On the Function and Structure of the Septal Pore of *Polyporus rugulosus*

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SUMMARY

Micrographs of serial sections of *Polyporus rugulosus* Lev. show minute and regularly spaced pore-cap perforations which are of similar dimensions to nuclear membrane pores. The presence of two types of pore apparatus in Basidiomycetes is established, and suggestions are made about the function and evolutionary development of the septal pores of Basidiomycetes.

INTRODUCTION

The septal pores of Basidiomycetes have been studied by several authors; although the general structures reported are similar there are differences in detail and different interpretations for function of this apparatus have been given. Moore & McAlear (1962) studied five Basidiomycetes and suggested that the pore apparatus has an occluding function, allowing only humoral continuity; Gibardt (1961) seemed to be in agreement, calling the pore cap a 'verschlussband'. Bracker & Butler (1963) showed micrographs of mitochondria passing through the pore of *Rhizoctonia solani* Kühn, and tentatively concluded that even nuclei could pass through this pore. In the present work the pore structure of *Polyporus rugulosus* has been examined in serial sections, longitudinal and transverse. The results indicate a variation in the structure and function of the pore apparatus in Basidiomycetes.

METHODS

Polyporus rugulosus Lev. was isolated from decaying timber and cultured on sterile cellophan discs on corn meal agar. Material was fixed in an aqueous unbuffered 2% (w/v) KMnO_4 solution at room temperature for 30 min., dehydrated in a graded series of ethanol + water mixtures and embedded in Epon 812 (Luft, 1961). Sections were cut on a Reichert Ultra-microtome and stained with lead hydroxide (Feldman, 1962). The grids used were carbon coated, and the specimens were viewed with a Siemens Elmiskop I, operating at 80 kV.

RESULTS

The terminology used by Bracker & Butler (1963) is used in the present descriptions.

In Pl. 1, fig. 1, a nearly median section of a septal pore is seen; it seems clear that the pore cap is continuous with the endoplasmic reticulum (ER) which lies parallel to the cross-wall. Small interruptions can be seen in the cap, suggesting the presence of openings.

Plate 1, fig. 2, shows the layered cross-wall extending into the septal swelling, being separated from the actual pore only by the ectoplast which is resolved as a double layer. Bracker & Butler (1964) interpreted such a pore as a fully open one in which the septal swelling has been depressed during active cytoplasmic streaming. In Pl. 1, fig. 2, some membranous matter can be seen in the subcap matrix, and is most probably in the process of passing through the pore.

Serial sections of pore, sections parallel to hyphal axis. Only six of nine sections are shown here. Pl. 1, fig. 3, represents the peripheral region of the pore apparatus and interrupted strands of endoplasmic reticulum (ER) can be seen lying parallel on both sides of the cross-wall. In Pl. 1, fig. 4, the perforated pore cap begins to appear. The swelling on the cross-wall appears in Pl. 1, fig. 5, and a large area of the pore cap can now be seen on both sides.

In Pl. 2, fig. 6, the very regular arrangement of the pore-cap perforations can be seen, the openings are very regular in shape and size, being $\pm 800 \text{ \AA}$ in diameter, and $\pm 500\text{--}700 \text{ \AA}$ apart. In Pl. 2, fig. 7, the actual pore opening is beginning to appear; Pl. 2, fig. 8, represents a median section, where it can be seen that the pore cap is continuous with the endoplasmic reticulum (ER). The pore-cap perforations now appear as lighter regions alternating with darker ones.

Sections of pore apparatus, sections transverse to hyphal axis. The apical part of the dome-shaped pore cap is seen in transverse section in Pl. 3, fig. 9; the regular spacing and size of the pore-cap perforations is again evident. An interesting comparison can be made between the dimension of the pore-cap perforation and mitochondrion size in Pl. 3, fig. 10, which is a lower magnification of an adjacent serial section.

The interrupted nature of the endoplasmic reticulum which is continuous with the pore cap could not be understood in longitudinal sections. Plate 3, fig. 11, is a cross-section very near the cross-wall, and it includes the endoplasmic reticulum (ER) referred to above. This is clearly reticulated, consisting of narrow tubules. Lamellations can be seen in the pore swelling, and the double ectoplast lines the pore.

Nuclear membrane. An almost tangential section of the nuclear membrane (Pl. 3, fig. 12) reveals pores, the dimensions of which are almost identical with the pore-cap perforations.

DISCUSSION

The general structure of the pore apparatus of Basidiomycetes, as suggested by Gibardt (1961), Moore & McAlear (1962), and Bracker & Butler (1963), is confirmed in the present work for *Polyporus rugulosus*: the actual pore opening is lined by the ectoplast, a barrel-shaped septal swelling is present, the pore is covered by a dome-shaped pore cap on both sides of the cross-wall, and the pore cap is regarded as a specialized region of the endoplasmic reticulum.

The *Polyporus* type of pore cap (with its regular small perforations) will not allow the passage of organelles which are much bigger than $800 \text{ \AA}$. In *Polyporus rugulosus* the mitochondria are remarkably large (Pl. 3, fig. 10) and one cannot visualize the movement of mitochondria through the pore. This was confirmed by studying vitally stained mycelia of this fungus and by phase microscopy. Rapid streaming was observed, mitochondria were recognized, but no migration of particles through the pore was seen. The pore cap of *Polyporus rugulosus* therefore acts

as a sieve, allowing only the migration of particles smaller than 800 Å or particles that can shrink or be compressed to this dimension. The pore-cap perforations are of almost the same diameter as the nuclear membrane pores. The pore cap therefore will maintain the same physical boundary between adjacent cells as that present between the nuclear sap and the cytoplasm. These minute pore-cap perforations can also be seen in the pore cap of *Polystictus versicolor* in the micrographs of Gibardt (1961). Some preliminary work has shown similar perforations in *Poria monticola*. These genera all belong to the Polyporaceae. Nuclear migration is obviously impossible through an intact Polyporus-type pore. It is suggested that in such Basidiomycetes functional diploidy is maintained, while humoral continuity exists between different cells. These results are in agreement with those of Moore & McAlear (1962) and Gibardt (1961).

In comparison, the situation in *Rhizoctonia* (Bracker & Butler, 1963) is very different: the perforations in the pore cap are irregular and much larger, while the mitochondria are smaller. The migration of mitochondria shown in the micrographs of Bracker & Butler (1963) can be confirmed by phase-contrast microscopy. In *Rhizoctonia* even the actual pore opening is larger, and in this Basidiomycete the pore cap does not have an occluding function; if nuclear migration occurs the fungus is not even a functional diploid. The *Rhizoctonia*-type of pore cap has recently also been shown to occur in *Exidia nucleata* (Wells, 1964).

There are therefore two types of pore apparatus in Basidiomycetes and although the present data are very inadequate, it would appear that modifications of septal pore structure within this group of fungi may have phylogenetic significance. At this stage it is impossible to say which type preceded the other; more information is necessary, including more about the possible function of the *Rhizoctonia*-type pore cap. If the function of the *Rhizoctonia*-type pore cap is indeed protection of a 'delicate' pore swelling (and we do not know whether the pore swelling is delicate), one might regard this as an initial evolutionary development. The Polyporus-type pore cap could then be interpreted as a further differentiation of the pore apparatus. It is tempting to believe this hypothesis because the Polyporaceae are considered by mycologists to be a much more advanced group than fungi like *Rhizoctonia* and *Exidia*.

Bracker & Butler (1963) suggested that the actual pore opening can be increased during rapid cytoplasmic streaming as a result of the depression of the septal pore swelling. One can understand such a depression of the pore swelling when mitochondria (or nuclei) pass through a pore and in doing so exert lateral pressure on the swelling, but in Polyporus the pore cap acts as a sieve which allows only the migration of particles much smaller than the actual pore opening. In Polyporus pores can be seen which would be interpreted by Bracker & Butler (1963) as widened pores, but we cannot visualize hydrodynamic forces which will exert unilateral forces to cause the lateral depression of the pore swelling. From some observations Bracker & Butler (1963) concluded that the septal swelling was of different chemical nature than the cross-wall, but nothing more is known about the substance of the pore swelling. We consider that septal pore swelling variation as due to rapid streaming to be unlikely. It may be due to structural variation, but it must be appreciated that the swelling will appear different in median and near-median sections.

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EXPLANATION OF PLATES

E, ectoplast; ER, endoplasmic reticulum; G, compound lipid granule; Mi, mitochondrion; N, nucleus; NM, nuclear membrane; NP, nuclear pore; PC, pore cap; PCP, pore cap pore; S, septal pore swelling; SP, septal pore; V, vacuole; XW, cross-wall.

PLATE 1

Fig. 1. Hypha with septum, section not median thus actual pore opening not showing. Small pores are apparent in the dark pore cap which is continuous with the endoplasmic reticulum. Compound granules (G), thought to be of lipid nature, are present in the cytoplasm. $\times 12,000$.

Fig. 2. Median section of 'fully open' pore. Lines and numbers refer to figs. 3-8. $\times 60,000$.

Figs. 3-5. These are the first three sections through the septal pore apparatus. Position of the sections are indicated in fig. 2. $\times 60,000$.

PLATE 2

Figs. 6-8. These are the following three serial sections, omitting a section between figs 6 and 7. Approximate position of sections indicated in Pl. 1, fig. 2. $\times 60,000$. The pore cap is thus shown to be a dome-shaped cap extending over the pore swelling, and having perforations of very regular size and distribution.

PLATE 3

Figs. 9, 10. Serial sections at different magnifications of the pore cap, transverse to hyphal axis. Note difference in size between pore cap perforations and mitochondria. Fig. 9, $\times 60,000$; fig. 10, $\times 36,000$.

Fig. 11. A transverse section very close to the cross-wall including the endoplasmic reticulum which is continuous with the pore cap. $\times 60,000$.

Fig. 12. An almost tangential section of the nuclear membrane. Compare size of nuclear pores with pore-cap pores in fig. 9. $\times 60,000$.