Morphogenesis and Ultrastructure of *Claviceps purpurea* During Submerged Alkaloid Formation

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Criteria for morphogenetic and ultrastructural distinction between conidia and chlamydospores of a submerged culture of *Claviceps purpurea* (Fr.) Tul. are described. Both the hyphae of the sphacelia (asexual) stage and the conidia contained granular cytoplasm. Cytoplasmic invaginations in vacuoles were transformed to electron-opaque bodies and disintegrated prior to germination. The budding of conidia had basipetal succession. The chlamydospores were formed by rounding up the terminal cells of filamentous hyphae. Homogeneous nonvacuolized cytoplasm with lipid droplets and lipid-forming bodies was characteristic of young chlamydospores. Cristate mitochondria did not appear in the chlamydospores before the alkaloid production phase. Simultaneously a specific organelle in the chlamydospores, a dense body, appeared to absorb intracellular lipids and form large deposits of phospholipid material. No germination of chlamydospores was observed. The ultrastructural pattern described for chlamydospores was also observed in hyphae with reduced proliferation during the alkaloid production phase.

Light-microscopy observations of submerged cultures of *Claviceps purpurea* (Fr.) Tul. revealed that a substantial number of ovoid cells did not germinate, even during alkaloid formation (13). These cells were concluded to be the plectenchymatic cells as described by Mantle and Tonolo (10), and ultrastructural criteria were sought for their differentiation from germinating ovoid cells, i.e., conidia.

MATERIALS AND METHODS

Our work was performed on a saprophytic strain Pla-4 of *C. purpurea* (Fr.) Tul. The cultivation and sampling of this strain were described in a preceding paper (11). Samples of mycelium taken for electron microscopy were fixed by glutaraldehyde with osmium tetroxide postfixation (15) and embedded in Vestopal W. Ultrathin sections were contrasted by lead citrate by the method of Reynolds (14) and viewed under an electron microscope (JEOL JEM 100B) at 80 kV.

RESULTS

Submerged culture of *C. purpurea* Pla-4 grown on simple synthetic medium consisted of hyphae (Fig. 1) and of ovoid cells (see Fig. 3 and 5). The diameter of hyphae was 1.5 µm. The ovoid cells were usually of dimensions of 2.5 by 5 µm; some of them, however, attained a length of up to 10 µm (see Fig. 6). The ultrastructure of the cytoplasm of ovoid cells provided a clue for their classification into two groups. The first group consisted of cells with granular cytoplasm (GC cells, see Fig. 3) that was also observed in filamentous hyphae. During budding, the wall of a GC cell was only 0.1 µm thick (Fig. 2); at later stages its thickness doubled (Fig. 3). The cytoplasm of GC cells contained numerous mitochondria and one to three vacuoles. As a rule, the vacuoles were characterized by invaginations of part of the cytoplasm still retaining tonoplast on its surface (Fig. 3). At later stages, these invaginations became homogeneously electron dense, rendering its unit membrane boundary invisible. Prior to the onset of germination of GC cells, the “vacuolar bodies” disintegrated (Fig. 4). Germination of GC cells was preceded by a thickening of the cell wall up to 0.3 µm, growth and fusion of vacuoles, and the formation of the germ tube (Fig. 4). Most GC cells germinated prior to the extensive appearance of fat droplets. As the pattern of budding was characteristic for fungal conidiogenesis (5), GC cells may be assumed to be identical with conidia. A remarkable feature of conidia is the vacuolar apparatus ultrastructure. The vacuolar apparatus contains complex concentric membranes and multivesicular bodies characteristic of fungi. The shape of fragments of vacuolar bodies during their disruption (Fig. 4) indicates their phospholipid character and the association of the conidial vacuolar apparatus with the cell membrane.
Ovoid cells of the second group differed from the conidia in their homogeneous cytoplasmic ultrastructure (HC cells, Fig. 5). In submerged C. purpurea cultures, ovoid cells prevailed in the period of alkaloid formation, e.g., starting from the seventh day of cultivation (13). In the course of experiments they did not germinate and their cytoplasm contained lipid deposits usually compared to the alkaloid formation in parasitic Claviceps cultures. HC cells were primarily dumb-bell shaped (8). Our observations showed that these cells were formed by a conversion of terminal hyphal cells (Fig. 5), which explains why some of them are considerably elongated (Fig. 6), and thus easily mistaken for "hyphae composed of short thick sclerotic cells" (10). Mantle and Tonolo (10) turned these ovoid cells with lipid inclusions "plectenchymatic cells;" we propose that these cells should be designated more accurately as "chlamydospores."

The penultimate hyphal cells bearing chlamydospores were generally autolyzed (Fig. 5). The cytoplasm of chlamydospores contained no vacuoles but it did contain numerous lipid droplets with nonmembraneous outline, which
Fig. 3. Conidia from 5-day-old C. purpurea culture. Abbreviations: N, nucleus; M, mitochondria; V, vacuole. Arrows indicate invaginations of cytoplasm into vacuoles. Marker bar = 1 μm.

Fig. 4. Germination of conidia from 5-day-old C. purpurea culture. Abbreviations: CW, cell wall; V, vacuole; MB, multivesicular body; GT, germ tube; BS, basal septum. Arrow indicates the fragments of vacuolar body. Marker bar = 1 μm.
was present prior to the separation of the chlamydospores from the mycelium. Lipid droplets were usually concentrated around an organelle which we call a "lipid-forming body" (Fig. 6). This lipid-forming body was electron transparent, 1 μm in diameter, without defined ultrastructure, and devoid of visible membraneous outline. The lipid-forming body was surrounded by tightly adhering lipid droplets which were subsequently released into the cytoplasm. Under a light microscope the clusters resembled voluminous lipid inclusions (10).

Other characteristic organelles of young chlamydospores included spherical electron-dense bodies, 0.3–1.0 μm in diameter, whose membrane boundaries were frequently penetrated by adhering lipid droplets (Fig. 7). In reference to the drop of the mycelial lipid curve observed...
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during the alkaloid production phase of C. purpurea (13), we named these organelles “lipid-absorbing bodies” (LA body). At least one LA body could be observed in each chlamydospore. At the beginning of alkaloid formation, on the 7th day of cultivation, an electron-transparent layer was formed beneath the membrane of most LA bodies (Fig. 7). This may be assumed to lead to the formation around the LA bodies of extensive spherical deposits of a material with an ultrastructure attributed generally to phospholipid inclusions (Fig. 8) (1). These deposits were named “lipid-absorbing vesicles” (LA vesicles) since their surface displayed a unit membrane penetrated by lipid droplets (Fig. 8). The interior of the LA vesicles was regularly observed to contain one to three LA bodies.

Cristate mitochondria did not appear in chlamydospores prior to alkaloid formation (Fig. 8 and 9). Upon the disappearance of most lipid droplets from the cytoplasm of chlamydospores, cristate mitochondria invaded the LA vesicles (Fig. 9). However, a thin layer of intervening cytoplasm was preserved in all cases. Beneath the cytoplasmic membrane of chlamydospores were regularly situated tiny vesicles (4), 0.15 \( \mu \text{m} \) in diameter (Fig. 9). The nuclei of chlamydospores usually exhibited a homogeneous nucleoplasm ultrastructure (Fig. 5); chlamydospores were often binucleate.

**DISCUSSION**

Observation of the chlamydospore cell organelles indicated that certain alterations in the ultrastructure, accompanying the development of a submerged culture of C. purpurea, can be interpreted as indicators of the physiological state of the culture. For instance, the function of lipid-forming bodies is clearly perceptible, since chlamydospores already contain lipid droplets prior to the appearance of other organelles such as dense bodies and cristate mitochondria. The determination of a decrease in the amount of intracellular lipids accompanying alkaloid formation (13) served as a basis for our concept of the function of dense bodies and phospholipid-containing vesicles in chlamydospores. We would like to postulate that these organelles absorb lipid droplets from the cyto-

**Fig. 7.** Chlamydospore from 7-day-old C. purpurea culture. Abbreviations: LAB, lipid-absorbing body; L, lipid droplets. Marker bar = 1 \( \mu \text{m} \).
plasm of chlamydospores and that they are related to the formation of alkaloids in C. purpurea, e.g., by mobilizing cell lipid reserves. The direction of the movement of lipid droplets across the membrane of LA bodies and LA vesicles, however, is merely a deduction, and further study of this phenomenon is necessary. Nevertheless, the present study harmonizes
with our previous conclusion (13) that alkaloids are synthesized only after conidia formation and growth stage. Also interesting was the phenomenon of the embedding of mitochondria into the LA vesicles. This phenomenon corresponds with the maximum of citrate synthase—-a key enzyme of the tricarboxylic acid cycle (13).

The ultrastructure of the cytoplasm of filamentous hyphae during the alkaloid formation was the same as in chlamydospores (Fig. 10). The homogeneous cytoplasmic matrix was vacuolized and contained numerous lipid droplets. The hyphae were 2 μm thick at this stage.

The ultrastructure of the vacuolar contents of C. purpurea conidia and its biochemical nature seemed to be very similar to those of vacuolar dense bodies found in the zoospores and oogonia of Saprolegnia (3), in the conidia and hyphae of Penicillium (2, 16), and in the hyphae of Phialophora dermatitidis (4). On the other hand, the dense bodies in the cytoplasm of C. purpurea chlamydospores may have a distinct and specific function in lipid metabolism. That we revealed dense bodies in proximity of lipid droplets, as also shown by Koltin et al. (9) when depicting the chlamydospores of the hemomycete Schizophyllum commune, indicates their rather general occurrence in fungal reproductive cells.

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LITERATURE CITED


Fig. 10. Segment of hypha from 9-day-old C. purpurea culture. Abbreviations: N, nucleus; M, mitochondria; V, vacuole; L, lipid droplets. Marker bar = 1 μm.