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THE MICROTUBULAR SKELETON IN DIFFERENTIATING ROOT TIPS OF RAPHANUS SATIVUS L.

J. DERKSEN[1]*, G. JEUCKEN[1], J. A. TRAAS[1] and A. A. M. VAN LAMMEREN[2]

[1] Department of Botany III, University of Nijmegen, Toernooiveld 1, 6533 ED Nijmegen, The Netherlands
[2] Department of Plant Cytology and Morphology, Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands

SUMMARY

Using immunofluorescence techniques, microtubules were studied in differentiating cells of *Raphanus sativus*. Within one cell large differences in microtubule patterns occur. The results indicate a relation between cell expansion and microtubule orientation: at elongating surfaces microtubules are found perpendicular to the direction of expansion at the cells surface. At non-expanding cell surfaces, randomly oriented microtubules are found. Possibly a relation exists between the rate of elongation and the density of the microtubules.

1. INTRODUCTION

Microtubules are regular constitutents of all eukaryotic cells (DUSTIN 1984). In plant cells microtubules are mainly studied in relation to cell wall synthesis (ROBINSON & QUADER 1982). In general their role in cell morphogenesis is supposed to occur via control of cellulose microfibril deposition in cell wall (HARDHAM et al. 1980, GUNNING & HARDHAM 1982, ROBINSON & QUADER 1982). However, they also can be directly involved in cell morphogenesis (GUNNING & HARDHAM 1982, ROBINSON & QUADER 1982, DUSTIN 1984).

Ultrastructural studies have shown a correlation between cell morphogenesis and various properties of the cortical microtubules, most strikingly between microtubule orientation and cell expansion (MARCHANT 1979, HARDHAM & GUNNING, 1979, HARDHAM et al. 1980).

The study of microtubules as coherent skeletons of cells during morphogenesis has become possible only recently by the development of immunofluorescence techniques (WICK et al. 1981). Immunofluorescence studies on intercalary growing higher plant cells have not only shown transverse arrays of microtubules, but also helical arrays with a variable pitch (TRAAS et al. 1984, LLOYD et al. 1985). The pitch of these helical arrays has also been related to cell expansion: either directly to the vector sum of axial and lateral expansion (TRAAS et al. 1984), or to a variable growth rate (LLOYD et al. 1985).

In order to test these possibilities we studied the early differentiation of rootlets of *Raphanus sativus*, when the small meristematic cells undergo typical changes

* To whom all correspondence should be sent.

in morphology. To obtain intact microtubular skeletons, immunofluorescence preparations were made from squashes (WICK et al. 1981, TRAAS et al. 1984). The origin of the cells could be determined from their morphological properties as seen in sectioned material. Observations on microtubules were also made directly on sections of poly-ethylene-glycol embedded material (VAN LAMMEREN et al. 1985).

2. MATERIALS AND METHODS

Seeds of *Raphanus sativus* L. were grown at room temperature on wet filter paper. We used 48 hrs old rootlets (see also: TRAAS et al. 1985).

Preparations were made from the part of the root which had not yet developed root hairs. Some preparations were made from the root hair zone to study the microtubules in trichoblasts. To study cell morphology, roots were fixed in 3% acroleine in 0.1M sodium phosphate buffer pH 6.8 and stained with Schiffs reagent (VAN DUYN 1961). After dehydration in a water-alcohol series, preparations were embedded in epon resin. Semi thin sections were embedded in entellan and photographed.

Immunofluorescence preparations were made of squashes of root tips as described by WICK et al. (1981), with some modifications (TRAAS et al. 1985). Immunofluorescence preparations of polyethylene-glycol embedded material were made as described previously (VAN LAMMEREN et al. 1985). Photographs were taken with a Leitz Orthoplan – Vario Orthomat combination equipped for immunofluorescence. Except in the root cap, in *Raphanus* roots the cell axis is always parallel to the root axis. In this study cell surfaces more or less parallel to the root axis are called parallel walls; all surfaces more or less perpendicular to the root axis are called transverse walls. Expansion at the cell surface, parallel to the cell axis is called axial expansion; expansion perpendicular to the cell axis is called lateral expansion. The notion trichoblast will not only be used for the root hair initiating cell, but also for the root hair carrying cells.

3. RESULTS

The morphological properties of root cap cells, epidermis cells including trichoblasts, cortex cells and cells of the pericycle were determined in cross and longitudinal sectioned roots.

Already within a few cells distance from the meristem most cells show a definite morphology. In *fig. 1* a typical cross section is shown and the various cell types are indicated. Within the tissue of the central cylinder morphological differentiation occurs only in the root hair zone.

In the root cap irregular shaped cells are found (*fig. 2a, b*). Cell divisions occur in all directions throughout the root cap. Near the meristem the cells are either disk-shaped with an irregular circumference (the circumferential surface) or cylindrical and elongated (*fig. 2*). The flat surfaces of these cells are perpendicular to the cell axis, but not to the root axis. As seen in squashed material

the flat surfaces show randomly distributed microtubules (*fig. 2 b-d*). The cylindrical cells and the circumferential side of the flat cells invariably show regular patterns transverse to the cells axis (*fig. 3 a-c*).

Older root cap cells become elongated, the cells covering the epidermis remain cylindrical (see also *fig. 1*). whereas the cells of the lower part of the root cap show diverse forms: varying from globular, spindle or disk-shaped to irregular, without a preferential direction of the cell axis. The microtubules in the older root cap cells are apparently less dense as compared to those of the circumferential surfaces of young cells (*fig. 3*). In the cylindrical cells they are mostly transverse to the long axis of the cell, in other cells they are mostly transverse to helical (*fig. 3*), but in some cells only irregular patterns occur (not shown). Full grown cells of the root cap are not stable and get lost during preparation.

Throughout the root part between meristem and root hair zone cells elongate. Near the root hair zone this elongation is less prominent. However, the vector sum of lateral and axial expansion of the cell surface remains almost perpendicular to the cell axis. Accordingly, the cortical microtubules at the parallel walls always are perpendicular to the cell and root axis (*figs. 4, 5*). At the transverse walls, hardly any cell expansion occurs and always random patterns are found (*figs. 4, 5*). Even in cortex cells (*fig. 5*) which also expand strongly in lateral direction, the transverse walls hardly expand: large intercellular lumina are formed (see also *fig. 1*).

However, the trichoblasts near the root hair zone (see also *fig. 1*) may show oriented microtubules at their transverse walls: their shape gradually changes from more or less square to wedge shaped (like the endodermis; see *fig. 4b-d*) and eventually the narrow part of the wedge shaped cell will widen and become

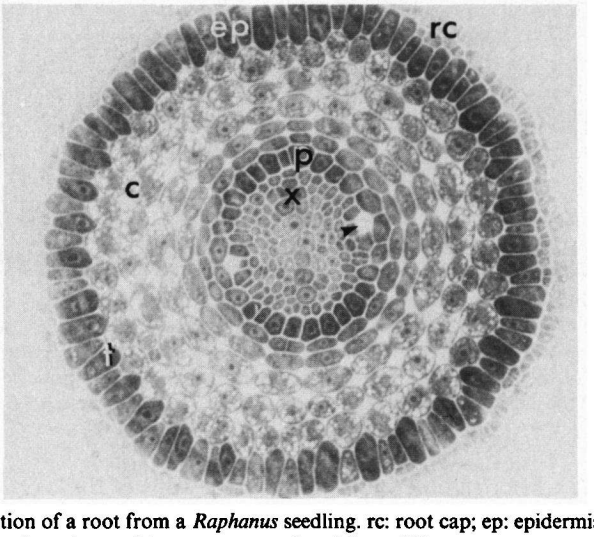

Fig. 1. Cross section of a root from a *Raphanus* seedling. rc: root cap; ep: epidermis, t: trichoblast; c: cortex; p: pericycle; pointer: phloem; x: protoxylem. Magn: 720 ×.

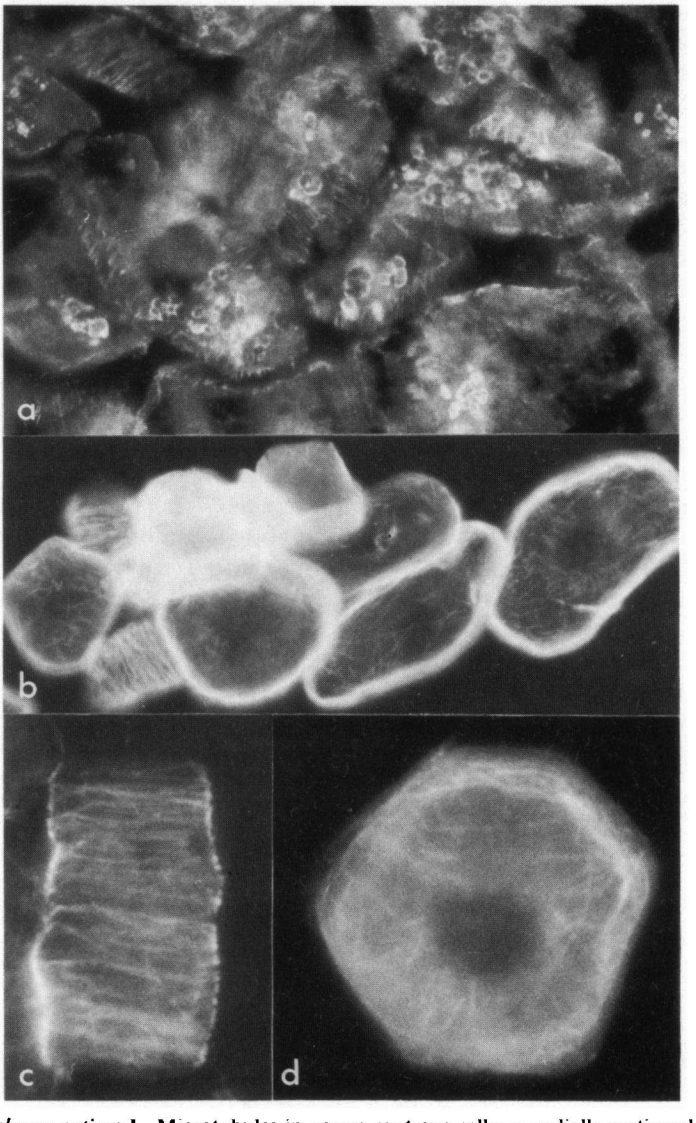

Fig. 2. *Raphanus sativus* L. Microtubules in young root cap cells; a: radially sectioned root cap; b: squashed root cap cells; c: elongating cells, d: flat cell. In contrast to other root cells, the cells of the root cap are variable in size and morphology, the axis of the cells mostly is not parallel to the long axis of the root. Cells of variable size and morphology are present and accordingly the orientation of the microtubules is highly variable. Magn., a,b: 800 ×; c,d: 1500 ×.

tapered (*fig. 4e*). Microtubules at the transverse walls of these cells remain randomly oriented, but occasionally they may show a preferential direction: parallel to the edges of the tapered part of the surface, transverse to the direction of

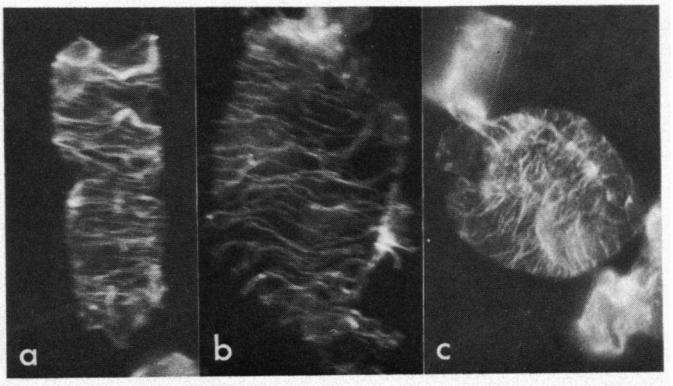

Fig. 3. *Raphanus sativus* L. Microtubules of older root cap cells; a: strongly elongated cell, b: spindle shaped cell, c: globular/disk shaped cell. Magn: 480 ×.

expansion of the wall. In elder trichoblasts, near the root hair zone, the transverse walls obtain a more pear shaped circumference and gradually increase their surface till an elliptical circumference is acquired.

At the tapering parts of these walls microtubules show random patterns, the remaining part of the wall, which undergoes stronger lateral extension, shows oriented microtubules perpendicular to the extension of the wall (*fig. 4f*).

The microtubules at the transverse walls are conspicuously less dense than at the parallel walls, indicating a relationship between microtubule density and the rate of cell elongation (*figs. 3, 4* and *6*; compare also *fig. 2*).

In the still slightly stretching trichoblast of the lower root hair zone, microtubules are found transverse to the cell axis, in the trichoblasts from the upper root hair zone that do not stretch anymore, microtubules become less strictly oriented and may even show random orientations (*fig. 6*).

In sections of poly-ethylene glycol-embedded material essentially the same observations were made (not shown).

Besides the differences in the patterns of cortical microtubules, no other differences or changes in microtubule organization could be observed in the various cell types.

4. DISCUSSION

The results indicate a relation between microtubule organization and cell morphogenesis. Clearly, the cortical microtubules during the early differentiation of root cells are perpendicular to the direction of expansion of the cell surface. These observations are in agreement with previous observations on expanding cells (MARCHANT 1979, GUNNING & HARDHAM 1982, WICK et al. 1981, TRAAS et al. 1984). At the parallel walls, which expand strongly in the direction of the cell axis, microtubules are always observed perpendicular to the cell axis. At the transverse walls of the cells which do not or expand little in all directions,

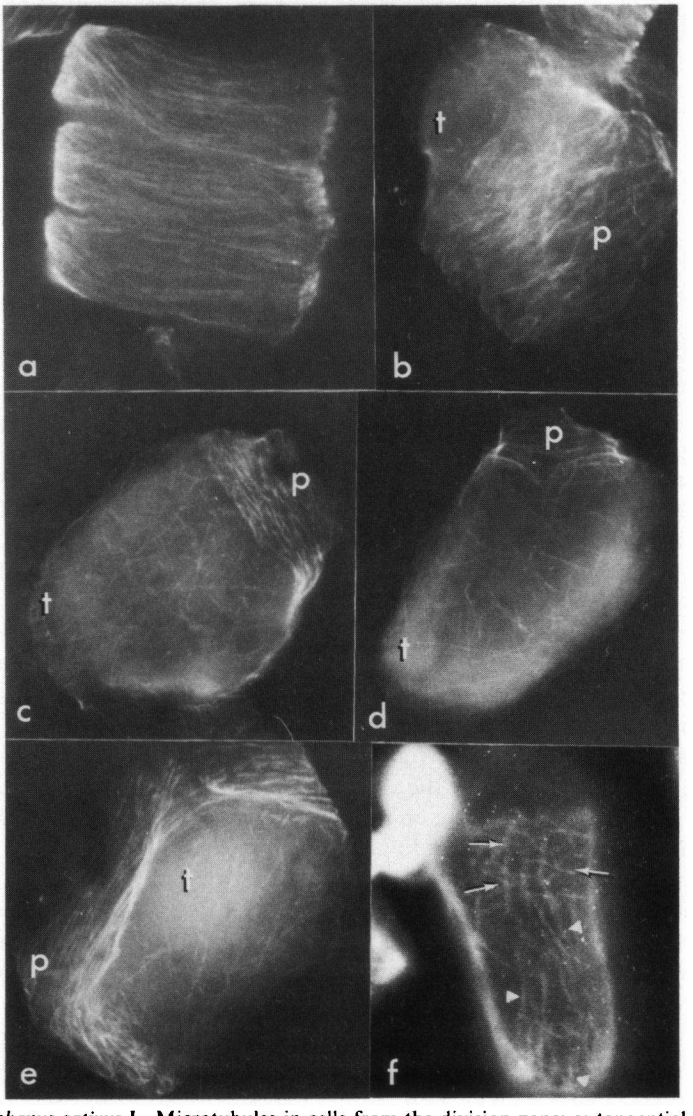

Fig. 4. *Raphanus sativus* L. Microtubules in cells from the division zone; a: tangential walls with the typically perpendicularly oriented microtubules; b, c: cells of endodermis, d: young epidermis and e: young trichoblast: perpendicular oriented microtubules occur at the parallel walls (p) and randomly oriented microtubules at the transverse walls (t); f: older trichoblast showing random and crossing microtubules (arrows), but also oriented microtubules (pointers) are visible at the transverse wall. Magn.: a-e: 1.440 ×; f: 960 ×.

random orientations are found. Similarly random orientations have been described for protoplasts and root hair tips (VAN DE VALK et al. 1980, TRAAS et al. 1985, see also the contribution of EMONS & DERKSEN in this issue). Thus

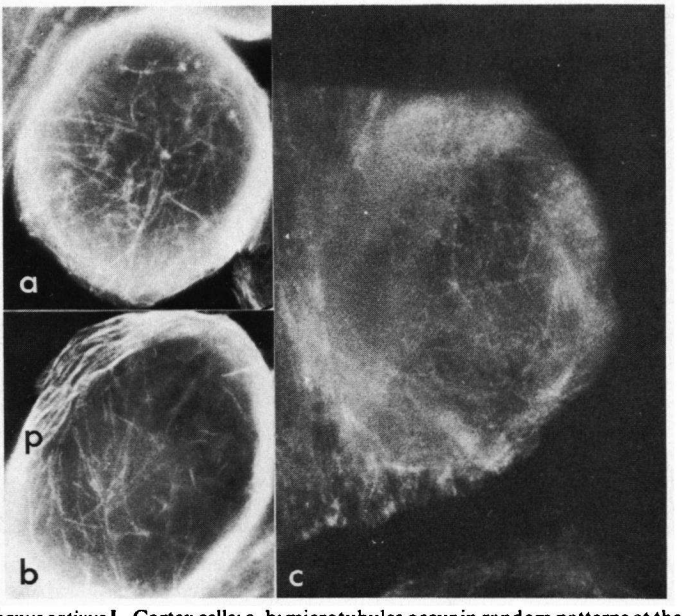

Fig. 5. *Raphanus sativus* L. Cortex cells; a, b: microtubules occur in random patterns at the transverse walls, in b the perpendicular orientation of the microtubules at the parallel walls (p) is visible; c: transverse wall of a fully expanded cortex cell showing randomly oriented microtubules. Magn.: 800 ×.

the relation between microtubule orientation and expansion at the cell surface as proposed previously (see f.e. GUNNING & HARDHAM 1982, TRAAS et al. 1984) clearly exists.

We especially consider the microtubules at the transverse walls of the trichoblasts and the loss of orientation of the microtubules in trichoblasts after ceasing of cell elongation important in this respect.

Whether microtubules limit cell expansion and thus play a direct and active role in cell morphogenesis, or whether their orientation is a result of the morphogenetic process remains to be established. However, present evidence (DUSTIN 1984, ROBINSON & QUADER 1982, GUNNING & HARDHAM 1982) indicates the first possibility. Also the high density of microtubules at the parallel walls as compared to the non-expanding transverse walls indicates an active function of microtubules in cell elongation (compare: HARDHAM et al. 1980, and GUNNING & HARDHAM 1980).

The results show that large differences in microtubule organization exist within a single cell or within a cell type. Such large differences have not been shown previously. In our view, the observation of such differences in microtubular organization within one single cell or within one cell type, is not consistent with the idea of a helix with pitches depending on the rate of elongation (c.f. LLOYD et al. 1985). Such a concept, which implies a more or less stable helix of interconnected microtubules (c.f. LLOYD et al. 1985), cannot be reconciled with current

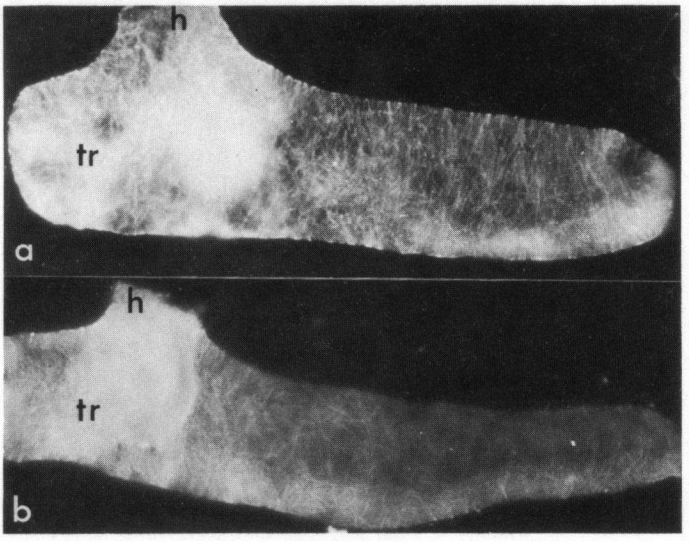

Fig. 6. *Raphanus sativus* L. Differentiated trichoblasts (tr) with hairs (h); a: from the lower root hair zone near the root tip with perpendicularly oriented microtubules; b: older trichoblast from the upper part of the root hair zone with more randomly oriented microtubules. Magn.: 480 ×.

models on the dynamics of microtubular skeletons (see f.e.: SCHULZE & KIRSCHNER 1986).

The changes in microtubule patterns are in strong contrast to those of the microfilaments that do not undergo essential changes during cell differentiation (DERKSEN et al. 1986).

In elongating cells there appears to be a relationship between the direction of the microtubules and the nascent cellulose microfibrils in the cell wall (HARDHAM et al. 1980; GUNNING & HARDHAM 1982). Since such a relationship is not always present in non-expanding cells (EMONS 1985, MIZUTA & WADA 1982, TRAAS et al. 1985, HAHNE & HOFFMANN 1985) and may even be restricted to expanding cells (see also the contribution of EMONS & DERKSEN in this issue), we believe cell expansion and co-alignment of microtubules and cellulose microfibril deposition to be related phenomena. Such a relationship, however, is not compatible with present models for the microtubular control of cellulose microfibril deposition (see: ROBINSON & QUADER 1982).

A model concerning the possible relationship between cell expansion and microtubular control of cellulose deposition will be presented in the near future.

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