

# CAROLINA Tips



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## Cells with “Personality”: *Physarum polycephalum*

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Imagine a cell so big that you don't need a microscope to see it. What if you could cut off pieces of the cell for closer study? And what if those pieces could be cultured to make more huge cells? Now imagine that the cell crawls like an amoeba and responds to environmental conditions by creeping toward a stimulus or by moving away, trying to escape. This cell is not the product of fiction; it is the plasmodial stage of the true slime mold *Physarum polycephalum*.

### *Physarum* Life Cycle

*Physarum polycephalum*, a true slime mold or myxomycete, lives in dark, humid areas such as under the bark of decaying trees or within the leaf litter of the forest floor. Although there are several stages of its life cycle, the

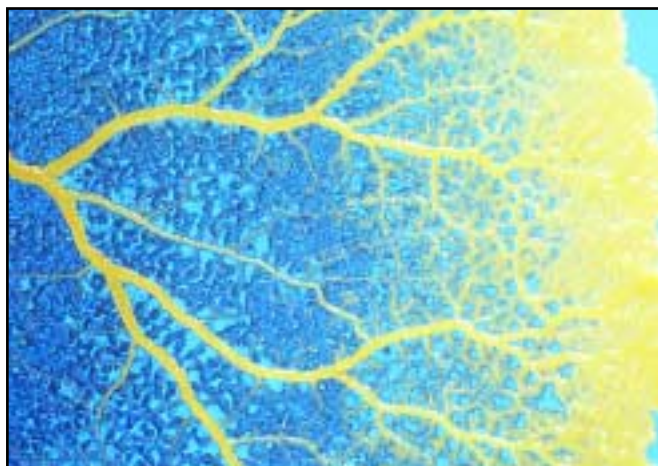


Figure 1 *Physarum* plasmodium.

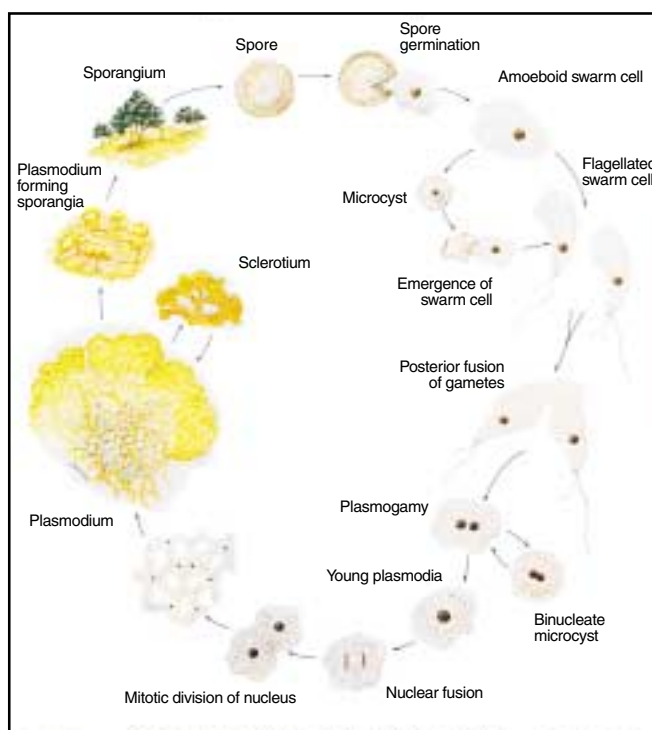


Figure 2 Life cycle of *Physarum polycephalum*.

plasmodial or vegetative phase is the most spectacular and the form in which you would most likely encounter *Physarum* in nature (Fig. 1).

The new plasmodium is a bright yellow, glistening mass (although other species of *Physarum* produce plasmodia of other dramatic colors). It resembles a giant amoeba. Sensitive to food and light, the plasmodium crawls in search of food, all the while attempting to avoid light. Plasmodia feed on bacteria, spores, and decaying organic material. Although composed of only one huge cell containing many nuclei, the plasmodium can get quite large—up to 30 cm in diameter!

*Physarum* does not spend its entire life cycle in the plasmodial stage (Fig. 2). If food becomes scarce, the plasmodium stops eating and initiates development.

Numerous fruiting bodies, or sporangia, form from the plasmodium. These sporangia are stalked and contain masses of resting structures called spores. When food becomes available again, and if humidity is high enough, spores germinate and release either amoeboid or flagellated swarm cells depending on environmental conditions. The swarm cells fuse together to produce a new, feeding plasmodium.

In addition to the sporangia, *Physarum* can make another type of resting structure. If the plasmodium dries out during feeding and migration, it will form a hardened multinucleate tissue called a sclerotium. If the sclerotium is moistened and fed, the plasmodium will resume growth.

### Culturing *Physarum*

*Physarum* is one of the easiest microbial eukaryotes to grow in culture. You can order the plasmodial stage or the sclerotial stage. The medium used is 2% non-nutrient agar, which you can either purchase or make yourself (20 g agar per liter of distilled or deionized water).

After you pour the 2% agar into petri dishes, let it harden and then sprinkle its surface with 25 to 30 flakes of unflavored oatmeal. Using a sterile scalpel or small spatula, cut out a small block of agar (about 1 cm<sup>2</sup>) on which some plasmodium is present. Place the agar block, plasmodium side down, on the agar-oatmeal dish. If you start your culture with a sclerotium, use sterile forceps to transfer the piece of filter paper (on which the resting structure resides) to the agar-oatmeal plate with the sclerotium facing down. (Sclerotia arrive attached to filter paper when you order them.) Wet the filter paper with a drop of sterile water.

Once you have prepared the cultures, seal the edges of the petri dishes with Parafilm® or plastic tape. Cover each dish entirely with aluminum foil because *Physarum* grows best in the dark. Incubation at room temperature is ideal. Once plasmodia begin to grow, subculture every 3 to 4 days unless you incubate at lower temperatures to slow growth.

### Cytoplasmic Streaming and Migration

One of the most dramatic and beautiful behaviors of *Physarum* is cytoplasmic streaming. Using a stereomicroscope it is easy to see the elaborate back-and-forth flow of the cytoplasm. Although the streaming probably serves a metabolic rather than motility function, it does demonstrate many of the same basic features of contractile mechanisms found in other eukaryotic cells.

An interesting way to investigate cytoplasmic streaming and migration is to expose plasmodia to different test chemicals (before using these or any other chemicals in this experiment, be sure to learn and talk about safe handling and disposal). Chemicals that result in effective tests include sodium azide (a metabolic inhibitor), lanthanum chloride (a calcium antagonist), barium chloride (a calcium competitor), and cytochalasin B (disrupts microfilaments). Dissolve the chemical to be tested into the 2% agar before it hardens. Place a piece of plasmodium on the experimental agar and monitor

cytoplasmic streaming and migration. This method can also be used to test the influence of pH, temperature, and humidity on cytoplasmic streaming and migration.

### Chemotaxis

One of the great features of *Physarum* is that the plasmodium is sensitive to its environment. Our students perform experiments to explore chemotaxis, the ability of a cell or organism to move in response to a chemical. They set up experiments in which a small amount of plasmodium (on a 1 cm<sup>2</sup> agar block) is deposited in the center of a petri dish containing 2% agar. The plasmodium is presented with a “choice” of a control (a plain agar block) or a material to be tested, by placing the choices at the edge of the agar on opposite sides of the plate (Fig. 3). The entire setup is then covered with aluminum foil.

Students return in 24 hours and record where the plasmodium is located. In our experiments, students have observed a positive chemotactic response (the plasmodium migrated toward each of these samples) to glucose, banana, oatmeal, corn flakes, and sugar pops. In contrast, plasmodia migrated away from sour candy and migrated randomly when presented with NaCl as the test substance. Depending upon the class goals, the chemotaxis experiment can be done simply (a choice between plain agar and an experimental material) or more elaborately (a choice between different experimental materials, or a choice between different concentrations of various chemicals).

### Phototaxis

*Physarum* is also responsive to light. Plasmodia exhibit negative phototaxis—they move away from light. To explore this phenomenon, place a small amount of plasmodium (on a 1 cm<sup>2</sup> agar block) in the center of a petri dish containing 2% agar. Cover the dish with aluminum foil and make a tiny pinhole on the edge of one side of the petri dish. Observe the culture in 24 hours to see where the plasmodium has migrated. It will usually move away from the light.

Our students used this method to test what happens if plasmodia are presented with an attractive food item in the light. Does the positive chemotaxis overpower the negative phototaxis? Also, by taping pieces of different colored cellophane over the pinhole, our students tested

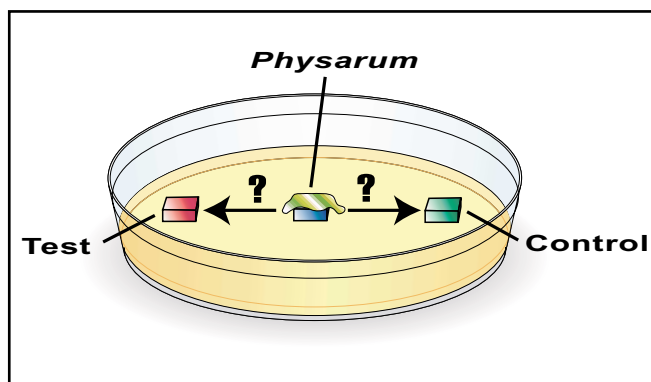


Figure 3 Diagram of chemotaxis experimental design.

whether the response to light is specific for a particular wavelength.

### Other Ideas

Because it is easy to culture plasmodia and investigate their migration, students can pursue many of their own ideas. For example, some of my students found an interesting way to study the relative effects of light/dark and gravity on plasmodia. They wanted to know whether, in nature, the movement of plasmodia away from the light was augmented by positive geotaxis, the movement toward greater gravitational forces.

To do this, they set up a series of cultures, each of which had a piece of plasmodium deposited in the center. These cultures were incubated in the dark with the petri dishes standing vertically on their sides (thus the agar was perpendicular to the horizontal incubation surface). They tested whether the plasmodia would crawl “up” or “down” (away from or toward the direction of gravity). Next, they did the same experiment but also exposed the plasmodia to a pinhole of light originating either below (“down”) or above (“up”). Another adaptation of this general method uses horizontal incubation to see if plasmodia migrate directionally if presented with a temperature gradient across the dish.

### Summary

The many advantages of *Physarum* for lab instruction include the ease of culture and manipulation. The plasmodial stage of the life cycle is especially intriguing, as it possesses many of the characteristics of eukaryotic cells but in an easier-to-see package. Moreover, the migration behavior and responsiveness of the plasmodium make it ideal for a variety of investigations and independent explorations by students.

### Further Resources

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- Lee, D. 1981. Slime mold: The fungus that walks. *National Geographic* 160(1): 131–136.
- Sauer, H. W. 1982. *Developmental Biology of Physarum*. Cambridge University Press, Cambridge.



### Physarum Culture Kit

For a class of 30. What is it? A protist? An animal? Despite its confusing taxonomic status, the creeping slime mold, *Physarum polycephalum*, is still an attention getter for your students. Students can follow through the life cycle observing the active plasmodial stage and the inactive sclerotial stage. With teacher instructions. *Kit contains card for prepaid delivery of perishable materials. Return card at least 2 weeks prior to requested delivery date to ensure prompt arrival of materials.*

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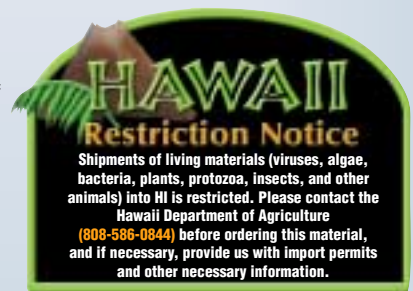
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