FUNCTION OF THE SEPTAL PORE APPARATUS IN *RHIZOCTONIA SOLANI* DURING PROTOPLASMIC STREAMING

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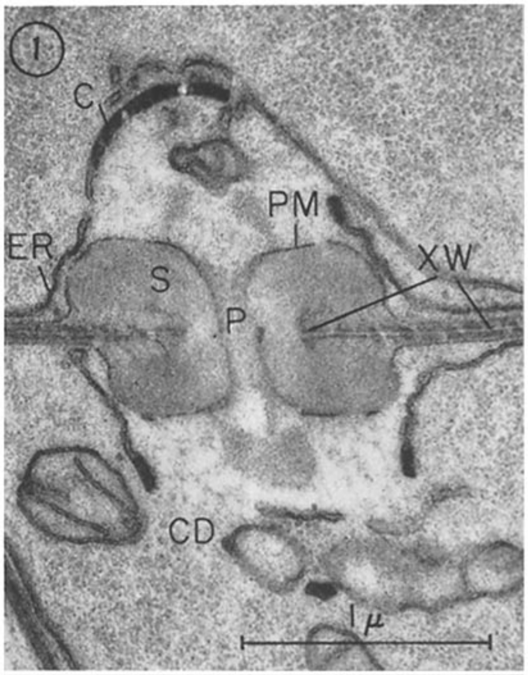
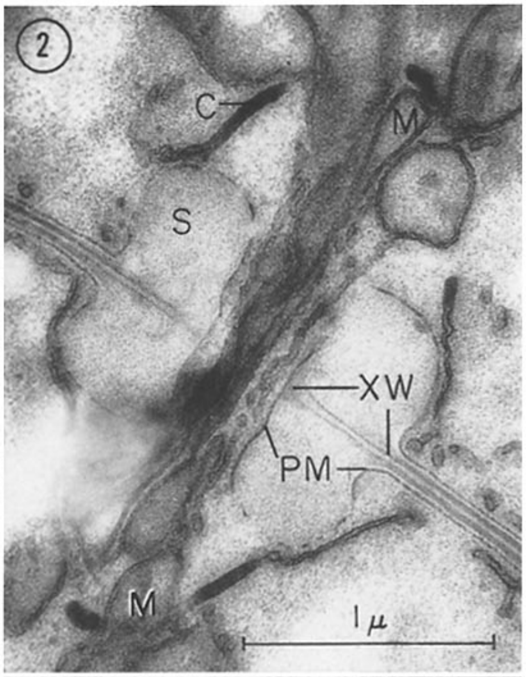
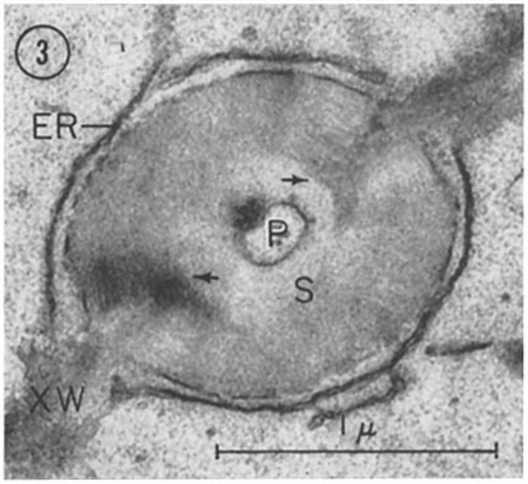
Protoplasmic streaming is regarded as an important means of food transport and translocation of cytoplasm in the filamentous fungi (1–3). In addition, streaming may play a vital role in the migration of nuclei through fungous thalli (1, 4). In the septate fungi, cross-walls possessing a single minute central pore, 0.1 μ diameter, are situated at intervals in the mycelium. Complex septal structure is typical of many Basidiomycetes (4–6) and strongly suggests a potential physical barrier to protoplasmic streaming and nuclear migration in the mycelium of these organisms (5). Nevertheless these phenomena have been reported in fungi possessing an intricate septal pore apparatus (1, 4, 6). Such an apparatus, described earlier (6), is found in *Rhizoctonia solani* Kuehn, a widespread plant pathogenic fungus. This report introduces evidence for a mechanism in *R. solani* which permits the septal pore to accommodate the passage of objects as large as 0.5 μ diameter, through an increase in the diameter of the pore.

For electron microscopy, the mycelium was fixed







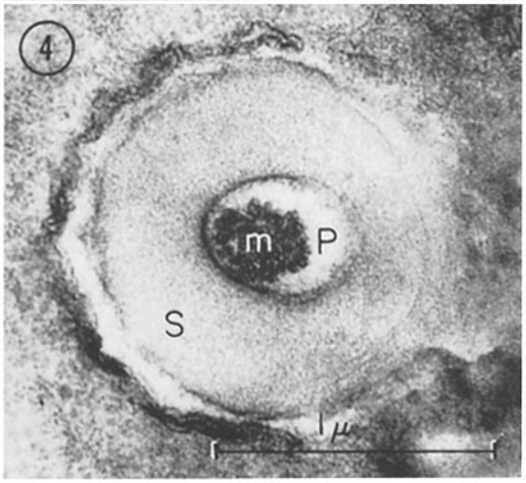


FIGURE 1 Longitudinal section of a hypha showing the septal pore apparatus in the absence of streaming. Cross-wall (*XW*), septal swelling (*S*), septal pore (*P*), septal pore cap (*C*), cap discontinuity (*CD*), endoplasmic reticulum (*ER*), plasma membrane (*PM*). × 33,000.

FIGURE 2 Longitudinal section showing the septal pore apparatus with constricted mitochondria (*M*) streaming up through the pore. The plasma membrane (*PM*) is pressed against the cross-wall (*XW*) as the pore is enlarged. × 33,000.

FIGURE 3 Cross-section of a hypha illustrating the septal pore apparatus. The septal swelling (*S*) is surrounded by endoplasmic reticulum (*ER*) forming the base of the septal pore cap. Arrows indicate the limit of projection of the cross-wall (*XW*) into the septal swelling. × 37,000.

FIGURE 4 Cross-section showing enlargement of the septal pore (*P*). A cluster of constricted membranes (*m*) is found within the pore. × 37,000.

Figs. 1 and 3 were originally published in Electron Microscopy, Proceedings of the 5th International Congress, Philadelphia, 1962; New York, Academic Press, Inc., 1962, **2**, UU-6.

Figure 5 Oblique section through the septal pore apparatus during vigorous streaming. Mitochondria (*M*) become constricted and deformed as they enter the pore (*P*). × 41,000.

in 1 per cent $KMnO_4$, embedded in Araldite, sectioned, and then examined in an RCA EMU-3E. The specimens shown here were all taken from cultures less than 24 hours old.

In *R. solani*, the septal pore apparatus lies at the center of the septum in the region of the septal pore, and the septum conforms in many respects to that found in other Basidiomycetes (5, 6). In the absence of streaming, the septal pore is about 0.1 to 0.2 μ in diameter (*P*, Figs. 1 and 3) and is surrounded by a doughnut-shaped septal swelling (*S*, Figs. 1, 3, and 6). In this situation the cross-wall as seen in longitudinal sections terminates within the swelling about 0.15 μ away from the septal pore (Fig. 1). In cross-section the limit of the projection of the cross-wall into the swelling can be seen (Fig. 3, arrows). The swelling clothes the ends of the cross-wall and delimits the size of the pore, making it a tube rather than a simple orifice. The pore is lined by the plasma membrane passing from cell to cell along the surface of the septal swelling. A thick, dome-shaped, electron-opaque cap covers the septal swelling and the pore (*C*, Figs. 1, 2, and 6). Large discontinuities in the cap permit a continuity of protoplasm from cell to cell (*CD*, Figs. 1, 2, and 5–7).

Although most electron micrographs show the diameter of the septal pore to be 0.1 to 0.2 μ, a number of micrographs indicate that at times it is much larger, reaching a diameter of over 0.5 μ (Figs. 2, 4, and 5). In such views, the pore nearly always houses membranes or organelles. Since in *R. solani* protoplasmic streaming can be observed in living specimens as a unidirectional mass flow from the older portions of the colony toward the hyphal tips (1, 6), it is logical to assume that Figs. 2, and 4–6 represent images of streaming protoplasm. In Fig. 2 the septal pore has been opened up in the presence of mitochondria until the plasma membrane surrounding the septal swelling is pressed against the ends of the cross wall. Fig. 6 illustrates diagrammatically the normal (dotted line) and expanded (solid line) phases of the septal pore. While the cross-wall is not absolutely rigid (1), it is nevertheless much more rigid than the septal swelling and resists any further opening of the pore. This allows the conclusion that the potential maximum diameter of the septal pore in *R. solani* is determined by the inner edge of the cross-wall.

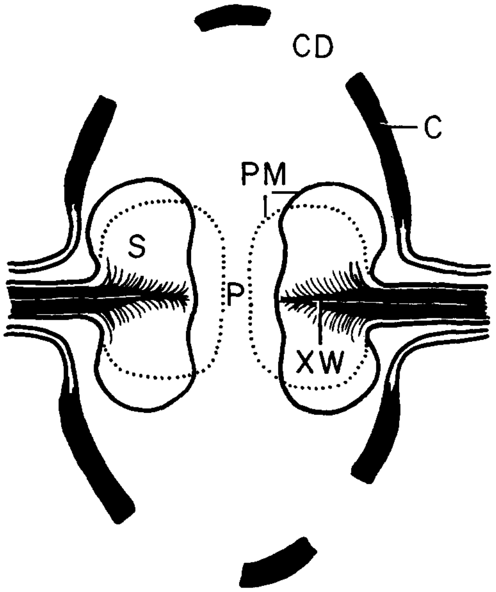


Figure 6 Diagram of the septal pore apparatus in longitudinal section showing it in the normal position (dotted line) and modified during protoplasmic streaming (solid line).

In addition to the flexibility of the septal swelling, plasticity of organelles is another important factor contributing to the streaming of protoplasm through a small pore. Shatkin and Tatum (7) showed a nucleus, caught in the septal pore of *Neurospora crassa* Shear and Dodge, which was constricted at its middle to a diameter of about 0.1 μ. Mitochondria and endoplasmic membranes may also undergo extreme constriction and deformation in *R. solani*. In Fig. 2, mitochondria are not only constricted but also greatly elongated as they move through the pore. This elongation is not surprising since the increased velocity of moving protoplasm within the pore would be expected to draw the mitochondria out to give the elongated profiles. Fig. 5 demonstrates how the mitochondria may be reduced to an aggregate of membranes as they undergo extreme deformation while in the septal pore. At least seven of the mitochondria in this micrograph are tapered to a point, as they approach the septal pore. In some cases mitochondria have been observed in adjacent cells as well as within the connecting pore. One does not commonly find severely constricted mitochondria away from a pore, indicating that they have regained their original shape after emerging from the pore.

We have observed a nucleus caught in a septal pore on only one occasion with the electron microscope. In Fig. 7 the nucleus is constricted in the pore and the nuclear membrane has ruptured in the lower cell near the union of two anastomosing hyphae. The role of protoplasmic streaming in nuclear migration is uncertain, but that nuclear migration is a common occurrence in a fungus possessing a Basidiomycetes-type septum has been well documented by Snider (4) for *Schizophyllum commune* (Fr.) Fr.

The septal pore cap appears to play an important role in protoplasmic streaming. The discontinuities in the cap are a prerequisite for mass flow through the mycelium. The cap maintains its characteristic dome-shaped form even under conditions of vigorous streaming (Figs. 2 and 5) and so appears to be quite rigid, possibly serving a protective function for the flexible septal swelling. These observations have been made with both

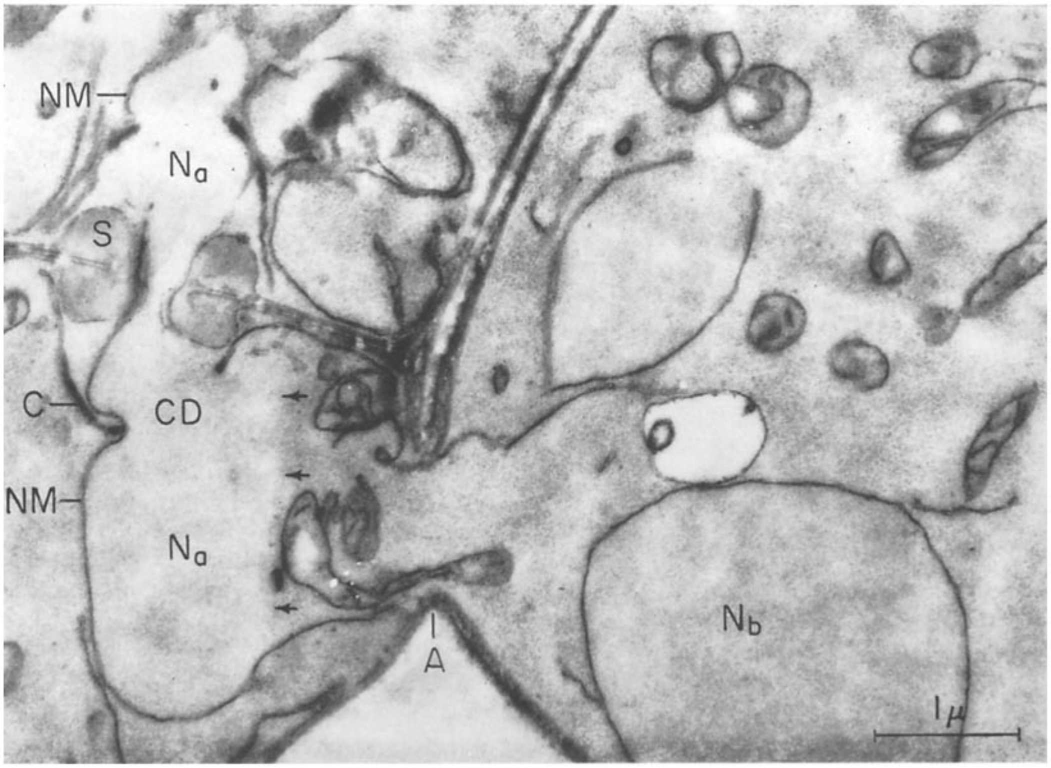


FIGURE 7 Section through two anastomosing cells in adjacent hyphae, with the region of anastomosis at *A*. The hypha on the left is in longitudinal section; the one on the right is in cross-section. A nucleus (*Na*) is caught in the septal pore and bridges two cells through cap discontinuities (*CD*). The nuclear membrane (*NM*) has ruptured in the cell at lower left; short arrows indicate the limit of the nuclear area. A second nucleus (*Nb*) lies in the hypha on the right × 19,000.

living and fixed specimens. Although the cap is commonly discontinuous above the septal pore, it is usually continuous at the base (Figs. 1 and 2) near the outer margin of the septal swelling. This base forms a rim around the sides of the swelling which prevents moving protoplasm from striking the swelling from the side, parallel to the cross wall. Organelles which are destined to pass through the pore must approach through the discontinuities in the cap, at a more acute angle than if the cap were not present (Figs. 2, 5, and 6). If the cap were completely lacking and organelles struck the swelling from the sides, this force could be sufficient to close or diminish the size of the pore by forcing the swelling toward the pore.

The results of this study indicate that the septum in *R. solani* is well adapted for protoplasmic streaming and does not offer a mechanical barrier. The fact that the septal pore is not fixed in size is significant with regard to the movement of protoplasm through the fungous thallus. Thus, our findings make feasible Buller's statement (1) that in *R. solani* "as the protoplasm came up to a septum it seemed to pass through it with greatest of ease."

SUMMARY

A study of the fine structure of the mycelium of the plant pathogenic fungus *Rhizoctonia solani* reveals that the diameter of the septal pore is increased during protoplasmic streaming. Despite its complex structure, the septum is well adapted to permit mass flow of protoplasm from cell to cell. Discontinuity of membranes and plasticity of organelles expedite protoplasmic movement through the septal pore.

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