Antonie van Leeuwenhoek 55: 237–244 (1989)

Hyphal fusion during initial stages of trap formation in *Arthrobotrys oligospora*

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Received 13 April 1988; accepted in revised form 1 August 1988

Key words: anastomosis, *Arthrobotrys oligospora*, Nematophagous fungus, trap formation

Abstract. Hyphal fusion during initial stages of trap formation by *Arthrobotrys oligospora* was studied by video-enhanced contrast and electron microscopy. Trap initials grew perpendicularly to the parent hypha, then curved around and anastomosed with a peg that developed on the hypha. Trap initials usually developed 40–140 μm apart while the anastomosis occurred 20–25 μm from the initial. Vigorous cytoplasmic movements in trap initials and developed traps corresponded to intense staining with fluorescein diacetate (FDA) of these cells. In addition, bundles of microfilaments were seen in developing loops of traps. On fusion organelle migration took place from the tip cell of the trap into the peg. Later on a septum was formed at the site of fusion.

Introduction

Hyphal fusions (anastomoses) are common among ascomycetous and basidiomycetous fungi and important for colony growth and three-dimensional development of mycelia (Buller 1931; Gregory 1984). Hyphal anastomosis is defined as complete fusion of both wall and cytoplasm and thus is distinct from mere contact and adhesion of hyphae. In spite of numerous observations of tip-hypha and tip-tip anastomoses it is not known what the causal factors are, what signals are transmitted and how they bring about this development (Gregory 1984; Jennings 1986).

The nematode-trapping fungus *Arthrobotrys oligospora* captures nematodes by means of three-dimensional adhesive network traps. The development of these traps is a result of numerous anastomoses of hyphal loops. In this paper we describe details concerning the formation of the first loop of the network of *A. oligospora,* studied by electronic contrast enhancement on a video system and by transmission electron microscopy. Previously, Higgins & Pramer (1967), using time lapse microphotography, showed that in *A. dacty-*

loides, a constricting-ring forming species, the tip fused with a bud of the stalk cell of the same trap. Barron (1981) confirmed this type of anastomosis in another constricting-ring fungus, *Dactylaria brochopaga*, and showed that a two-step fusion took place with subsequent septum formation.

Materials and methods

A. oligospora Fres. (ATCC 24927) was grown on thin layers (about 1 mm) of either dilute corn meal agar (Difco CMA 1:10; Nordbring-Hertz 1977) or on a low nutrient trap-inducing medium (LNM + phenylalanyl-valine; Nordbring-Hertz 1973) containing 0.8 or 1.0% agar on microscope slides. When necessary, trap formation was induced by addition of a drop of a nematode suspension (20–50 nematodes/drop) to 3–4 d cultures.

Video-enhanced contrast microscopy (Wyss & Zunke 1986) was used to visualize details of hyphal fusion. Fungal hyphae were stained with fluorescein diacetate (FDA) according to the method of Söderström (1977). Growth of the fungus and preparation and selection of the material for electron microscopy were as previously described (Nordbring-Hertz et al. 1984; Veenhuis et al. 1985a, b).

Results and discussion

Initial stages of trap development did not differ significantly if trap formation was induced by peptides or by living nematodes. Trap initials could often be seen by light microscopy along a hypha. The intervals between trap initials varied but typically were in the range 40–140 μm. Each initial arose perpendicularly to the parent hypha. It subsequently arched backwards or forwards and anastomosed with a peg, usually formed by the neighbouring cell 20–25 μm from the initial. Thus, the distance between trap initials was always larger than the diameter of the loop. These results were obtained from a large number of estimations using the light microscope on different occasions.

Time lapse light microscopic studies showed that under the present experimental conditions the formation of the first loop took about 1–2 h (Nordbring-Hertz et al. 1986). The details of the last 15 min of the anastomosis (Fig. 1) were studied using video-enhanced contrast microscopy. During this period a hyphal peg grow to meet the approaching tip of the curving branch (Figs. 1a-c), and a tip-tip anastomosis took place (Figs. 1d-f). The loop, typically consisting of three cells (Figs. 1a-c), showed vigorous cytoplasmic movements. After fusion an intense traffic of cytoplasmic constituents was initially visible between the two fused cells (Figs. 1e, f). Subsequently a cross wall was formed at

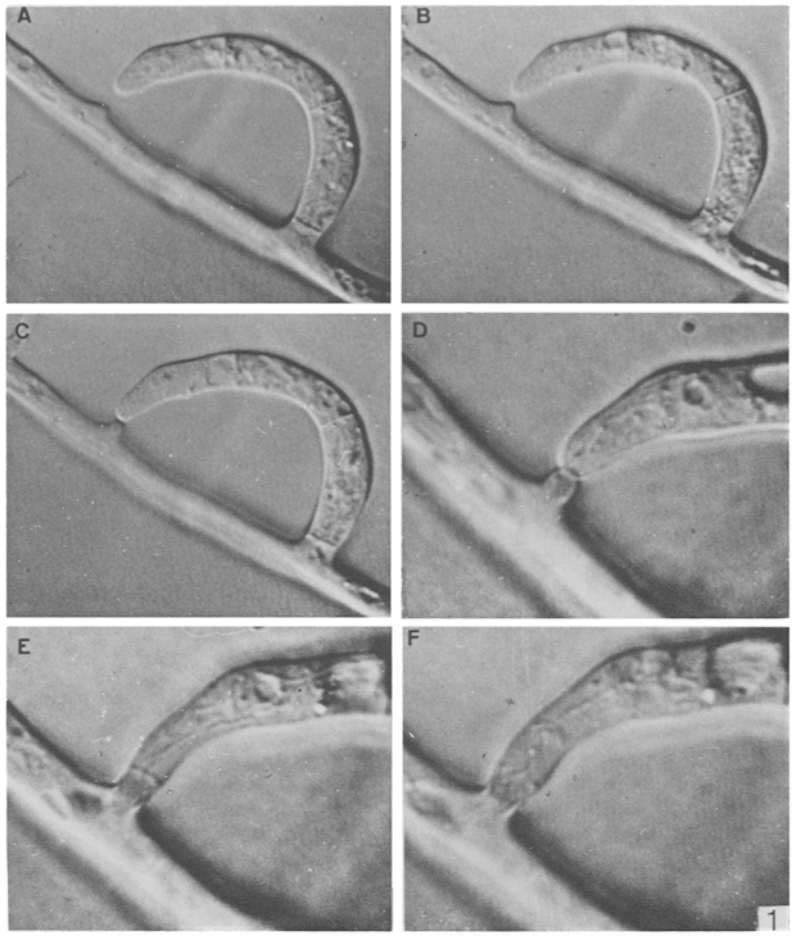

Fig. 1. Video-enhanced contrast microscopy of the last 15–20 min of the fusion between the tip cell of the trap and a hyphal peg. Time sequence: 0 (*A*), 3.5 (*B*), 6.5 (*C*), 9.5 (*D*), 12.5 (*E*) and 16.5 (*F*) min (*A–C* 2400 ×; *D–F* 4800 ×).

the original site of fusion (light microscopy, not shown; electron microscopy, see below). The trap then developed into a three-dimensional network by the formation and fusion of newly developed loops (Nordbring-Hertz et al. 1986).

The vigorous cytoplasmic movements in the trap cells coincided with brilliant staining of traps and trap initials by fluorescein diacetate (FDA) as previously reported (Nordbring-Hertz 1977). In the present study we confirmed that brilliant staining was evident not only in the trap cells and trap initials but also in the parent hypha immediately before a trap initial was visible. On the other hand, there was no strong FDA staining at the distance of

20–25 μm from the initial where the hyphal peg started or in the peg itself (data not shown). This would indicate differences between the trap tip and the peg originating from the basal hypha.

Trap cells of *A. oligospora* are characterized by the presence of numerous dense bodies (Veenhuis et al. 1985b). Electron microscopic observations showed that these organelles had already developed in the initial stages of trap development (for mechanisms of dense body development see Veenhuis et al. 1984, 1985b). They were also found in the hyphal cell from which the trap initial developed (data not shown). They were lacking, however, in all other vegetative cells including the ones neighbouring the trap initial cell. The hyphal peg meeting the tip of the trap also lacked dense bodies, indicating that it is not, per se, a trap cell up to the moment of contact and fusion. These observations support those made using light microscopy.

Another remarkable feature in developing traps was the presence of many microfilaments (Fig. 2). These often occurred in large bundles, sometimes of considerable diameter (Fig. 2 inset, arrow). They were lacking in mature traps.

At the moment of contact of the developing loop and the meeting hyphal peg both cells had relatively thin cell walls. We have not observed any signs of active hydrolysis of either cell wall (Figs. 3 and 4) comparable to that observed during development of the nematode-penetrating tube (Veenhuis et al. 1985a). Two stages of fusion are shown in Figs. 3 and 4. Since dense bodies were lacking in the hyphal pegs prior to fusion, their presence in these cells after fusion suggests that they originated from the developing trap by migration (Fig. 3). When fusion was complete the actual site of fusion could still be recognized by, in section, dense spots in the outer layer of the cell wall (Fig. 3, arrows). At this spot a new cross wall subsequently developed, separating the two cells again (Fig. 5). The formation of this cross wall was as in normal vegetative cells; it was initiated by the formation of thin – probably chitinous – layer (Kreger-van Rij & Veenhuis 1969), subsequently covered at each side by a broader amorphous layer (Fig. 5, arrow). The central pore of these walls was generally covered by Woronin bodies characteristic of ascomycete-type hyphae (Markham & Collinge 1987).

Different types of cell-cell interactions have previously been observed in this strain of *A. oligospora*. The trap cell fusion is characterized above all by the pronounced migration of numerous cytoplasmic components – i.e., dense bodies – as evidenced by video-enhanced-contrast microscopy (Fig. 1); their presence in these cells was confirmed by transmission electron microscopy (Fig. 3). Trap cell fusions differed from anastomosis of vegetative cells (Veenhuis et al. 1985c) with respect to the different cytoplasmic organelles which take part, especially dense bodies (Veenhuis et al. 1985a, b). Trap cell anastomosis was also completely different from another type of trap cell-cell interac-

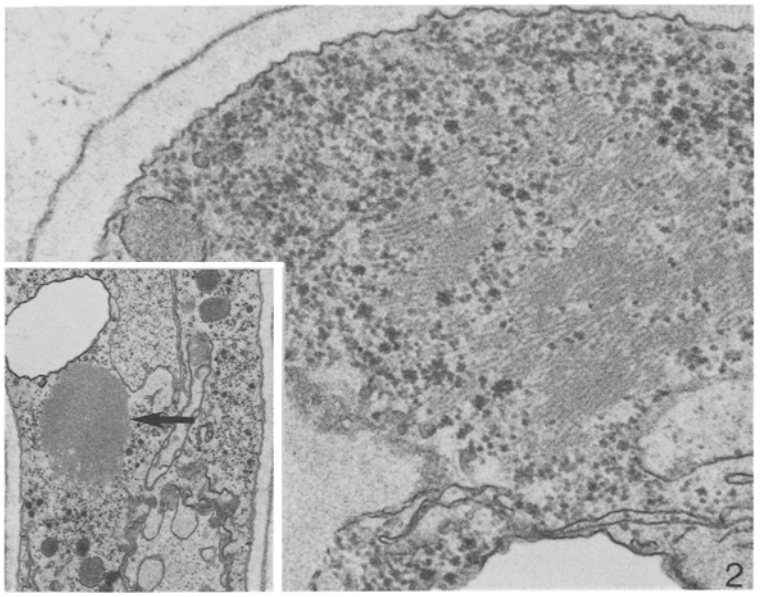

Fig. 2. Details of developing trap cell showing the presence of many microfilaments (Fig. 2 41000 ×), often arranged in bundles (Fig. 2, inset, arrow; 11000 ×). (aldehyde-$KMnO_4$).

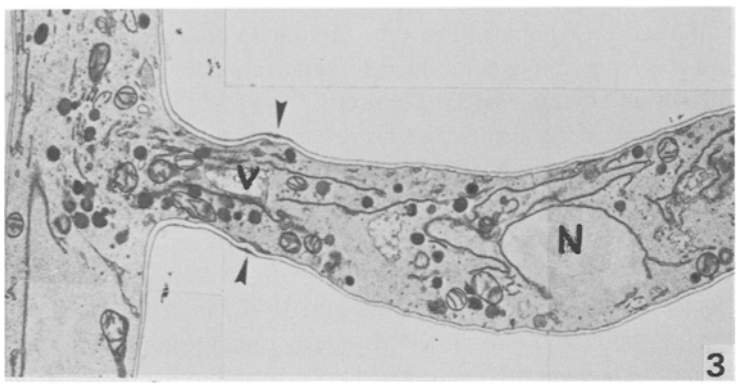

Fig. 3. Survey micrograph of the trap tip-peg fusion (fusion place marked by arrows) of a developing trap (see Fig. 1). After completion of fusion protoplasmic streaming into the meeting peg of the parent hypha occurred, leading to the migration of dense bodies into this cell. (10000 ×).

All electron micrographs were made of $KMnO_4$-fixed cells, unless otherwise stated. Abbreviations: N – nucleus; V – vacuole; W – Woronin body.

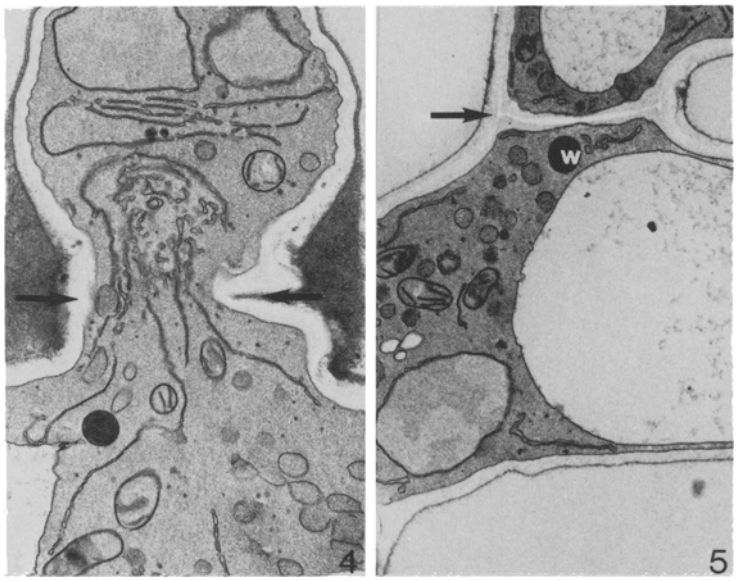

Fig. 4. Partly completed anastomosis (arrows) of trap tip and meeting peg (15000 ×).

Fig. 5. Completed anastomosis in a developing trap (13000 ×). In the cross wall formed at the original site of fusion the electron light chitinous central layer can be seen (arrow).

tion occurring only in the presence of nematodes and considered as a nematode-fungus interaction side-effect (Veenhuis et al. 1985c). In this type of cell-cell interaction a penetration tube similar to that in nematode-fungal interaction was formed by one of the cells penetrating the other cell; fusion of their cell walls and cytoplasm did not occur.

The underlying mechanisms of trap cell anastomosis are still unknown. Chemotropism has been suggested to occur before hyphal fusion. As pointed out by Gooday (1975) a chemotropic response must involve recognition of a chemical gradient at the growing apex itself to result in oriented growth. So far, we have no idea what molecules could be involved in forming a gradient necessary to explain bending at a distance of 10–20 μm or the directed tip-tip growth during the last 15 min period. It is worth mentioning that heavy trap formation and numerous anastomoses also occur in liquid culture with vigorous air bubbling (Friman et al. 1985) and it is difficult to envisage how a chemical gradient is maintained in this system for such periods.

The significance of the microfilaments is also still unclear. Their abundance, however, suggests a distinct function during trap development; in this respect it can be envisaged that the filaments play a role in curving of the developing loop directing it to the meeting branch tip cell.

Acknowledgements

We thank Prof. Urs Wyss and Dr Ulrich Zunke, Institute of Phytophatology, University of Kiel, for providing facilities and help with video-enhanced microscopy and Klaas Sjollema and Jan Zagers for skillful assistance in preparation of the electron micrographs.

This study was supported by a grant from the Swedish Natural Science Research Council to BN-H.

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