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Development and differentiation in the water mold, *Blastocladiella emersonii*

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The water molds, aquatic members of the fungi known as phycomycetes, are a richly varied group of organisms. Simple in structure, their life histories are truly amazing, often belying this simplicity. Their variety ranges from delicate zoospores, swimming by means of flagella, to thick-walled resistant spores or sporangia, from simple sack-like thalli to the extended branching mycelium of a typical mold, from syngamy by means of motile isogametes, to typical oögamy to fusion of whole thalli or specially differentiated parts of thalli, gametangia. Their life cycles may be primarily haploid, primarily diploid, or consist of equal alternations between haploid and diploid states. As a group they are a rich mine of interesting organisms for study. They are interesting and challenging because their special characteristics allow an insight into problems of general biological interest. One of these is the problem of cellular differentiation and development.

The problem of development is central in biology. Most organisms start life as a single "undifferentiated" cell. In time this cell grows and divides, in the case of multicellular organisms, and new structures appear until finally the state that we recognize as a mature organism is attained. This process may be as "simple" as

the production of a new virus particle from coat protein and nucleic acid core or as complex as the production of multicellular, multi-organged body of a phanerogam or mammal.

Inherent in the problem of development is the problem of differentiation, for in most cases the initiating cell of the sequence, the zygote, or spore is quite unlike the final products. Differentiation can be stated as a problem of differential growth. In the course of the orderly increase of the parts of the organism, the various components of growth, size, material, and number of cells increase at differing rates according to the plan of development programmed in the genes of the organism. The final product depends on the balance of these various rates. Thus, if the rate of cell size increase is large compared to cell division, the tissue will be composed of a few large cells, or the rate of water uptake may be large relative to the increase in protoplasm and the cells may become vacuolate.

As one proceeds down the scale of organizational levels from organism to organ to tissue to cell to sub-cellular parts, the processes of development and differentiation are explained in terms of the events of the next lower level of organization until finally the explanation concerns the interactions of atoms within the structure of mole-

cules which result from special electronic configurations of the bonds that hold the molecules together, and one begins to wonder whether he is a biologist or a physicist. Even so, each level of inquiry has its contribution and is necessary to our complete understanding of the process. Approaching the problems of development at the cellular level of organization is particularly rewarding to the biologist. The cell is the fundamental unit of organization of the organism. From the cell one can look upwards and see the organization of cells into tissues and organs and downwards into the biochemical mechanisms underlying the behavior of the cell.

In addition to the description of the events within the cell during its development and the relationship the cell has to its neighbors in the tissue and organ, one is interested in the causes of the events within the cell. The cell does what its genes tell it to do, but cells with the same genes do different things. Thus, the causes of development lie outside of the cell as well as within. Can we identify some of the environmental factors which affect the development and differentiation of the cell?

This brings us back to the water-molds, and more specifically to the mold known as *Blastocladiella emersonii*. *Blastocladiella* is a particularly good organism for studying the processes of differentiation and development at the cellular level. Life for a *Blastocladiella* begins with a motile spore. The spore is about $7 \times 9 \mu$, and has a single posterior flagellum which propels it forward quite vigorously. The spore lacks a cell wall and is capable of ameboid movement when in

contact with a solid substrate. The internal architecture of the spore is unique (Reichle and Fuller, 1967). The single nucleus, located in the posterior portion of the cell near the flagellum is covered over its anterior surface by a large, conspicuous, cup-shaped nuclear cap. At the base of the nucleus, extending up one side of the spore is a single mitochondrion. The mitochondrion surrounds the basal body of the flagellum and is attached to it by means of banded rootlets. In suitable nutrient conditions the spore ceases swimming, retracts its flagellum, and forms a cell wall about itself. At this time the nuclear cap disintegrates, releasing its store of ribosomes to the cytoplasm and the mitochondrion presumably breaks into smaller units as well. The encysted spore grows a slender germ tube which develops into a much branched rhizoidal system that contains cytoplasm but lacks nuclei. The cyst wall enlarges and the nucleus divides many times until a large, 50 to 200 μ diameter, multinucleate single-celled thallus with a basal tuft of rhizoids is formed. When the thallus has reached its maximum size, the protoplasm collects toward the upper end of the thallus and the rhizoids and basal portion of the thallus become vacuolate and devoid of protoplasm. A cross wall forms dividing the thallus into two cells and the apical portion containing protoplasm develops into a sporangium. Each nucleus within the sporangium will become the focal point for the formation of a new zoospore. Each thallus forms only one sporangium and the entire protoplast is converted into spores. However, *Blastocladiellas* are capable of form-

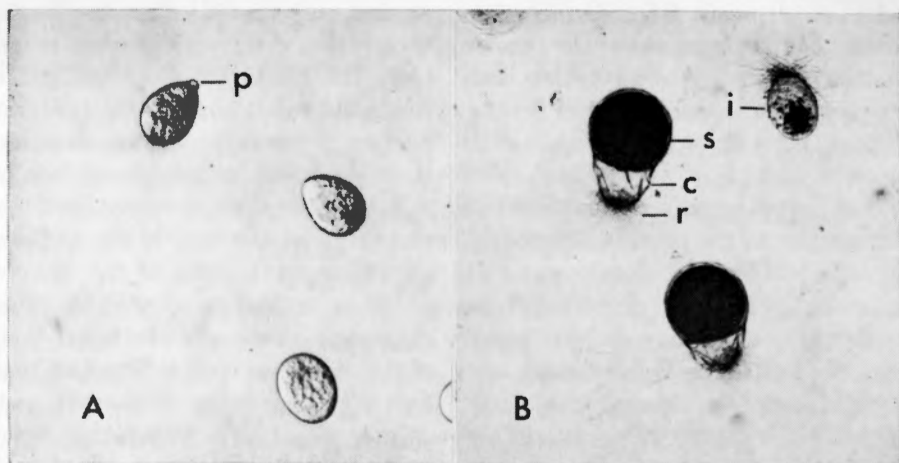


Figure 1. Thalli of *B. emersonii*. A. Ephemeral sporangiate thalli, one with discharge papillum, p; basal cell and rhizoids not visible in the photograph. B. Resistant sporangiate thalli, sporangium, s, basal cell, c, rhizoids, r, immature thallus, i. Note outer wall of the thallus is distinct from the sporangium wall at maturity. Magnification 170x for both.

ing two distinctly different kinds of sporangia (Fig. 1). One kind is colorless, thin-walled and ephemeral (Fig. 1A). It requires about twenty hours to develop from a zoospore and cleaves out new spores immediately upon reaching its full size. One or more refractile papillae appear on the wall which dissolve forming pores through which the spores escape. The other kind of sporangium is orange-brown, has a thick wall, is dormant and resistant to cold, drying, and lack of nutrients. It requires about seventy hours to develop, and after this time no further visible changes take place until it is placed in conditions suitable for germination. At germination the outer wall cracks and a thin inner wall protrudes through the crack. Refractile discharge papillae are formed on this protruding wall. The protoplast cleaves out zoospores as described before and these escape through the pores formed by the papillae. Thus

Blastocladia can proliferate rapidly under good growing conditions, but when the environment becomes inhospitable it can produce a resistant sporangium which is dormant and can tide the organism over to better days.

What are the environmental factors which lead to the formation of the ephemeral sporangiate or resistant sporangiate *Blastocladias*? The study of this question began more than fifteen years ago when Dr. E. C. Cantino first isolated the organism now known as *Blastocladia emersonii*. Cantino (1951) found that zoospores streaked out onto an agar medium containing peptone, yeast extract, and glucose developed into ephemeral sporangiate plants and discharged their spores into the tiny droplet of water that collected in the crevice between the thallus and the agar medium. These spores were unable to disperse and therefore germinated in this droplet and formed a

colony of densely packed thalli. Some of these second-generation *Blastocladia* formed ephemeral sporangia which released zoospores, and the colony increased in size and density. However, many of the second- and third-generation thalli did not cleave and discharge zoospores, and after three to five days Cantino noted that they were forming the orange-brown, thick-walled resistant sporangia. Thus resistant sporangi were formed in the crowded colonial environment. Since carbon dioxide was an important factor in the differentiation of sporangia in a close relative, *Blastocladia* (Emerson and Cantino, 1948), he modified the carbon dioxide relationships in the environment by incorporating sodium bicarbonate into the medium. Under these conditions more than 95 per cent of the thalli that grew from zoospores streaked onto the medium developed resistant sporangia, and only a few developed ephemeral sporangia resulting in colonies. Since the addition of bicarbonate causes the medium to become more basic, the relationship between pH, bicarbonate, and carbon dioxide concentration was investigated (Griffin 1965). These experiments showed that bicarbonate *per se* was effective in stimulating the formation of resistant sporangia, and pH and carbon dioxide had no effect on the differentiation of sporangia. In the course of these experiments an additional unrelated factor causing the formation of resistant sporangia was discovered, potassium ion. With a relatively high concentration of potassium chloride in the medium the thalli all developed resistant sporangia, with lower concentrations they developed ephemeral sporangia. Further experi-

ments (Griffin, unpublished) show that potassium is not specific, but chlorides of sodium and ammonium work equally effectively.

Is this information of value to you as a science teacher? I would like to suggest incorporating experiments with *Blastocladia* into your biology laboratory. The organism is easy to grow, only simple equipment is required, and the experiments are compact enough that many students can participate firsthand. The lessons to be learned are many. The student will be introduced to the marvelous world of the water-mold, that never-never land between plants and animals. They will learn sterile technique and other general methodology needed for handling micro-organisms. They will gain experience in experimental method in understanding the relationship between treatments and controls in an experiment. They will learn some basic developmental biology concerning cause and effect relationships of the environment and the behavior of the cell in differentiation.

Following are specific directions for growing *Blastocladia* and suggestions for experiments concerning the control of differentiation:

Materials needed:

Aluminum foil

Alcohol

Alcohol lamps or bunsen burners

Erlenmeyer flasks, 125 ml and 250 ml

Petri plates

Streaking rods—these are made from 4 mm glass rods five inches long with the terminal inch bent to an angle like a hockey stick.

Pasteur pipets—these can be made by plugging both ends of a five-inch length of glass tubing (fire pol-

ished) and sterilizing in an oven for two hours at 170 C. These can be stored until needed. At this time draw the center into a coarse capillary and separate the two halves by melting the capillary through the center. Break the heat-sealed tips with sterile forceps and the pipet is ready for use.

Inoculating loop

Sterile water in test tubes

Media:

<i>Constituent</i>	<i>Amt. g/liter</i>
Yeast extract (Difco)	1.25
Peptone (Difco)	1.25
Glucose	3.0
Agar (Difco-Bacto)	15

This basal medium is suitable for growing the ephemeral sporangiate plants and producing zoospores. It can be modified for the production of resistant sporangia by the addition of the appropriate amount of salt, see below. The medium must be sterilized in an autoclave or pressure cooker at fifteen pounds pressure for twenty minutes.

Procedure:

The essence of the procedure is this: Zoospores are streaked onto agar medium in petri dishes and the *Blastocladiellas* are observed daily for three days. The plates without added salt or with low salt will show mature ephemeral sporangiate thalli discharging zoospores after one day, and colonies after two or three days. The plates with 60 millimolar salt will show immature thalli after one or two days and mature resistant sporangia after three days.

Preparation of Zoospore Inoculum. Make up the basal medium as directed above. Melt the agar by heating to boiling and mix it thoroughly.

While the agar is still molten dispense it in 15 ml aliquots into 125 ml erlenmeyer flasks and cap these with aluminum foil and sterilize. When these have cooled to room temperature cut a small piece of agar containing thalli from your stock culture and put it on the medium. Flood with sterile water and recap with the aluminum foil. Care and cleanliness are required to prevent contamination with bacteria and molds. Put this in a cool place, 65 - 70 F. The next day, twenty-four hours later, examine the culture for the presence of *Blastocladiella* and bacteria in the water. It may take from one to four days for a suitable growth of *Blastocladiella*, depending on the age of the stock culture. As soon as thalli are noticeable, pour off the free water and let the culture incubate for twenty-four hours. At this time flood the culture with sterile water and pour some of the resulting suspension of spores into a fresh flask of medium. Swirl the suspension over the agar to completely wet the surface then pour off the excess and incubate this flask for twenty-four hours. This process must be repeated every twenty-four hours to maintain the culture for zoospore production. These cultures are your source of zoospore inoculum. With some care you should obtain a suspension that is very turbid with zoospores each day. It is wise to carry two flasks in parallel in case one gets contaminated.

Experimental Procedure. Prepare the medium in 100 ml amounts in 250 ml erlenmeyers making some with 0, 30, 60, and 90 millimolar KCl (NaCl or NH₄Cl). Cap the flasks with aluminum foil and sterilize. Sterilize

enough petri plates for the experiment. Each flask of medium is enough to pour five or six plates.

To inoculate the plates, place one small drop of a turbid spore suspension in the center of the plate with a sterile pasteur pipet. Add two or three drops of sterile water to this. Sterilize the streaking rod by dipping it into alcohol and flaming the alcohol off. Cool the short streaking end by resting it against the agar for a few moments before spreading the spores suspension. Carefully spread the spore suspension over the surface of the medium. Incubate the plates in a cool place and make daily observations.

Stock cultures on slants can be obtained by writing to me. Please indicate the date you would like to re-

ceive the culture. This should be at least one week prior to doing the experiment to allow time for building up an inoculum. Also, please allow two weeks notice for preparation of the culture.

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