

# Chapter 1

## A Practical Guide to the Use of Cellular Slime Molds for Laboratory Exercises and Experiments

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1

## Contents

Introduction.....	2
Background.....	2
Cell Culture.....	3
Stock Cultures.....	4
Life Cycle Plates.....	4
Growing and Harvesting Cells for Experiments.....	5
Aggregation-Competent Cells.....	6
Development Cultures.....	6
Clearing Contaminated Cultures.....	7
Experiments.....	7
Choice Between Developmental Pathways: Macrocysts vs. Fruiting Bodies.....	8
Food Preferences in Feeding Cellular Slime Mold Amoebae.....	10
Chemotaxis.....	11
Phototaxis.....	13
Summary.....	15
Literature Cited.....	16
Appendix A: Additional References.....	17
Appendix B: Recipes.....	18
Appendix C: Materials.....	19

## Introduction

One of the challenges in the design and implementation of laboratory exercises is to find suitable live materials for study. One wishes to choose an organism: (a) which is easily handled and maintained, (b) for which a rich source of biological phenomena can be demonstrated, (c) which is amenable to experimental work at both introductory and advanced levels, and (d) which is relatively inexpensive. The cellular slime molds fulfill these requirements admirably. During the course of their life cycle, they undergo many of the same phenomena that must occur for the development of more complex organisms: chemotaxis, cell migration, morphogenesis, and changes in gene expression. However, since cellular slime molds are microorganisms, it is easy to grow and manipulate them in culture. The goals of this chapter are to provide instructors with the information necessary for the “care and feeding” of the cellular slime mold, *Dictyostelium discoideum*, and to present a few examples of laboratory exercises using this organism.

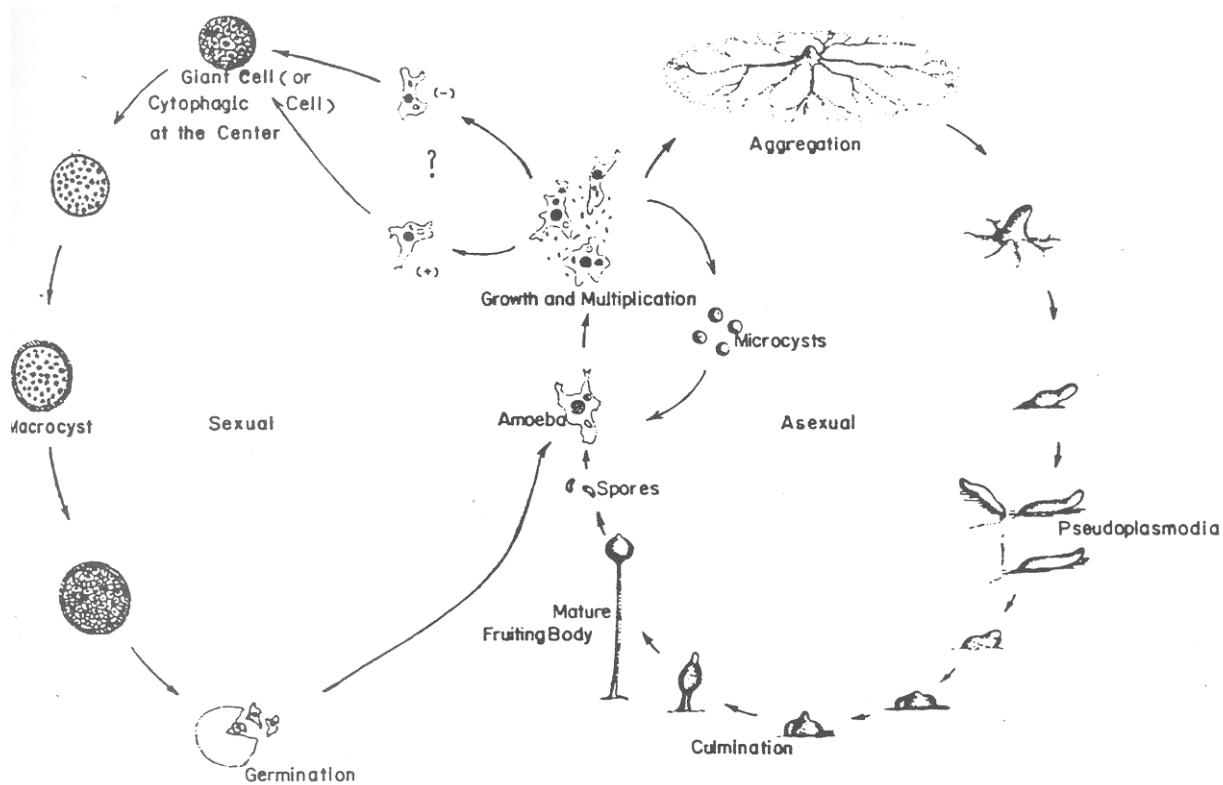
## Background

The life cycle of *D. discoideum* consists of two possible developmental pathways, each with a very different outcome: asexual development results in the formation of a fruiting body and sexual development produces macrocysts (Figure 1.1). Development starts when independently-feeding amoebae run out of food. As long as nutrients are available, amoebae will continue to eat and divide. When food is depleted, cell division ceases and development begins (reviewed in Bonner, 1982).

During asexual development, starving amoebae aggregate to central collection points thus producing a multicellular structure. This aggregate undergoes a series of shape changes resulting in the formation of a fruiting body, a ball of spores on top of a cellular stalk. If food becomes

available, these spores germinate to release amoebae thereby beginning the cycle again (Bonner, 1944).

During sexual development, starving amoebae of opposite mating type fuse to form a giant cell. These giant cells attract other amoebae and eat them. Protective walls are laid down around the giant cell, thus producing a macrocyst. Eventually, the giant cell, within the macrocyst, undergoes meiosis and then several rounds of mitosis so that upon germination of the macrocyst, a new population of haploid amoebae are released (Erdos et al., 1973; Filosa and Dengler, 1972).



**Figure 1.1.** Life cycle of the cellular slime mold, *Dictyostelium discoideum*. See text for details.

### Cell Culture

Since *D. discoideum* is a microorganism, it can be cultured and maintained using relatively standard methods. This is particularly helpful to the instructor because preparation of live material is fairly straightforward and stock cultures can be stored over long periods of time. This is also helpful to the student because failed experiments can be repeated without too much extra work and more importantly, new experimental questions can be pursued without delay. The methods described in this section detail how to: (a) culture and maintain stock cultures, (b) prepare “life cycle” plates for demonstration, (c) grow and harvest cells for experimental work, and (d) prepare “aggregation competent” cells, (e) culture cells for development, and (f) clean contaminated cultures.

## Stock Cultures

For establishment and subculture of stock cultures, it is essential that aseptic technique be used throughout these procedures. Stock cultures of *D. discoideum* and *Klebsiella aerogenes* are maintained on lactose-peptone agar (2LP). *K. aerogenes* is a bacterium that is used as a source of food for the cellular slime mold amoebae. One to three days before stocks are to be transferred, a broth culture of *K. aerogenes* is prepared; approximately 50 ml of Sussman's Medium (SM/2) broth is inoculated with a loopful of *K. aerogenes* (maintained as a stock on 2LP). Incubation is at 20–24°C.

To prepare stock plates of *D. discoideum*, two successive inoculations are done. First, 0.5 ml of *K. aerogenes* suspension is pipetted onto 2LP agar. Second, a loopful of *D. discoideum* spores is added to the plate. In order to disperse the spores and bacteria, the suspension is spread over the agar using a glass elbow (a glass rod bent at a 90° angle). Stacks of plates should be taped to retard drying. Incubation is at 20–24°C in the light. Stock cultures can be stored at 20–24°C for up to 1 month (although it is best to subculture every 2 weeks), in the refrigerator for 4–6 months, or desiccated on silica gel for years; lyophilized samples are essentially immortal (Raper, 1984).

## Life Cycle Plates

One of the most interesting aspects of cellular slime mold biology is the asexual life cycle. For introductory labs in which one might want to demonstrate this life cycle and for advanced labs where students may need to gain skill in the recognition of various morphological stages, it is very helpful to prepare “life cycle” plates.

The procedure for preparing life-cycle plates are as follows:

1. Inoculate plate with bacteria.
2. Spread bacteria with glass elbow.
3. Add a loop of spores to center of plate.
4. Tape shut.
5. Incubate.

Approximately 3–5 days before the plates are needed, inoculate 2LP with 0.5 ml of *K. aerogenes* cell suspension prepared as described above. Using a glass elbow, spread the bacterial suspension on the agar. Next, deposit a loopful of spores into the center of the plate; do not spread them around. Tape the plate shut and incubate at 20–24°C in the light. The spores deposited in the center of the plate will germinate, and the resulting amoebae will feed on the bacteria. As food becomes scarce, amoebae will migrate out as a ring towards the greater quantity of bacteria present on the rest of the plate. Any amoebae left behind will starve and thus initiate their development. Successive rings of feeding amoebae will continue to migrate out towards the edge of the dish and other groups of amoebae will be left behind to develop. The result is that the amoebae that have developed for the longest amount of time will be in the center of the dish and the vegetative, feeding amoebae will be at the periphery. In between, stages of the entire life cycle will be present. The life cycle plates work best if the feeding amoebae have moved to a position approximately 2 cm from the edge of the petri dish. If the cultures seem to be developing too fast for your needs, simply put the plates in the refrigerator and they will go into “suspended animation.” You can retrieve them before your scheduled lab. For the preparation of life cycle plates, it is a good idea to maintain aseptic technique.

## Growing and Harvesting Cells for Experiments

To prepare cells for use in experiments, one uses the same culturing technique employed for the preparation of stock cultures. Incubation is at 20–24°C for 34–38 hours, in the light for experiments involving fruiting body development, and 20–22 hours in the dark for those concerned with macrocyst production. Culturing and harvesting 22 hours later is obviously not a problem but a 34- to 38-hour incubation can be quite a challenge. Happily, one can put the cultures into the refrigerator for hours so that cell harvest can be done at a more convenient time. For example, I will inoculate cultures at 4 p.m. on Day 1. On Day 2, I will incubate these cultures in the refrigerator for 8 hours; as far as the cells are concerned, this time does not count. Incubation at 20–24°C is resumed and cells can be harvested at 12 p.m. on Day 3. The number of cultures prepared depends on the requirement of a particular experiment. For planning, you can estimate that every petri dish (diameter = 8.5 cm) will yield  $1 \times 10^7$  to  $5 \times 10^7$  cells depending upon the length and temperature of incubation.

To harvest cells, pipet or pour approximately 10 ml of cold Bonner's Salt Solution (BSS) onto a culture. Gently dislodge the cells, using the glass elbow, and pour the resulting cell suspension into the next culture. Dislodge the cells in that culture with the glass elbow, pour the suspension into the next plate; repeat until all plates have been scraped. The cell suspension is poured into a sterile centrifuge tube and placed on ice. Next, wash the culture plates that have just been harvested by pipetting approximately 5 ml of BSS onto one of the cultures. Again, gently dislodge any cells that might remain and pour the suspension into the next dish. Repeat until all plates have been washed and combine this suspension with the one collected above. It is important to keep this cell suspension on ice as much as possible during these procedures. The number of tubes needed will depend upon the number of plates to be harvested. Plates are best harvested in groups of 10 to 15, each group producing 15 ml of crude cell suspension.

The cell suspension collected still has too many bacteria in it for use in an experiment. In order to separate the amoebae and bacteria, the cell suspension is subjected to differential centrifugation. Using a clinical, table-top centrifuge (or whatever you like), spin the cell suspensions at 1500–2000 rpm for 4 minutes. Discard the supernatant and resuspend the cell pellet in cold BSS. Vortex gently. Spin again. Discard supernatant, resuspend the pellet; repeat this 3–5 times until the supernatant is fairly clear. Resuspend cells in cold BSS, determine cell density (using a hemocytometer or Coulter Counter) and adjust the cell density to whatever you require. At the end of the cell harvest, you will have a suspension of clean, vegetative amoebae that when cultured on a solid substrate, will initiate development and complete the life cycle in 24 to 36 hours.

A summary of the procedure for cell harvest and quantitation of cell density is as follows:

1. Inoculate bacteria and spores.
2. Incubate 34–38 hours.
3. To harvest:
  - (a) Wash cells off plates.
  - (b) Centrifuge, discard supernatant, resuspend cells, ..., until clean.
4. To count:
  - (a) Dilute a small aliquot.
  - (b) Count using a hemocytometer.
  - (c) Resuspend cells to appropriate density.

## Aggregation-Competent Cells

Newly-starved amoebae must go through some very important molecular and biochemical changes before they are able to commence aggregation and hence, morphogenesis. This interval is called interphase (Wier, 1977). For some experiments, you may not want to wait for cells to complete interphase, you might prefer to start your experiment with cells that are closer to beginning morphogenesis; these are called “aggregation-competent” cells. The usefulness of aggregation-competent cells is especially great in any experiment studying chemotaxis, morphogenesis or any particular multicellular stage of the life cycle.

To prepare aggregation-competent cells, *D. discoideum* spores are grown and harvested using the methods described above. The resulting cell suspension is adjusted to a cell density of  $2 \times 10^8$  cells/ml and 1 ml is pipetted onto a petri dish containing non-nutrient agar (NNA). The cells are spread over the plate using a glass elbow. The cultures are incubated at 20–24°C in the light for 2–4 hours. They are placed in the refrigerator overnight (15–18 hours) and restored to room temperature approximately 2–3 hours before the cells are to be used. Such cells will begin aggregation in 1–4 hours.

The procedure to prepare aggregation competent cells is as follows:

1. Grow and harvest cells.
2. Adjust cell density.
3. Place cells on non-nutrient agar.
4. Incubate 2–4 hours at room temperature.
5. Refrigerate overnight (15–18 hours).
6. Return to room temperature.

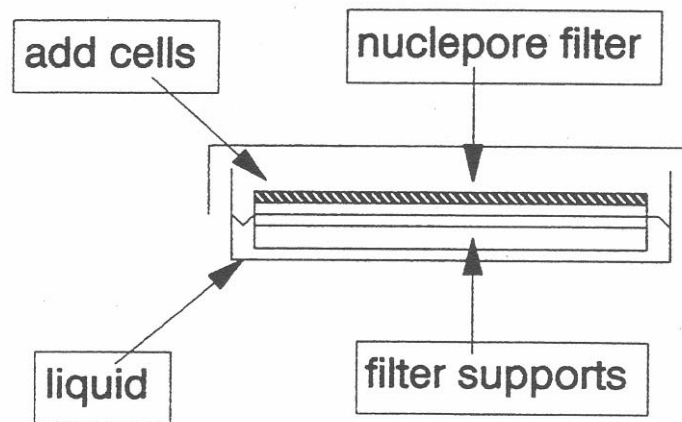
## Development Cultures

There are two principle methods used to develop amoebae in culture. Both permit clear observation and study of morphogenesis as well as investigation of the effects of various chemical parameters on development.

With the first method, vegetative or aggregation-competent cells are deposited onto petri dishes containing NNA. An optimal number of cells to deposit on a standard size petri dish would be  $1 \times 10^8$ . To test the effects of a specific chemical, dissolve it in the agar before the plates harden (see the instructions for Chemotaxis Experiment). To see whether the effects of a particular chemical are reversible, harvest the cells from the dish and replate them on fresh NNA.

The second method uses a different solid substrate (Figure 1.2). Black nuclepore filters are placed on top of two supporting filter pads and all are placed in a small sterile petri dish. The pads are wetted with approximately 0.5 ml of BSS. Gently pipette 250  $\mu$ l of a suspension of vegetative or aggregation competent cells ( $2 \times 10^8$ /ml) onto the nuclepore filter; use the pipet tip to spread the cells to cover the filter. After the cells have soaked into the filter, add more BSS to the petri dish so that the filter pads are thoroughly soaked and the bottom of the dish is covered with liquid. To test the effects of a particular chemical, substitute it for the BSS. To examine the timing of sensitivity to a chemical or the reversibility of the effect, you can transfer the nuclepore filter to a new dish containing filter pads soaked with BSS.

Interesting chemicals to test include transcriptional inhibitors, translation inhibitors, metabolic poison, drugs that effect the cytoskeleton, and certain ions.



**Figure 1.2.** Diagram of set-up for development on filters. See text for details.

### Clearing Contaminated Cultures

It is a good idea to maintain several stock plates of each strain or species of cellular slime mold that you carry. Even so, sometimes a culture can become contaminated. When this happens, the first thing to attempt is a subculture from a non-contaminated region of the plate. If this fails, there is one last trick that you can try before you reorder stocks. This method takes advantage of the fact that the slug stage of the life cycle is phototactic.

Inoculate one edge of a 2LP plate with approximately 50  $\mu$ l of *K. aerogenes* suspension. Deposit a small loop of spores from your contaminated culture right into the *K. aerogenes* on the plate. Do not spread. Tape the culture shut and very carefully cover the plate with aluminum foil. On the side of the dish, directly opposite the position where you inoculated the plate (180° from the inocula) make a small hole in aluminum foil. The hole should be about the size of the head of a pin and it must be on the side and not on top (see Figure 1.8). When the spores you inoculated germinate and the amoebae deplete the *K. aerogenes* available, the life cycle will be initiated. The slug stage will migrate in response to the light (your contaminant will not). Consequently, the slugs will migrate to the pinhole, and you will have uncontaminated fruiting bodies. This works best if you give the culture 1 week to clean itself.

### Experiments

Described on the following pages are protocols for four experiments which each illustrate an important biological phenomenon. For each experiment, information relevant to the instructor is presented and the student instructions are delineated. In addition, several possible variations or extensions of each of these experiments are provided along with the instructional level intended for each experiment.

## Choice Between Developmental Pathways: Macrocyts vs. Fruiting Bodies

### *Instructor's Information*

One of the most important, unanswered questions in biology is how do embryonic cells choose particular developmental pathways. One aspect of this question is to ask: What are the cues or signals that influence the choice between, for example, becoming liver vs. brain, flower vs. root, or spore vs. amoeba? This experiment addresses the question of how do cellular slime mold amoebae decide whether to mate and produce a macrocyst or develop asexually and construct a fruiting body. While not very much is known about how *D. discoideum* chooses mating vs. fruiting, experiments have shown that certain physical conditions are important. For instance, light, lack of moisture, presence of phosphate ions, absence of a compatible mate and warmer temperatures favor fruiting body formation while the converse enhances macrocyst production (reviewed in Raper, 1984).

The protocol outlined below provides instructions for preparing cultures and suggests some ideas for experiments. Students can set up their experiments using stock plates of *D. discoideum* NC4 and V12 as sources of spores or you may provide them with spore suspensions.

To prepare spore suspensions, harvest the fruiting bodies from stock plates, within 1 or 2 hours before the beginning of your lab. For a class of 20, with students working in five teams of four, you will need three stock plates of *D. discoideum* NC4 and three of V12.

To harvest the spores, pipet approximately 15 ml of BSS onto a plate of spores. Gently dislodge the fruiting bodies with the glass elbow and pour the suspension into the third plate of the same strain. After dislodging the fruiting bodies from this third plate, pour the spore suspension into a sterile beaker or flask. Pipet another 15 ml of BSS onto the first harvested plate, gently swirl with the glass elbow, and pour into the second plate and into the third. This wash should be combined with the spore suspension. Finally, bring the spore suspension volume up to approximately 50 ml with BSS. Repeat the same washing and harvesting steps for the other *D. discoideum* strain.

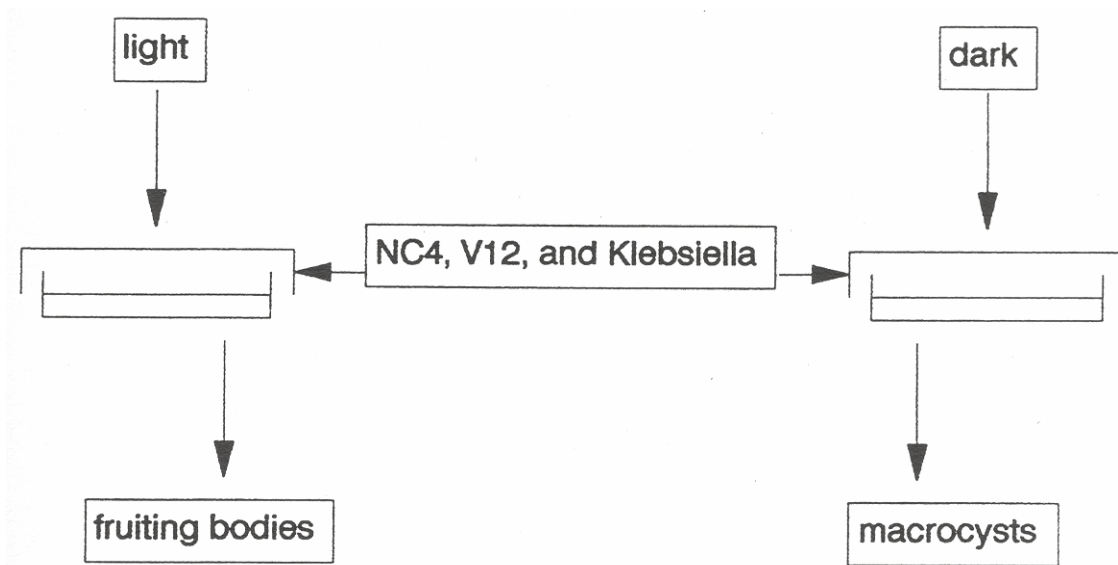
In the form presented here (see Figure 1.3), this exercise is suitable for a course in introductory biology and, with minor variations, cell or developmental biology. Such variations might include (a) starting the experiment with washed amoebae of each mating type rather than with spores; and (b) designing more complex experiments to study this question.

### *Student Methods*

A general protocol for preparing your cultures is described below. You will undoubtedly deviate from this procedure according to the specific needs of your experiments but this description should serve as a guideline.

1. *Maintain sterile technique throughout this experiment.*
2. Each group will be provided with lactose-peptone agar (LP; for growth and development of *D. discoideum*), a suspension of *Klebsiella aerogenes* (for *D. discoideum* to eat), separate suspensions of spores of NC4 and V12 (opposite mating types), and Bonner's Salt Solution (BSS; for adjusting moisture level).
3. To prepare a culture of *D. discoideum* for development, put the following on an LP plate: 0.5 ml *Klebsiella*, 0.25 ml NC4 spores, 0.25 ml V12 spores, and 2 ml BSS. Gently rock plate back and forth to distribute liquid. Tape shut. Incubate in the light; for incubation in the dark, cover the petri dish with aluminum foil.



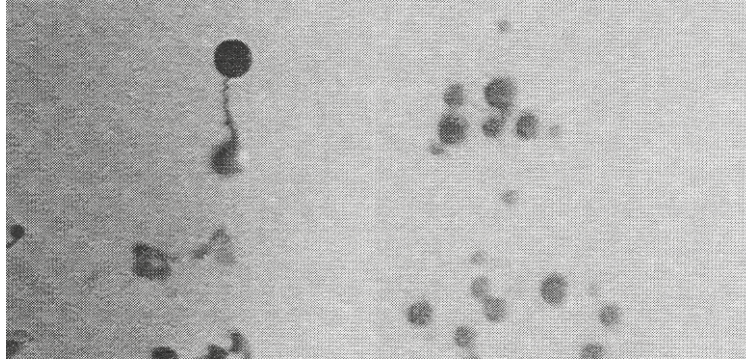


**Figure 1.3.** Set-up for developmental choice experiment. See text for details.

4. For your experiments, you may choose one of the following ideas or you may pursue a question of your own:
  - Light vs. dark
  - Temperature
  - Different wavelengths of light (wrap the petri dishes in cellophanes of different colors)
  - Different moisture contents (adjust the amount of BSS added)
  - Relative importance of light and moisture
  - Effect of phosphate
  - pH
  - Amount of time in light vs. in dark
  - Requirement for presence of *both* mating types
5. Data collection and analysis:
  - (a) Using a dissecting scope, observe your cultures and record the types of structures that developed: fruiting bodies, macrocyts, indeterminate (Figure 1.4).
  - (b) Try to assess relative amounts of each type of structure with the following scale: **0**: none; **+**: a few; **...++++**: lots.
  - (c) Record any pertinent observations.

20X (*left*); macrocyts, 40X (*right*).

**Figure 1.4.** Sample results from the developmental choice experiments: fruiting bodies,



**Figure 1.4.** Sample results from the developmental choice experiments: fruiting bodies, 20X (*left*) macrocysts, 40X (*right*)

### Food Preferences in Feeding Cellular Slime Mold Amoebae

#### *Instructor's Information*

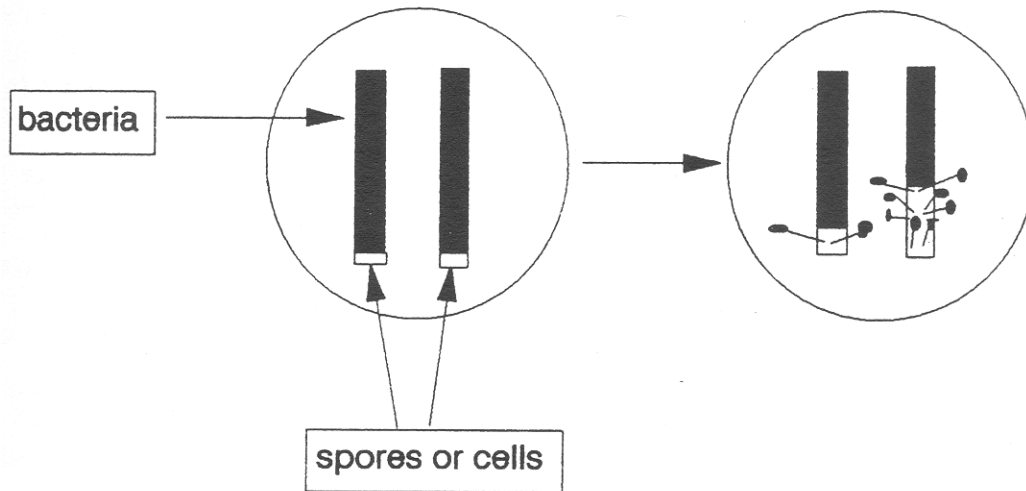
Examination of soil samples collected from a variety of locations reveal that different species of cellular slime mold exist very close together in nature and in all probability, inhabit the same physical space (Buss, 1982; Kuserk, 1980). The question arises then, How do these organisms partition their niches? At least for some cases, it has been determined that different species of cellular slime mold feed on different soil bacteria (Horn, 1971). Therefore, the similarity of the physical space occupied is not important; the different species avoid direct competition by having different food preferences. The experiment outlined below describes a simple method to study this behavior (Figure 1.5).

This experiment is appropriate for an introductory biology lab or, with minor modifications, a course in ecology. Such modifications include increasing the number of bacterial species and cellular slime mold species tested, or examining the results of direct competition by mixing cellular slime mold species. Suitable bacterial species to use include any soil bacteria. Cooperative cellular slime mold species include *D. discoideum*, *D. mucoroides*, *D. rosarium*, *D. giganteum*, *Polysphondylium violaceum*, and *P. pallidum*.

#### *Student Methods*

1. On Day 1 of the experiment, you will prepare the bacterial streaks. Pipet 1 ml of sterile SM/2 into a sterile test tube. Inoculate the SM/2 with 2–3 loops of bacterial Species 1. Vortex to suspend the cells. Using a sterile loop, “paint” a thin line of the bacterial suspension on a petri dish of 2LP agar. Prepare two plates in this manner.
2. Repeat step 1 for bacterial Species 2 and 3.
3. On Day 3, deposit, at one end of the streak of bacteria Species 1, 25  $\mu\text{l}$  of a suspension of cellular slime mold spores ( $1 \times 10^7/\text{ml}$ ) or starved vegetative amoebae ( $1 \times 10^7/\text{ml}$ ). Repeat for bacteria Species 2 and 3.

4. Repeat step 3 using a different species or strain of cellular slime mold.
5. Determine the rate of feeding by measuring the movement of the feeding front. Measurements should be taken daily for 7 to 10 days. Class data can be pooled.



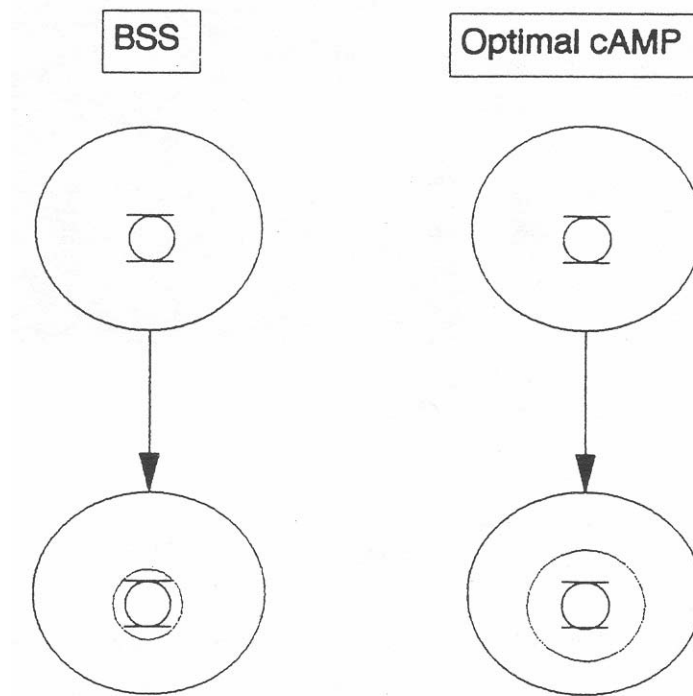
**Figure 1.5.** Diagram of set-up for food preference experiments. See text for details.

## Chemotaxis

### *Instructor's Information*

One of *D. discoideum*'s main claims to fame is that the process of cell aggregation is mediated by chemotaxis to cyclic AMP (cAMP; Konijn et al., 1967). The protocol outlined below describes a straightforward quantitative assay. Aggregation-competent amoebae are deposited as drops onto agar containing various concentrations of cAMP. The assay takes advantage of the fact that aggregating *D. discoideum* amoebae release an extracellular phosphodiesterase which degrades cAMP. This degradation of cAMP will be greatest in the positions where amoebae are located. The amoebae will migrate away, in a concentric ring, from the spot where they were initially deposited. The amoebae will degrade the cAMP present in this new area and will migrate out further. The consequence is that if cells are placed on agar containing a concentration of cAMP appropriate to elicit a chemotactic response, the cells will migrate out in a ring and onto the agar. One can measure the distance migrated as a function of time to quantitate the chemotactic response (Figure 1.6).

This experiment is suitable for a cell biology or developmental biology courses. Related questions include testing cAMP analogues and related molecules, determining the chemotactic response of various developmental stages (e.g., vegetative amoebae, slugs, etc.), or investigating the species specificity of the chemotactic response (not all cellular slime molds use cAMP as a chemoattractant; reviewed in Raper, 1984).



**Figure 1.6.** Diagram of set-up and hypothetical results for the chemotaxis experiments. See text for details.

### *Student Methods*

1. You will each receive two plates of NC4 aggregation-competent amoebae. These cells have already been washed clean of bacteria and will be ready for action. Each plate contains  $2 \times 10^8$  cells.
2. Place 3 ml of cold BSS (Bonner's Salt Solution) onto one plate. Gently dislodge the cells with a glass spreader (remember to dip in alcohol and flame first). Pour the cell suspension into the second plate, gently dislodge these cells and decant into the conical, screw cap centrifuge tube. You should have 3 ml of a cell suspension of  $1.5 \times 10^8$  cells/ml. Place 1 drop on a slide and make sure you have plenty of cells. Keep the cells on ice.
3. Meanwhile, someone should prepare a cAMP dilution series. You will be given 1.5 ml of  $2 \times 10^{-3}$  M cAMP. You will use this to make a set of cAMP dilutions.
  - (a) Take 0.5 ml  $2 \times 10^{-3}$  M cAMP and place in one of the seven small test tubes. Add to it 4.5 ml of BSS. This will result in a 1:10 dilution  $\rightarrow 2 \times 10^{-5}$  M cAMP.
  - (b) Take 0.5 ml  $2 \times 10^{-4}$  M cAMP and place it in a second test tube. Add to it 4.5 ml of BSS. This will result in another 1:10 dilution (1:100 overall)  $\rightarrow 2 \times 10^{-5}$  cAMP.
  - (c) Repeat these steps to make  $2 \times 10^{-6}$ ,  $2 \times 10^{-7}$ ,  $2 \times 10^{-8}$ ,  $2 \times 10^{-9}$ , and  $2 \times 10^{-10}$  M. (You will eventually have eight different cAMP concentrations.)

4. Once the dilutions are done, you can prepare your cAMP agar. In the water bath in the lab, there will be flasks of 3% agar (one per group). To prepare your cAMP agar do the following:

Take your nine tiny petri dishes and prepare as follows:

- (a) 1 ml agar + 1 ml BSS = BSS Agar (no cAMP control)
  - (b) 1 ml agar + 1 ml  $2 \times 10^{-3}$  M cAMP =  $10^{-3}$  M cAMP agar
  - (c) 1 ml agar + 1 ml  $2 \times 10^{-4}$  M cAMP =  $10^{-4}$  M cAMP agar
- And so on until  $10^{-10}$  M cAMP agar.

*Hints:* Add cAMP to petri plates first starting with 0 cAMP (BSS) and working up to higher concentrations; if you do this you will only need one pipet.

Add agar after you have obtained cAMP into all the plates. *Do not get the agar until you are ready for it because you don't want it to solidify too soon.* Measure the agar with a 5 or 10 ml pipet so you can get it into the dishes quickly. Be sure to mix plates *gently* by swirling or rocking.

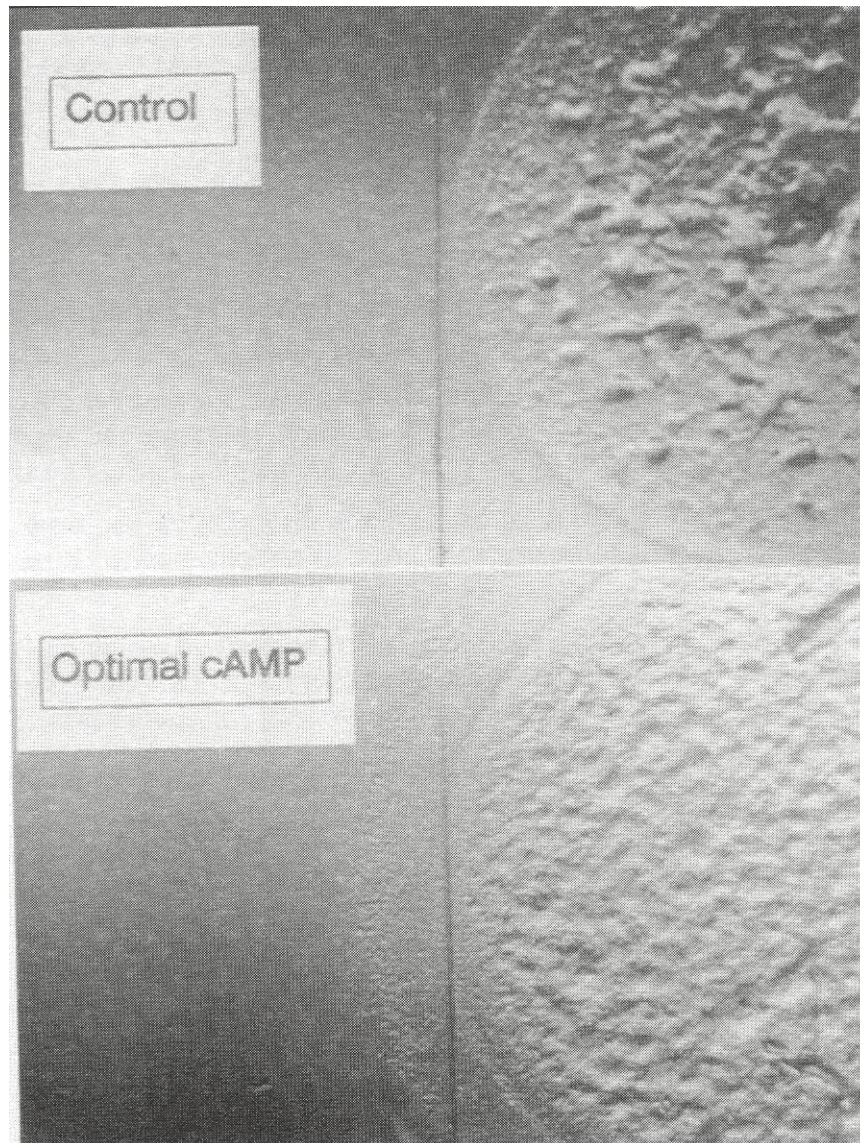
5. After the agar has hardened, add 20  $\mu$ l of cells to each dish. Let the drop dry a little before you move the dish or cover it. When the drop has dried (it no longer runs), mark the border of the drop by scratching a line on the petri dish bottom.
6. You will follow the movement of the cells across that border and, using an ocular micrometer, measure it at appropriate time intervals. Measuring every 1–2 hours for the next 8 hours would be ideal. You are trying to determine the optimal [cAMP] for chemotaxis (Figure 1.7).
7. Also, you will record what the development of the cultures looks like at each cAMP concentration.
8. In order to test whether the response to cAMP is specific, follow steps 3–7 substituting AMP for cAMP.

## Phototaxis

### *Instructor's Information*

Another interesting cell behavior exhibited by cellular slime molds is phototaxis. While individual amoebae are phototactic during various periods of development (reviewed in Loomis, 1982; Raper, 1984), this phenomenon is easiest to demonstrate and study in slugs. After aggregation of amoebae to form the pseudoplasmodium, the aggregate undergoes morphogenesis to form a migratory, multicellular structure called a slug. Migrating slugs will travel in search of light, optimal temperature and dryness; it is thought that this stage is important for locating an appropriate site for fruiting body development (Bonner et al., 1982). The simple experiment outlined below demonstrates the phototaxis of *D. discoideum* slugs to white light.

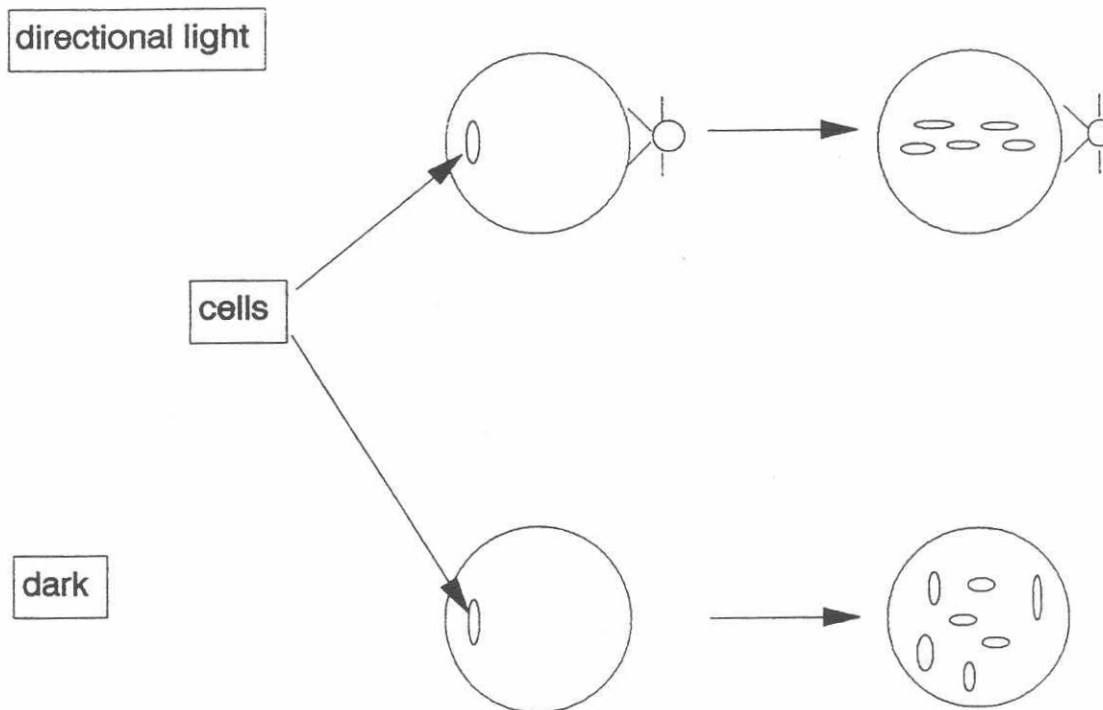
This experiment is suitable for introductory biology as described below. It is also a good exercise to do along with the chemotaxis experiment to make a cell behavior unit for courses in cell or developmental biology. Related questions include determining the optimal wavelength for phototaxis, studying the species specificity of the response, and examining the effects of relevant physical or chemical parameters on this behavior (e.g., pH, ammonia, moisture; Bonner et al., 1982, 1988).



*Student Methods*

1. You will each receive one plate of NC4 aggregation-competent amoebae. These cells have already been washed clean of bacteria. Each plate contains  $2 \times 10^8$  cells.
2. Place 2 ml of cold BSS (Bonner's Salt Solution) onto the plate. Gently dislodge the cells with a glass spreader (remember to dip in alcohol and flame first). Decant the cell suspension into a conical, screw cap centrifuge tube. Keep the cells on ice.

3. Take six non-nutrient agar plates and set-up duplicates as follows:
  - (a) 50  $\mu$ l cells on one side of the dish. Tape shut. Incubate in the light.
  - (b) 50  $\mu$ l cells on one side of the dish. Tape shut. Incubate in the dark (wrap plate in foil).
  - (c) 50  $\mu$ l cells on one side of the dish. Tape shut. Wrap plate in foil but make a small hole (size of the head of a pin) in the foil on the side of the dish directly opposite the cells. This hole should be on the side and not on the lid so that cells perceive light from the side and not above.
4. Observe in 18–20 hours for slug development. Measure (a) the distance migrated for at least 10 slugs per dish and (b) determine the slug orientation (which way are they pointing) (Figure 1.8).



**Figure 1.8.** Diagram of set-up for the phototaxis experiment. See text for details.

### Summary

This chapter presents methods to enable you to use cellular slime molds as an experimental system for laboratory instruction. I have outlined several examples of experiments that have been tested on students (and which work most of the time!). All information regarding recipes and suggested sources of materials are found in the appendices.

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APPENDIX A  
*Additional References*

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APPENDIX B  
*Recipes*

**Bonner's Salt Solution (BSS)**

CaCl <sub>2</sub>	0.3 g
KCl	0.75 g
NaCl	0.6 g

Add distilled or deionized H<sub>2</sub>O up to 1 liter. Autoclave 20–25 minutes, slow exhaust (liquid cycle).

**Lactose-Peptone Agar (2LP)**

Lactose	2 g
Peptone	2 g
KH <sub>2</sub> PO <sub>4</sub>	0.28 g
Na <sub>2</sub> HPO <sub>4</sub>	0.28 g
Agar	15 g

Add distilled or deionized H<sub>2</sub>O up to 1 liter. Autoclave 20–25 minutes, slow exhaust (liquid cycle). One liter of agar is sufficient for 30 petri dishes (8.5 cm in diameter).

**Non-Nutrient Agar (NNA)**

Agar	20 g
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Add BSS up to 1 liter. Autoclave 20–25 minutes, slow exhaust (liquid cycle).

**Sussman's Medium (SM/2)**

Glucose	0.5 g
Peptone	0.5 g
Yeast extract	0.05 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.05 g
KH <sub>2</sub> PO <sub>4</sub>	0.1 g
K <sub>2</sub> HPO <sub>4</sub>	0.05 g

Add distilled or deionized H<sub>2</sub>O up to 100 ml. Autoclave 20–25 minutes on slow exhaust (liquid cycle).

## APPENDIX C

### Materials

#### Cultures

*Dictyostelium discoideum*, strains NC4 and V12 and other cellular slime mold species, can be obtained from:

American Type Culture Collection (ATTC)  
12301 Parklawn Dr.  
Rockville, MD 20852  
(301) 881-2600

*Dictyostelium discoideum* can also be purchased from:

Carolina Biological Supply Co.  
2700 York Rd.  
Burlington, NC 27215  
(919) 584-0381

*Klebsiella aerogenes* (*Enterobacter aerogenes*) and *Escherichia coli* B/r (another suitable food source for *D. discoideum*) are available from: American Type Culture Collection (ATTC)

*K. aerogenes* and *E. coli* (non-mucoid) can be purchased from: Carolina Biological Supply Co.

#### Supplies

*Petri dishes:* I generally use standard size petri dishes (8.5 cm in diameter; e.g., Falcon 1029) for stock cultures, life-cycle plates, growing and harvesting cells, preparation of aggregation-competent cells, and for the following experiments: Choice Between Developmental Pathways, Food Preferences, and Phototaxis. For the Chemotaxis experiment and for Cell Development on filters, I use small petri dishes (3.5 cm in diameter; e.g., Falcon 1008) so that I can conserve chemicals. Suggested sources: VWR, Fisher, or Falcon. All of these experiments can be done on smaller dishes provided the volumes of cell suspension applied are adjusted taking into account the differences in the surface area of the variously sized petri dishes.

*Filters:* Nuclepore, Polycarbonate Membrane Filter, Black.  
Pore size: 0.2 or 0.4  $\mu\text{m}$ . Size: 25 mm.  
Suggested sources: VWR, Fisher.

*Pads:* Filter Paper: 3-mm Whatman.  
Size: 2.5 cm  
Suggested sources: VWR, Fisher.

*Glass Elbows:* You can make your own or purchase Bacti-Spreaders from Carolina Biological Supply Co.

*Loops:* Nichrome Wire Inoculating Loop.  
Suggested source: Carolina Biological Supply Co.

*Conical Centrifuge Tubes:* VWR 15-ml sterile centrifuge tubes with sterile, polypropylene caps. (These can also be purchased from other sources.)