A Novel Apical Corpuscle in Hyphae of *Mucor rouxii*

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Median longitudinal sections of germ tube apices of *Mucor rouxii* revealed the presence of a single, roughly hemispherical, electron-dense organelle, in intimate contact with the apical cell wall. Conceivably, this "apical corpuscle" may be responsible for the emission of the germ tube and its continued apical growth.

**Materials and Methods**

*M. rouxii* IM-80 was employed. Spores (sporangiospores) were obtained from a 4- to 7-day-old culture grown in bottles containing solid YPG (yeast extract-peptone-glucose-agar) medium (2). The spore suspension was washed three times with distilled water and was inoculated into liquid YPG medium to a final concentration of $10^4$ to $2 \times 10^4$ spores/ml. Cultures were grown in 250-ml Erlenmeyer flasks containing 50 ml of medium. The flasks were covered with cotton plugs and were incubated aerobically on a reciprocating shaker at 29 °C for 4 to 5 hr, until the majority of the spores showed an incipient germ tube. The cells were washed three times by centrifugation in cold 0.2 M potassium phosphate buffer (pH 7.0) and then were fixed in 1% KHMnO$_4$ at room temperature for 20 to 30 min. They were subsequently washed with the above buffer solution until the supernatant fluid was colorless. Some specimens were prefixed in the aforementioned buffer containing 3% glutaraldehyde, but no improvement in intracellular detail was detected. After fixation, the cells were embedded in agar blocks to facilitate handling and then were dehydrated for 10 min each in 25, 50, and 100% acetone, followed by three 15-min washings with 100% acetone. The agar blocks were subsequently embedded in an Epon-Araldite resin mixture (5) and were sectioned (approximately 600 A) with a glass knife in a LKB Ultrotome. Sections were poststained with lead citrate for 30 sec (13). Electron micrographs were made with an RCA microscope model EMU-3B (Fig. 1) and with a Hitachi HU-11 microscope (Fig. 2, 3).

**Results**

To increase the probability of obtaining and locating longitudinal sections of hyphal apices, incipient germ tubes, rather than fully developed hyphae, were selected for study. Such sections were not common but could be recognized readily by their characteristic outline (round cells with a small protuberance). The use of young cells also insured that the observed apices corresponded to actively growing hyphae.

The cytoplasm of the incipient hyphal tube contained a variable number of vesicular elements of the endoplasmic reticulum which were located next to the cytoplasmic membrane (Fig. 1–3). These peripheral structures, however, were not confined to the germ tube but occurred throught the outer cytoplasmic regions of the germinating spore. Occasionally, mitochondrial sections and cisternal rings (3) were seen in the apical cytoplasm. In cross section, the wall of the germ tube was structurally similar to, and continuous with, the vegetative wall (Fig. 1) that was formed de novo under the spore wall during the first stage of spore germination (in preparation).

The only truly distinctive feature of the hyphal apex was a single electron-dense corpuscle, frequently seen in median longitudinal sections. This corpuscle was consistently found near the point of maximal curvature of the apical dome, inside an inner bulge of the cell wall (Fig. 1–3). It was not seen anywhere else on the surface of the germinating spore. The appearance of the corpuscle varied from section to section. In some sections, it exhibited a semicircular profile, with a maximal diameter of about 0.2 μ, and was only partially surrounded by cell wall material (Fig. 3). In other sections, the corpuscle was much smaller and was completely surrounded by the
FIG. 1–3. Different views of the apical corpuscle of aerobically germinated spores of *Mucor rouxii*. Insert on upper left is a high magnification view of germ tube (GT) apex of Fig. 1; AC, apical corpuscle; ER, endoplasmic reticulum; G, glycogen-like bodies; M, mitochondrion; N, nucleus; Nu, nucleolus; PM, plasma membrane; SW, spore wall; V, vacuole; VW, vegetative wall.
cell wall (Fig. 1, 2). This variable appearance may simply be the result of sections made through different planes of the corpuscle with the geometry illustrated in Fig. 4; the corpuscle is represented as a hemispherical particle with its base oriented toward the wall and its dome in close contact with the cytoplasmic membrane. The apical corpuscle appeared to be extracytoplasmic; we could not determine whether there was any direct channel of communication between it and the interior of the cell. Some, but not all, sections exhibited a narrow transparent space separating the corpuscle from the surrounding wall.

**DISCUSSION**

In a study of mold-yeast dimorphism of *M. rouxii*, the hypothesis was advanced that hyphal morphogenesis is a consequence of the localization (polarization) of cell wall synthesis to a small region of the cell surface, the hyphal apex (1). The possibility that a cytoplasmic organelle is responsible for this localization was also mentioned. The findings reported in this paper lend credence to this possibility, with the modification that the apical organelle is seemingly extracytoplasmic and is in more intimate contact with the growing cell wall than was originally postulated. In contrast to reports describing different degrees and types of intracytoplasmic vesiculation in the hyphal apices of various fungi (4, 8, 9), we have found no such evidence of cytoplasmic differentiation in the germ tube apices of *M. rouxii*. Lomasomes, multivesicular structures which are located between the cytoplasmic membrane and the cell wall in a variety of fungi (10) and are suspected of having a role in cell wall synthesis (14), were also absent.

Recent autoradiographic studies have confirmed that cell wall synthesis in hyphae of *M. rouxii* is strongly localized in the apical dome (unpublished data). Although the evidence is circumstantial, we believe that the apical corpuscle may be directly involved in the mechanism which confines cell wall synthesis to the apical region, and thus may be responsible for germ tube protrusion and its continued elongation via tip growth. However, the possibility that the apical corpuscle is not involved in apical morphogenesis but is involved in an accessory function related only to germ tube protrusion (e.g., dissolution of the spore wall) has not yet been discounted.

The only previous evidence of a subcellular structure uniquely associated with hyphal tips of fungi is Brunswik's description of "Spitzenkörper," which were considered to have a role in the apical growth of *Coprinus* (cf. Girbardt (7)). By phase microscopy, "Spitzenkörper" were detected in hyphal apices of higher fungi (6, 8), but not in *Mucor* (8). In the electron microscope, the "Spitzenkörper" were resolved as a conglomeration of small cytoplasmic vesicles (8) and are therefore entirely different from the apical corpuscle of *M. rouxii*. Apparently, there have been no previous reports of cellular structures similar to the apical corpuscle in hyphal tips of fungi. The only structures observed in electron micrographs of fungi which bear some resemblance to the apical corpuscle are the so-called Woronin bodies. These electron-dense spherical bodies are frequently found in the cytoplasm of Ascomycetes in the vicinity of septa (11) and hyphal tips (8). The possibility that apical corpuscles may be derived from Woronin-like bodies deserves further consideration. Future investigations should also reveal whether the apical corpuscle is an organelle unique to *Mucor* and allied fungi, or if it occurs universally throughout the fungi.

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**LITERATURE CITED**


