Acta Bot. Neerl. 39(1), March 1990, p. 49–52

BRIEF COMMUNICATION

Simultaneous visualization of cytoskeletal elements and cellulose microfibrils in cortex cells of tobacco explants

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SUMMARY

A method is described for the simultaneous visualization of membrane-bound cytoskeletal elements and cellulose microfibrils in cortex cells of tobacco explants. The method consists of sectioning polyethylene glycol embedded material, dry-cleaving and electron microscopy.

Key-words: cytoskeleton, cellulose microfibrils, PEG, tobacco explants.

INTRODUCTION

Various techniques exist for the separate visualization of cytoplasmic elements, e.g. cytoskeleton and organelles, and cellulose microfibrils (for review see Robards 1985). Sassen & Wolters-Arts (1986) showed cortical microtubules (MT) and cellulose microfibrils (CMF) in the same preparation in stamen hairs of *Tradescantia virginiana* after freeze substitution and sectioning.

However, when ultra-thin sections are used, the area of the cell that can be examined is very small. Microtubules, microfilaments, coated pits, mitochondria, endoplasmic reticulum and cellulose microfibrils have been demonstrated over a much larger cell area using the dry-cleaving method for plant cells (Traas 1984). Since then, many cell types have been examined with this method: various cell types in roots (Traas *et al.* 1984; Traas *et al.* 1985; Emons & Traas 1986), pollen tubes (Derksen *et al.* 1985), protoplasts (Emons & Traas 1986) and seed hairs (Quader *et al.* 1986). Recently, Traas & Derksen (1989) have shown MTs and CMFs in root hairs in the same preparation after dry-cleaving.

For the purpose of visualizing cytoskeletal elements and cellulose microfibrils in cells with thick walls, such as the cells of tobacco explants, the dry-cleaving technique cannot be used as such. Wolosewick (1980) introduced the water-soluble embedding medium polyethylene glycol (PEG) for electron microscopy in animal tissue studies. Later Hawes *et al.* (1983) and Hawes & Horne (1985) used the PEG technique for electron microscopic studies on plant material. The PEG technique can also be used successfully for immunocytochemistry on plant material (Van Lammeren *et al.* 1985, Wilms & Derksen 1988).

This paper describes a method based on a combination of PEG embedding and dry-cleaving, which allows the visualization of the distribution and orientation of cytoplasmic

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elements (e.g. microtubules, filaments, coated pits) and of the cell wall in cortex cells of tobacco explants.

MATERIAL AND METHODS

Tissue strips (0·6 × 7 mm) were cut from flower stalks of *Nicotiana tabacum*, and cultured for 4 h on Murashige and Skoog (M&S) medium (Murashige & Skoog 1962), supplied with 10^{-7} M naphthalene acetic acid (NAA), 10^{-6} M benzylamino-purine (BAP), 125 mM glucose and 1% agar, as previously described by Wilms & Sassen (1987). On this medium, cortical microtubules in cortex cells of tobacco explants have been shown to change their orientation within 6 h from transverse to parallel to the long cell axis (Wilms & Derksen 1988).

Explants were fixed in 4% *p*-formaldehyde and 0·25% glutaraldehyde in a microtubule stabilizing buffer with 0·1% tannic acid and 0·01% Triton X for 2 h at room temperature. The MT stabilizing buffer (pH 6·8) contained 50 mM phosphate buffer, 10 mM EGTA, 5 mM $MgSO_4$ and 10% DMSO. After rinsing in buffer for 2 h, preparations were embedded in aqueous PEG 1500, as described by Wilms & Derksen (1988). Longitudinal sections, 5–15 μm thick, were cut with a steel knife on a Reichert hand microtome. Sections were placed in water to dissolve PEG and attached to poly-L-lysine coated nickel foldover (oyster) grids. The grids were snapped together, post-fixed in 0·5% OsO_4 for 1 h, rinsed in water for 1 h, stained with 0·5% uranyl acetate for 1 h and, after rinsing in water for 2 h, they were dehydrated slowly in ethanol. The oyster grids were opened after critical-point drying and both halves were examined in a Jeol JEM 100 CX electron microscope at 60–80 kV. In order to visualize cellulose microfibrils, the opened grids were shadowed with platinum at an angle of 45° and reinforced with carbon.

RESULTS AND DISCUSSION

The overall appearance of the cells embedded by the PEG method was largely identical to that of cells after dry-cleaving (Traas 1984). The ultrastructure of the cells was well preserved; microtubules, microfilaments and coated pits were observed in the preparations (see also Hawes & Horne, 1985). Microtubule preservation was improved by the addition of tannic acid to the fixative (Traas 1984). In contrast to Hawes & Horne (1985), we used 15-μm thick sections instead of 0·25–1 μm thick sections. Furthermore, we cleaved these sections by opening the oyster grids instead of using normal grids without cleaving the preparation. The advantage of our method is that relatively thick sections can be used, which allow large parts of the cells to be observed. Microtubules, with a diameter of 28 nm, are shown in Fig. 1a, b, d and f; smaller filaments with diameters of 9–14 nm in Fig. 1c and coated pits in Fig. 1d. Cellulose microfibrils are shown in Fig. 1e and f. Figure 1f simultaneously visualizes microtubules and cellulose microfibrils.

In cells of freshly cut explants, the orientation of microtubules and cellulose microfibrils was transverse to the long cell axis (Fig. 1a and e). After explanation, the orientation of microtubules and cellulose microfibrils changed from transverse to parallel to the long cell axis, within 2–6 h. Whenever microtubules and cellulose microfibrils were observed simultaneously, their orientations were identical (Fig. 1f) (see also Wilms & Derksen 1988; Wilms 1989).

PEG embedding and sectioning, followed by dry-cleaving, appears to be a suitable technique for the examination of cytoskeletal elements and cellulose microfibrils in cells

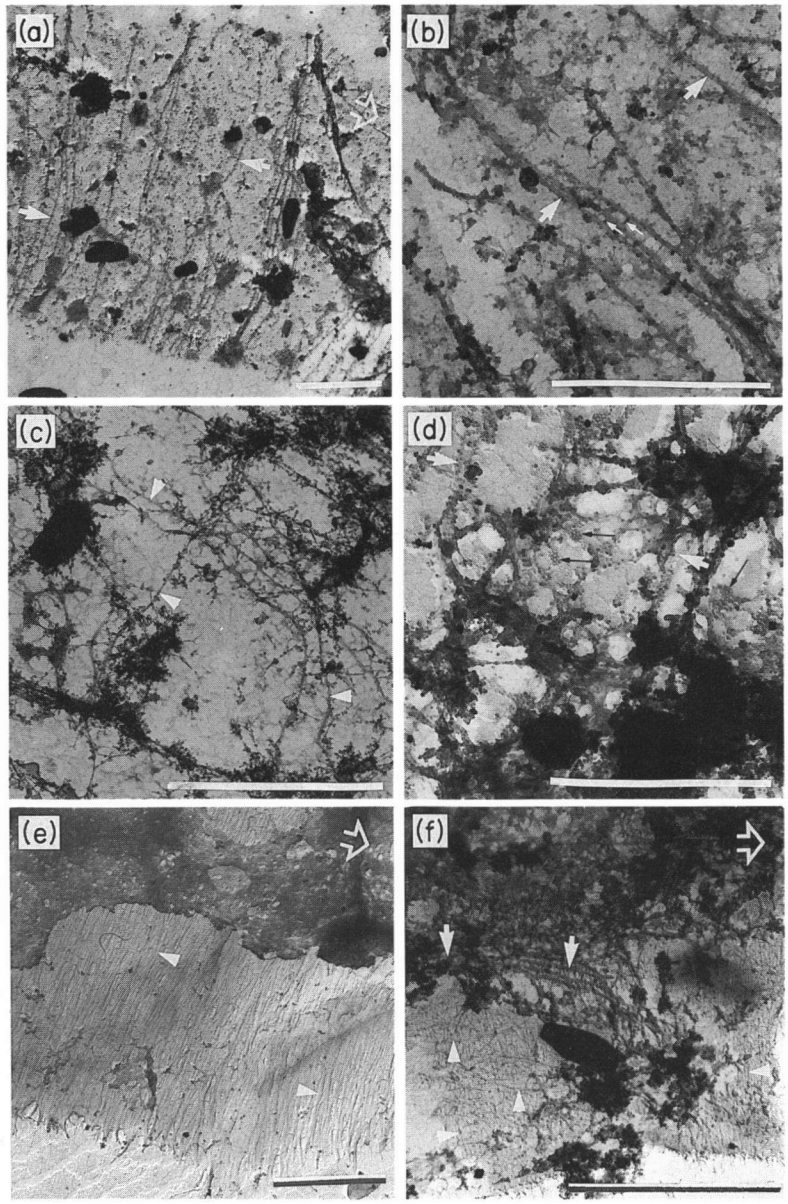

Fig. 1. Electron micrographs from PEG-embedded, sectioned and dry-cleaved cortex cells of tobacco explants. (a) Preparation after shadowing with platinum and carbon (Pt/C), showing transverse-oriented cortical microtubules. Open arrow: long cell axis; arrow: microtubule. Bar: 1 μm. (b) Preparation before Pt/C treatment, showing cortical microtubules and connections between the microtubules. Arrow: microtubule; small arrow: connection between microtubules. Bar: 1 μm. (c) Preparation before Pt/C treatment, showing a network of filaments. Arrowhead: microfilament. Bar: 1 μm. (d) Preparation after Pt/C treatment, showing cortical microtubules, coated pitts and coated vesicles. White arrow: microtubule; small black arrow: coated pitts/vesicles. Bar: 1 μm. (e) Preparation after Pt/C treatment, showing transverse-oriented cellulose microfibrils. Open arrow: long cell axis; arrowhead: cellulose microfibril. Bar: 1 μm. (f) Preparation after Pt/C treatment, showing longitudinally oriented cortical microtubules and longitudinally oriented cellulose microfibrils. The underlying layers of transverse-oriented microfibrils are also visible. Open arrow: long cell axis; arrow: microtubule; arrowhead: cellulose microfibril. Bar: 1 μm.

with thick walls. Compared to the more conventional sectioning of resin and PEG-embedded material for EM or HVEM (cf. Hardham & Gunning 1979; Hawes & Horne 1985), our technique of sectioning not only allows the visualization of substantially larger parts of the membrane areas, but also the simultaneous studying of the cytoplasm and cellulose microfibril deposition in the wall.

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