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Ultrastructure of Mitochondria and Crystal-containing Bodies in Mature Ballistospores of the Fungus *Basidiobolus ranarum* as Revealed by Freeze-etching

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By using the freeze-etch technique, a regular pattern on both sides of the outer mitochondrial membrane can be demonstrated in mature spores of *Basidiobolus ranarum*. Bands consisting of 5 to 10 parallel ridges, each of which is 20 nm wide, envelop the outer as well as the inner side of this membrane. Hexagonal crystals, probably representing stored protein, are enclosed in special bodies with a very smooth surrounding unit membrane. The crystals are formed by parallel rods which consist of globular subunits, 6.5 to 7.0 nm wide.

Several investigators have pointed out that it is difficult to obtain satisfactory chemical fixation of fungal material, especially spores (4). Owing to the low permeability of most fungal cell walls for osmium tetroxide, potassium permanganate has been commonly used as a fixative (1, 3-6). Good preservation of membranous structures can be achieved in this way. On the other hand, potassium permanganate destroys the ribosomes and causes considerable changes in the cytoplasm. Better fixation could be obtained with glutaraldehyde-acrolein followed by osmium tetroxide (7).

A new and excellent tool for the study of fungal cytology is the freeze-etching technique (11). The usefulness of freeze-etching in the study of yeast cells has been demonstrated by Moor (10, 12). Recent studies on the ultrastructure of *Penicillium megasporum* conidiospores and conidia (8, 9, 14, 15) have already proved the advantages of this method over the conventional fixation procedures for fungal spores. This paper describes and discusses the ultrastructure of the mitochondria and crystal-bearing bodies of mature ballistospores of the fungus *Basidiobolus ranarum* as revealed by freeze-etching.

MATERIALS AND METHODS

Cultures of the fungus *Basidiobolus ranarum* (strain isolated by C. Robinow) were grown on cornmeal agar for 3 to 5 days. On this medium, the fungus produces a great number of ballistospores (aplanospores). The discharged spores were collected on a thin collodion film which was spread over a liquid yeast-glucose medium (1.5% glucose, 0.5% yeast extract) containing 15% glycerol as a freeze protection reagent. Small droplets of the harvested spores in the collodion were frozen in Freon 22 and then frozen-etched in a Balzers unit (Balzers AG, Lichtenstein). The electron micrographs were taken with a Philips 100 or a Philips 200 electron microscope.

RESULTS

Ballistospores are easily identified under a light microscope by their pyriform shape and by the dense and granular appearance of their cytoplasm (Fig. 1a). Figure 1b shows a cross fracture through a whole spore. The spore shape is remarkably well preserved after the physical fixation in Freon 22 followed by freeze-etching. Even at the relatively low magnification (× 10,000) in Fig. 1b, several structural details of the spore cytoplasm can be recognized. Numerous lipid granules (storage material) were identified by their smooth fracture planes. Surface views of several small vacuoles were detected as well. A great number of small vesicles accumulated preferentially along the spore periphery under the cell wall.

The mitochondria show wide variation in size as well as in shape. Spherical, elongated, and triangular shapes were found side by side (Fig. 2). Cross sections of mitochondria show clearly the invagination of the inner unit membrane (Fig.

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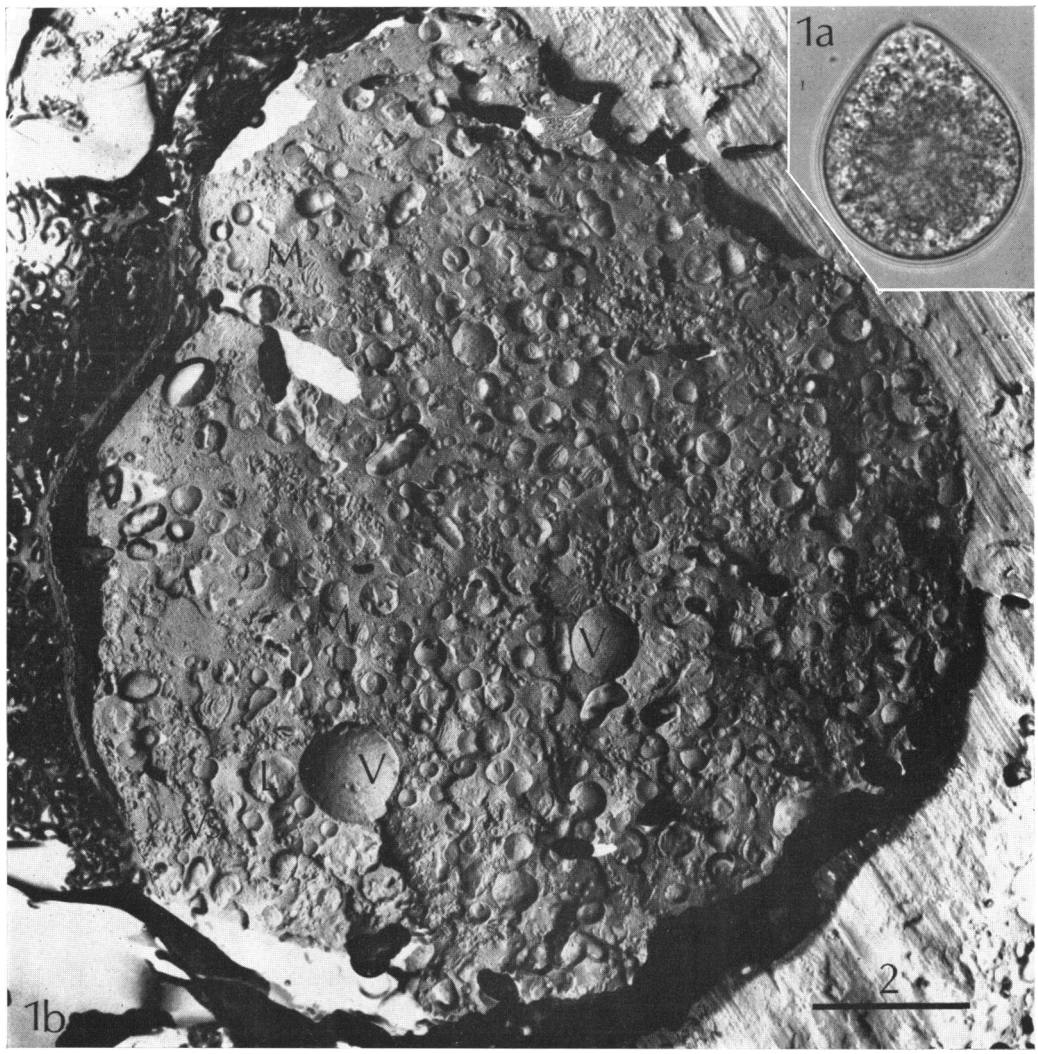

FIG. 1a. *Light micrograph of a typical pyriform ballistospore.* × *350.*

FIG. 1b. *Cross fracture through a ballistospore. The cytoplasm is densely packed with different cell organelles. L, lipid; V, vacuole; Vs, vesicles; M, mitochondria.* × *10,000.*

3 and 5). The cristae were often arranged in stacks. In Fig. 6, outer and inner surfaces of these parallel lamellae are exposed. A few scattered particles are attached to the outer smooth side of the membrane facing the perimitochondrial space, whereas the inner surface, facing the stroma, is relatively densely packed with globular units (oxisomes). A strikingly regular pattern not previously seen in frozen-etched preparations lends a sculptured appearance to the outer mitochondrial membrane (Fig. 1b, 2, 4, and 5). The "finger-print"-like structure appeared on the outer (Fig. 2 and 4) as well as on the inner (Fig. 2 and 5) side of the membrane. Bands consisting of 5 to 10 parallel ridges, each approximately 20 nm wide, enveloped the outer mitochondrial membrane on both sides. The ridges appeared to have a fine granular substructure, but as the diameter of these particles is almost the same as the carbon-platinum grain (2 nm) of the shadowing, it was difficult to measure them accurately.

Frequently, six to eight crystal-containing bodies were observed in a single cross-fracture plane through a whole, mature ballistospore. A very smooth unit membrane separates the ground cytoplasm from the matrix of the crystal body

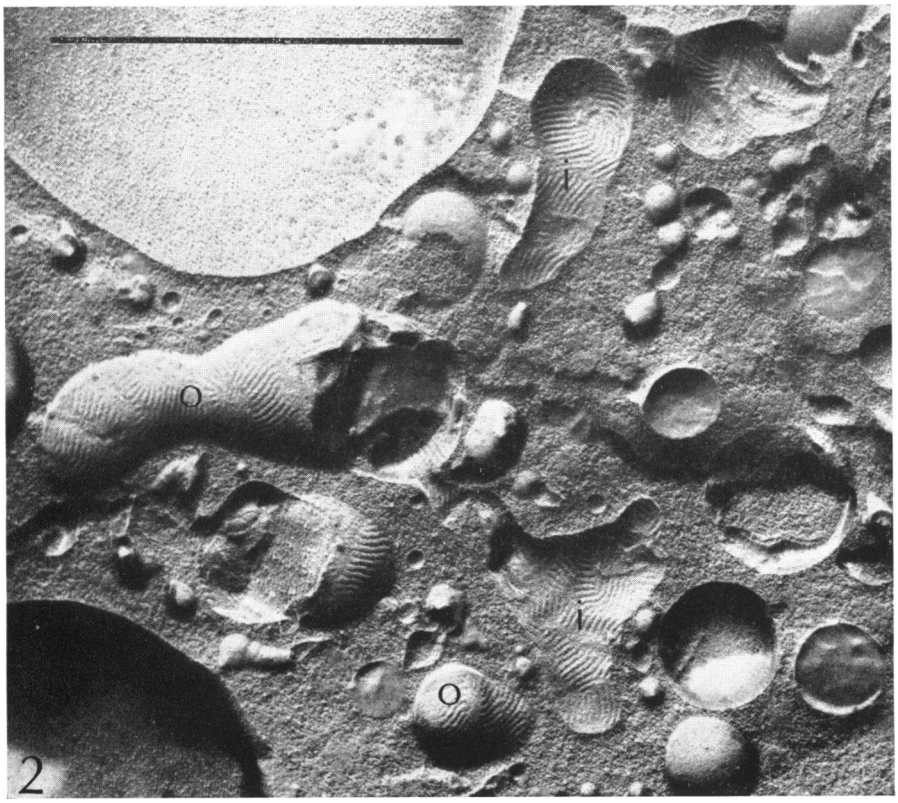

FIG. 2. *Spherical, elongated, and triangular mitochondria are side by side. Inner (i) and outer (o) surfaces of the outer mitochondrial membrane with a "finger-print"-like pattern are visible.* × *55,000.*

(Fig. 7). No difference in the granularity of the crystal matrix and the ground cytoplasm was observed. In Fig. 7, the fracture plane is almost at a right angle to the main axis of the crystal. As the etching depth was very shallow, only the regular hexagonal pattern, but no crystal edges are visible. Figures 8 and 9 show two different fractures through the crystal after longer exposure to etching. The arrows point to regions where clearly parallel rods (crystal units) can be recognized. The rods appear to consist of globular subunits with a diameter of 6.5 to 7.0 nm.

DISCUSSION

In 1963, Moore and McAlear (13) examined the mitochondrial micromorphology of more than 50 genera of *Eumycetes*. They found wide variation of mitochondrial structure in different fungi, but concluded that fungal mitochondria showed no special features that distinguished them from the mitochondria of algae, higher plants, and metazoa. Freeze-etch studies of yeast cells (12) confirmed the general ultrastructure of fungal mitochondria as revealed earlier by chemical fixation. In addition, it was possible with this method to show the surface of the outer mitochondrial membrane. In yeasts (12), as well as in onion root tips (2), the appearance of the outer mitochondrial membrane is rough and shows cracks or tiny pores. However, it has not yet been possible to demonstrate a regular pattern on or in this membrane. Because the oxidation processes occur on the inner mitochondrial membrane, it is difficult to explain the parallel ridges as surface-enlarging structures. These ridges are probably not involved in physiological functions and merely give greater rigidity to the mitochondria. Mitochondria with this "finger-print"-like structure have not yet been observed in vegetative hyphae of *Basidiobolus*.

The crystals described here are probably stored protein. The unit membrane surrounding them could be regarded as a possible barrier against intracellular proteases. The first step in the mobilization of the protein might be the breakdown of the protecting envelope. In this connection,

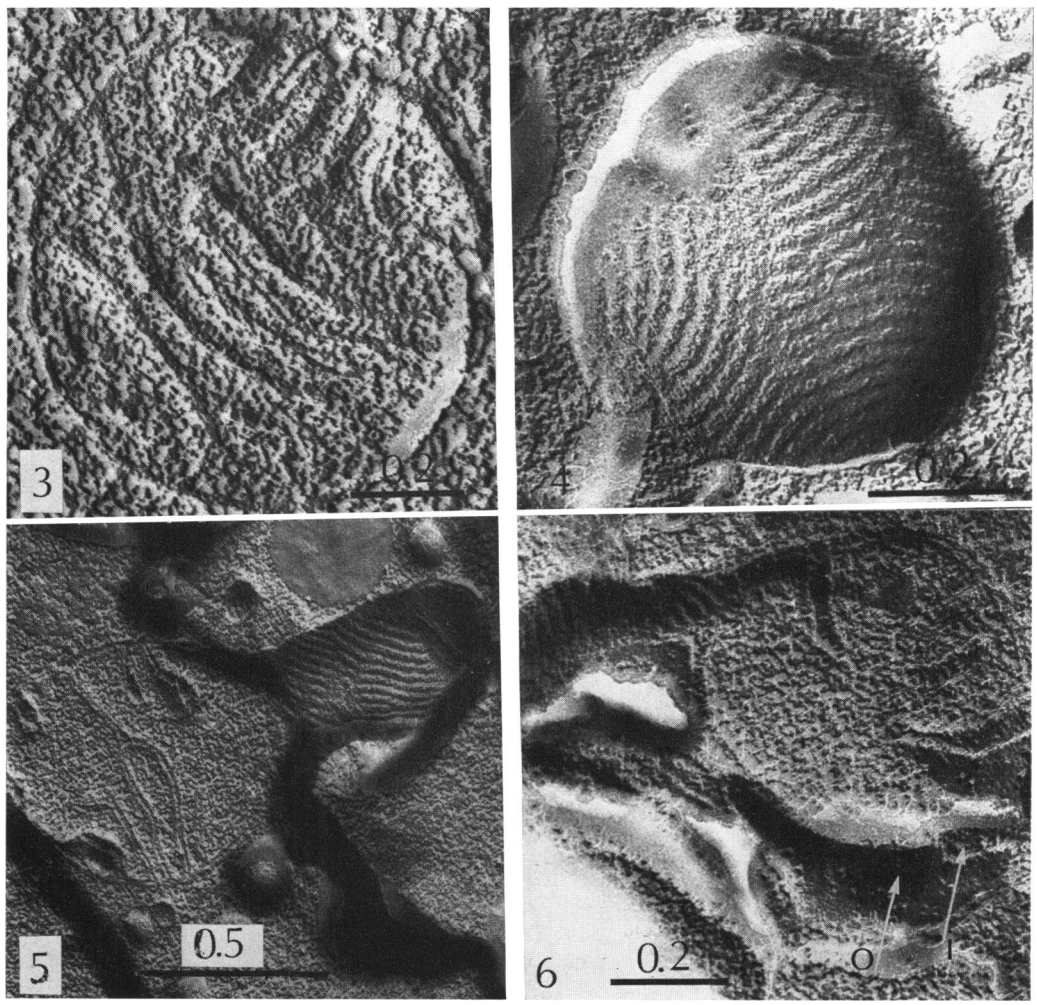

FIG. 3. *Cross section of a mitochondrion showing the cristae mitochondriales.* × *78,000.*

FIG. 4. *Surface view of the outer side of the outer mitochondrial membrane. Note the regular pattern formed by ridges 20 nm wide.* × *100,000.*

FIG. 5. *"Budding" mitochondrion. Inner surface of the outer membrane is in the "bud" region. Invaginations of the inner membrane are visible in the cross fracture.* × *50,000.*

FIG. 6. *Inner (i) and outer (o) surfaces of the cristae are exposed (arrows). Only the stroma facing side is densely covered with oxisomes. Part of the "finger-print"-like structure of the outer mitochondrial membrane appears at the right.* × *84,000.*

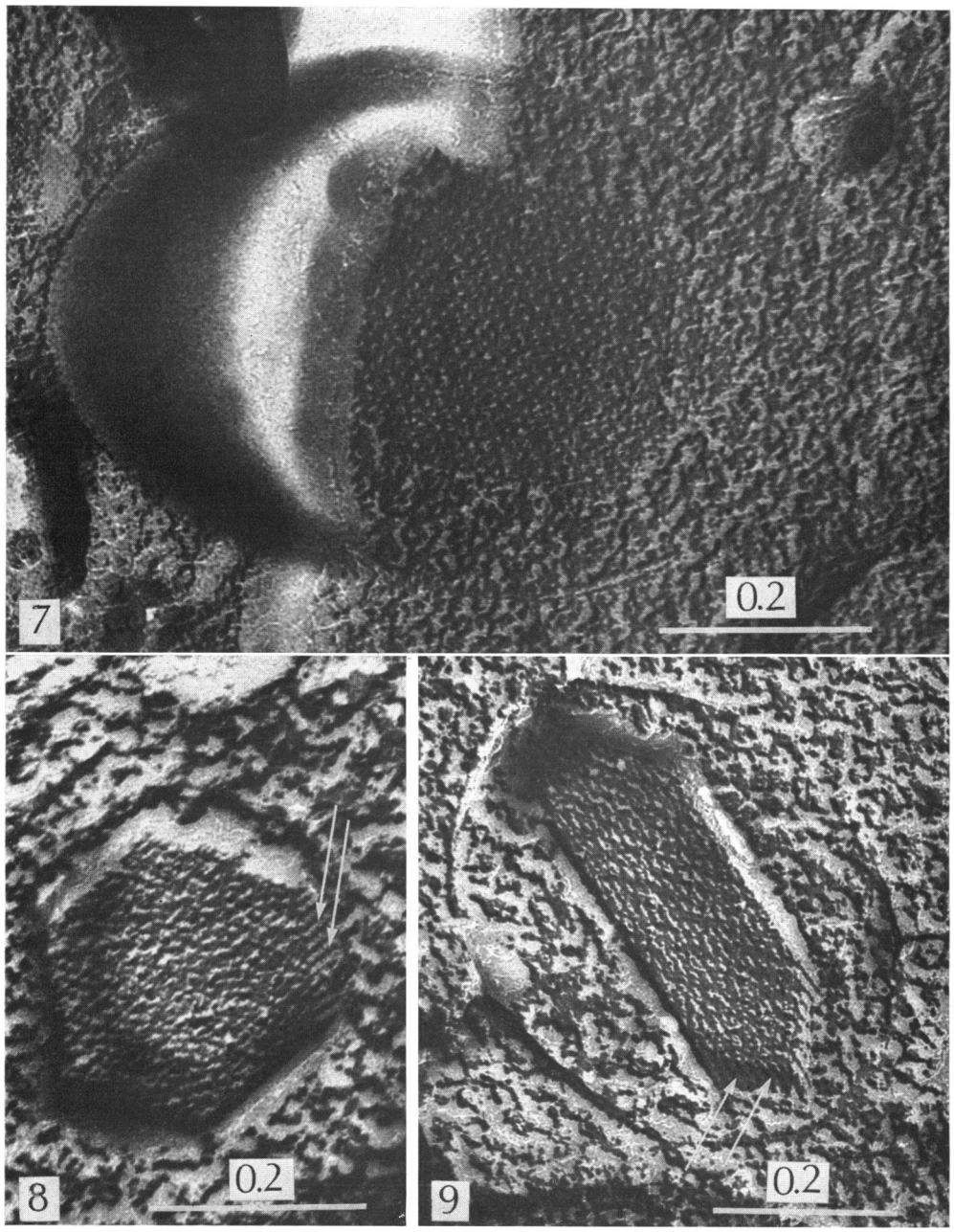

FIG. 7. *Crystal-containing body with a smooth unit membrane. Owing to the low etching depth, only the hexagonal pattern of the crystal and no crystal edges are visible (fracture plane almost at right angles to the main axis of the crystal).* × *146,000.*

FIG. 8 and 9. *Two different fractures through crystals. Arrows point to arrays of parallel rods, consisting of globular subunits 6.5 to 7.0 nm in diameter. Fig. 8,* × *146,000; Fig. 9,* × *125,000.*

it is of interest that similar crystals lacking a membranous envelope have been seen several times in germ tubes of *Conidiobolus* (C. F. Robinow, *personal communication*), a fungus to which *Basidiobolus* is closely related.

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