

Ultrastructure of Septal Complex in *Filobasidiella neoformans* (*Cryptococcus neoformans*)

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Electron microscopy of *Filobasidiella neoformans*, the perfect state of *Cryptococcus neoformans*, revealed basidiomycete doliporesepta between hyphal cells and also between clamp connections and adjacent cells. The pore-occluding material was a heterogeneous flattened plate with dark margins and a lighter center, as seen in the species of *Filobasidium*. Representative basidiomycete parenthesomes were lacking, and endoplasmic reticulum was seen in the dolipore region.

The genus *Filobasidiella* Kwon-Chung gen. nov. (2) was established to accommodate the perfect state of *Cryptococcus neoformans*, *Filobasidiella neoformans*. It is the second genus belonging to the family *Filobasidiaceae* Olive (6) in *Ustilaginales*. *F. neoformans* is characterized by a hyaline mycelium, which consists of dikaryotic hyphae with clamp connections and an elongate basidium bearing terminal sessile basidiospores in chains. The characteristics of the mycelium and basidium resemble those in the genus *Filobasidium* Olive (6), the type genus of *Filobasidiaceae*. *Filobasidiella*, however, differs from *Filobasidium* by the formation of chains of small basidiospores by continuous basipetal budding.

The imperfect states of two *Filobasidium* species, *Filobasidium capsuligenum* and *F. floriforme*, share similarities with *C. neoformans*. They are white to cream-colored, inositol-assimilating, starch-forming yeast (9).

Moore and Kreger-van Rij (4) studied the ultrastructure of both *Filobasidium* species and found representative basidiomycete dolipores, with some variation in parenthesome morphology. This is, to date, the only report of a dolipore-parenthesome septum from *Ustilaginales* (3). Before the perfect state of *C. neoformans* was discovered, Cutler and Erke (1) studied the ultrastructure of hyphae produced by a single strain of the species. They showed that the hyphal septum consisted of a solid wall with no pore. The clamp-like structure was also seen to be separated from the adjacent cell by a solid wall, hence the evidence of true clamp connections was lacking.

We have studied the ultrastructure of the septal complex in *F. neoformans* and found

representative basidiomycete dolipores but no clear parenthesomes.

F. neoformans strains B-3501 (α type) and B-3502 (a type) were mixed on malt extract agar plates and incubated for 10 days at 25 C. Pieces of agar block containing mycelium material were transferred to malt extract broth and incubated for 5 days at 25 C to increase hyphal formation. The hyphal masses obtained by centrifugation were prepared for sectioning according to methods by Cutler and Erke (1), Moor and Kreger-van Rij (4), and Popkin et al. (7). The last method was the most satisfactory, with a slight modification, and the protocol was as follows. Primary fixation was performed with 2.5% glutaraldehyde buffered in 0.2 M sodium cacodylate, pH 7.4 (Tousimis Research Corp., Rockville, Md.). After two successive washings in sodium cacodylate buffer, secondary fixation was in 1% osmium tetroxide in Veronal acetate buffer (10). The fixed cells were washed two times in Veronal acetate buffer at 25 C for 10 min and once in 0.5% uranyl acetate, in Veronal acetate buffer, overnight at 4 C. Final embedment was in Spurr medium (mixture "C," moderately soft) (11). All sectioning was performed with an LKB Ultratome III and a DuPont diamond knife. Ultrathin sections were placed on 300-mesh naked copper grids and stained for 3 min with alkaline lead citrate (8). Micrographs were recorded on Kodak electron microscopy sheet film no. 4489 with a Hitachi HU-11C electron microscope operated at 75 kV.

Figure 1 shows the septal complex of *F. neoformans*. Dolipores are seen in the hyphal septum (Fig. 1a) as well as in the septum between a clamp connection and an adjacent cell (Fig. 1b).

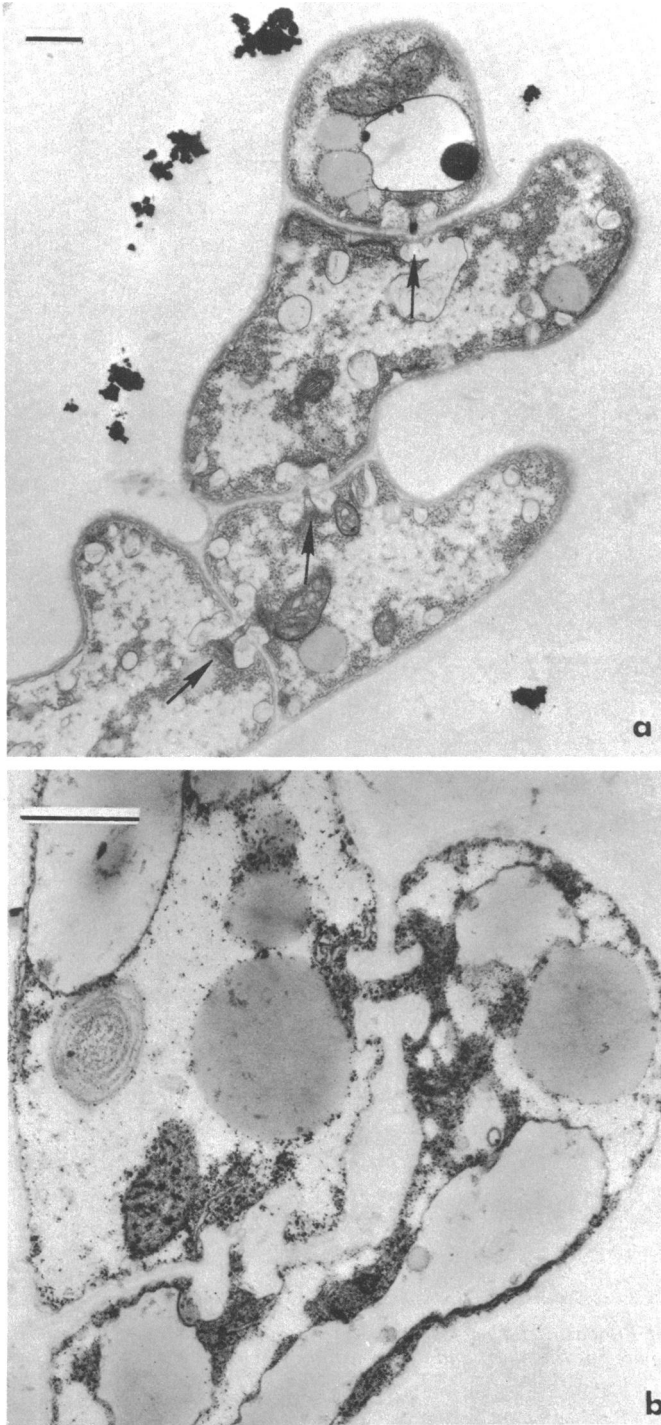


FIG. 1. Ultrathin sections of hyphae of *Filobasidiella neoformans*. Note the dolipore septa between hyphal cells (arrows) in (a) and between clamp connection and hyphal cell in (b). Bar, 0.5 μ m.

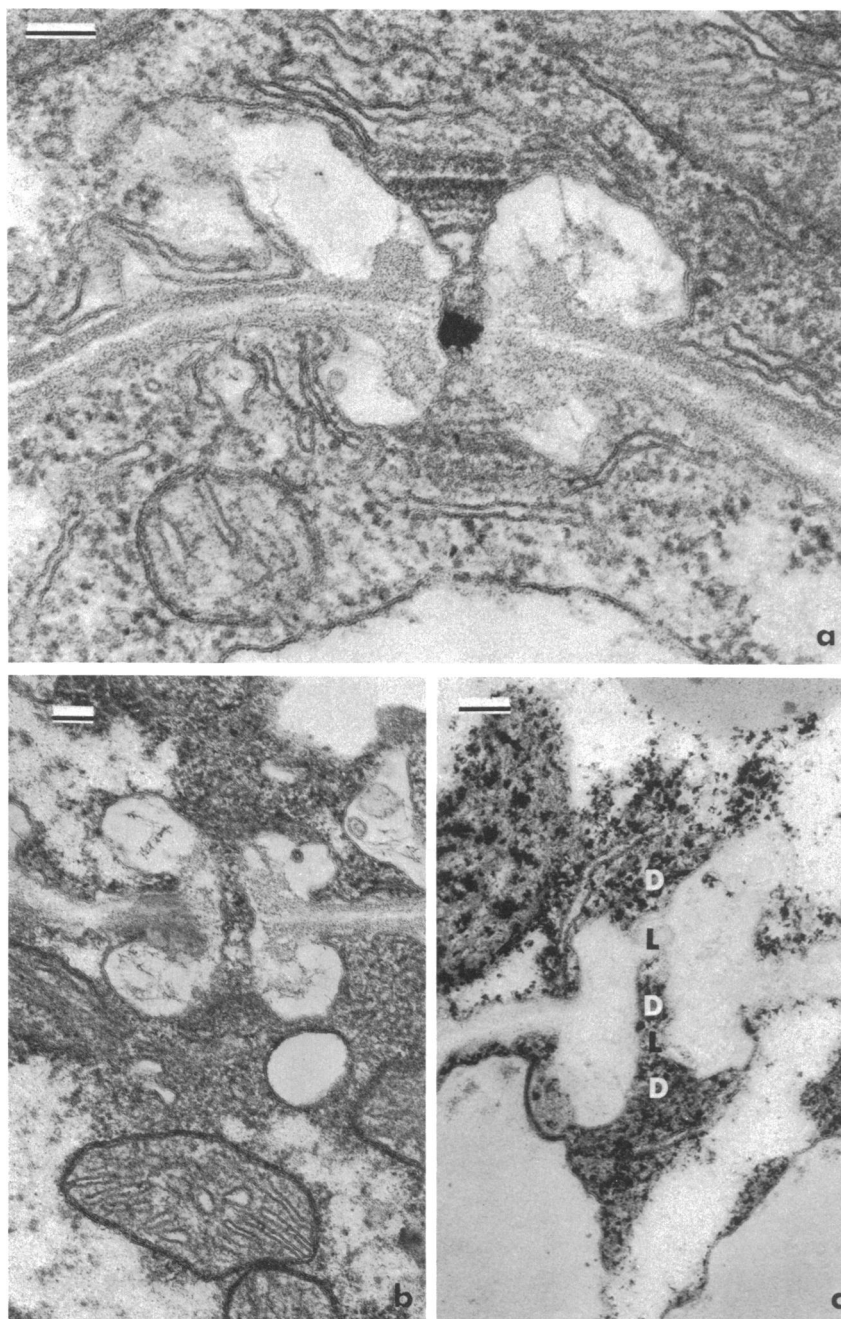


FIG. 2. Dolipores of *Filobasidiella neoformans*. (a) Note dark heterogeneous banding and endoplasmic reticulum around the pore openings; (b) and (c) show dark (D) and light (L) bands in the channel. Bar, 0.5 μm .

The dolipore channel exhibits symmetrical bands of dark and light substance (Fig. 2c), as seen in two species of *Filobasidium* (4). The dark outer band is composed of heterogeneous

layers (Fig. 2a and 2b). Some fragments of hyphae showed solid walls with no pores between hyphal cells or between clamp connections and adjacent cells (Fig. 3a and 3b). The lack of pores

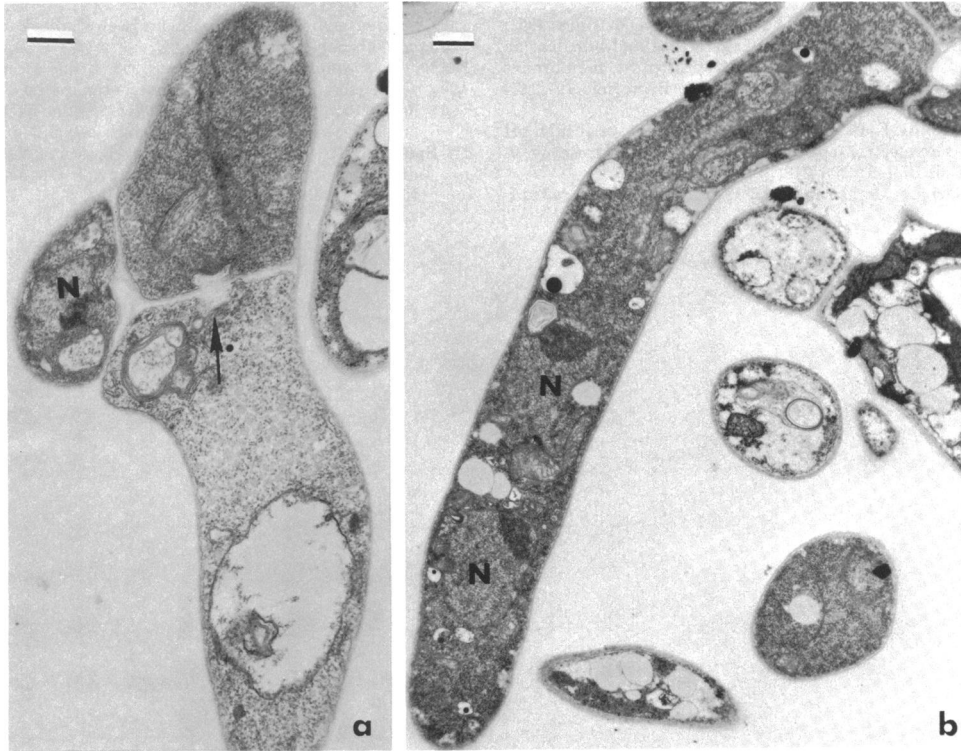


FIG. 3. Clamp connection and hyphal cells of *Filobasidiella neoformans* with nuclei. Note a nucleus (N) within the clamp connection and a dolipore septum without channel (arrow) in (a); dikaryotic hyphal cell in (b). Bar, 0.5 μ m.

in the septa shown in Fig. 3b may represent a vigorously growing young cell. Figure 3a shows evidence of a dolipore apparatus between hyphal cells but with no channel, possibly a result of the plane of sectioning. The septal complex of *F. neoformans* appears to resemble that of *F. floriforme* more closely than that of *F. capsuligenum*, since it lacks a parenthesome. The clamp connections were occasionally found to contain a nucleus (Fig. 3a), and many hyphal cells were dikaryotic (Fig. 3b). These results support the taxonomic position of *F. neoformans* in *Filobasidiaceae* of *Heterobasidiomycetes*. The dolipore septum of the basidiomycetes has been considered as significant as the basidium in characterizing this class of fungi (4). The micromorphology of the dolipore septum appears to be an important feature for the understanding of phylogenetic relationships within the class. The dolipore with dome-shaped, entire, or perforated parenthesome is found only in the *Homobasidiomycetes* and the basidiocarpic mycelium of the jelly fungi (*Heterobasidiomycetes*) (5). The dolipore septum has still not been found in the order *Uredinales*

and has been reported only in three species from *Ustilaginales*. All three species are in the family *Filobasidiaceae*, and their septa either lack parenthesomes or exhibit a modification in parenthesome structure.

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