

Chapter 9

Pollen Tube Formation and the Central Dogma of Biology

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Reprinted from: Scott jr. R. J. 1995. Pollen Tube Formation and the Central Dogma of Biology. Pages 121–134, *in* Tested studies for laboratory teaching, Volume 16 (C. A. Goldman, Editor). Proceedings of the 16th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 273 pages.

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Introduction

This exercise was developed with two major goals in mind. The first goal was to provide an experiment which uses a dynamic example of plant development. Students often expect experiments involving plants to be less interesting than those using animals. This is frequently because there is less activity for them to observe in botanical experiments. In this exercise, the rapid elongation of pollen tubes, which students observe and measure, provides a very interesting phenomenon upon which to focus. The second goal of this exercise is to reinforce the concepts of genetic information flow. An understanding of these processes, which include transcription (the copying of DNA into RNA), translation (the production of specific proteins using the information in mRNA), and the action of gene products, is essential to an understanding of modern biology. Each of these three aspects of genetic information flow is specifically illustrated by this exercise.

This exercise could be used at various levels within a biology curriculum. If presented in a simplified fashion, elements of it could even be used in an introductory course. However, the student outline as presented here is probably too complicated for this use. Alternatively, this exercise could be used as is, in an upper-level genetics course, in a plant physiology or a plant morphology course, or even in a cell biology or a developmental biology course.

For specific aspects of this exercise, the organization is flexible. The individual instructor can tailor the specific procedure to meet his or her objectives and resources. For instance, the measurement of pollen tubes can be achieved using a computer-aided measurement system or alternative “low technology” methods (see the Notes for the Instructor section and appendix for details). Also, the timing of the measurements can be adjusted to fit the needs of a particular laboratory session.

Materials

Flowers (see Notes for the Instructor section and Appendix A for details)
 Petri plates, 35 × 10 mm (4 per group)
 Markers, wax pencils or permanent marker pens (1 per group)
 Plain pollen tube medium (at least 2 ml per group)
 Pollen tube medium with 30 µg per ml actinomycin D (at least 3 ml for every three groups)
 Pollen tube medium with 200 µg per ml cycloheximide (at least 3 ml for every three groups)
 Pollen tube medium with 20 µg per ml cytochalasin B (at least 3 ml for every three groups)
 Graduated pipets, 1 or 2 ml (2 per group)
 Forceps for holding flowers (1 per group)
 Laboratory counting devices (1 per group)
 Compound microscopes (1 per group)
 Microscope slide and cover slip (1 or more per group)
 Microscope with a measuring system (see Notes for the Instructor section for details)

Student Outline

Introduction

In this laboratory exercise, you will be studying the phenomenon of pollen tube growth and its relationship to a set of concepts which have become known as the “central dogma” of biology.

Pollen Tube Growth

The growth of pollen tubes is a fascinating phenomenon which has served as a model system for research. Pollen grains are small structures (usually ca. 10–50 μm in diameter) which contain either two or three 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 in which the pollen nuclei travel to the ovary of the flower.

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 which has yielded valuable insights relates to the relative roles mRNA transcription and protein translation in the process of pollen tube growth. In this lab exercise, you will be studying the relationships between these phenomena by measuring the growth of pollen tubes under several conditions which inhibit these processes.

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 60s many of the details of this process became known. The concepts which describe how information stored in the DNA is used in the cell have become known collectively as the “central dogma” of biology (it should be noted that 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,” that RNA is copied from DNA and not the other way around, was shown to be less than universal with the discovery of RNA viruses).

The discoveries of the 1950s and 60s provided the following general picture of information flow in cells. 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. All of these processes taken together are often referred to as the “central dogma” of biology, which can be summarized diagrammatically as shown in Figure 9.1.

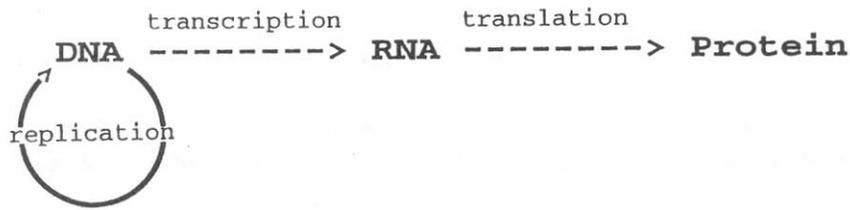


Figure 9.1. A schematic overview of the events collectively known as the “central dogma” of biology.

Much additional information is also available regarding the details of these processes and many techniques have been developed to study the relationships between them. One technique which has been useful in defining the relative importance of each of these processes during growth and development is the use of biochemical inhibitors. Various inhibitors are available which have relatively specific capacities to block certain biochemical processes. For instance, *actinomycin D* is a substance which binds tightly to DNA double helixes and prevents transcription. This 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 which 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 which have been treated with actinomycin D, cycloheximide, and cytochalasin B to determine the relative roles of the processes that each inhibitor affects in the process of tube growth.

Procedure

Your ultimate goals for this exercise are twofold:

1. To characterize normal rates of germination and pollen 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 exercise 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. The initiation of the various treatments should be staggered in time so that available equipment can be used most efficiently.

For each treatment follow these steps:

1. Obtain two 35 × 10 mm petri dishes for each condition.
2. Add 2 ml of the appropriate medium (i.e., plain medium or medium with one of the three inhibitors) to *one dish only*.

Note: The inhibitors used in this experiment have toxic effects, handle them with care. Avoid contact with the skin.

3. Use the demonstrated technique to add pollen from an optimum number of flowers to the 2 ml of medium. Record the time of pollen addition as “time 0”.
4. Suspend the pollen grains in the medium and remove 1 ml of pollen suspension. Place this sample into the second petri dish.
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.

Germination counts should be made for 50–100 randomly selected pollen grains viewed using a compound microscope. Make a wet mount of pollen grains by suspending the grains in the medium and removing a small amount to add to a slide (replace depleted medium in the dish as necessary; use appropriate medium only). To randomly count pollen grains, scan the slide at an appropriate magnification and consider each pollen grain viewed. Use care to correctly assess whether germination has occurred. Ask for assistance if needed.

Pollen tube measurements will be made using the technique demonstrated by the instructor. 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 randomly select pollen tubes, consider each pollen grain viewed and measure 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.

6. Following collection of all data, the entire class and the instructor will discuss appropriate ways of handling and interpreting the data which you collect.

Notes for the Instructor

Materials

The items described below are needed to conduct this experiment. There is some flexibility regarding the number of students involved in each activity. Therefore you must determine the numbers of certain items that you will need, based on the way that you set up the experiment. More detailed notes on some materials appear in Appendix A.

Pollen tube measuring station: The number of stations available will be the major limiting factor with regard to the number of students that can participate in this exercise. If only one of these stations is available, you will have to set up a staggered schedule for measuring pollen tubes cultured under various conditions. The appendix contains a discussion of various types of pollen tube measuring stations which can be used.

Compound microscopes, slides, and cover slips: One compound microscope is needed for each student who will be taking germination counts. It is also extremely helpful if each of these students has a *laboratory counting device* to help keep track of germination counts.

Several packs of 35 × 10 mm disposable petri dishes: The number of dishes needed will depend on the number of groups performing the experiment. Each group will need a total of four plates (two for pollen in plain medium and two for pollen in medium with one of the inhibitors). I use Falcon #1008 dishes.

Pollen tube growth medium: Plain pollen medium (i.e., without inhibitors) and media with the three inhibitors are needed for this experiment. Plain medium is prepared first and then media supplemented with inhibitors is made by adding stock solutions of the inhibitors to the plain medium. Specific instructions for preparation of these media are given in Appendix A.

Flowers: The type of flowers used as a pollen source is an important element for the success of this experiment. Pollen from many flowers fails to germinate *in vitro*. Other types of pollen will germinate but only form short pollen tubes. I have successfully used pollen from five types of plants: the wild flowers *Melilotus officinalis*, *M. alba*, and a species of *Tradescantia*, the ornamental *Gibasis geniculata*, and a cut flower called “sweet pea” purchased at florist shops. Additional notes on the use of these flowers are given in Appendix A.

Procedure

Setting Up the Pollen Cultures

Each group of students should observe the growth of pollen tubes under control conditions (i.e., in plain medium) and also in the presence of one of the three biochemical inhibitors (either actinomycin D, cycloheximide, or cytochalasin B). The inhibitor conditions should be assigned in such a way that the effect of each inhibitor is studied by at least one group. (*Note:* The inhibitors are toxic substances and should be handled with care.) For each of the two conditions, students should set up two petri plates containing the appropriate medium and pollen. One plate will be used for obtaining data on the germination rate and the other will be used for obtaining measurements of pollen tubes.

Once the conditions are set up, each petri plate should contain 1 ml of medium. This is just enough to cover the bottom of the plate, ensuring that adequate gas exchange will occur throughout the culture. However, instead of adding 1 ml to each of two plates and adding the pollen separately, the students are instructed to first add 2 ml of medium to one plate and add pollen to this solution. After a sufficient amount of pollen has been added and thoroughly suspended (using a pipet), 1 ml of pollen suspension is removed and added to the second plate. This is done to ensure that the pollen in both plates represents a homogeneous mixture.

Pollen should be added to the medium in such a way to ensure that as much pollen as possible is present in each petri plate. Different flowers require slightly different techniques. Additional details on techniques for several types of flowers are given in Appendix A.

Characterizing the Germination Rate

Each group should characterize the germination rate for its control culture of pollen grains. This is most easily done by observing wet mounts prepared from the pollen cultures at set time intervals. A new wet mount should be made for each measurement because pollen in wet mounts may not receive sufficient oxygen to continue normal germination and growth. The wet mounts should be made in such a way that the cover slip floats on a large drop of solution. If too little solution is used, the pollen grains will be smashed by the cover slip. To take germination counts students should count a large number of pollen grains (a total of 50 works well), keeping track of the number which have germinated. This process is made much easier if students are supplied with some type of laboratory counting device. At the end of the experiment, each group should have values for the percentages of germinated pollen grains over a range of predetermined times. The percentages from the various groups can be averaged to give a more accurate representation of the actual germination process.

Measuring Pollen Tubes

While one of the members of each group is taking germination counts, another member should be taking measurements of pollen tube lengths. These jobs should be rotated so that each member of the group gets an opportunity to do each job. The student measuring pollen tubes should measure as many tubes as possible in a short period of time (5 minutes or less works well). The students should be instructed to measure all of the tubes that they see, and not just the very long ones. This ensures that all students are measuring the tubes in the same manner. Several possible methods of measuring pollen tubes are discussed in Appendix A.

Timing of the Experiment

The time intervals at which germination counts and tube measurements should be taken is flexible. I have students take these measurements every 20 minutes, however, shorter or longer intervals could be used. If the number of pollen tube measuring stations available is a limiting factor, it will be necessary to stagger the times at which the students initiate their experiments. This way, each group will be taking measurements at different times (i.e., at different “clock times”) but at the same number of minutes from initiation of the cultures.

Characterization of the Effects of Biochemical Inhibitors

In addition to the control cultures, each group should be responsible for one of the three biochemical inhibitors. These cultures should be initiated slightly after the control cultures are initiated. Since equipment is generally limiting, I do not have students make an ongoing characterization of germination and tube growth for these cultures. Instead, I have them make these characterizations at a time equivalent to the last time point at which the control culture were characterized. These data are then compared to the data from the control condition.

APPENDIX A *Additional Information*

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Types of Pollen Tube Measuring Stations

Computer-Aided Measurement

Several types of computer measurement systems are available. These systems can be used for many other types of experiments besides the one described here. The system that I use allows measurements to be made from images seen through a microscope. In this system, a drawing tube (also called a camera lucida) allows one to see the image to be measured superimposed upon an electronic measuring pad. The image is then traced with an electronic cursor and the length of the tracing is stored in an attached computer. The components of this type of system are:

- (a) A microscope with a drawing tube attachment. Either a high power dissecting microscope (ca., 60X) or a compound microscope can be used. I use a dissecting microscope because this enables one to measure the pollen tubes without removing them from the petri plate (i.e., without making a wet mount).
- (b) A computer loaded with measurement software. I use Sigma Scan by Jandel, however other programs would work equally as well.
- (c) An electronic digitizing pad which is hooked up to the computer.

Another type of computer-aided measuring system uses a microscope attached to a video camera which is interfaced with a computer. This type of system employs software which automatically measures and records images observed on the video screen. Many companies including the Jandel Scientific (65 Koch Rd., Corte Madera, CA 94925-9924) sell this type of imaging system.

Alternatives to Computer-Aided Measurement

If computer-aided measuring devices are not available, there are several possible alternatives. The simplest possibility would be to have students estimate pollen tube lengths directly from their microscopic observations. This process could be facilitated by the use of ocular micrometers in the microscopes. The major difficulty that would occur using this approach would be the measurement of curved or sinuous pollen tubes. Pollen tubes growing *in vitro* seldom grow in a perfectly straight line. This tendency becomes more and more pronounced as the tubes continue to elongate.

Another alternative would involve the use of microscopes with attached drawing tubes, but without the computer-aided measuring devices. If such microscopes are available, students could draw the images of the pollen tubes on pieces of paper and then measure the drawn images. The advantage of this approach is that curved or sinuous pollen tubes could be drawn and accurately measured by placing a piece of string on top of the drawn image and then measuring the string.

A similar approach to the one described above could be used if a projecting microscope or a microscope with a video camera and screen were available. In either case, students could superimpose pieces of string over the images of pollen tubes (either projected on a screen or seen on a video monitor) and measure the lengths of the tubes from the pieces of string.

Potential Problems Associated with Measuring Pollen Tubes

The typical problems associated with measuring pollen tubes are the same as those usually encountered when students use microscopes. If students are unable to see pollen tubes, the problem is often one of inappropriate use of the microscope.

Another problem which may occur when measuring pollen tubes involves the accuracy of the measurements which are made. In all systems for measuring pollen tubes described above, microscopes are used to make the measurements. It is therefore necessary to perform some type of calibration routine before meaningful measurements can be made. Calibrating these systems is generally simple, however, problems arise when students (or instructors) forget that the system is calibrated for one particular magnification. Always remember that if the magnification level is changed, the system must be recalibrated.

Pollen Media Preparation

Plain Medium

One liter of plain pollen tube medium contains the following: 100 g of sucrose, 0.1 g of H_3BO_3 , and 0.3 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ made to 1,000 ml with distilled water. These components should be carefully weighed, since variations in the formulation can inhibit pollen tube formation.

This medium should be sterile filtered and kept in a sterile container if long term storage is necessary. Otherwise, use it quickly and keep it free from contamination. When this medium is autoclaved some of the components apparently become complexed and the medium no longer supports good pollen tube formation. Small volumes can be filtered using a sterile syringe with a disc-shaped syringe filter (i.e., Acrodisc #4192). Larger volumes can be filtered through sterile suction filtration devices (i.e., Nalgene filter unit #121-0020). Sterile solutions can be collected in autoclaved glass containers or pre-sterilized plastic containers (i.e., Falcon sterile tubes #2098).

When this plain medium is used as the plain pollen tube growth medium, 0.4% dimethyl sulfoxide (DMSO) should be added as control since the media with inhibitors contains DMSO (see below). *Note: DMSO has some harmful effects, direct contact with the body should be avoided.*

Media with Biochemical Inhibitors

Caution: Each of the three biochemical inhibitors has toxic effects. They should be handled with special care during the preparation of the media. Once they are in solution, they are less dangerous. Avoid contact with the skin and also avoid inhaling dust from the powdered inhibitors. You may wish to provide gloves for students to wear during the preparation of pollen cultures with media containing inhibitors.

The inhibitors are prepared as described below. Stock solutions are first prepared in DMSO because the inhibitors have limited solubility in aqueous solutions. For best accuracy, microliter volumes (μl) should be added using a micropipet. For each inhibitor solution, the indicated volume of stock solution is added to pollen tube medium made according to the directions given above.

Actinomycin D Medium

Stock solution: Add 100 μl (0.1 ml) of DMSO to 1 mg of actinomycin D.

30 $\mu\text{g}/\text{ml}$ solution: Add 30 μl of stock solution to 10 ml of plain pollen tube medium.

Cycloheximide Medium

Stock solution: Add 1 ml of DMSO to 10 mg of cycloheximide.

200 $\mu\text{g}/\text{ml}$ solution: Add 200 μl of stock solution to 10 ml of plain pollen tube medium.

Cytochalasin B Medium

Stock solution: Add 100 μl of DMSO to 0.5 mg of cytochalasin B.

20 $\mu\text{g}/\text{ml}$ solution: Add 40 μl of stock solution to 10 ml of plain pollen tube medium.

Flowers Used as Pollen Sources

I use pollen from the common wild flowers *Melilotus officinalis* (yellow clover) and *M. alba* (white clover). These plants grow abundantly in many parts of the country during the summer. Pollen from *Melilotus* works extremely well because it produces very long pollen tubes and exhibits a very high percentage germination. *Melilotus* pollen must be incubated in pollen tube media for at least 2 hours in order for differences in pollen tube lengths to be observed.

The common ornamental plant *Gibasis geniculata* (the “bridal veil” plant) also produce pollen which gives good results like those obtained with *Melilotus*. Pollen from a flower called “sweet pea,” available in florists shops during the winter months, also gives results similar to those obtained with *Melilotus* pollen.

I have also obtained good results with the pollen of a wild species of *Tradescantia* (“spiderwort”) obtained locally. Pollen from these plants germinated and grew very quickly. Very long pollen tubes (over 1 mm) were present in plain medium after 1 hour and differences between the control condition and the conditions with the inhibitors could readily be observed at that time. If *Tradescantia* pollen is used, meaningful measurements cannot be obtained after 1.5–2.0 hours because the tubes in control medium begin to coil once they reach a certain length. It is unknown if pollen from all species of *Tradescantia* will perform equally well. Both Ward’s and Carolina Biological Supply Company carry live specimens of *Tradescantia*. If these plants are to be used for this experiment it is suggested that they be purchased well in advance so that the plants can become established in a green house and the pollen can be tested.

For all of the plants described above, it is necessary to ensure that the flowers used for this experiment have anthers which have gone through anthesis (“pollen shedding”). For *Melilotus*, this is done simply by selecting flowers in the appropriate developmental stage. The flowers of this plant grow on a spike, with the youngest, unopened flowers on the top and the oldest, senescent flowers on the bottom. Flowers with highly viable pollen are selected simply by using only open flowers from the middle of the spike. For the flowers of *Gibasis*, sweet pea, and *Tradescantia* the anthers should be visually inspected to determine if pollen is present and old, withered flowers should not be used. For *Tradescantia*, special care should be taken because even fresh-looking flowers may have anthers with virtually no pollen remaining on them.

To add the maximum amount of pollen to a culture, different techniques are needed for different types of flowers. Pollen from flowers with exposed anthers, such as those of *Gibasis* and *Tradescantia*, can be added to the medium by immersing the flower or individual anther in the medium (using forceps). To add as much pollen as possible, the flowers or anthers should be ground against the side or bottom of the petri plate. *Melilotus* produces small flowers with a keel petal which encloses the anthers. To expose the anthers, the flower can be pinched with a forceps at the base of the keel petal, causing the keel to open. The pollen is then added to the culture as described above. The sweet pea flower also has a keel petal, but the flower itself is much larger. The anthers of this flower should be dissected out and immersed into the pollen medium. After the pollen is added, the solution should be suspended by aspirating with a pipet and the culture should be viewed with a microscope to verify the presence of sufficient pollen.

It may be possible to freeze certain flowers and use the pollen later. I have obtained pollen exhibiting varying degrees of viability after several days to 1 month of frozen storage, using flowers of *Melilotus*, *Gibasis*, and *Tradescantia* (prolonged storage appears to substantially reduce viability; frozen storage of sweet pea flowers has not yet been tested). For these experiments, I have frozen flowers in air-tight microcentrifuge tubes (i.e., Fisherbrand #50-407-5) in a constant temperature deep freeze (at -70°C) and in a freezer unit of “household”-type refrigerator-freezer. Since variable results have been obtained, this technique is not recommended as a reliable means of storing pollen for future use. However, further experimentation with frozen storage of flowers may lead to a reliable method.

Interpretation of Results

In this section typical results for pollen from the types of flowers noted above will be described and discussed. Figures 9.2 to 9.4 are graphical representations of actual data my students have collected using *Melilotus* pollen. When I conduct this experiment with students, I leave some time at the end of the lab period to discuss the trends with them. I generally have them post their data on the board as they collect it, and then I use these data to sketch graphs of the results on an overhead projector.

Germination Patterns

Generally speaking, pollen from most flowers germinates over a period of time in such a way that a sigmoidal pattern is observed (Figure 9.2). However, some pollen may germinate so rapidly that the initial lag period is not evident. After students collect data on germination from their control conditions, the nature of this pattern should be discussed. They should realize that this pattern demonstrates the heterogeneous nature of the pollen grain population which they are studying. Some pollen germinates very early and grows for a long time, while others germinate later and grow for a shorter time. This observation becomes important when they begin to study the data collected from the tube length measurements.

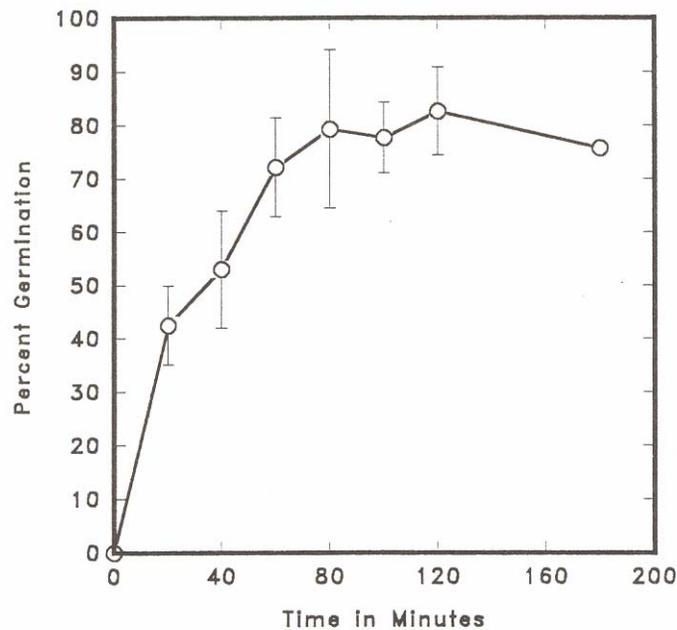


Figure 9.2. Average percent germination (\pm SD) over time for pollen grains of *Melilotus officinalis* cultured in plain medium for a total of 3 hours.

Pollen Tube Lengths

Generally, pollen tubes grow in such a way that the increase in average length is proportional to the increase in time (Figure 9.3). It is also noteworthy that the variation (shown as standard deviation in Figure 9.3) in average tube lengths increases greatly over time. This observation provides a good opportunity to discuss variability in a heterogeneous population. It also provides a graphic illustration of how insights gained from one aspect of an experiment (i.e., germination measurements) can help to explain observations derived from another aspect of an experiment (i.e., tube length measurements).

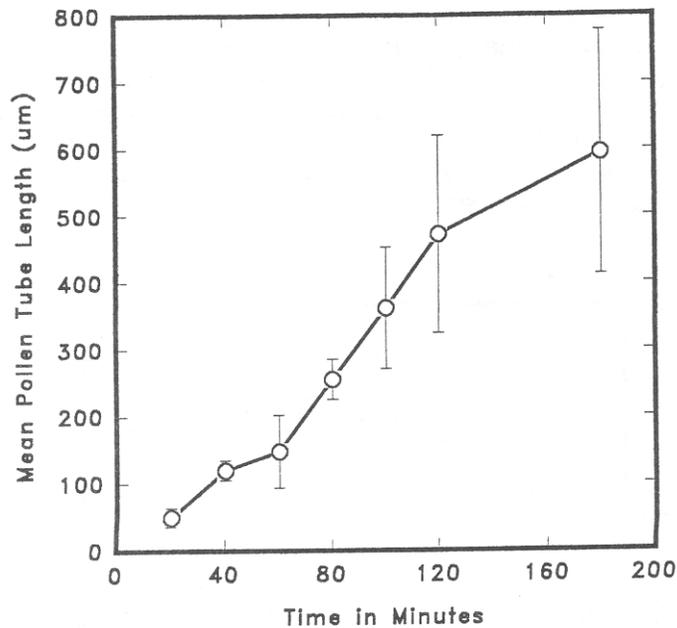


Figure 3. Average pollen tube length (\pm SD) over time for pollen grains of *Melilotus officinalis* cultured in plain medium for a total of 3 hours.

Effects of Biochemical Inhibitors

After discussing how pollen normally germinates and forms pollen tubes *in vitro*, students can better understand how the biochemical inhibitors effect these processes. It should be recalled that actinomycin D blocks transcription, cycloheximide blocks translation, and cytochalasin B blocks the polymerization of actin microfilaments. I usually discuss these relationships at the beginning of the lab period and ask students to make predictions about how these inhibitors will effect germination and tube growth. At the end of the period when we discuss their results, I remind them of their predictions and we see how accurate they were.

Each of the inhibitors somewhat reduces the ultimate percentage of germinated pollen grains (data not shown). Actinomycin D has the least effect on germination, reducing it only slightly as compared to the control. Cycloheximide reduces germination significantly, but a large number of pollen grains still germinate (as many as 50% in some experiments). And cytochalasin B prevents virtually all pollen grains from germinating. Occasionally a very small number of germinated pollen grains are seen in this medium. However the number is so small (usually less than 1%) that these may actually be grains that germinated on the stigma of the plant and were inadvertently introduced into the medium in this condition.

The trends for the average pollen tube lengths in different media are similar to those for germination (Figure 9.4). Pollen grains cultured in actinomycin D have tubes which are somewhat shorter than those of the controls. The tubes of pollen cultured in cycloheximide are even shorter. And those of pollen cultured in cytochalasin B are very short (it should also be noted that this measurement represents the length of the tubes of the few germinated pollen grains out of several hundred grains observed).

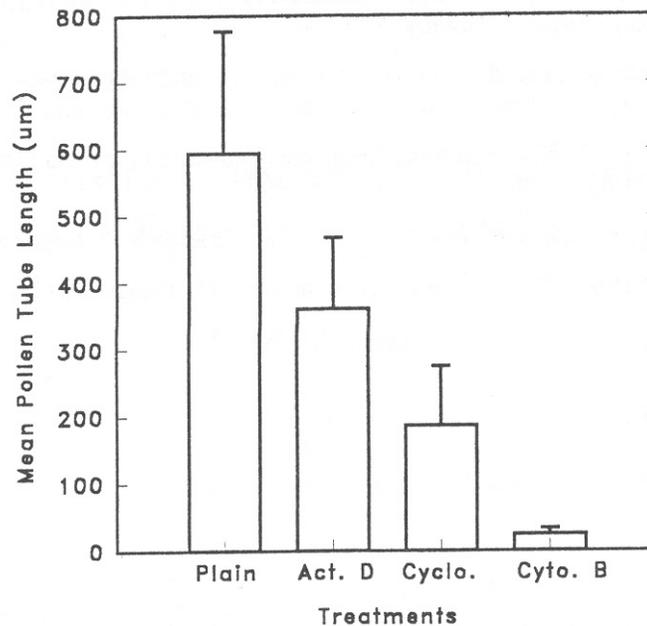


Figure 9.4. Average lengths (\pm SD) of pollen tubes of *Melilotus officinalis* after 3 hours of culture in plain medium, and media containing actinomycin D (Act. D), cycloheximide (Cyclo.), and cytochalasin B (Cyto. B).

The effects the inhibitors on both germination and tube growth can be readily understood with reference to the concept of genetic information flow (“the central dogma”). Students often assume that all three of the inhibitors will completely prevent germination. However, when they see the data, they can be directed towards making some insightful conclusions. The first point that we generally discuss is the significant amount of growth in cultures which contained actinomycin D. I usually ask them why so much growth occurred if no *new* mRNAs were being produced. Eventually, some bright student volunteers the idea that perhaps *new* mRNAs might not be needed for initial growth, perhaps there is a stockpile of pre-made mRNAs already present in the pollen grains. This turns out to be the correct explanation as verified by experiments described by Mascarenhas (1966, 1971). Similar situations involving the presence of pre-made or “maternal” mRNA exist in many systems which undergo rapid growth and development. Examples include their presence in many animal eggs including those of sea urchins and frogs and also in fern spores. Maternal mRNAs allow initial growth to occur rapidly and by the time they are used up, newly transcribed mRNAs are generally present to support continued growth and development.

Cycloheximide, which inhibits translation, greatly reduces both percentage germination and also tube length. It would be possible to hypothesize that a certain quantity of pre-made proteins exist which support this limited amount of growth even in the presence of cycloheximide. However, this appears not to be the case, instead, the growth which occurs seems to result from translation of pre-made mRNAs during the time it takes for cycloheximide to penetrate the pollen grain and stop translation (Mascarenhas, 1971).

Cytochalasin B is the most effective of the three inhibitors. It almost completely blocks germination and tube growth (the very small number of grains which appear to germinate in this medium may represent an artifact of the experimental set up, see above). This inhibitor is also the most specific of the three, blocking the function of one single gene product. Apparently this inhibitor functions quickly and completely blocks actin polymerization. Since no actin can polymerize, microfilaments are not formed and no germination occurs.

Literature Cited and Related References

- Mascarenhas, J. P. 1966. Pollen tube growth and ribonucleic acid synthesis by vegetative and generative nuclei of *Tradescantia*. *American Journal of Botany*, 53(6):563–569.
- . 1971. RNA and protein synthesis during pollen development and tube growth. Pages 201–222, *in* Pollen: Development and physiology. (J. Heslop-Harrison, Editor). Butterworth and Co., London.
- Mascarenhas, J. P., and E. Bell. 1970. RNA synthesis during development of the male gametophyte of *Tradescantia*. *Developmental Biology*, 21:475–490.
- Schimpf, D. J. 1992. Rapid germination of pollen *in vitro*. *The American Biology Teacher*, 54(3):168–169.
- Steer, M. W., and J. M. Steer. 1989. Pollen tube tip growth. *New Phytologist*, 111:323–358.