Selective Transport of Nutrients via the Rhizoids of the Water Mold *Blastocladiella emersonii*

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Previous work in this laboratory demonstrated that the rhizoids of *Blastocladiella emersonii* grow chemotropically toward a source of P_i and thus provided preliminary evidence that, in addition to serving as a holdfast, the rhizoids absorb nutrients. To further examine the role of the rhizoids in nutrient uptake, we devised a technique to introduce a barrier between the rhizoids and the thallus so that these cell compartments could be studied independently. Cells were grown on polycarbonate membrane filters in such a way that all of the thalli were on one side of the filter and essentially all of the rhizoids were on the opposite side. Nutrient uptake into the rhizoids and the thallus was measured by floating the filters bearing cells on radioactive medium so that only one side of the filter contacted the label. Mineral oil was used to block the diffusion of the label through the unfilled pores in the filter. This technique permitted us to establish clearly that the rhizoids absorb all seven of the nutrients tested. In addition, we found that some nutrients, specifically P_i and amino acids, appeared to be preferentially taken up via the rhizoids, whereas K^+ , Rb^+ , and Ca^{2+} entered the thallus and rhizoids equally. Cells grown in the presence of the microtubule synthesis inhibitors nocodazole and carbendazim elaborated only a stunted rhizoid system, so we examined their ability to accumulate the two classes of compounds. As expected, these cells were severely inhibited in P_i and amino acid uptake but retained normal uptake of K^+ , Rb^+ , and Ca^{2+} .

Most eucaryotic cells are inherently polarized: appendages, internal organelles, and physiological functions occupy defined positions and confer directionality upon the whole cell. Mycelial fungi supply a striking example, for the hyphae that comprise the mycelium elongate only at their tips. The materials required for growth, such as precursors of new plasma membrane and cell wall, are transported unidirectionally down the hyphae to the terminal growth zones. We have chosen to investigate an aspect of functional polarity in the water mold *Blastocladiella emersonii*. This large, unicellular organism consists of two distinct compartments, a thallus and a rhizoid system, that can be studied separately in hope of elucidating their specialized functions.

B. emersonii is classified as a chytridiomycete and, like other members of its order, begins its life cycle as a small, posteriorly flagellated zoospore (12). When placed in growth medium, the zoospore rounds up, retracts its flagellum, and germinates. The most striking feature of germination is the outgrowth of a germ tube that gives rise to the anucleate rhizoid system; the spore body is destined to become the coenocytic thal-

lus. The cytoplasm is continuous throughout the cell and lacks obvious barriers such as septae. When the vegetative cell reaches a limiting size or is starved for nutrients, sporulation ensues; each nucleus and the cytoplasm surrounding it are reorganized into a zoospore.

Our investigations focused on the localized uptake of nutrients by the rhizoids. The suggestion that *Blastocladiella* rhizoids are capable of nutrient uptake arose from studies on chemotropism conducted in this laboratory. Harold and Harold (7) showed that in phosphate-deficient growth medium, rhizoids grow chemotropically toward any source of P_i (see Fig. 1). Cells that respond to the gradient grow much larger than do phosphate-starved cells further from the P_i source; presumably, this growth stimulation results from the ability of the rhizoids to absorb P_i .

These findings encouraged us to devise a technique to demonstrate that rhizoids are indeed capable of transporting P_i and other nutrients. By growing the cells across a polycarbonate membrane filter so that the thalli were on one side of the filter and nearly all of the rhizoids were on the opposite side, we were able to show that the rhizoids do take up nutrients and that P_i

and some amino acids are apparently taken up preferentially through the rhizoid system. By contrast, K^+ and Ca^{2+} enter the rhizoids and thallus equally. Experiments utilizing the antitubulin drugs nocodazole and carbendazim demonstrated that cells grown in the presence of these drugs possessed a very stunted rhizoid system (7). Concomitantly, they lost the ability to transport the nutrients that preferentially enter the rhizoid, but retained the capacity to transport K^+ and Ca^{2+} .

MATERIALS AND METHODS

Organism and media. Previous publications have described the maintenance of *B. emersonii* L-17 as resistant sporangia, the harvesting of zoospores from sporangia on peptone-yeast extract-glucose plates, and the growth of the organism on a defined medium (DM2 medium) (7, 11, 21, 25). The concentrations of nutrients in modified DM2 medium (17) were further adjusted as follows: amino acids—2.72 mM glutamic acid, 435 μ M methionine, 400 μ M each leucine, isoleucine, lysine, threonine, and valine, 200 μ M each arginine, glycine, phenylalanine, serine, and tyrosine, 100 μ M histidine, and 40 μ M tryptophan; salts—10 mM $MgSO_4 \cdot 7H_2O$, 1.0 mM $CaCl_2$, 5.0 mM NH_4NO_3 , 10 mM NaCl, and 1 mM Na_2HPO_4 ; and trace metals—4.0 μ M $FeSO_4 \cdot 7H_2O$, 0.6 μ M $CuSO_4 \cdot 5H_2O$, 1.2 μ M $ZnSO_4 \cdot 7H_2O$, 1.3 μ M $MnSO_4 \cdot H_2O$, and 0.12 μ M thiamine-HCl. The medium was made up in 1.0 mM Tris-maleate buffer. The pH was adjusted to 6.8 with KOH, after which KCl was added to a total of 25 mM K^+ . Finally, 10 mM glucose was added. In many experiments the medium was made up to contain a reduced concentration of a particular nutrient, as indicated by a subscript; for example, DM2P₂₅ stands for DM2 medium containing 25 μ M P_i .

Chemotropism. Granules of insoluble calcium phosphate (hydroxylapatite) were sterilized in water, and a few drops of this slurry were added to 10 ml of sterile 0.2 M poly-L-lysine, which was then poured into a 100-mm tissue culture dish (Falcon 3003). The slurry was swirled and poured off, and the dish was allowed to dry. DM2P₀ (DM2 medium lacking added phosphate; 10 ml) was added to the dish and inoculated with either 1.8×10^5 or 3.6×10^5 zoospores. The dishes were incubated overnight at 24°C without agitation. This entire procedure was performed aseptically.

Oriented growth on membrane filters: the floating garden. To interpose a barrier between thalli and rhizoids, cells were grown through the pores in membrane filters. Again, sterile conditions were maintained throughout the procedure.

An inoculator was constructed by drilling 40 wells, 25 mm in diameter and 10 mm deep, in a sheet of Plexiglas. Short lengths of aluminum tubing were cut from a shower curtain rod (25-mm diameter) and machined until they fit snugly into the wells in the Plexiglas. A polycarbonate membrane filter (Nucleopore Corp.; 25-mm diameter, 5.0- μ m pores, 10- μ m thickness) was placed in the bottom of each well and held in place with an aluminum stack.

A concentrated inoculum of fresh zoospores was diluted to 5×10^4 zoospores per ml with DM2P₂₅. A

portion (2 ml) of this suspension was immediately pipetted onto the top of each of the 40 filters. The inoculator was placed in the incubator at 24°C for 2 h, during which time the zoospores settled, germinated, and attached to the filters. The medium was then aspirated, the stacks were removed, and the filters were floated (germlings up) on the surface of DM2P₂₅ (90 ml in a 150-mm petri dish). Ten filters were placed in each dish. These polycarbonate filters float on the surface with only a very thin layer of medium above the filter, hence the term "floating garden." The dishes were incubated overnight (14 to 16 h) at 24°C, permitting the germlings on the upper surface of the filters to grow rhizoids down through the pores into the medium. The thalli remained above the plane of the filter.

Diffusion measurements. As the uptake of nutrients into rhizoids or thalli was measured by exposing one side of the filter or the other to medium containing radioactive label, it was necessary to prevent the label from diffusing through the pores to the opposite side. A thin layer of mineral oil was shown to block diffusion.

A hole (1-cm diameter) was cut in the bottom of a 35-mm tissue culture dish (Falcon 2002), and a filter bearing cells was glued (General Electric silicone glue and sealant) to the bottom of the dish so that the hole was covered and the rhizoids dangled beneath the dish. A second 35-mm dish was filled to the brim with radioactive medium, and 40 μ l of mineral oil (Nujol) was pipetted onto the surface; the oil spreads rapidly over the medium, forming a film approximately 40 μ m thick. The first dish with the attached filter was placed on top of the second dish so that the bottom of the filter came into contact with the oil layer, creating a junction free of air bubbles. To begin the experiment, 0.5 ml of nonradioactive medium was pipetted onto the exposed upper surface of the filter (1-cm diameter). Samples were taken by withdrawing 10 μ l of the medium above the filter and counting the radioactivity. Any label found above the filter had to diffuse through the pores.

A similar procedure was used to measure diffusion through the filter when the thalli were placed down into the radioactive medium. However, as all measurements of nutrient uptake into the thallus were made by spreading the 40 μ l of mineral oil directly onto the rhizoids (see below), diffusion through the filter had to be measured in an analogous fashion. The mineral oil was spread onto the rhizoids, and the filter was attached to the diffusion apparatus in the thallus-down orientation; 0.5 ml of nonradioactive medium was placed above the oil layer to initiate the experiment. Again, 10- μ l samples were withdrawn from the medium in the upper chamber and counted.

Nutrient uptake. By taking advantage of the ability of a mineral oil layer to block diffusion through the filter, we were able to measure uptake into either rhizoids or thalli. In all filter assays, the nutrient of interest was present in limiting concentrations. Uptake into the rhizoids was measured by dangling these filaments down through an oil layer into radioactive medium. A filter bearing vegetative cells was lifted from the floating garden and placed, rhizoids down, on tissue paper for 10 s to absorb the medium trapped in the rhizoid mass. The filter was then floated, rhizoids down, on a film of mineral oil approximately 40 μ m

thick (40 μ l of oil) that was spread over the surface of 3.0 ml of radioactive medium in a 35-mm tissue culture dish. Rhizoids are approximately 150 to 200 μ m long, so we assume that they extended down through the mineral oil into the radioactive medium. In addition to preventing the label from contacting the thalli, the mineral oil probably caused a slight underestimation of uptake into the rhizoids, as a 40- μ m length of each rhizoid was immersed in oil and out of contact with the medium. Each filter was washed twice with nonradioactive medium on a Millipore filtration apparatus, and the radioactivity was counted.

Attempts to measure uptake into the thallus in the same fashion did not give satisfactory results. Thalli were typically 50 to 80 μ m long, so less than half the surface could penetrate the oil layer to contact the radioactive medium below. A thinner oil layer allowed some label to diffuse across the filter, so we dismissed this approach. To circumvent this problem, 40 μ l of mineral oil was placed directly onto the rhizoids and gently spread over the filter with a rubber policeman; the filter was immediately floated, thallus down, on radioactive medium. The oil on the upper surface prevented label from diffusing into the rhizoid mass. However, any rhizoids within the pores came into contact with the medium, resulting in some overestimation of uptake into the thallus.

The possibility that the cells were damaged by placing the filters on tissue paper to absorb the medium from the rhizoid mass or by directly spreading mineral oil onto the rhizoids was examined. To demonstrate that placing the filters, rhizoids down, on tissue paper did not disrupt these filaments, we measured the uptake of P_i into cells with and without medium absorption. Filters bearing cells either were removed from the gardens and floated, rhizoids down, on DM2P₂₅ containing $^{32}P_i$ or were removed from the gardens, placed, rhizoids down, on tissue paper for 10 s, and then floated, rhizoids down, on the radioactive DM2P₂₅. Painting the rhizoids with a rubber policeman was also shown not to harm the cells. Control filters were simply placed on tissue paper for 10 s and floated, thallus down, on the radioactive DM2P₂₅. The test filters were mock painted with DM2P₂₅ as follows: after the medium from the rhizoid mass was absorbed, 40 μ l of DM2P₂₅ was spread over these filaments with a rubber policeman; this medium was then absorbed as usual, and the filter was floated, thallus down, on the radioactive medium.

Uptake into the entire cell was also measured to confirm that it equalled the sum of the uptake into rhizoids and thalli. This measurement was made by submerging an entire filter in 3 ml of radioactive medium in a 35-mm tissue culture dish.

Control experiments were conducted to measure the binding of radioactive tracers to the filters. Filters were inoculated with DM2P₂₅ lacking zoospores and were incubated overnight in floating gardens. These blank filters were subjected to the radioactive uptake assays exactly as were the experimental filters bearing cells; the time course of filter binding was followed for 12 min under conditions analogous to rhizoids-down, thallus-down, and whole-cell assays. Regardless of the technique used to measure nutrient uptake, the filter binding of all radioactive tracers except $^{45}Ca^{2+}$ was negligible for the duration of the assays. $^{45}Ca^{2+}$ binding equalled approximately 20% of the label taken up

by the cells, and this background was subtracted from all $^{45}Ca^{2+}$ uptake measurements.

Microtubule inhibitor studies. A liquid culture was started in a Spinner flask containing 500 ml of DM2 medium inoculated with 4×10^4 zoospores per ml. All glassware was pretreated with silane (Prosil-28). This culture was grown for 6 h at 24°C with continuous aeration, after which three 20-ml aliquots were pipetted into 125-ml Erlenmeyer flasks. Nocodazole and carbendazim were added in 20 μ l of dimethyl sulfoxide (Me_2SO) to give final concentrations of 5 and 10 μ M, respectively; controls received 20 μ l of Me_2SO . The cultures were then grown for an additional 12 h on a reciprocating shaker. Cells were collected on Sargent-Welch filter paper (grade S32915G), washed twice, and resuspended in 20 ml of fresh medium containing a reduced concentration of the nutrient of interest. Antitubulins and Me_2SO were again added to the cultures, and samples were taken for protein measurement. At 1 h after resuspension, the radioactive tracer was added to each culture, and 1.0-ml samples were filtered on 0.45- μ m membrane filters (Millipore Corp.). The filters were soaked in a solution of the substrate before use to reduce background binding. Each filter was washed once with 4 ml of nonradioactive medium, and the radioactivity was counted.

Protein assay. Cells were collected on Sargent-Welch paper, washed, and extracted with 0.1 N NaOH in a boiling-water bath. The extracts were acidified, and protein was determined by the Coomassie blue method of Bradford (4).

Materials. Reagents were purchased from standard suppliers, often Sigma Chemical Co. Nocodazole and cytochalasins were purchased from Aldrich Chemical Co., and carbendazim was a gift from H. C. Van der Plas, Landbouwhogeschool, Wageningen, The Netherlands; inhibitors were dissolved in Me_2SO . Radioactive compounds were purchased from New England Nuclear Corp.

RESULTS

Chemotropism. Our first experiments were aimed at confirming the chemotropic growth of rhizoids toward a source of P_i . Cells grown in the absence of P_i were able to detect a P_i gradient emanating from hydroxylapatite granules and to orient rhizoid growth (Fig. 1). The cells with chemotropic rhizoids were significantly larger than cells further from the granules, strongly suggesting that rhizoids take up P_i . Furthermore, some rhizoids that initially grew away from the granules were able to redirect their growth so that the filament bent and began growing toward the granule. As expected, the presence of sufficient P_i in the medium blocked rhizoid chemotropism. In control experiments, neither rhizoids nor thalli grew chemotropically toward the granules when these were made to serve as the sole source of Ca^{2+} in calcium-limited medium.

Floating gardens. To prove that rhizoids take up P_i , it was necessary to separate the rhizoids functionally from the thalli so that each structure could be studied independently. Severing the

FIG. 1. Chemotropic growth of rhizoids toward a source of P_i . The calcium phosphate granule in the middle of the field was the only source of P_i for cells grown in DM2P₀. The background spots are poly-L-lysine attached to the tissue culture dish. The thick, elongated rhizoids grew directly toward the granule. Bar, 100 μ m.

FIG. 2. Oriented growth of *B. emersonii* on poly-carbonate membrane filters. Cells were grown as described in the text. This edge-on view of a filter (10 μ m thick) shows that all the thalli remained above the filter, whereas all the rhizoids grew through the pores into the medium below the filter. Bar, 100 μ m.

FIG. 3. Diffusion of radionuclides through filter pores in the presence and absence of mineral oil. Filters bearing cells were placed, rhizoids down, on the surface of radioactive DM2 medium (○) or on a layer of mineral oil (40 μ m deep) spread over the surface of the radioactive medium (●). Samples (10 μ l each) were taken from the DM2 medium above the filter (initially nonradioactive). (A) $^{32}P_i$ diffusion. The DM2 medium below the filter contained 50 nCi of $^{32}P_i$ per ml. (B) $^{42}K^+$ diffusion. The DM2 medium below the filter contained 35 nCi of $^{42}K^+$ per ml.

cells near the thallus-rhizoid junction proved to be lethal, so we introduced a physical barrier between the two halves of the cell, much as Robinson and Jaffe did with germinating *Pelvetia* eggs (16). When a filter bearing 2-h-old germlings attached to one surface was floated on P-limited DM2 medium, the rhizoids grew down through the pores into the medium below, leaving the thalli on the upper surface; thalli are too large to fit through the pores (Fig. 2). The rhizoids presumably grew down into the medium to procure nutrients.

Since the pores in the filter permitted the diffusion of material from one side of the filter to the other, we employed a mineral oil layer to make the barrier between the two halves of the cells more complete. A film of mineral oil only 40 μ m thick blocked the diffusion of radionuclides through the pores in the filter. Figure 3 shows the effect of the mineral oil layer on the diffusion of $^{42}K^+$ and $^{32}P_i$. When a filter bearing oriented, vegetative cells was placed, rhizoids down, on top of radioactive medium, the label diffused through the pores and was recovered above the filter in significant amounts after 10 min. The presence of the thin film of mineral oil between the medium and the filter reduced the diffusion of both radionuclides to nearly undetectable levels. Similar results were obtained when the mineral oil was spread directly onto the rhizoids and diffusion was measured in the thallus-down orientation.

Nutrient uptake. By use of mineral oil as a diffusion barrier, the ability of the rhizoids to

concentrate nutrients was clearly demonstrated (Fig. 4). Potassium, P_i , and glutamic acid were taken up by the rhizoids at constant rates for at least 10 min. At most, 10% of the label taken up by the rhizoids could be attributed to diffusion through the filter followed by absorption by thalli. This figure was determined by estimating the maximum amount of substrate available to the thalli by diffusion through the filter (Fig. 3).

Note that, although the thalli also accumulated all three nutrients, both P_i and glutamic acid apparently entered the cell preferentially through the rhizoid system. Potassium entered rhizoids and thallus at equal rates. Under our conditions, the surface areas of the thallus and the rhizoids were approximately equal (determined from a series of measurements on representative cells). It therefore appears that the rate of potassium uptake per unit of surface area was roughly the same in the two cell compartments, whereas the rates for glutamic acid and P_i were greater in the rhizoids than in the thallus.

Phosphate uptake into the entire cell was determined by submerging filters bearing cells in medium containing $^{32}P_i$. The total uptake was approximately equal to the sum of the uptake into the rhizoids and into the thallus (Fig. 4B). The total uptake was measured for all the nutrients under consideration and, in all cases, was found to be greater than the uptake into the

rhizoids or into the thallus alone (data not shown). The total uptake did not always equal the sum of the uptake into the rhizoids and thallus, but, as the three measurements were made by using different techniques, quantitative agreement was not to be expected. Nevertheless, the fact that no serious discrepancies were found reassures us that our methods for measuring differential uptake into the thallus and rhizoids were reasonably valid.

Additional experiments provided further evidence that the techniques employed did not damage the cells. Cells in which the medium was absorbed from the rhizoid mass by placing the filters on tissue paper accumulated as much P_i as control cells not exposed to the absorption technique. Furthermore, cells whose rhizoids had been mock painted with DM2P₂₅ also took up P_i as well as did controls when the cells were placed, thallus down, on radioactive medium. Hence, neither medium absorption nor painting of the rhizoids reduced the ability of the cells to transport P_i .

There is some reason to believe that most of the P_i uptake into the rhizoids occurred near the rhizoid tip. When the depth of the oil layer, through which the rhizoids had to penetrate to contact the radioactive medium, was increased from 40 to 100 μm , the uptake of P_i was unaffected (data not shown). Hence, the upper 100 μm of

FIG. 4. Nutrient uptake into rhizoids (●), thalli (○), and whole cells (△). After overnight growth on DM2P₂₅, filters were removed from the floating gardens and placed, rhizoids down or thallus down, on radioactive medium in the presence of mineral oil. Whole-cell uptake was measured by submerging the filters in radioactive medium. After being washed, each filter was counted for radioactivity; each point represents the uptake into one filter. (A) K⁺ uptake from DM2P₂₅K₂₅ containing 35 nCi of $^{42}\text{K}^+$ per ml. DM2P₂₅K₂₅ was made by setting the pH with NaOH and then adjusting the monovalent salt concentration with NaCl. (B) P_i uptake from DM2P₂₅ containing 5 nCi of $^{32}P_i$ per ml. (C) Glutamic acid uptake from DM2P₂₅ (no amino acids except 10 μM glutamic acid) containing 10 nCi of [^{14}C]glutamic acid per ml.

the rhizoid probably transported very little P_i .

Figure 4 suggests that there may be two classes of metabolites, those that preferentially enter the rhizoids and those that are absorbed uniformly over the entire surface. To test this hypothesis, we assayed the uptake of more nutrients. In Fig. 5, the patterns of uptake for a number of metabolites are expressed as the rhizoid preference ratio (R/T; see legend to Fig. 5), which serves as an index of the degree to which uptake is localized. P_i and the amino acids all had R/T values greater than 1, but within this group the R/T values ranged from a low of 1.5 for lysine to a high of 3 for glutamic acid. The uptake of Rb^+ (an analog of K^+) and Ca^{2+} mirrored that of K^+ (R/T = 1). If more metabolites had been examined, the graph in Fig. 5 might have been transformed into a continuum. Nevertheless, the results of studies with antitubulin drugs confirmed the existence of two discrete patterns of uptake.

Inhibition of nutrient uptake by antitubulins. Harold and Harold (7) found that cells grown in the presence of the microtubule synthesis inhibitors nocodazole and carbendazim are small and possess short, stubby rhizoids. We therefore examined the uptake of the various metabolites by cells grown in the presence of antitubulins; uptake was expressed per milligram of cell pro-

FIG. 5. Rhizoid preference ratio for the two classes of nutrients. R/T, Ratio of the rate of uptake into rhizoids to the rate of uptake into thalli. Rates of uptake were determined from the slopes of the lines in graphs similar to those in Fig. 4. Floating gardens were grown on DM2P₂₅ and transferred to the following media for uptake measurements: K^+ , DM2P₂₅K₂₅ containing 35 nCi of $^{42}K^+$ per ml; Ca^{2+} , DM2P₂₅Ca₁₀ containing 200 nCi of $^{45}Ca^{2+}$ per ml; Rb^+ , DM2P₂₅Rb₁₀ (all K^+ replaced with Na^+) containing 25 nCi of $^{86}Rb^+$ per ml; Lys, DM2P₂₅ (no amino acids except 10 μ M lysine) containing 10 nCi of [^{14}C]lysine per ml; P_i , DM2P₂₅ containing 5 nCi of $^{32}P_i$ per ml; Glu, DM2P₂₅ (no amino acids except 10 μ M glutamic acid) containing 10 nCi of [^{14}C]glutamic acid per ml; Met, DM2P₂₅ (no amino acids except 10 μ M methionine) containing 25 nCi of [^{35}S]methionine per ml.

tein to allow for the inhibition of growth. As expected, cells grown overnight in micromolar concentrations of nocodazole or carbendazim accumulated very little P_i or glutamic acid (Fig. 6B and C). The inhibition of P_i uptake was independent of P_i concentration over the entire range tested (50 μ M to 1.0 mM). Potassium uptake, however, was the same in treated cells as in the controls (Fig. 6A). The distinction between the two classes of compounds, suggested by the floating garden experiments, was magnified in these antitubulin experiments (Fig. 7).

We would emphasize that antitubulins interfered with transport only if the cells were grown in the presence of the drugs. Exposure of 15-h-old cells to nocodazole (20 μ M) or carbendazim (80 μ M) for 2 h just before use did not affect the uptake of $^{32}P_i$. Cytochalasins A and B were entirely ineffective in blocking uptake, whether the cells were grown in their presence overnight or exposed to higher concentrations for 2 h. Taken together, these findings suggest that elaboration of a normal rhizoid system is required for the uptake of P_i ; once formed, the rhizoids retain their transport capacity even in the presence of the inhibitors.

Antitubulins may well have multiple effects on cell function, and the inhibition of rhizoid growth may not be solely responsible for the reduced uptake of P_i and amino acids. Cells grown in the presence of antitubulins were probably not energy depleted, as they respired as rapidly as did the controls ($Q[O_2]$ [protein] = 1.2 and 1.3 ml of O_2 h⁻¹ mg⁻¹, respectively). Furthermore, the treated cells continued to synthesize protein for the duration of the experiment, but at slower rates than untreated cells (data not shown). Nevertheless, the cells grown in the presence of the drugs had severely altered morphologies, and an investigation of all the possible effects of the antitubulins on nutrient uptake is not within the scope of the present research.

DISCUSSION

In the laboratory *B. emersonii* can grow attached to solid surfaces, and we presume that the same mode of growth occurs in nature. The rhizoids serve as the holdfast and have been assumed to absorb nutrients (5). However, prior to this report there has been little direct evidence to support this hypothesis.

To our knowledge, the only relevant data have come from studies on nutrient uptake by the haustoria of fungi that are parasitic on plants. The haustorium is a specialized structure, resembling a rhizoid, that penetrates the cell wall of the host plant, bringing host and parasite into close contact (6). By growing host cells in labeled medium and then infecting the host with

FIG. 6. Effect of antitubulins on nutrient uptake. Cells were grown overnight in DM2 medium containing 5 μ M nocodazole (●), 10 μ M carbendazim (△), or Me₂SO (○). Stock solutions of the antitubulins in Me₂SO were diluted 1:1,000 into the medium. To measure uptake, cells were collected by filtration and suspended in fresh media as indicated below; radioactive substrate was added 1 h later. (A) K⁺, DM2K₂₅ containing 50 nCi of ⁴²K⁺ per ml. The pH of DM2K₂₅ was adjusted with NaOH, and the monovalent salt concentration was manipulated with NaCl. (B) P_i, DM2P₅₀ containing 10 nCi of ³²P_i per ml. (C) Glu, DM2Glu₁₀₀ containing 10 nCi of [¹⁴C]glutamic acid per ml.

fungal spores, transfer of labeled material from the host to the fungus has been demonstrated for ³²P and ³⁵S (13), carbohydrates (20), and amino acids (15). Transfer was assumed to occur via the haustoria, but release of label into the extracellular space followed by fungal uptake was not ruled out. Extending these earlier findings, we provide direct evidence that rhizoids do indeed take up nutrients and that they appear to do so selectively.

This conclusion rests on two lines of evidence: floating garden assays and experiments utilizing antitubulin drugs. The floating garden assays, conducted in the presence of mineral oil, provided evidence that both rhizoids and thalli are capable of accumulating all the nutrients tested. Moreover, the results suggest that P_i and amino acids are preferentially taken up via the rhizoids, whereas K⁺ and Ca²⁺ uptakes are more uniformly distributed.

For several reasons, we are reasonably confident that the floating garden assays measured the active transport of nutrients across the plasmalemma of the thallus and rhizoids. The possibility that the radioactive label was bound to extracellular sites seems unlikely; controls demonstrated that blank filters bound negligible amounts of label (except ⁴⁵Ca²⁺), and experiments utilizing respiratory inhibitors indicated that the binding of ³²P_i and ⁴²K⁺ to nonexchangeable sites on the cell surface was minimal. To wit, hydroxyquinoline-*N*-oxide and sodium azide severely reduced the uptake of these two radioactive tracers in vegetative cells grown

in suspension culture (data not shown). This effect of the respiratory inhibitors also suggests that the transports of P_i and K⁺ are active processes. Furthermore, we found that after 30 min of uptake in cells grown on filters, both cell compartments and whole cells continued to accumulate all the nutrients (except Ca²⁺) against concentration gradients (data not shown), pro-

FIG. 7. Effect of antitubulins on nutrient uptake. This is a composite based on several experiments of the kind illustrated in Fig. 6. Relative uptake is the ratio of the rate of uptake by antitubulin-grown cells to the rate of uptake by control cells, multiplied by 100. Cells were grown on DM2 medium except as noted below. Media used for uptake: K⁺, DM2K₂₅ containing 50 nCi of ⁴²K⁺ per ml; Ca²⁺, cells grown on DM2Ca₂₀₀, uptake in DM2Ca₂₀₀ containing 1.0 μ Ci of ⁴⁵Ca²⁺ per ml; Rb⁺, DM2P₂₅Rb₂₀₀ (all K⁺ replaced with Na⁺) containing 20 nCi of ⁸⁶Rb⁺ per ml; Lys, DM2 medium containing 100 nCi of [¹⁴C]lysine per ml; P_i, DM2P₅₀ containing 10 nCi of ³²P_i per ml; Glu, cells grown on DM2Glu₁₀₀, uptake in DM2Glu₁₀₀ containing 10 nCi of [¹⁴C]glutamic acid per ml; Met, DM2 Met₁₀₀ containing 100 nCi of [³⁵S]methionine per ml.

viding further evidence that these nutrients are actively transported. Ca^{2+} uptake rapidly reached a plateau at an internal concentration 1/10 that of the extracellular medium, which is consistent with the hypothesis that Ca^{2+} is actively excluded from the cells. However, some nutrients, especially P_i , may be sequestered in an insoluble form or in organelles, significantly lowering their free internal concentrations. Van Brunt et al. recently provided additional evidence supporting an active transport mechanism for K^+ uptake in *B. emersonii* (24).

The suggestion that the nutrients tested in the floating garden assays fall into two discrete classes was reinforced by microtubule inhibitor studies. The treated cells took up K^+ and Ca^{2+} as well as did untreated cells but were markedly deficient in their ability to transport P_i and amino acids. There is now good evidence that antitubulin drugs interfere with the assembly of fungal microtubules (8, 14, 18). Nocodazole and carbendazim also cause *B. emersonii* to elaborate only short, stubby rhizoids in comparison to the bushy rhizoid mass of control cells, and we believe that the abnormal rhizoids are at least partially responsible for the reduction in P_i and amino acid uptake. However, nocodazole and carbendazim may cause additional lesions in the cell, not directly related to the reduced rhizoid mass, that result in the selective inhibition of uptake. For example, the few remaining rhizoids may not be competent to translocate nutrients to the thallus, as intact microtubules are required for intracellular trafficking in many diverse cell types (9). The inhibition of P_i and amino acid uptake is so severe (Fig. 6 and 7) as to suggest that microtubules may be required even for transport of these metabolites into the thallus. We are reluctant to speculate about possible reasons but would draw attention to previous observations that suggest a relationship between microtubules and metabolite uptake (23, 26). Although the experiments utilizing microtubule inhibitors are inconclusive with respect to the cause of the selective inhibition of nutrient uptake, the results are consistent with the suggestion that the rhizoids are the major site of P_i and amino acid uptake.

This localization of functions appears to make ecological sense. In ponds, most of the P_i and amino acids are likely to be tied up in macromolecules contained in organic debris. Potassium, on the other hand, is soluble and present in pond water at a concentration near 30 μM ; calcium levels are 10-fold higher, whereas the concentration of P_i is only 1 μM (2). We therefore speculate that the rhizoids serve the complementary functions of attachment and procurement of those scarce nutrients that are bound up in organic matter.

The localization of transport processes in particular regions of a single cell is a well-documented but poorly understood aspect of physiology. Epithelial cells, for example, are well known to effect the movement of NaCl from the mucosal to the luminal side of the cell. The mucosal membrane is thought to possess Na^+ channels that increase the local Na^+ permeability, whereas the luminal membrane has a high concentration of the Na^+/K^+ ATPase that extrudes Na^+ , thus creating a Na^+ flow through the cell. We know of no clear precedent from the fungal world, with one exception: Slayman and Slayman (19) reported that the membrane potential declines toward the tip of *Neurospora* hyphae, suggesting differential localization of an ion pump or an ion leak. In all cases, the mechanisms by which pumps and leaks become functionally segregated remain unknown.

We believe that tip growth provides the means whereby transport systems for P_i and amino acids are concentrated in the rhizoids. Fungal tip growth depends on membrane-bound vesicles that are transported down the hyphae and accumulate in the apical region; the fusion of the vesicles with the plasma membrane adds new material to the hyphal tip (5). Rhizoids also elongate at the tip, and the characteristic apical vesicles have been identified (3). We speculate that the proteins which comprise the P_i and amino acid transporters are selectively incorporated into the membranes of vesicles destined for the rhizoid tip and thus become localized.

The P_i and amino acids that enter the rhizoid must be translocated to the thallus and incorporated into macromolecules. Fungal hyphae translocate nutrients at rates of 2 to 3 cm/h via cytoplasmic streaming (5). If a similar mechanism operates in rhizoids, the P_i and amino acids could be moved from the rhizoid tips to the thallus in a matter of minutes. Although cytoplasmic streaming has recently been observed in *B. emersonii* (R. L. Harold, personal communication), we would note that the uptake of P_i was resistant to cytochalasins which inhibit streaming in many organisms (1). The mechanism by which nutrients are carried through the rhizoids into the thallus remains to be clarified. We wonder whether the transcellular electrical current observed in *B. emersonii* by Stump et al. (22) has any bearing on nutrient transport or on the localization of transport systems at the rhizoid tip (10).

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