The Mesenchyme-Like Layer of the Fiber Cells of *Trichoplax adhaerens* (Placozoa), a Syncytium

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Trichoplax, Fiber Cell Syncytium

The fiber cells of the middle layer of *Trichoplax adhaerens* are interconnected by slender extensions. Newly formed connections after mechanical disruption of the tissue studied in ultrathin sections revealed cytoplasmic continuity between the cell bodies, suggesting a syncytial organisation of the fiber cell layer. The slender extensions connecting the cell bodies are traversed by microtubules and microfilaments. The structure of rare osmiophilic cell contacts suggests a stage in the fusion of adjacent cell membranes.

Introduction

The fiber cells of *Trichoplax adhaerens* (Placozoa) form a mesenchym-like layer in the interspace between the dorsal and the ventral epithelium (Grell and Benwitz, 1971). In contrast to the epithelia, the fiber cells are tetraploid (Ruthmann and Wenderoth, 1975; Ruthmann, 1977). Their long, arborizing and contractile extensions are traversed by microtubules and microfilaments (Thiemann and Ruthmann, 1989) and establish multiple connections both with the cell bodies and extensions of other fiber cells and with the epithelia. The resulting three-dimensional meshwork, demonstrated by scanning electron microscopy (Rassat and Ruthmann, 1979), may be instrumental in the coordination of movement. This task has formerly been ascribed to specialized osmiophilic cell contacts (Grell and Benwitz, 1974) that are, however, too rarely encountered to fulfill such a function. When whole *Trichoplax* are forced through a small constriction pipette after incubation in calcium-free sea water, a method originally employed by Ruthmann and Terwelp (1979) to study the capacity for reaggregation, the tissue is disrupted and the isolated fiber cells are roundish and without extensions. These reappear gradually after the cells have settled on a substrate. Occasionally, some fiber cell extensions seem to remain intact and give the impression of a syncytium (Fig. 3 in Thiemann and Ruthmann, 1989). As this might be of interest with respect to the question of coordination including movement, we studied such extensions by various methods of electron microscopy. A syncytial organisation would imply cytoplasmic continuity within the thin bridges connecting the cell bodies while mere cell contacts should be revealed by the presence of closely apposed membranes.

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Materials and Methods

Trichoplax adhaerens was cultured as described by Grell and Benwitz (1971). To obtain isolated fiber cells, the organisms were washed thrice for 5 minutes in calcium-free artificial sea water containing 0.5 mM EGTA and then gently dispersed with a 5 µl constriction pipette (Thiemann and Ruthmann, 1989). After settling on thin thermanox plates in a moist chamber for 30 minutes, the samples were treated with hypertonic sea water at 1.5 times normal salinity for 1 minute and then fixed by feeze-substitution. Hypertonicity prevents the formation of ice crystals without affecting structural preservation aside from a slight shrinkage. Shock freezing was carried out by dipping the samples rapidly into nitrogen-cooled propane (−180°C), followed by substitution in ethanol at −70°C for 4 days. The tissue was then

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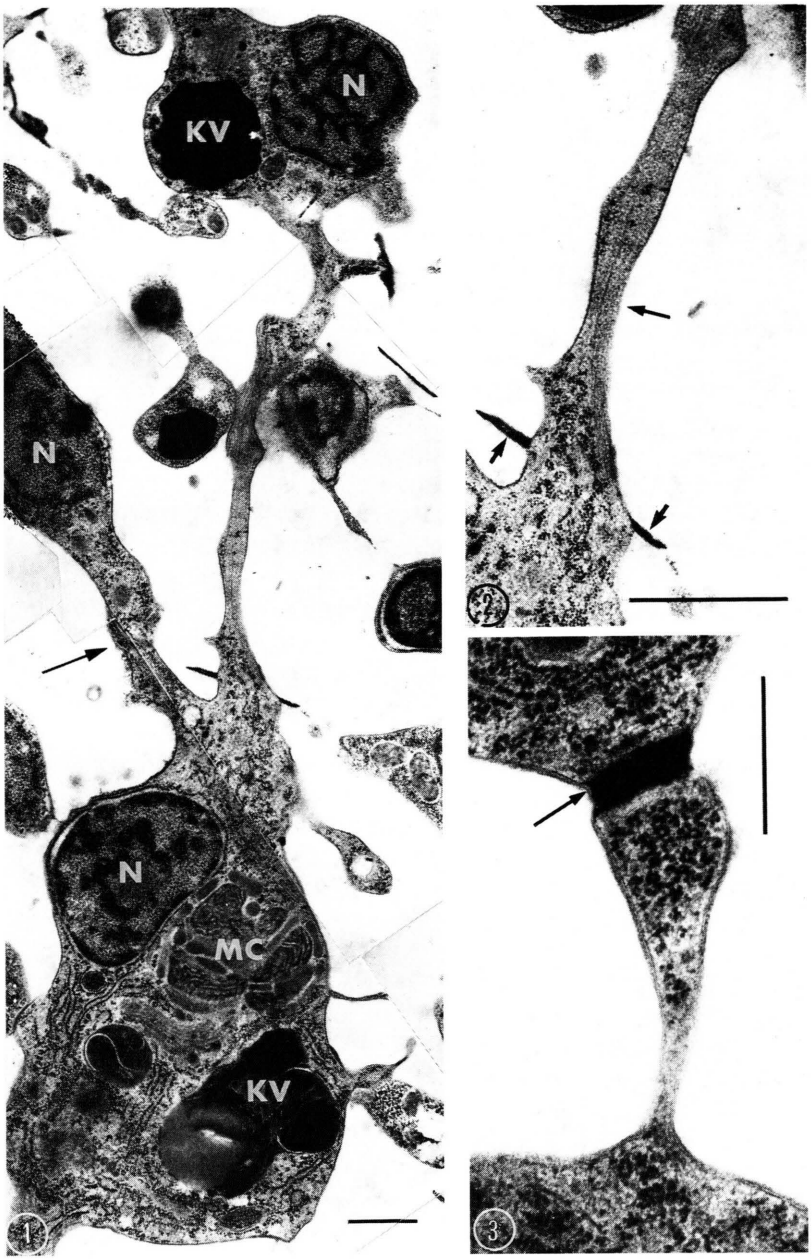

Fig. 1. Two interconnected fiber cells after tissue dispersal, sectioned parallel to the supporting thermanox plate. A third cell in close contact (arrow) but without cytoplasmic continuity. N: nuclei, KV: "concrement vacuoles", MC: mitochondrial complex. Bar: 1 μm.

Fig. 2. Enlargement of Fig. 1 showing cytoplasmic continuity and microfilaments (large arrow). Short arrows: attachment sites to the substrate. Bar: 1 μm.

Fig. 3. Osmiophilic plate at the contact site between two fiber cells. Arrow: note continuity between the outer halves of the cell membranes. From whole *Trichoplax*. Bar: 0.5 μm.

All figures from *Trichoplax* after freeze-substitution.

fixed with 1% OsO_4 in ethanol where it blackens slightly. Ultrathin sections were prepared for transmission electron microscopy after epon embedment.

Results

Sections of flat embedments after the dispersed cells had settled for about 30 minutes on the thermanox plates to establish contacts with the substrate provide clear evidence of cytoplasmic continuity between fiber cells. Fig. 1 shows two fiber cells, each with its own set of organelles, connected by a cytoplasmic bridge traversed by fibrillar components of the cytoskeleton (Fig. 2). It is fastened to the substrate by dense lateral outgrowths (arrows, Fig. 2). A similar, but thicker and branched outgrowth is shown in the upper part of Fig. 1. True connections between adjacent fiber cells by cytoplasmic strands are to be distinguished from apparent or doubtful connections of the kind shown in Fig. 1 (arrow). Separating cell membranes seem to be present at this point, but they may be inclined relative to the plane of sectioning and therefore not noticeable. To be confident of true cytoplasmic continuity between adjacent cells, we therefore payed special attention to the uninterrupted course of cytoskeletal fibrils in serial sections. Scanning electron micrographs of material prepared as described above and of the intact middle layer of *Trichoplax* tissues that show a network of fiber cells do not permit a distinction between true and apparent interconnections. However, a number of interconnections seemed traversed by continuous fibrils that persisted after treatment with the detergent Brij 58 that destroys membranes (not shown).

The specialized connections between fiber cells originally described by Grell and Benwitz (1974) and later also identified by scanning electron microscopy (Rassat and Ruthmann, 1979) are very rarely encountered in ultrathin sections. Their most prominent feature is a dense osmiophilic plate at the point of cell-to-cell contact. It is of varying thickness (110–150 nm) and a width of 200–300 nm which is slightly less than the width of the fiber cell extension itself. In scanning electron micrographs it can be identified by this constriction at the plane of contact between the cells. The use of freeze-substitution in this study permitted the demonstration that the outer leaflets of the cell membranes are continuous between both cells whereas the inner leaflets merge into the dense osmiophilic plate (Fig. 3).

Discussion

The cell bodies of fiber cells isolated by the mechanical disruption of *Trichoplax* tissues in calcium-free sea water adhere firmly to the substrate (glass or thermanox plates) as shown by interference microscopy with reflected light and by scanning and transmission electron microscopy (Thiemann and Ruthmann, 1989). Most if not all pre-existing extensions are disrupted and withdrawn during the isolation procedure, but new ones soon protrude. These attach at their free tips and at intermediate points to the substrate and form actin-containing adhesion plaques (Thiemann and Ruthmann, 1989). In addition, the extensions can evidently fuse with other fiber cells giving rise to a syncytial organization via cytoplasmic strands, suggesting the presence of a more or less extended syncytium in the middle layer of mesenchyme-like fiber cells of *Trichoplax*. The latter have previously been shown to be interconnected by an extensive network of branched cytoplasmic extensions that also establish contacts with the cell bodies of other fiber cells and with both epithelia (Grell and Benwitz, 1971; Rassat and Ruthmann, 1979). A syncytium in the middle layer would also be in accord with the occasional observation of two or even three nuclei in the same fiber cell. As in the case of cells isolated on slides, it may form by the fusion of fiber cell extensions with each or with the cell bodies of other fiber cells. It might be maintained after mitoses of the tetraploid fiber cells (Ruthmann and Wenderoth, 1975; Ruthmann, 1977) by a wide separation of the daughter nuclei leading to long cytoplasmic strands between them. The constant changes of shape of the whole organism may be the result of local contractions within the three-dimensional syncytium of fiber cells whose slender, anastomosing interconnections have been shown to be traversed by prominent unidirectional bundles of actin filaments (Thiemann and Ruthmann, 1989). The dense connecting discs once thought to be involved in transmitting impulses

for the contractions in the manner of synapses conducting in both directions (Grell and Benwitz, 1974) are much too rarely found to fulfill such a task. Instead, the discs may represent a very transitory stage of a cell contact preceding the fusion of two fiber cells of the syncytium.

Grell K. G. and Benwitz G. (1971), Die Ultrastruktur von *Trichoplax adhaerens* F.E. Schulze. Cytobiologie **4**, 216–240.

Grell K. G. and Benwitz G. (1974), Spezielle Verbindungsstrukturen der Faserzellen von *Trichoplax adhaerens* F.E. Schulze. Z. Naturforsch. **29c**, 790–791.

Rassat J. and Ruthmann A. (19799, *Trichoplax adhaerens* F.E. Schulze (Placozoa) in the Scanning Electron Microscope. Zoomorphologie **93**, 53–72.

Ruthmann A. and Wenderoth H. (1975), Der DNA-Gehalt der Zellen bei dem primitiven Metazoon *Trichoplax adhaerens* F.E. Schulze. Cytobiol. **10**, 421–431.

Ruthmann A. (1977), Cell differentiation, DNA content and chromosomes of *Trichoplax adhaerens* F.E. Schulze. Cytobiol. **15**, 58–64.

Ruthmann A. and Terwelp U. (1979), Disaggregation and Reaggregation of Cells of the Primitive Metazoon *Trichoplax adhaerens* (Placozoa). Differentiation **13**, 185–198.

Thiemann M. and Ruthmann A. (1989), Microfilaments and microtubules in isolated fiber cells of *Trichoplax adhaerens* (Placozoa). Zoomorphology **109**, 89–96.