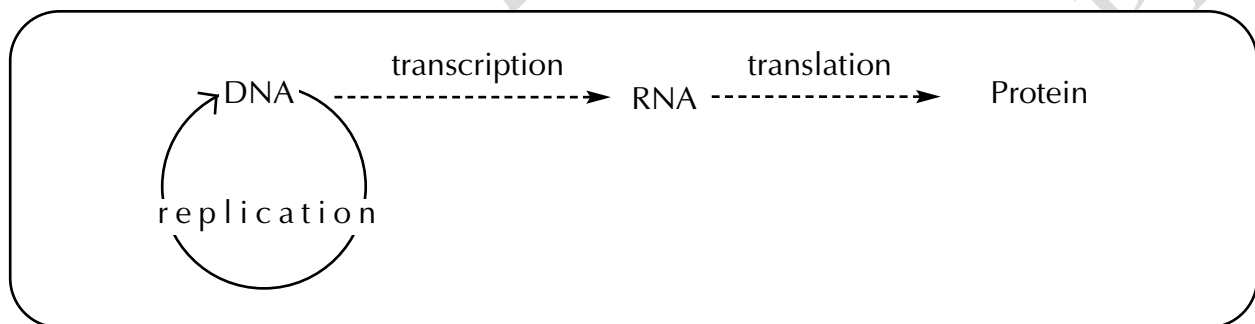


Figure 13.1 – A representation of the model for the flow of information assumed by the central dogma of biology. See the text for a detailed description.

PROCEDURE

Your ultimate goals for this experiment are two-fold:

1. To characterize normal rates of pollen germination and tube elongation over time during a period of several hours.
2. To determine the effects of each of the three biochemical inhibitors after several hours of exposure.

This experiment will be conducted in small groups. Each small group will characterize the normal rate of pollen tube growth for a sample of pollen (these data will be pooled at the end of the lab) and also the effects of one of the inhibitors. Prior to initiating the experiment, the entire class should establish a schedule for initiating and characterizing the various treatments.

For each treatment, follow these steps:

Step 1: Obtain two 35 x 10-mm petri dishes for each condition (i.e., two dishes for plain medium, and two dishes for medium containing the assigned inhibitor).

Step 2: Add 2 ml of the appropriate medium to *one of the two* petri dishes for each condition. *Note:* The inhibitors used in this experiment have *toxic* effects; handle them with care. Avoid contact with the skin.

Step 3: Use the demonstrated technique to add pollen from individual flowers or batches of stored pollen to the 2 ml of medium. Record the time of pollen addition as “time 0.”

Step 4: Suspend the pollen grains in the medium and remove 1 ml of pollen suspension. Place this sample into the second petri dish. Swirl the dish to make the solution cover the bottom. An aliquot of 1 ml of solution will barely cover the bottom of the petri plate; this provides optimum conditions for diffusion of oxygen into the solution. It may be necessary at several points throughout the procedure to add one or two additional drops of the appropriate medium to your pollen culture. However, avoid adding any more drops than necessary to keep approximately 1 ml of solution in the dish.

Step 5: At time points designated by the instructor, germination counts should be established and recorded from one petri dish, and pollen tube lengths should be established and recorded from the other dish.

Note: Germination counts and tube length measurements should be made at each designated time period for the control cultures. Due to time limitations, you only need to take these measurements for the cultures containing the inhibitors at the end of the lab period.

Germination counts should be made for 50 to 100 randomly selected pollen grains viewed using a compound microscope or a high-powered dissecting microscope. If a compound microscope is used, the pollen in the dish can be viewed directly at 100x (i.e., using a 10x objective), without making a wet mount slide. Do this by removing the petri dish lid and placing the dish directly on the microscope stage. Use care to ensure that the lens in the objective does not touch the surface of the solution in the dish. To count pollen grains randomly, scan the plate and consider each pollen grain viewed. Use care to correctly assess whether germination has occurred. Ask for assistance if needed.

Pollen tube measurements may be made using several different techniques, depending on what

equipment is available in your lab. If a video imaging system is available, measurements can be made quickly and easily. Otherwise, your instructor will show you how to make measurements using a device such as an ocular microscope.

For each time point, at least 10 randomly selected tubes should be measured (the more the better) in a period of time not exceeding about 5 minutes. To select pollen tubes randomly, carefully scan the bottom of the petri dish, considering each pollen grain viewed and measuring any tubes present. Pollen tubes may or may not be present at the first, and possibly the second, time point. Be sure to check with the instructor if you are uncertain regarding whether you are accurately identifying pollen tubes.

ANALYSIS

Following collection of all data, results should be pooled so that the entire class has access to all of the data. The germination and tube length data for the control conditions should be determined for each group, and then averaged for the entire class (also calculate and record the standard deviation for each data point). If more than one group tested a given inhibitor, these data should also be combined.

Once the data are pooled, you should prepare three figures and a table as described below, and answer the questions that follow. Hand in the figures and your answers for your lab assignment. Your instructor may choose to discuss the trends in the data with you during a later class or laboratory meeting.

Figure 1: This figure should show the percentage germination (\pm SD) at each of the designated time periods for the control condition.

Figure 2: This figure should show the average tube length (in micrometers; \pm SD) at each of the designated time periods for the control condition.

Figure 3: This figure should show the average lengths (in micrometers; \pm SD) at the end of the experiment (at X minutes) for the control condition and for each of the three conditions with the inhibitors. This figure should be presented as a bar graph.

Table 1: This table should show the germination data (percentage germination \pm SD) at the end of the experiment (at X minutes) for the control condition and for each of the three conditions with the inhibitors.

Q1 Describe the trends you observe in the Figure 1 that you just created, and discuss what these trends indicate about the biological processes that occurred during the growth of the pollen tubes. In your discussion, you should address the following questions:

A. What is the general shape of the curve associated with the rate of pollen tube germination?

B. Why is such a curve produced when the data are plotted (i.e., what seems to be happening in the population of pollen grains being studied)?

C. How variable are the data represented by this figure?

Q2 Describe the trends you observe in Figure 2, and discuss what these trends indicate about the biological processes that occurred during the growth of the pollen tubes. In your discussion, you should address the following questions:

A. What is the general shape of the curve associated with the rate of pollen tube germination?

B. Why is such a curve produced when the data are plotted (i.e., what seems to be happening in the population of pollen grains being studied)?

C. How variable are the data represented by this figure? Are the data in Figure 2 more variable than the data in Figure 1? If so, why may this be the case?

- Q3** How do the final data (germination and tube length) for the control condition and those for the various inhibitors compare? Were the results what you expected they would be? How might you account for the differences between these various conditions?