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MICROFIBRILS, MICROTUBULES AND MICROFILAMENTS OF THE TRICHOBLAST OF EQUISETUM HYEMALE

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SUMMARY

The cell wall of the trichoblastic part of the root hair cell is helicoidal like the cell wall of the root hair proper. The rotation mode of the helicoid is counter-clockwise and the angle between microfibrils in adjacent lamellae is approximately 40°.

During trichoblast elongation cortical microtubules lie perpendicular to the axis of elongation. During root hair initiation and root hair growth cortical microtubules align according to the long axis of the forming hair, not in parallel with the microfibrils. The freeze-substitution technique reveals microtubules in the apical dome of the forming hair, where they lie in random orientations enabling isodiametric expansion. Microtubules in these cells function in morphogenesis, but not in microfibril orientation.

F-actin cables, microfilaments, are present in the trichoblast; they form a network in the cell interior. They do not coalign with the microfibrils nor with the microtubules.

1. INTRODUCTION

In many plant cells, nascent microfibrils and cortical microtubules have been found in parallel orientations and therefore microtubules have been hypothesized to orientate the nascent microfibrils. It is, however, not known how they do so; and several hypotheses have been proposed (HEATH & SEAGULL 1982).

The coalignment between these structures has especially been studied in enlarging cell surfaces (HARDHAM 1982, GUNNING & HARDHAM 1982) and in xylem elements, in which local wall thickening occurs (FALCONER & SEAGULL 1985).

Absence of paralellism has been explained by pointing out that microtubules were already in the position of the expected new microfibril orientation (NEWCOMB & BONNETT 1965, SEAGULL & HEATH 1980).

Recent investigations on root hairs with helicoidal walls, however, did not allow for such an explanation (EMONS 1982, EMONS & WOLTERS-ARTS 1983, MEEKES 1985, TRAAS et al. 1985). In these cells, microtubules align longitudinally while in time and in place the adjacent microfibrils attain all different orientations with respect to the long axis of the hair. Therefore, the microtubules cannot orientate the microfibrils by their own direction. It has to be emphasized that root hairs are tip-growing cells (SIEVERS & SCHNEPF 1981) and that the helicoidal wall is a secondary wall deposited in the non-enlarging hair tube (EMONS & WOLTERS-ARTS 1983).

F-actin strands, microfilaments, have recently been shown, by means of the F-actin specific probe rhodamine-phalloidin, to be a normal component of plant cells (PARTHASARATHY 1985, PARTHASARATHY et al. 1985, DERKSEN et al. 1986, PIERSON in press). They are involved in cytoplasmic streaming (PESACRETA & PARTHASARATHY 1984, PARTHASARATHY et al. 1985, DERKSEN et al. 1986) and may interact with microtubules (POLLARD et al. 1984).

We studied microfibrils, microtubules and microfilaments in trichoblasts of *Equisetum hyemale* to investigate their possible interactions. A root hair is a protuberance of an epidermic cell. This cell is called a trichoblast. In this paper the term trichoblast is used for the epidermis cell before, during and after root hair formation. As fibril lamellae of the root hair seem to originate from this part of the cell (EMONS & WOLTERS-ARTS 1983) a clue for the microfibril orientating mechanism might be found in the trichoblast.

2. MATERIAL AND METHODS

The microtubules of the cytoplasm were visualized by immunofluorescence (WICK & DUNIEC 1983, TRAAS et al. 1985).

The microfibrils of the cell wall were visualized by thin-sectioning (EMONS & WOLTERS-ARTS 1983) and by dry-cleaving (SASSEN et al. 1985).

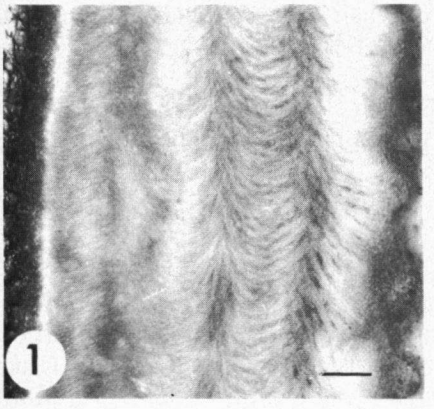
Microfibrils and microtubules were also visualized with the freeze-substitution procedure. For freeze-substitution, pieces of roots with hairs were placed on pieces of boiled dialysis tubing as large as a grid. These pieces were rapidly frozen by plunging them in liquid propane, cooled by liquid nitrogen. They were transferred to a substitution fluid composed of anhydrous aceton containing 2% OsO_4 and 0.1% uranyl acetate precooled at $-78\,^{\circ}C$ in a metal vial. The metal vials, containing the specimens were transferred to a freeze-drying device held at a temperature of $-80\,^{\circ}C$ ($\pm$ 5 °C) by liquid nitrogen during 20 h. The material was brought to room temperature in this apparatus very slowly during another 6 h. At room temperature the specimens were rinsed with anhydrous aceton several times, infiltrated with spurr's resin and embedded as a flat layer. Under a light microscope individual hairs, showing no evidence of gross ice

Fig. 1. Thin section of the cell wall of a trichoblast with short growing root hair, prepared by freeze-substitution and on-block staining with uranyl acetate, showing the arcs of the helicoidal cell wall. Width of parabolae in a micrograph depends on obliqueness of sections. 13.200 ×, bar: 0.5 μm.

Fig. 2. Thin section of the cell wall of a trichoblast with a long growing root hair, prepared by dissolving the wall matrix prior to embedding and staining of the sections with permanganete. In cells with longer root hairs more helicoidal arcs are present in the proximal part of the hair than in cells with shorter root hairs. 27.300 ×, bar: 0.5 μm.

Fig. 3. Surface view of the innermost layer of the cell wall prepared by dry-cleaving and shadowing, showing the three last-deposited microfibril lamellae. Arrows indicate the direction of the three last-deposited lamellae. Looking from the cytoplasm, going from former (a) to latter (b resp. c) deposited lamellae, the rotation mode of the helicoid is counter-clockwise. The angle between consecutive lamellae is approximately 40°. 27.000 ×, bar: 0.5 μm.

Fig 4. Thin section of tip of very young root hair, prepared by freeze-substitution. Small arrows indicate microtubules. Microtubules occur in random orientations. 13.200 ×, bar: 0.5μm.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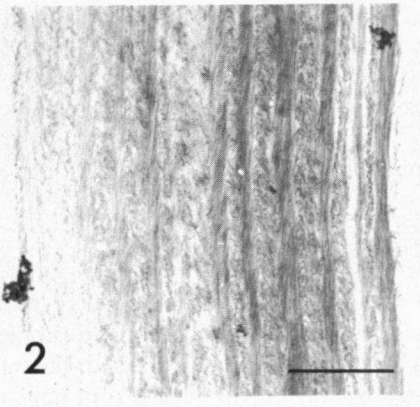
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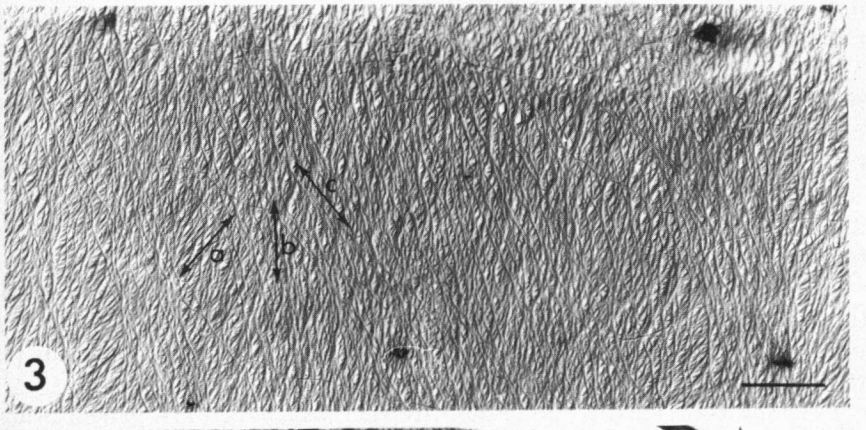
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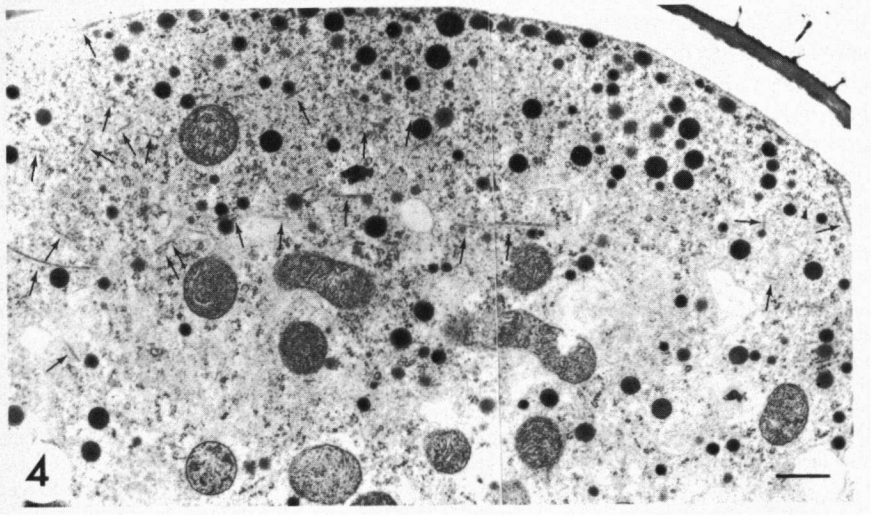
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crystal damage, were selected. The selected hairs were sectioned tangentionally with a Sorvall Porter Blum MT 5000 onto formvar coated grids and stained with uranyl acetate/lead citrate.
Sections were examined with a Philips EM 201 electron microscope.

The microfilaments of the cytoplasm were visualized using rhodamine labelled phalloidin (DERKSEN et al. 1986).

3. RESULTS

Freeze-substitution gave good preservation of the cytoplasm of the root hair, in which the longitudinal microtubule alignment could be ascertained (EMONS in preparation). As is expected, the microfibrils of the cell wall were not visualized by this method in the root hair, but the method did visualize microfibrils in the trichoblast cell wall. Sofar, in this part of the cell we did not succeed to preserve the cytoplasm with cortical microtubules.

The cell wall of the trichoblast is clearly of the helicoidal type (*fig. 1*). This is also shown by thin-sectioning of material from which matrix substances has been dissolved (*fig. 2*). Dry-cleaving shows the last-deposited microfibrils in surface view (*fig. 3*). The angle between microfibril orientations in adjacent lamellae is aproximately 40°. Looking from the cytoplasm and going from former to latter deposited lamellae the rotation of the helicoid is counter-clockwise. Microfibrils within a lamella are not contiguous.

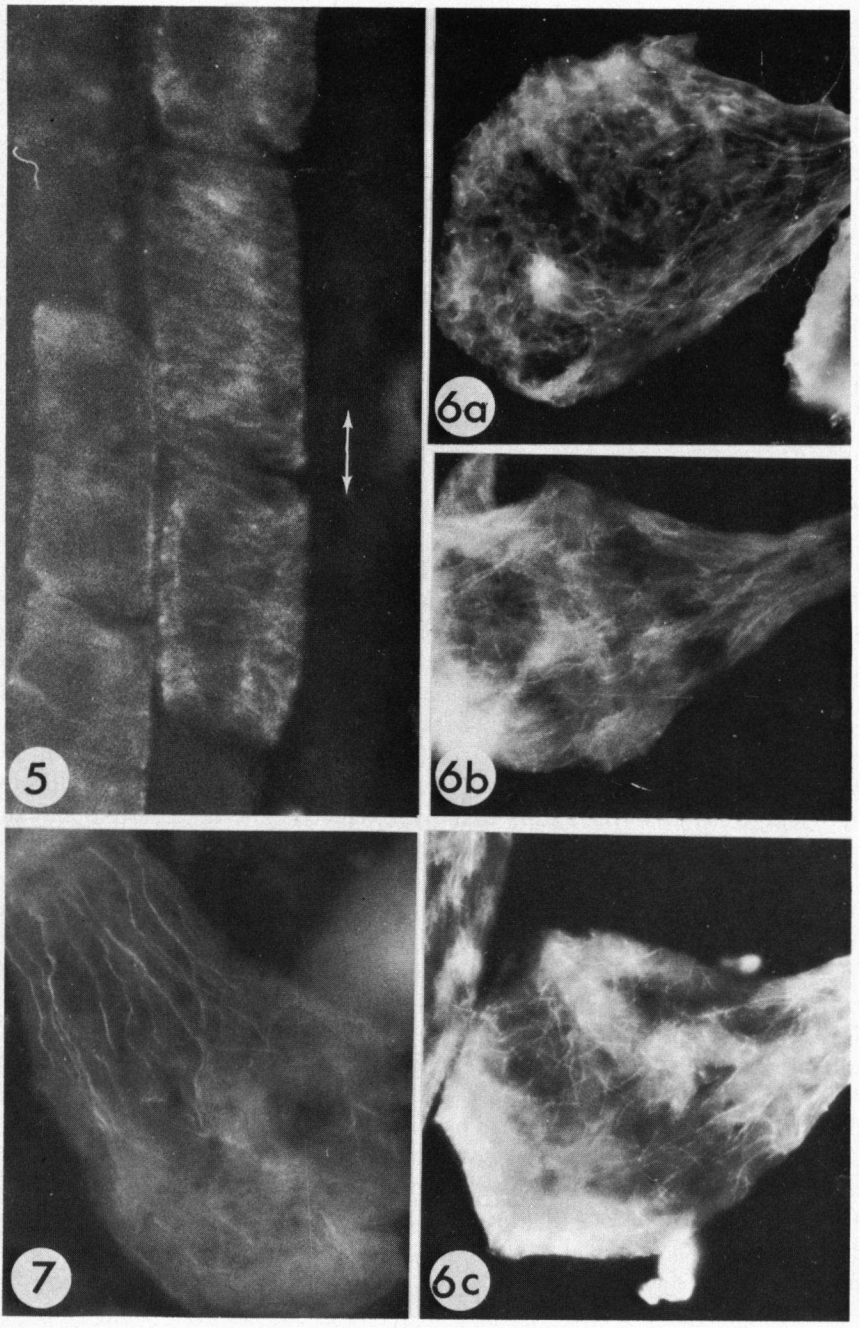
The three different methods employed show that the cell wall texture of the trichoblast is helicoidal with the same angle between fibril orientations of successive lamellae and with the same rotation of the helicoid as in the root hair cell wall.

Fig. 5 shows the microtubule pattern in epidermis cells before root hair formation. *Figs. 6a, b, c* show the microtubule pattern in (6a) a trichoblast with a short protuberance, (6b) a trichoblast with a growing root hair and (6c) a trichoblast with a full-grown root hair. In elongating epidermis cells before root hair initiation microtubules align transverse to the axis of elongation of the root and of the epidermis cell (*fig. 5*). In a trichoblast with short protuberance (*fig. 6a*) the original microtubule alignment in the tangential wall is lost; alignment in the radial walls is still transverse to the axis of elongation of the root (*fig.*

Fig. 5. Microtubule alignment of undifferentiated epidermis cells during cell elongation, visualized by immunofluorescence. Cortical microtubules align transverse to the axis of elongation. As to microtubule alignment trichoblasts and atrichloblasts are the same. Arrow designates long axis of root, which is the axis of elongation of the cell. 1000 ×.

Fig. 6. a, b, c. Microtubule alignment in trichoblasts during root hair growth, visualized by immunofluorescence. Microtubules mainly occur in the cortical cytoplasm. a) trichoblast, which has a short protuberance, b) trichoblast, which has a growing hair, c) trichoblast, which has a full-grown hair. During hair growth microtubules lie in the direction of the long axis of the hair; after growth this alignment is more or less lost in the trichoblastic part of the cell. 960–1000 ×.

Fig. 7. Microfilament alignment in a trichoblast, bearing a growing hair, visualized using the F-actin specific probe rhodamine-phalloidin. Microfilaments occur at different levels in the cell, not only in the cortical cytoplasm. 1200 ×.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6a
6b
7
6c

6a). Microtubules protrude into the forming hair and lie in the direction of the long axis of the hair. The same microtubule pattern is conserved during root hair growth (*fig. 6b*). In *fig. 6c* it can be seen that in a trichoblast with a full-grown hair all regular microtubule alignment is more or less lost. Microfibril deposition in the trichoblast goes on during all these stages (*figs. 1* and *2*).

Freeze-substitution reveals a random microtubule orientation in the tip of the protuberance (*fig. 4*).

Fig. 7 reveals the alignment of F-actin cables, microfilaments, in a trichoblast with a young growing root hair. These microfilaments are situated in the cell interior unlike microtubules, which are present predominantly in the cortical cytoplasm. Throughout hair growth microfilaments conserve this alignment. They do not coalign with the microfibrils of the cell wall.

4. DISCUSSION

4.1. Visualization of microfibrils by means of freeze-substitution

Lead citrate and uranyl acetate stains have no chemical affinity for cellulose. The image of cell walls after this staining is often unreliable (NEVILLE & LEVY 1984). COX & JUNIPER (1972) have reported that uranyl acetate and lead citrate stain cellulose microfibrils, but that this staining is physical in nature and therefore removed by washing of the material on the grids. On-block staining would reveal microfibrils. The dark threads seen by freeze-substitution of material on-block stained with uranyl acetate (*fig. 1*) occur in the same pattern as in thin sections from which matrix material has been dissolved (*fig. 2*), as in shadowed dry-cleaved material (*fig. 3*) and as in freeze-etched material (EMONS 1985) and are therefore interpreted as cellulose microfibrils.

In freeze-fractured preparations microfibrils of root hairs of *Equisetum hyemale* measured 8.5 nm (± 1.5 nm) including shadow deposit (EMONS 1985). In dry-cleaved preparations (*fig. 3*) microfibrils measure 8 nm (± 1 nm) including shadow deposit. However, in thin sections of material from which the cell wall matrix has been dissolved (fig. 2) their diameter is much smaller (approximately 3.5 nm). The diameter in the helicoidal layer of the wall prepared with freeze-substitution is much larger (compare *fig. 1* and *2*), which could point at the possibility that a hydrophylic sheath of less-crystalline glucans, which surrounds the crystalline core, is positively stained.

Freeze-substitution of on-block stained material is one of the means to elucidate the debate on microtubule and microfibril coalignment (EMONS 1982, EMONS & WOLTERS-ARTS 1983, LLOYD & WELLS 1985, TRAAS et al. 1985), because artefacts of chemical fixation are ruled out and microtubules and microfibrils may be visualized at the same time in the same preparation.

4.2. Microtubules and morphogenesis

In a review article, CORMACK (1949) stated that there was general agreement

that retardation in vertical elongation of the trichoblasts is a prerequisite for epidermal cells to give rise to root hairs.

It has become known since then that microtubule orientation is at least one of the factors that determine the orientation of cell elongation (GUNNING & HARDHAM 1985, HARDHAM 1982, BUSBY & GUNNING 1984). In cells expanding uniformly in one direction microtubules lie perpendicular to the axis of elongation (GUNNING & HARDHAM 1982, HARDHAM 1982, BUSBY & GUNNING 1984, SASSEN & WOLTERS-ARTS this issue). This can also be seen in epidermis cells before root hair initiation (*fig. 5*). In isodiametrically expanding cells, as protoplasts, microtubules lie in random orientations (MARCHANT 1979, LLOYD et al. 1980, VAN DER VALK et al. 1980). The random microtubule orientations occur also in the expanding tip of *Equisetum hyemale* root hairs (*fig. 4*). (cf. DERKSEN et al. this issue).

Cells, which have completed elongation, never form hairs (CORMACK 1949). In these cells microtubules occur in random orientations (data not shown). It seems that a microtubule alignment perpendicular to the axis of elongation of the trichoblast, i.e. according to the long axis of the protuberance, is one of the prerequisites needed to form a hair, but it is not the root hair initiating factor as atrichoblasts have the same microtubule pattern and do not form hairs. In *Equisetum hyemale* the trichoblast stops elongating at the onset of root hair formation.

Our observations clearly indicate a morphological role of the cortical microtubules: at the time that the protuberance forms microtubule alignment along the tangential wall of the trichoblastic cell changes and microtubules lie in the direction of the long axis of the protuberance. At the tip of the protuberance they span the hemisphere and lie in random orientations allowing for isodiametric expansion. The crucial question therefore is: 'What factors determine microtubule orientation?'

It further remains to be explained how transverse hair expansion is inhibited in tip-growing cells, which have axial microtubule alignment.

4.3. Microtubules and microfibril orientation

Cortical microtubules lie against the plasma membrane. Across the membrane, microfibrils are deposited. During root hair growth, microfibril deposition occurs along the whole *Equisetum hyemale* root hair and the cell wall of the trichoblast thickens also (compare *figs. 1* and *2*). As in the hair, microfibrils in this basal part of the cell are deposited according to a helicoid, showing parabolae in thin sections. (*figs. 1* and *2*). Thus, lamellae with microfibrils in subsequently differing orientations are deposited while microtubules remain aligned in one direction only. This corroborates the statement that microtubules do not orientate the nascent microfibrils in a helicoidal cell wall (EMONS 1982, EMONS & WOLTERS-ARTS 1983).

LLOYD & WELLS (1985) have suggested that phosphate buffer somehow would disorientate transversely oriented microtubules in root hairs. TRAAS et al. (1985), however, did not find any differences in microtubule pattern between cells pro-

cessed in phosphate buffer and cells processed in Pipes buffer recommended by LLOYD & WELLS (1985).

Microtubules and microfibrils influence plant cell morphogenesis. Microtubules constitute the inner skeleton, with a role in maintenance and alteration of cell shape, comparable to their role in animal cells (erythrophores: OCHS 1982; amoebae: UYEDA & FURUYA 1985). Microfibrils constitute the outer skeleton of a plant cell, with a role in more permanently shaping cells. This outer skeleton is transient in growing cells or cell parts and permanent in full-grown cells or cell parts. An enlarging cell in a transient wall needs the microtubules as the inner cytoskeleton and to alter the axis of elongation. Absence of coalignment of microfibrils and microtubules has also been shown in *Valonia* (ITOH & BROWN 1984). MIZUTA & WADA (1982) concluded that microtubules do not orientate the nascent microfibrils, because anti-microtubule agents did not affect microfibril orientation in *Boergesenia*. HAHNE & HOFFMANN (1985) have found that in mesophyll cells microtubules are present during cell division and cell enlargement but not during cell wall deposition.

As microtubules and microfibrils have some functions in common, microtubules and microfibrils lie in parallel in enlarging cells, but microtubules do not directly orientate the nascent microfibrils.

4.4. Microfilaments

The orientation of F-actin cables, microfilaments, visualized with rhodamine labelled phalloidin, differs from the orientation of microtubules and is not correlated to the orientation of nascent microfibrils. We conclude that the actin cables observed are not involved in cell morphogenesis nor in microfibril orientation.

In freeze-substituted root hairs of *Equisetum hyemale* microfilaments are often seen along the microtubules (EMONS in preparation). These filaments are not visualized with the rhodamine labelled phalloidin. They may interact with microtubules (POLLARD et al. 1984).

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