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## Scanning electron microscopy of the septal pore cap of the basidiomycete *Schizophyllum commune*

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As part of a comparative study of the structure and function of pore structures in heterobasidiomycetous yeasts, dikaryotic hyphae of *Schizophyllum commune* were subjected to chemical fixation, freeze fracturing, maceration, and freeze substitution, and were subsequently prepared for scanning electron microscopy. The interior of the hyphal cell was visualized and revealed the perforated septal pore cap or parenthesome, mitochondria, vacuoles, and tubular endoplasmic reticulum. The septal pore cap showed connections with tubular endoplasmic reticulum. This tubular endoplasmic reticulum covered the dolipore septal surface. The results presented here complement and extend the ultrastructural image of the septal pore cap obtained from transmission electron micrographs.

*Key words*: septal pore cap, *Schizophyllum commune*, freeze fracture, maceration, scanning electron microscopy.

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En tant que partie d'une étude comparative des structures et des fonctions des structures des pores chez les levures hétérobasidiomycètes, des hyphes dikaryotiques de *Schizophyllum commune* ont été soumis à une fixation chimique, une cryofracturation, une macération et une cryosubstitution et ils ont été subséquemment préparés pour étude en microscopie électronique à balayage. L'examen interne des cellules hyphales a révélé le pore du dome septal perforé ou parenthesome, les mitochondries, les vacuoles et le réticulum endoplasmique tubulaire. Le pore du dome septal a présenté des raccordements avec le réticulum endoplasmique tubulaire et ce réticulum endoplasmique tubulaire recouvrait la surface septale du doripore. Les présents résultats ajoutent aux micrographies ultrastructurales déjà obtenues par microscopie électronique des informations complémentaires sur le pore du dome septal.

*Mots clés* : pore du dome septal, *Schizophyllum commune*, cryofracturation, macération, microscopie électronique à balayage.

[Traduit par la Rédaction]

The dolipore septa of the Basidiomycetes show a complex ultrastructure as compared with the septa of the Ascomycetes. Buller (1933) first noticed hemispherical pads on either side of

septa of *Rhizoctonia solani*; however, Girbardt (1958) visualized the septal pore caps of *Polystictus versicolor* for the first time with the transmission electron microscope (TEM). Since then, many septa and septal pore caps have been described (Bracker 1967; Traquair and McKeen 1978). The septal pore cap or

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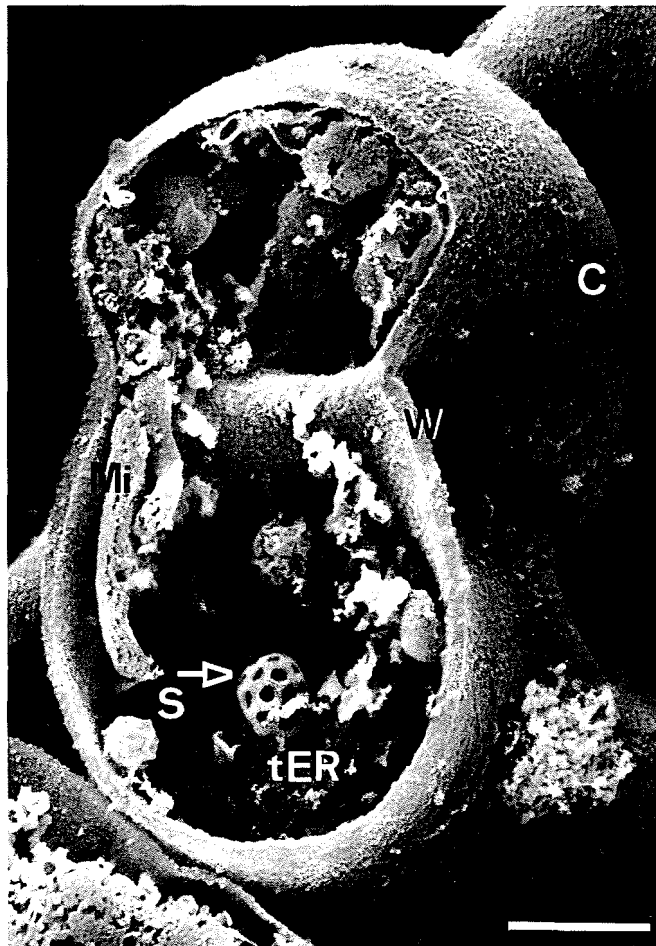


FIG. 1. The interior of a hyphal cell of *S. commune* next to a clamp connection after freeze fracturing, maceration, freeze substitution, and subsequent processing for scanning electron microscopy. The septal pore cap (arrow) is perforated, and shows its position within the cell amongst other organelles. Mi, mitochondrion; tER, tubular endoplasmic reticulum; C, clamp; S, septum; W, cell wall. Scale bar = 1  $\mu$ m.

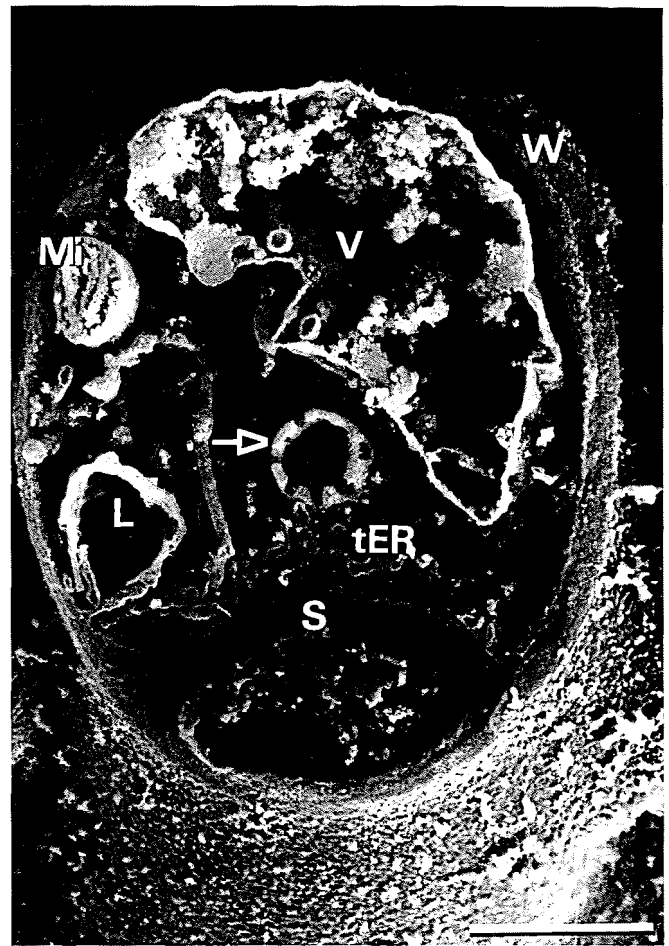


FIG. 2. *Schizophyllum commune* hyphal cell revealing the septal pore cap partly transversely fractured (arrow) in relation to other cellular organelles. V, vacuole; Mi, mitochondrion; tER, tubular endoplasmic reticulum; L, membranous loculus; S, septum; W, cell wall. Scale bar = 1  $\mu$ m.

parenthesome is a pair of membranous, dome-shaped structures (Moore and McAlear 1962) and can be perforate, nonperforate, vesiculate, or ampullate (Moore 1979). The taxonomy of Basidiomycetes is based in part on these pore cap variations (Moore 1978, 1980; Khan and Kimbrough 1982; Van der Walt and Von Arx 1985; Suh et al. 1993).

A better understanding of the ultrastructure of the septal pore cap can be obtained by combining transmission and scanning electron microscopy. Methods have been described to study the internal cell organization with the scanning electron microscope (SEM) in animal tissue (Haggis and Bond 1978; Tanaka 1981; Tanaka and Naguro 1981) and in plant tissue (Barnes and Blackmore 1984, 1986; Van Aelst and Wilms 1988). This SEM preparation method comprises chemical fixation, cryoprotection, freezing and fracturing, thawing, maceration by chemical etching, postfixation, dehydration at room temperature, critical point drying, and finally gold sputtering. Plant tissue is subjected to prolonged maceration as compared with animal tissue (Barnes and Blackmore 1984). Another way to improve this preparation for SEM is to use the freeze-substitution method to circumvent the dehydration at room temperature, since dehydration of tissue performed at room temperature leads to shrinkage of structures (Boyde 1980; Howard and O'Donnell 1987).

Dikaryotic hyphae of *Schizophyllum commune* CBS 340.81  $\times$  341.81 were cultivated in yeast extract – malt extract (YM) broth for 3 days in a Gio gyratory shaker at 175 rotations/min and 25°C. The YM broth contained 1% (w/v) glucose, 0.3% (w/v) malt extract, 0.3% (w/v) yeast extract, and 0.5% (w/v) peptone in distilled water. The 3-day-old hyphal globules of about 3 mm in diameter were fixed for 16 h at 4°C in 2% (v/v) glutaraldehyde (Polysciences, Inc., 8% glutaraldehyde EM grade) in 50 mM sodium cacodylate, pH 7.4, rinsed in 66 mM phosphate buffer (PB), pH 7.4, and postfixed for 16 h at 4°C in 1% (w/v) osmium tetroxide in PB. After being washed three times in PB, the samples were immersed in a series of 15, 30, and 50% (v/v) aqueous dimethyl sulfoxide (DMSO), for 15 min each. To remove excess DMSO, a hyphal globule was blotted on Whatman filter paper 541, and subsequently frozen by being placed on a metal block cooled with liquid nitrogen (-170°C). The stainless steel metal block, 4 cm in diameter and 4.5 cm high, was placed in a polystyrene foam box, 26 cm wide  $\times$  30 cm long  $\times$  17 cm high, which was filled with liquid nitrogen up to the surface of the metal block. The frozen fungal globule was cracked into two fragments by placing a cooled single-edge razor blade on it, then slamming a hammer on top of the razor blade. Subsequently, the two fragments were thawed 10–20 s in 50% (v/v) DMSO, and

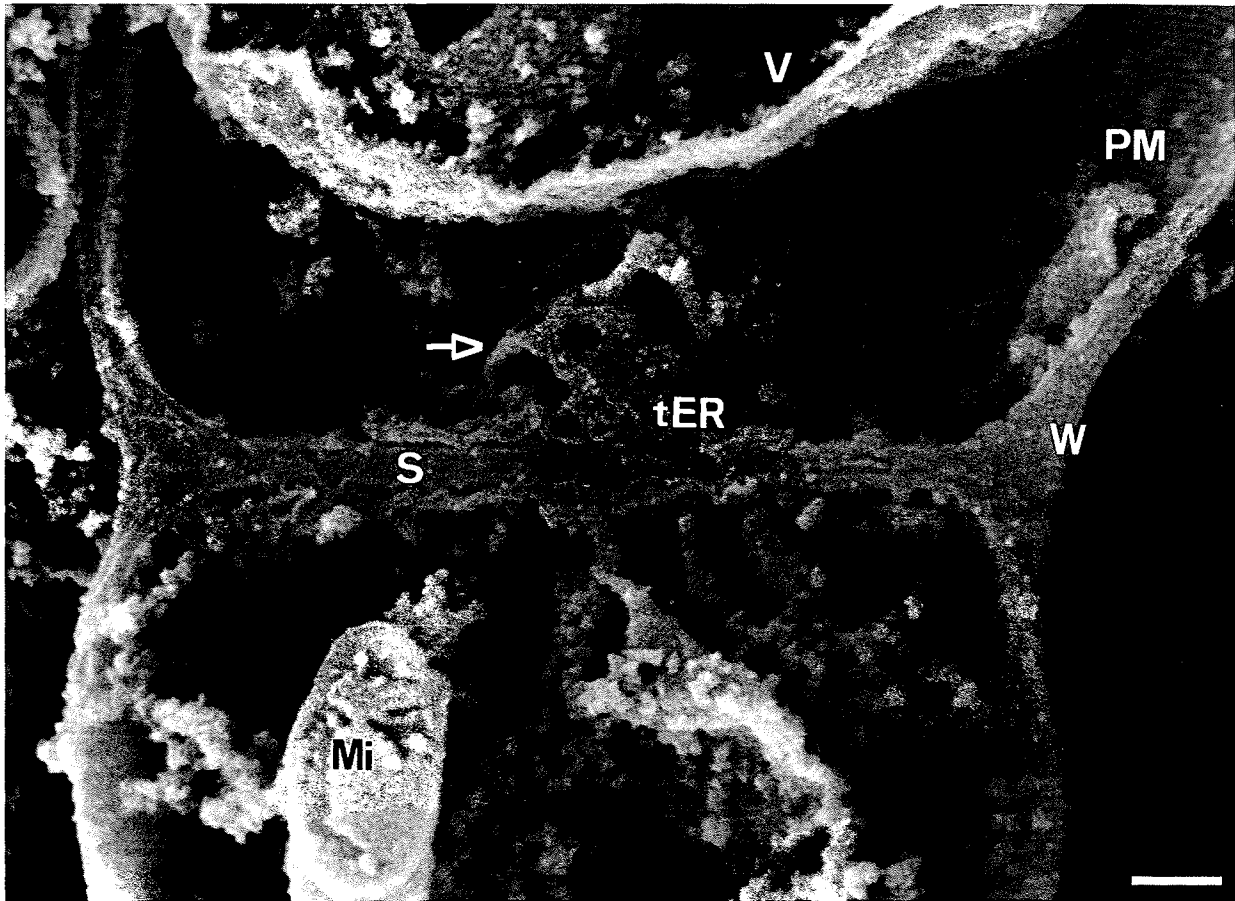


FIG. 3. *Schizophyllum commune* hyphal cells separated by the septum (S). The septal pore cap (arrow) is connected (arrowhead) with the tubular endoplasmic reticulum (tER). Tubular elements are present at the upper part of the pore cap as well. Note the faintly visible pore cap at the lower side of the septum behind tubular structures and a mitochondrion (Mi). W, cell wall; PM, plasma membrane; Mi, mitochondrion; V, vacuole. Scale bar = 200 nm.

washed three times in PB. Then the thawed fragments were macerated for 5 days at 4°C in a solution of 0.2% (w/v) osmium tetroxide in PB. Each day the fragments were transferred into fresh maceration solution. After maceration the fragments were placed in a solution of 1% (w/v) osmium tetroxide – PB for 1 h at 4°C, washed in distilled water, and subsequently transferred to a 2% (w/v) aqueous tannic acid (Mallinckrodt) solution. The vials containing the fragments in the tannic acid solution were slowly rotated for 4 h at room temperature. The tannic acid solution was changed four times with fresh solution during this rotation period. The fragments were thoroughly washed in distilled water and were again put in a solution of 1% (w/v) osmium tetroxide – PB for 1 h at 4°C. After being washed six times in distilled water, the fungal fragments were immersed in 30% (v/v) aqueous *N,N*-dimethylformamide (Meissner and Schwarz 1990) for 30 min and cryofixed in liquid propane (KF 80 Reichert-Jung, Vienna). The frozen fragments were freeze substituted for 2 days at –90°C in methanol (CS auto Reichert-Jung, Vienna). After the temperature was gradually increased (5°C/h) from –90°C to room temperature, the methanol was substituted by anhydrous acetone. The fragments were dried in a critical point dryer (CPD 020 Balzers) with carbon dioxide and mounted on a specimen holder with conductive carbon cement (Neubauer chemikalien). After drying, the specimen holder with the fungal fragments was put in an Oxford cryotransfer system (CT 1500 HT) and subsequently magnetron sputter coated (Denton) at 10<sup>-2</sup> Pa. A repro-

ducible coating thickness of 2 nm was achieved (Cressington MTM 10). The coated fungal fragments were examined in a field emission SEM (JSM 6300F, Jeol) at an acceleration voltage of 5 kV.

We scanned the fractured hyphal globules at low magnification of 3500×, and if clamp structures were present in the fracture planes we also scanned at a magnification of 16 000×. The septal pore cap or parenthesome could be observed. Figure 1 shows parts of the interior of a clamp connection and a hyphal cell with a perforated septal pore cap, which is in agreement with the TEM studies on *S. commune* by Moore and Patton (1975) and by Patton and Marchant (1978). Figure 2 shows the spatial position of the septal pore cap in relation to other cellular organelles such as a mitochondrion, a vacuole, the invaginated plasma membrane forming an extracytoplasmic loculus (Wells 1965), and the endoplasmic reticulum. This endoplasmic reticulum is tubular and is situated adjacent to the septum. The apex of this septal pore cap has been removed, which allows visualization of the holes at the base of the septal pore cap only, and reveals the dolipore as well. The pore occlusion, frequently observed in thin sections, is not visible, but may have been lost during the maceration procedure. In Fig. 3 the septum separates two neighbouring hyphal cells. The tubular endoplasmic reticulum along the septum and the septal pore cap can be observed. Tubular elements are visible at the apex of the septal pore cap. The diameter of the holes in the cap is about 100 nm, which is in agreement with the TEM studies of Patton and Marchant (1978).

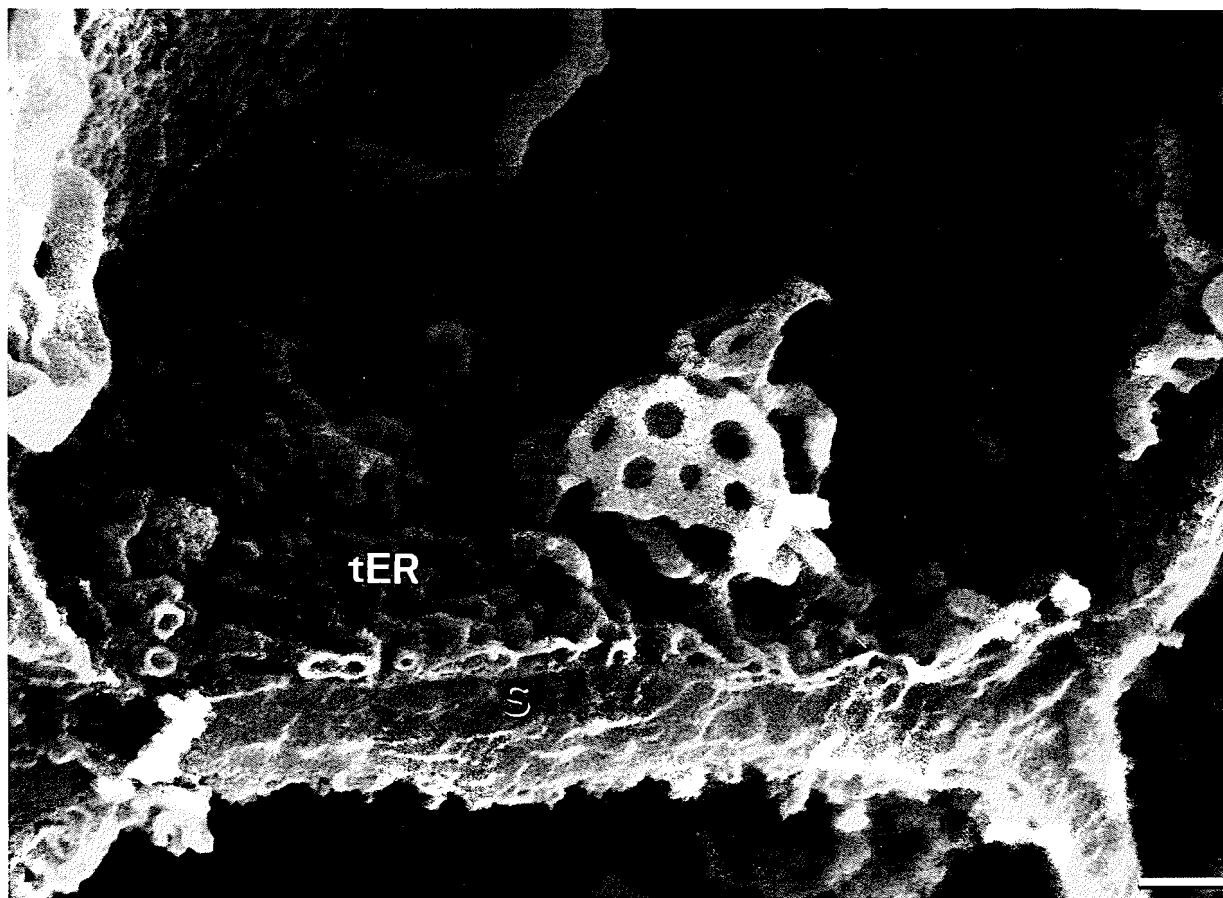


FIG. 4. The perforated septal pore cap of *S. commune* is connected with tubular endoplasmic reticulum (tER). This tER covers the dolipore septal surface. The cap holes at the apex are larger than those occurring at the base. Tubular elements are also present at the upper part of the pore cap. S, septum. Scale bar = 200 nm.

Further, a faintly visible pore cap can be seen at the lower side of the septum behind tubular elements and a mitochondrion. This pore cap does not represent the opposite part of the upper pore cap, and most likely is positioned between the leading hypha and a clamp connection or a side branch. Figure 4 shows a septal pore cap with larger holes at the apex than at the base. Tubular endoplasmic reticulum covers the septum and shows connections with the base of the septal pore cap only. Tubular elements are present at the apex of the pore cap as well. The diameter of the tubular endoplasmic reticulum varies between 40 and 60 nm. In accordance with the studies of Wilsenach and Kessel (1965), the septal pore cap of *Polyporus rugulosus* was connected with the endoplasmic reticulum, which was found to be a reticulated endoplasmic reticulum, consisting of narrow tubules. Most likely the wall endoplasmic reticulum in *Polyporus biennis* is tubular as well and is not a fenestrated, smooth endoplasmic reticulum skirt as described in the earlier studies of Moore and Marchant (1972) and Moore (1985).

In our first attempts to study the hyphal cell interior, we applied the freeze-fracturing and maceration methods developed for plant tissue (Barnes and Blackmore 1984). Though the septal pore cap of *S. commune* was visible, mitochondria and other organelles appeared to be collapsed, possibly because of shrinkage. Therefore, we changed methods from dehydration at room temperature to the freeze-substitution method. Although the observed structures are found to be better preserved after the freeze

substitution, the preparation method for SEM may cause significant deviations of the cellular fine structure from the in vivo situation. However, the SEM method permits visualization of the internal organization of the hyphal cell and the spatial position of the septal pore cap. The procedure described here is a modification of that of Barnes and Blackmore (1984). The major modifications are maceration in 0.2% osmium tetroxide - PB, 4 h in 2% tannic acid, and freeze substitution in methanol at  $-90^{\circ}\text{C}$ , and 2 nm platinum coating of the fragments by magnetron sputtering is important. These modifications resulted in a better preservation of the subcellular structure of the hyphal cell. Baba and Osumi (1987) reported the use of the freeze-substitution method for both TEM and SEM studies of yeast cells. Their TEM and SEM studies resulted in new concepts of the ultrastructure of the yeast cell.

On the basis of the combination of chemical fixation, freezing, freeze fracturing, maceration, freeze substitution, critical point drying, magnetron sputtering, and scanning electron microscopy, this study reveals a new, three-dimensional view of the septal pore cap of *S. commune*. The results presented in our study complement and extend findings on the ultrastructural features of the septal pore cap of *S. commune* shown after thin sectioning and TEM (Moore and Patton 1975; Patton and Marchant 1978). Both SEM and TEM methods may yield important clues about the biological importance of the septal pore cap and its role in the phylogeny of basidiomycetous yeasts.

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