Structural differences between two types of basidiomycete septal pore caps

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The septal pore cap (SPC) of *Trichosporon sporotrichoides* CBS 8245 is vesicular-tubular, connected with flat-tubular endoplasmic reticulum (ER), and stains densely with zinc/iodine/osmium tetroxide, as does the ER. The SPC of *Schizophyllum commune* CBS 340.81 is more complex, about 600 nm in diameter, with perforations of 80–120 nm diameter, and stains less densely with zinc/iodine/osmium tetroxide than the ER. In high-pressure frozen and freeze-substituted hyphae of *T. sporotrichoides* the ER is present parallel to the dolipore septa, and electron-dense material occurs opposite the septal pore channel; the SPC rarely showed smooth vesicular-tubular membranes, suggesting that this is an ephemeral function of the SPC. The SPC of *S. commune* has a smooth outer and inner membrane, which enclose a matrix with a palisade-like substructure. A thin layer of electron-dense material covers the inner surface of the SPC of *S. commune*, from which beaded filamentous structures connect the SPC and the pore-occluding material. These filamentous structures may maintain the intracellular position of the SPC and possibly play a role in plugging the septal pore channel. The septal pore swellings of *T. sporotrichoides* contain more 1,6- β -glucan than the septum, and intracellular glucans are also present near the septal pore channel. This cytosolic 1,6- β -glucan in *T. sporotrichoides* may serve as a matrix to keep the tubular membranous structures of the SPC together. In contrast, 1,6- β -glucan is not observed in the SPC and in the pore-occluding material of *S. commune*, and hyphal septa of this species show less labelling of 1,6- β -glucan than the septal swelling. The evolutionary transition from simple to more complex types of SPCs may have resulted in a requirement for different components to maintain the morphological integrity and cell biological function.

Keywords: 1,6- β -glucan, high-pressure freezing, *Schizophyllum commune*, septal pore cap, *Trichosporon sporotrichoides*

INTRODUCTION

The septal pore cap (SPC) or parenthosome (Bracker, 1967) occurs only in the Basidiomycota (Moore, 1985), and provides useful markers for taxonomy and phylogeny of these fungi (Tu & Kimbrough, 1978; Khan & Kimbrough, 1982; Van der Walt & Von Arx, 1985; Suh *et al.*, 1993; McLaughlin *et al.*, 1995; Moore, 1996). The SPC was visualized for the first time by Girbardt (1958)

in *Trametes versicolor* (L.: Fr.) Pilát (cited as *Polystictus versicolor*). Since then several types of SPC have been distinguished, viz. perforate, pauciperforate, non-perforate, vesiculate, cupulate and ampullate (Khan & Kimbrough, 1982; Moore, 1985). However, a number of basidiomycetes lack an SPC, e.g. those belonging to the Cystofilobasidium branch (Fell *et al.*, 1995). Moreover, the SPC may disappear in some fungi following certain morphogenetic triggering, for example: during the process of conversion of dolipore septa into tapered or uniformly thick septa as observed in *Coprinus lagopus* (Casselton *et al.*, 1971) and *Schizophyllum commune* (Marchant & Wessels, 1974), where nuclear transport

Abbreviations: ER, endoplasmic reticulum; PCTE, polycarbonate track etching; SPC, septal pore cap; ZIO, zinc/iodine/osmium tetroxide

between neighbouring cells is facilitated; during spore maturation in *Pleurotus broussonetiae* Pat. (cited as *Antromycopsis broussonetiae*; Moore, 1977); during septal schizolysis (Tsuneda *et al.*, 1993); in damaged hyphae of *Trametes versicolor* (cited as *Coriolus versicolor*; Aylmore *et al.*, 1984); or in old lysed cells of *Rhizoctonia solani* (Butler & Bracker, 1970).

The ultrastructure of the SPC has been elucidated to a certain extent, but its function remains largely unknown and is a matter of speculation. The perforate SPC of *R. solani* is about 1.8–2 µm in diameter and has large perforations of about 600–800 nm diameter (Müller *et al.*, 1998). The *R. solani* SPC may serve to protect the swellings of the dolipore septum during protoplasmic streaming, while allowing the passage of mitochondria and other small cell constituents (Bracker & Butler, 1964). On the other hand, SPCs in, for example, *Coprinus stercorearius*, *Polyporus rugulosus*, *Agaricus bisporus* and *Schizophyllum commune* have smaller perforations and may act as a sieve (Wilsenach & Kessel, 1965; Ellis *et al.*, 1972; Thielke, 1972; Müller *et al.*, 1994, 1995). Occluding material in the entrance of the septal pore channel may act as a valve and seems to be involved in basidiocarp formation (Flegler *et al.*, 1976). This occluding material has a proteinaceous nature, as it can be digested by trypsin and chymotrypsin (Flegler *et al.*, 1976). The SPC may release this proteinaceous material to plug the pore opening rapidly (Markham, 1994), suggesting a repository function for the SPC. In *Pisolithus arhizus* (cited as *P. tinctorius*), filaments occur between the inner surface of the SPC and the entrance of the septal pore channel (Orlovich & Ashford, 1994), possibly facilitating transport of cell constituents towards the septal pore channel. The non-perforate SPC in *Auricularia auricula-judae* effectively controls cytosolic streaming and may prevent passage of large organelles through the septal pore channel (Lü & McLaughlin, 1991). On the other hand, Patrignani *et al.* (1984) found that only septal pore channel occlusions, and not the cupulate SPC, can prevent the migration of organelles in *Tremella mesenterica*.

To understand the structure and function of the basidiomycete SPC, we compared the SPCs of an anamorphic yeast-like fungus, *Trichosporon sporotrichoides* CBS 8245 (Van Oorschot) Van Oorschot & De Hoog (Tremellales; Fell *et al.*, 1995) and a homobasidiomycete, *Schizophyllum commune* CBS 340.81 Fr.: Fr. (Schizophyllales), which belong to two different phylogenetic lineages. Several preparation methods were adopted for the electron-microscopic analysis: freeze fracturing and cytosolic maceration (Müller *et al.*, 1994, 1995); high-pressure freezing and freeze substitution; preferential staining of the endoplasmic reticulum (ER) and the SPC with zinc/iodine/osmium tetroxide (ZIO) (Hawes, 1991); sugar staining with alkaline bismuth (Shinji *et al.*, 1975, 1976); and immunogold labelling of 1,6-β-glucan. In addition, the SPCs of *S. commune* were studied in protoplasts. This combined approach has shown marked differences between the two types of SPC and resulted in a more complete picture of these SPCs.

METHODS

Strains, media and culture conditions. *Trichosporon sporotrichoides* strain CBS 8245 and *Schizophyllum commune* strain CBS 340.81 were maintained on YPGA (0.3% w/v, yeast extract, 0.5% w/v, peptone, 1% w/v, glucose, 2% w/v, agar) at 10 °C. Hyphal cells were scraped from a slant culture and grown for 3 d at room temperature between two polycarbonate track etching (PCTE) filters (Poretics; 0.6 µm pore size, 37 mm filter size) as described by Wösten *et al.* (1991).

Scanning electron microscopy. Colonies grown between PCTE filters were fixed in 2% (v/v) glutaraldehyde (EM grade 8%; Polysciences) in 50 mM sodium cacodylate buffer, pH 7.4, for 16 h at 4 °C. The fixed samples were postfixed with 1% (w/v) osmium tetroxide buffered with 66 mM phosphate buffer, pH 7.4, for 16 h at 4 °C. Freeze-fracturing, cytosolic maceration and further processing for scanning electron microscopy were done as described by Müller *et al.* (1994). The platinum-coated fungal fragments were examined in a field-emission scanning electron microscope (JSM 6300F, JEOL) at an acceleration voltage of 7 kV.

High-pressure freezing and freeze substitution. Peripheral mycelial parts of colonies cultured between PCTE filters were placed in specimen holders with 1-hexadecene (Müller & Moor, 1984; Studer *et al.*, 1995) and subsequently frozen in a high-pressure freezer (Leica EM HPF) and used for freeze substitution. Hyphal material was transferred in liquid nitrogen to a CS auto-substitution chamber (Reichert-Jung) at –90 °C containing a mixture of 1% (w/v) osmium tetroxide, 3% (v/v) glutaraldehyde and 0.5% (w/v) uranyl acetate in methanol (complex freeze-substitution medium) according to Müller *et al.* (1980). For immunogold labelling, the substitution chamber contained a mixture of 0.2% (w/v) uranyl acetate and 0.01% (v/v) glutaraldehyde in methanol. After 5 d, the temperature was raised from –90 °C to 4 °C (for immunogold labelling, to –40 °C) at a rate of 10 °C h^{–1}, for low temperature embedding and further processing with Lowicryl HM20 (Müller *et al.*, 1991). Specimens substituted with complex freeze-substitution medium were rinsed with methanol, followed by anhydrous acetone. After raising the temperature to room temperature, the specimens were infiltrated and embedded with Spurr's resin (Spurr, 1969), and polymerized for 24 h at 60 °C. Sections (80 nm) of hyphae substituted in complex freeze-substitution medium were mounted on 1.1% (w/v) pioloform and carbon-coated single-hole copper grids, dried for 16 h, and stained with 3% (w/v) aqueous uranyl acetate for 45 min and with lead citrate for 8 min, according to Venable & Coggeshall (1965).

Preferential staining. Colonies cultured between PCTE filters were fixed in 3% (v/v) glutaraldehyde (EM grade 8%; Polysciences) buffered with 50 mM sodium cacodylate buffer, pH 7.4, for 1 h on ice. After washing in the sodium cacodylate buffer and in distilled water, the colonies were immersed in unbuffered ZIO for 3 h at 37 °C (Hawes, 1991). The ZIO-stained hyphae were processed further as described by Müller *et al.* (1995).

Sugar staining. After freeze substitution, embedding in Spurr's resin, ultramicrotomy and polymerization, the 80 nm sections of hyphal material were stained with alkaline bismuth for 3 h at 37 °C according to Shinji *et al.* (1975).

Immunogold labelling of 1,6-β-glucan. Lowicryl HM20 sections of hyphal material were incubated with affinity-purified polyclonal antibodies (1.66 µg ml^{–1}) recognizing 1,6-β-

Fig. 1. Scanning electron micrographs of hyphae of *T. sporotrichoides* CBS 8245 and *S. commune* 340.81 revealing the intracellular organization. (a) Side view of an SPC of *T. sporotrichoides* CBS 8245, which consists of smooth vesicular-tubular membranous structures. The septum (S) is cross-fractured and a remnant of ER is connected to a tubular structure of the SPC. (b) Part of a longitudinally fractured hypha of *T. sporotrichoides* at the position of a septal pore channel (arrow), which is covered by the SPC. The smooth tubular structures are cross-fractured. The small tubular structures (small arrowheads) have a diameter of 30–40 nm and are located between the inflations of the dolipore septum, and the larger ones (large arrowheads) have a diameter of 80–120 nm. (c) Side view of a perforate SPC (arrow) of *S. commune* with different hole diameters. Plate-like ER is connected to the SPC (small arrowheads). In the connection area of the ER and the SPC, irregularly shaped holes are present (large arrowhead). (d) Part of a longitudinally fractured *S. commune* hypha with perforate SPCs on either side of the dolipore septum. The figure clearly shows the connection (large arrowhead) of plate-like ER (small arrowheads) to the basal part of the SPC. Bars, 250 nm.

glucan (Montijn *et al.*, 1994; Lu *et al.*, 1995). The antigen-antibody complexes were visualized with goat anti-rabbit antibodies ($1 \mu\text{g ml}^{-1}$) conjugated with 10 nm gold particles (5×10^{12} particles ml^{-1} ; Aurion).

Formation of *S. commune* protoplasts. Twenty-five millilitres of protoplasting medium (2 g citric acid l^{-1} , 0.5 M MgSO_4 , pH 5.8, set with Tris; De Vries & Wessels, 1972) and 250 mg Novozym 234 (Novo) were added to 5 g (wet weight) hyphae and incubated in a shaker incubator at 100 r.p.m. for 4 h at

30 °C. Protoplast formation was checked by light microscopy every 30 min. After 4 h, the protoplasts were collected in a 50 ml Falcon centrifuge tube (Becton Dickinson Labware) and centrifuged at 300 *g* for 10 min. The supernatant was centrifuged at 14000 *g* in a Sorvall RC-5 Superspeed Refrigerated Centrifuge (DuPont Instruments) for 45 min at 4 °C. The volume of the 14000 *g* pellet was about 2 ml and contained about 8×10^7 protoplasts. These protoplasts were chemically fixed with 2% (v/v) glutaraldehyde in MgSO_4 buffer (2 g citric

Fig. 2. For legend see facing page.

acid I^{-1} , 0.5 M $MgSO_4$, pH 5.8, set with Tris) for 30 min, washed twice with $MgSO_4$ buffer and postfixed with 1% (w/v) osmium tetroxide in $MgSO_4$ buffer for 1 h. The protoplasts were washed twice with $MgSO_4$ buffer and three times with distilled water. The protoplasts were embedded in 2% (w/v) low-melting-point agar (type VII low-gelling temperature; Sigma). Blocks of about 2 mm³ were cut with a razor blade and dehydrated through a series of 50% to 100% acetone. After a step in acetone + dimethoxypropane [1 ml acidic dimethoxypropane (four drops 37% HCl in 50 ml dimethoxypropane), 100 ml acetone], the material was infiltrated and embedded in Spurr's resin (Spurr, 1969), polymerized for 16 h at 65 °C, cut into 80 nm sections with a Diatome diamond knife on a Reichert Jung Ultracut E ultramicrotome and picked up on 150 mesh copper grids. The grids had been covered with a Formvar film (1%, w/v, Formvar in chloroform) and carbon coated. The sections were stained for 10 min with 4% (w/v) uranyl acetate, followed by 90 s with 2.66% (w/v) lead citrate (Reynolds, 1963).

Transmission electron microscopy. Sections were viewed in a Philips EM420 electron microscope. Micrographs of the SPC were taken at an acceleration voltage of 80 kV.

RESULTS

Scanning electron microscopy of the SPC

Longitudinal fracturing and subsequent processing revealed the intracellular organization of hyphal cells of *T. sporotrichoides* CBS 8245 and *S. commune* CBS 340.81. In *T. sporotrichoides*, an aggregate of tubular, globular and sausage-shaped membranous structures covered the dolipore septum (Fig. 1a), forming an SPC. These structures were connected to flat-tubular ER. In median fractures, the SPC consisted of cross-fractured smooth tubular membranous structures varying in width between 40 and 120 nm (Fig. 1b). The smaller tubular structures were located between the larger ones and the rounded inflations of the dolipore (Fig. 1b). The SPC of *S. commune* revealed round, or ellipsoid, apical perforations of 110–120 nm diameter, and smaller basal perforations of about 80 nm diameter. The SPC was connected to plate-like ER, and irregularly shaped holes were present in the connection area (Fig. 1c). A near-

Fig. 2. High-pressure freezing and freeze substitution of hyphae of *T. sporotrichoides* CBS 8245 (a, b) and *S. commune* CBS 340.81 (c, d, e, f) showing the SPC as it occurs *in situ*, and the SPC in an *S. commune* protoplast after conventional chemical fixation (g, h). (a) Part of a *T. sporotrichoides* hypha showing no SPC but electron-dense material opposite the septal pore channel (small arrowheads). Bar, 250 nm. (b) Obliquely sectioned vesicular-tubular structures of the SPC of *T. sporotrichoides*. The septal pore channel appears to be open at the tubular side (arrow). Small membranous structures are present in the septal swelling (arrowheads). Bar, 250 nm. (c) Perforate SPC of *S. commune* on either side of the dolipore septum. The septal pore channel is closed by electron-dense occluding material (arrowheads). Bar, 250 nm. (d) A perforate SPC of *S. commune* on either side of an open septal pore channel. Occluding materials (arrowheads) appear to be connected to beaded filamentous structures (small arrows). The plate-like ER is connected to the basal part of the perforate SPC (arrow). In the mid-point of the septal pore channel, a transverse line of electron-dense material separates electron-dense material of fluffy appearance. Bar, 250 nm. (e) Higher magnification of (d). A thin layer of electron-dense material (small arrowheads) covers the inner side of the SPC. Beaded filamentous structures (arrows) connect the inner side of the SPC with plug material (large arrowhead). Bar, 100 nm. (f) Higher magnification of a perforate SPC of *S. commune*, showing a layered structure. Two unit membranes confine SPC matrix material, which is separated by a thin electron-dense line (vertical arrow) and is made up of small palisade-like structures (horizontal arrows). Bar, 100 nm. (g) *S. commune* protoplast after chemical fixation showing that the structural relationships of the SPC appear to be intact. Besides the SPC (arrow), other organelles like a mitochondrion (Mi), a vacuole (Va) and lipid bodies (LB) are present. Bar, 1 µm. (h) Higher magnification of the SPC shown in (g). The perforate SPC confines occluding material (arrowhead). Bar, 250 nm.

Fig. 3. ZIO staining of the ER and the SPC in *T. sporotrichoides* CBS 8245 and *S. commune* CBS 340.81. (a) The ER (small arrowheads) parallel to the septum is connected to the SPC (large arrowheads) of *T. sporotrichoides*. The SPC is as densely stained as the ER. Bar, 250 nm. (b) Only the unit membranes (large arrowheads) of the SPC of *S. commune* and the ER (small arrowheads) are densely stained. Bar, 250 nm.

median fracture of an *S. commune* hyphal cell clearly showed that the perforations in the cap were smaller than mitochondria, which measured 300 µm in diameter, and vacuoles, while the ribosomes were small enough to easily pass through the SPC (Fig. 1d).

Transmission electron microscopy of the SPC

In most observations after high-pressure freezing, freeze substitution and longitudinal sectioning, the dolipore of *T. sporotrichoides* lacked an SPC. In *T. sporotrichoides*, electron-dense material was present near the opening and the mid-point of the septal pore channel (Fig. 2a). The septal pore channel contained longitudinal fibrils. Only rarely, vesicular-tubular membranous structures formed an SPC in *T. sporotrichoides* hyphae (Fig. 2b). Also, small membranous structures were present in the annular swelling (Fig. 2b). As observed by scanning electron microscopy, the smallest vesicular-tubular structures of the SPC were situated between the larger ones and the septal swelling. The vesicular-tubular SPC of *T. sporotrichoides* contained electron-dense material. The septal pore channel had a connection with the cytosol and contained more electron-dense material in its mid-region than at its entrance (Fig. 2b). In *S. commune*, the SPC was always present. Pore-occluding material blocked the septal pore channel (Fig. 2c); when the channel was open, a ring of electron-dense material was present (Fig. 2d) and the channel was about twice as wide as when closed. Electron-dense material filled the closed septal pore channels, and was located at the mid-point of the open septal pore channel (Fig. 2d). The SPC was connected to plate-like ER (Fig. 2d) and consisted of

an outer and an inner membrane, enclosing a matrix (Fig. 2e, f). In this matrix, palisade-like structures were often present (Fig. 2f). A thin layer of electron-dense material covered the inner surface of the SPC (Fig. 2e, f) and beaded, electron-dense, filamentous structures connected this inner surface of the SPC to the pore-occluding material (Fig. 2d, e, f). Based on the intactness and the regular appearance of the beaded-filamentous structures, these structures appeared to reflect the *in vivo* situation after cryofixation and freeze substitution. The filamentous structures were best observed when the septal pore channel was not completely occluded (Fig. 2d, e).

In sections, five out of 100 protoplasts of *S. commune* showed an SPC (Fig. 2g, h) and the SPC ultrastructure remained intact. The SPC contained putative remnants of the pore-occluding material.

In hyphae of *T. sporotrichoides* and *S. commune*, the connection of the ER to the SPC was clearly visible after ZIO staining, indicating the presence of calcium affinity sites (Gilloteaux & Naud, 1979). In *T. sporotrichoides*, the ER near the dolipore septum was continuous with the aggregate of membranous vesicular-tubular structures that formed the SPC. This simple SPC was as densely stained as the ER (Fig. 3a). In *S. commune* (Fig. 3b), only the outer and the inner membranes of the SPC and the ER lumen were electron-dense.

Polysaccharides with 1,2-glycol groups like glucan and mannan can be stained with alkaline bismuth (Shinji *et al.*, 1975, 1976). After alkaline bismuth staining, *T. sporotrichoides* hyphae showed a more densely stained dolipore swelling than the septum (Fig. 4a), while in *S.*

Fig. 4. Alkaline bismuth staining and immunogold labelling of sugar components in hyphae of *T. sporotrichoides* CBS 8245 and *S. commune* CBS 340.81. (a) Part of a hypha of *T. sporotrichoides* with electron-dense alkaline bismuth staining of the swelling of the septum and electron-dense material opposite the septal pore channel (arrowheads). (b) In *S. commune*, except for the central chitin plate, the dolipore septum is equally electron-dense. The occluding material (arrows) is also stained. (c) Location of 1,6- β -glucan in *T. sporotrichoides*. The septal swelling and the cytosolic electron-dense material (arrowheads) are abundantly labelled with gold particles. Arrows indicate the ER. (d) Location of 1,6- β -glucan in *S. commune*. The septal swelling is abundantly labelled with gold particles. No gold particles are located in the occluding material (arrowheads). Bars, 250 nm.

commune, all wall structures except the central chitin plate appeared equally electron-dense (Fig. 4b). Electron-dense material was present at the entrance and in the septal pore channel of *T. sporotrichoides* (Fig. 4a), whereas in *S. commune* the pore-occluding material gave a more electron-dense staining than the septum (Fig. 4b).

Immunogold labelling of 1,6- β -glucan showed clear differences between the SPCs of the two species. The dolipore swelling in *T. sporotrichoides* was more

intensely labelled with gold particles than the remainder of the septum (Fig. 4c), which agreed with our alkaline bismuth staining results. The dolipore swelling of *S. commune* was more densely labelled with gold particles than the septum (Fig. 4d). Gold particles in the occluding material at both sides of the septal pore channel in *T. sporotrichoides* indicated the presence of cytosolic 1,6- β -glucan in this species (Fig. 4c), whereas in *S. commune* neither the SPC nor the occluding material was gold labelled (Fig. 4d), indicating the lack of 1,6- β -glucan in these subcellular structures.

DISCUSSION

The structure of the SPC differs among the evolutionary lineages of the Basidiomycota. As we have shown, the SPC of *T. sporotrichoides* CBS 8245 consists of smooth vesicular-tubular membranous structures and a supporting electron-dense layer containing 1,6- β -glucan. In cell walls of *Saccharomyces cerevisiae*, 1,6- β -glucan has a cross-linking function, thus increasing cell wall rigidity (Kapteyn *et al.*, 1994, 1996). We hypothesize that the cytosolic 1,6- β -glucan-containing layer opposite the entrance of the septal pore channel of *T. sporotrichoides* may act as a matrix to keep the membranous structures together, thus forming a simple SPC. In addition, 1,6- β -glucan in the cell wall may support the rigidity of the septal swellings in hyphae with either simple or more complex SPCs.

1,6- β -Glucan was not detected in the SPC or in the pore-occluding material of *S. commune*. The integrity of the SPC after protoplast formation suggests that this structure is internally rigid. We favour a role of the filamentous structures connecting the inner surface of the SPC and the pore-occluding material to maintain the intracellular position of the SPC in *S. commune* hyphae. In *Pisolithus arhizus*, similar filamentous structures are thought to anchor the base of the SPC to the dolipore swelling, to maintain its shape and to guide the movement of cytosolic streams (Orlovich & Ashford, 1994). A possible alternative function of these filamentous structures in maintaining the shape of the cap, as proposed by these authors, seems to be unlikely as we showed that free-lying SPCs in *S. commune* protoplasts maintained their dome shape, and, after scanning electron microscopy, these SPCs did not reveal any filamentous structures. Therefore, maintenance of the shape of the SPC in *S. commune* hyphae may be solely accomplished by its matrix, which is made up of palisade-like structures of unknown composition, as was also described in the SPC matrix of *Pleurotus cystidiosus* (Moore & Patton, 1975).

The filamentous structures connecting the SPC with the pore-occluding material may also play a role in plugging the entrance of the septal pore channel, thus regulating intercellular communication (Flegler *et al.*, 1976, Orlovich & Ashford, 1994). Plugged septal pore channels of *S. commune* are narrower than non-plugged ones. The transverse electron-dense material at the mid-point of the septal pore channel of *S. commune* is probably involved in the dynamics of opening and closing the septal pore channel. In *T. sporotrichoides* the filamentous structures connecting the SPC and the occluding material were not observed, indicating that the process of plugging must be different in this species.

The plug may originate from the cytosol (Aylmore *et al.*, 1984) or from the SPC (Markham, 1994). We hypothesize that the ER may be another repository of the plug, as the ER is connected to the occluding material via the SPC and the filamentous structures. Consequently, precursors of the occluding material may be synthesized elsewhere in the cell or in the ER, then

processed in the ER, transported through the ER to the SPC and subsequently relocated through the inner membrane of the SPC. The processed precursors could be transported, via the filamentous structures, to the entrance of the septal pore channel to form a plug, probably with other components from the cytosol.

In *T. sporotrichoides*, the SPC and the ER did not show differences in ZIO deposits, while in *S. commune* only the inner and the outer membranes of the SPC and the ER showed ZIO deposits. ZIO-stained SPCs were also reported in the agaric *Amanita rubescens* (Hawes, 1981). This suggests that there is no structural or functional differentiation between the SPC and the ER in *T. sporotrichoides*, whereas the SPC of *S. commune* may be functionally different from the ER. As ZIO stains calcium-affinity sites (Gilloteaux & Naud, 1979), the ER of *S. commune* and the ER as well as the SPC of *T. sporotrichoides* may act as a sink for calcium ions, which may play an important role in cell signalling (Sitia & Meldolesi, 1992).

ZIO staining in ascogenous hyphae of *Sordaria humana* showed the continuity between the ER and the complex septal pore structures (Beckett, 1981; Read & Beckett, 1996), consisting of multilayered membranes from which membranous cisternae arise. No differences in ZIO deposits were observed between the ER and the cisternae. This is similar to the ER connected to the simple SPC in *T. sporotrichoides*. Therefore, there may be no difference between the calcium-affinity sites, as detected by ZIO staining (Gilloteaux & Naud, 1979), in the complex septal pore structures of the ascogenous hyphae of *S. humana*, and the calcium affinity sites present in the ER and the simple SPC of the basidiomycetous hyphae of *T. sporotrichoides*. In contrast, the presence of calcium-affinity sites seems to differ between the ER and the more complex SPC of *S. commune*.

With the scanning electron microscope, plate-like ER along the septum is seen in monokaryotic hyphae of *S. commune*, while the ER is tubular in dikaryotic hyphae (Müller *et al.*, 1994). Although septa of dikaryotic hyphae were found to be much more resistant to enzymic dissolution than those of monokaryotic hyphae of *S. commune* (Casselton *et al.*, 1971; Wessels & Marchant, 1974), the function of the different types of ER observed in these hyphae remains unclear.

The ultrastructure of the SPC of *T. sporotrichoides* and *S. commune* has been repeatedly studied after chemical fixation (e.g. Guého *et al.*, 1992; Jersild *et al.*, 1967; Mayfield, 1974; Moore & Patton, 1975; Van der Valk *et al.*, 1977). Guého *et al.* (1992) reported that the SPC was almost absent in *T. sporotrichoides* and was present only as remnants of ER opposite the entrance of the septal pore channel. However, we have shown that a simple SPC exists in this species, consisting of smooth vesicular-tubular membranous structures. As this simple SPC was only rarely observed, this SPC may occur in *T. sporotrichoides* only during certain developmental stages. Although the morphology of the SPC is better preserved by the combined use of cryofixation and

freeze substitution (e.g. Hoch & Howard, 1981; Howard & O'Donnell, 1987; Lü & McLaughlin, 1991; Orlovich & Ashford, 1994), ZIO staining, freeze-fracturing and cytosolic maceration may show other details of the SPC which cannot be seen after cryo-fixation and freeze substitution. We favour a combination of different preparation methods for electron microscopy to obtain a more complete picture of the SPC.

The ultrastructure and function of the SPC are still under debate. As the membranes of the *S. commune* SPC enclose a layered matrix, this raises the following questions: how are the matrix layers deposited, and what is the biochemical nature of the matrix? Answers to these questions will contribute to the understanding of the function of the SPC. Also, detailed comparative and functional studies of other types of SPC among the basidiomycetes may provide clues to their function and phylogenetic importance. The evolutionary change from the putatively simple SPC of *T. sporotrichoides* to the more complex SPC present in higher basidiomycetes may be the result of structural adaptations to a changed function. In this view, the function of the SPC is strongly correlated with its morphology (Ellis *et al.*, 1972) or its location in the hyphal filament (Patrignani *et al.*, 1984). It is most likely that the SPC is involved in cell-cell contact and maintaining homeostasis.

The results of our combined studies suggest that different components are required to maintain the integrity of the SPC in basidiomycetes belonging to different phylogenetic lineages. This may be accomplished by the presence of cytosolic 1,6- β -glucan, the direct connection of the SPC with the ER, the differentiation between the SPC and the ER with respect to calcium-binding sites, or the occurrence of a distinct SPC matrix enclosed by an outer and an inner membrane. Moreover, we propose a role of the beaded filamentous structures in transport of cytosolic components to form a plug.

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