LIPID TURNOVER DURING MORPHOGENESIS IN THE WATER MOLD BLASTOCLADIELLA EMERSONII*

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SUMMARY. The lipid content of Blastocladiella emersonii zoospores is 5 pg/cell or about 13% of dry weight. Within the first few minutes of germination 60-70% of total zoospore lipid is lost, with neutral lipid, glycolipid and phospholipid fractions decreasing to about the same extent. These changes in lipid content precede the breakdown during germination of the complex and extensive membrane system of zoospores. During growth, which immediately follows germination, net phospholipid synthesis resumes so that total lipid is maintained at 6% of dry weight, but net synthesis of neutral and glycolipid does not begin until induction of sporulation. During sporulation the phospholipid level decreases so that the distribution of lipid among the three fractions approaches that found in zoospores. These changes in lipid content suggest that zoospore membranes containing neutral and glycolipids are synthesized de novo during spore formation.

The life cycle of Blastocladiella emersonii (1) can be divided into four stages (Fig. 1): I. the zoospore stage, II. the germination stage, III. the vegetative cell stage and, IV. the sporulation stage. As a motile, uninucleate, metabolically active zoospore, Blastocladiella neither grows nor divides. Populations of zoospores can be maintained as such for long periods, or at any time the entire population can be induced to differentiate synchronously into vegetative plants capable of vigorous growth and nuclear division (2-4). Conversely, replacing the growth medium with a buffer solution at any time after germination is finished and up to 15 hours after growth commences causes vegetative cells to release spores within about four hours (2,5,6). Previous studies, based largely on microscopic observations of germinating and sporulating cells, have noted the

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extent to which these structural changes involve alterations in the extensive and complex membrane system of zoospores (2,7-10). At least three membrane-related structures are unique to zoospores (Fig. 1). These are:

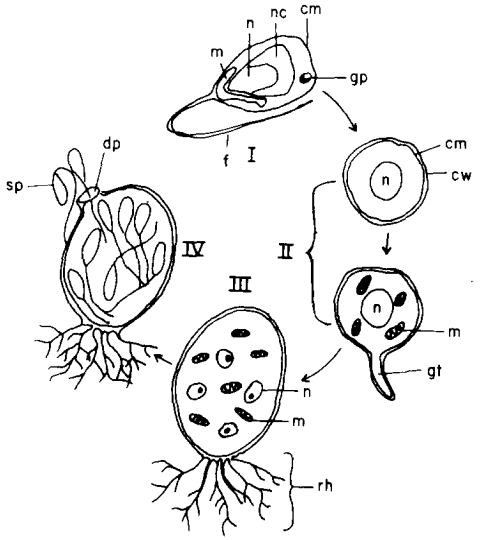

Fig. 1. Life cycle of B. emersonii. I, spore stage, II, germination stage showing two intermediate cell types (2); III, vegetative or growing cell stage; IV, sporulation stage. Stage II requires 40-60 min. Stage III normally lasts about 15-20 hours, depending upon growth conditions. Stage IV requires three-and-one-half to four hours. CM, cell membrane; F, flagellum; GP, gamma particle; M, mitochondrion; NC, nuclear cap; N, nucleus; CW, cell wall, GT, germ tube; Rh, rhizoid or holdfast system; DP, discharge papillum; SP, spore. For details see (2) and references therein.

γ-particles, vesicles of uncertain function (11) localized in the cytoplasm of zoospores (2,8,12-14); the nuclear cap, an organelle lying atop the zoospore nucleus and containing all of the cell's cytoplasmic ribosomes (15); and the single flagellum (8,12). These organelles disappear during germination (2,7,9,10) and are synthesized again during spore formation following a period of vegetative growth (13).

The ability to control both germination and sporulation in the laboratory and the synchrony of both processes are potential advantages in studying

membrane biosynthesis and degradation in Blastocladiella. There is, however, no published data regarding possible changes in lipid content or metabolism at specific developmental stages. This preliminary communication focuses on changes in lipid content occuring during morphogenesis and the possible relationship between these changes and membrane metabolism.

MATERIALS AND METHODS

Cells and media. Propagation of Blastocladiella, the procedure for obtaining large quantities of zoospores from nutrient agar plates and the composition of synthetic growth medium, will be described elsewhere.[1]

Cells were grown at 27° in 2 L Erlenmeyer flasks in a gyrorotatory shaker (New Brunswick #G25). One liter of synthetic medium was inoculated with 1-2 x 10^9 spores and grown for the appropriate period of time. After four hours cells were collected on Sargent #500 filter paper, washed with 100 ml of sporulation solution, suspended in 1 liter of sporulation solution, and incubated at 27°. This exchange causes cells to release spores within 4 hours. For lipid analysis, cells from growth medium or sporulation solution were isolated either by centrifugation at 1000 x g and 4° for 15 min or by filtration. The latter process separates plants from spores which pass through the filter. Cells were suspended in a small volume of water, lyophilized and weighed.

Lipid extraction and analysis: Lipid was extracted from dried cells by the method of Folch et al. (16). No additional lipid was removed after further extraction following sonication as described by Bertsch et al. (17). Lipid was fractionated into neutral, glyco- and phospholipid classes by silicic acid chromatography as described by Dittmer and Wells (18). All lipid classes were quantitated gravimetrically. From 100 μg to 8 mg of lipid were available for each sample. The data of Fig. 2 and 3 represent the average of 2-5 determinations.

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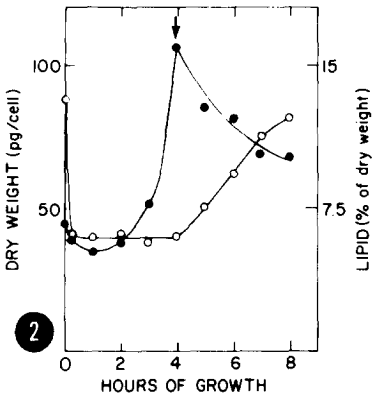
[1] Silverman, Her and Sun, in preparation.



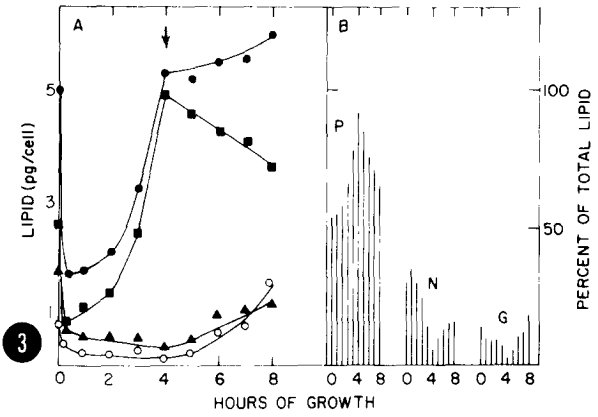


Fig. 2. Dry weight per cell and lipid as percent of dry weight throughout the life cycle. The arrow indicates the point at which sporulation was induced. Dry weight per cell (●—●); lipid percent of dry weight (O—O).

Fig. 3. (A) Lipid content per cell. Total lipid (●—●); phospholipid (■—■); neutral lipid (▲—▲); glycolipid (O—O). (B) Lipid class as percent of total lipid. P, phospholipid; N, neutral lipid; G, glycolipid.

RESULTS AND DISCUSSION

Zoospores contain approximately 13-14% of dry weight as lipid (19) (Fig. 2). The distribution of this material among three lipid classes is 55% phospholipid, 30% neutral lipid and 15% glycolipid (Fig. 3). Upon germination dry weight and lipid content of the zoospore decrease about 22% and 67%, respectively (Figs. 2 and 3). A similar change in dry weight has been observed by Lovett (20). The loss of lipid, occurring mainly within the first 15 min of germination, involves all three classes, phospholipids falling to 30% and neutral and glycolipid to 40% of their respective levels in the zoospore (Fig. 3). This loss is not due to lipolysis during the isolation procedure since the amount of lipid isolated from a mixture of spores and 15 min-cells is equal to the sum of the amounts isolated from the individual cell types.

These quantitative changes in lipid content occur rapidly and precede many of the morphological changes associated with germination. By 15 min

these cells still retain all of the readily observable structural features of zoospores. They are still motile and still contain well-defined nuclear caps, as indicated by aceto-carmine staining (2). Our results show that quantitative changes in lipid content precede the disappearance of these organelles. One explanation for this is that substantial lipid is removed from these membranes before observable breakdown, as has been found to occur in other systems (21).

After the first 15 min of germination lipid synthesis in Blastocladiella is balanced with growth so that the total lipid content remains at 6% of dry weight (Fig. 2). The composition of this material is clearly different from that of zoospores. Nearly all of the lipid synthesized during growth is phospholipid (Fig. 3A), principally phosphatidylcholine and phosphatidylethanolamine, as found by thin layer chromatography. These components also predominate among zoospore phospholipids. There is virtually no net change in the neutral or glycolipid content during growth, so that the contribution of these components to total lipid content steadily declines (Fig. 3B).

At four hours the medium was changed to sporulation solution to induce zoospore formation. The dry weight per cell decreases almost immediately and continues to decrease throughout the period of sporulation (Fig. 2). Spore release was first observed at three-and-one-half hours. The 8 hour points represent the lipid content of spore-containing plants and plant ghosts since free spores are removed by the filtration procedure. Although the lipid content as percent of dry weight increases nearly two-fold in this interval, the actual lipid content per cell increases by only 13% (Fig. 3A), the difference being attributed to the loss of dry weight (Fig. 2). The 13% increase in total lipid includes a net decrease in phospholipid and increases both in neutral and glycolipid, so that the lipid composition of sporulating cells approaches that of spores (Fig. 3B). Although net phospholipid synthesis ceases during spore formation, $^{32}P_i$ is still incorporated

into phospholipid, indicating that turnover still occurs.[2] The greater proportion of neutral lipid in spores than in sporulating cells (30% and 16%, respectively) suggests that relatively more of this material is incorporated into spores than of the other two lipid classes, but the total amount of lipid ultimately incorporated into spores is not known. In any event, the accumulation of glycolipid and neutral lipid during sporulation suggests that spore membranes containing these lipids arise by *de novo* synthesis during spore formation rather than by rearrangement of cell components synthesized during growth.

Analysis of lipids by thin layer chromatography (data not shown) indicates that the same neutral and phospholipid components appear to be present throughout the life cycle. In contrast, marked changes occur in the types of glycolipids. The detailed lipid composition of plants and spores is presently under investigation.

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