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# Microtubular cytoskeleton and root morphogenesis

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## Abstract

Although the microtubular cytoskeleton of plant cells is important in maintaining the direction of cell growth, its natural lability can be harnessed in such a way that new growth axes are permitted. In these circumstances, the system which fabricates the cytoskeleton is presumably responsive to morphogenetic information originating from outside the cell. Spatial patterns of hormonal and metabolic signals within the tissue or organ that house the responsive cells are one possible source of this information. However, a contrasting source takes the form of biophysical information, such as the supracellular patterns of stresses and strains.

We examined the microtubular cytoskeleton in roots of tomato and maize to test the assumption that the cortical microtubular array of each cell would have a particular orientation relative to the cell's position within the growth field of the root apex. Accordingly, each intracellular cortical array was mapped to the overall pattern of cells within the apex. In certain areas of the meristem, the arrays seemed to be more variable than elsewhere. These are sites where morphogenetic decisions are taken, usually involving a change in the plane of cell division. Roots which have suffered disturbance to their physical structure (e.g. removal of the root cap), or which had been exposed to low temperatures or treated with certain chemicals (e.g. inhibitors of nuclear division or DNA synthesis), exhibited new patterns of microtubular arrays which in turn predicted novel patterns of cell division. In all these circumstances, the arrays showed consistent alterations within distinct regions of the root - e.g. in the quiescent centre and also in a group of cells just behind the quiescent centre, at the boundary between cortex and stele. These altered arrays indicate that there are supracellular domains in which the microtubules respond to morphogenetic signals. Studies such as these reinforce the concept of microtubule lability and the inherent responsiveness of the microtubular system to external and internal stimuli. However, at present there is no indication of how the morphogenetic programme of the root is set up in the first place. Probably, this is established and stabilized early in embryogenesis and is then perpetuated by the prevailing metabolic and biophysical conditions. The microtubules of the cytoskeleton can be regarded as intracellular automata which not only participate in mitosis and cytokinesis but also ensure the realization of an organogenetic programme. Should the root confront circumstances which temporarily destabilize this programme, the prevailing growth field is sufficiently robust to ensure that the microtubular system is attracted back to the stable, pre-existing state capable of reinstating normal morphogenesis.

## Introduction

The puzzle of plant morphogenesis was clearly evident to J W von Goethe, one of the foremost universalists of his time. His concept of 'transformation', as outlined in Metamorphose der Pflanzen of 1790, can be construed as an attempt to understand how the form of any one plant part may literally transform into another. Whereas form is a somewhat abstract and idealized notion that harks back to the Platonic tradition, the plant organ possessing that form is somewhat more definite, even though the definition and classification of such organs continue to have a subjective basis (Hay and Mabberley, 1994). Therefore, alongside any ontogenetic transformation of organs in time and space,

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there are also the internal events that comprise organ development. Generally, development is thought of in terms of gain or loss of metabolic processes as the organ in question passes from one morphological state to another. Thus, there are evidently two inextricably linked aspects to the morphogenetic puzzle: the unfolding of organic form and the simultaneous acquisition of organ identity and function. Both await substantial clarification.

What is required in the case of morphological transformation are clear concepts for the organic basis of form. Given that the plant world is hierarchically organized, and each level in the hierarchy is supported by a canonical set of sub-systems (Barlow, 1987), then the form of an entity at one level of organization (level $n$, say) can be understood as an emergent property: that is, form is the outcome of interactions between sub-systems which support the immediately lower organizational level (level $n$-1). Specifically, in the case of organs (e.g. level $n$), form is governed by the properties of two sub-systems, the boundary and the supporter (Barlow, 1994), which belong to the cellular level, $n$-1, and which, in this case, are represented by the epidermis and its cuticle (and sometimes pericycle) and by the network of parenchymatous cells, respectively (Table 1). The same also holds true at the cellular level itself where the respective boundary and supporter sub-systems correspond with cell wall (extracellular matrix) and cytoskeleton (microtubules and microfilaments), these being entities of the sub-cellular, macromolecular level of organization ($n$-2) which exists at the interface between living and non-living material. Since it is not possible within a living system to pass to a yet lower level, wall and cytoskeleton must, if a reductionist concept of morphogenesis is admitted, represent the two most fundamental effectors of morphogenetic processes.

## Microtubules and morphogenesis

The plant cytoskeleton may be said to have been established with the observations of Ledbetter and Porter (1963) that groups of microtubules are located under the plasma membrane in the cortical cytoplasm (hence the term cortical microtubules, or CMTs), and that they run parallel to the principal direction of the most recently deposited cellulose microfibrils in the cell wall. In the intervening 30 years, much evidence has been adduced that this co-orientation of MTs and microfibrils is not one that occurs by chance, but that in all probability it is evidence of a structural/functional relationship between the two classes of macromolecules (Cyr, 1994; Giddings and Staehelin, 1991). The consensus view is that the CMTs are associated not only with localized sites of cell wall extension but also with a corresponding pattern of wall synthesis that involves the deposition of cellulose microfibrils. It is the orientation of the CMTs which predicts the orientation of the microfibrils and this then influences the orientation of the subsequent pattern of wall extension. Thus, if we consider a hypothetical cuboid cell (Figure 1) in which each of the walls is associated with CMTs and that the CMTs along four 'side' walls are oriented in the same direction parallel to the upper and lower edges of the walls, then extension of the cell would be anisotropic and in a direction perpendicular to the orientation of these parallel CMTs (Figure 1b). The random orientation of the CMTs and of the corresponding set of microfibrils on the two 'end' walls of the cell might modify the overall pattern of three-dimensional volumetric growth of the cell, but would not change the predominant direction of growth. However, given the apparent independence of CMT orientation at certain of the facets of a cell (Ishida and Katsumi, 1992), or even at different sub-domains of a wall facet, the pattern of cell wall growth can be further modified and different cell shapes can arise as a consequence (Figures 1a,c). Since these modifications trace to the details of CMT orientation, it may be concluded that CMTs are directly involved in cellular morphogenesis (Table 1) and hence contribute a means of influencing the unfolding form of organs.

A second system of MTs occupies the interior of the cell. This endoplasmic microtubular (EMT) system spans the cytoplasmic space between the surface of the nucleus and the cell cortex (Baluška et al., 1992). Unlike the CMTs, the EMTs do not seem to have any direct role in the orientation of cell growth. One of their functions may have to do with intracellular signalling and intracellular (or even intercellular) transport. Because MTs generally are in dynamic equilibrium with a pool of unpolymerized tubulin dimers, the system of EMTs is ideally poised to be continually making and breaking these channels of communication as the dynamics of the situation dictate. The attachment of EMTs to the nuclear envelope would also seem significant in this respect since, if they do have the above-mentioned communicative functions, they would be in an ideal position to relay information to and from the ultimate informational store embedded within DNA and chromatin. A second function of

*Table 1.* Contributions of two sub-systems (i and ii) which support the cellular level of organization (level $n - 1$) to the emergent properties of morphogenesis at the next, higher, organ level (level $n$) of plant organization

| Level | Sub-systems | Representatives | Morphogenetic programme |
|---|---|---|---|
| Cell ($n - 1$) | i) Boundary; | i) Cell walls; | Cell wall differentiation; |
| | ii) Supporter | ii) MTs | Cell shape |
| Organ ($n$) | i) Boundary; | i) Epidermis and pericycle; | Organ differentiation; |
| | ii) Supporter | ii) Cell network | Organ shape |

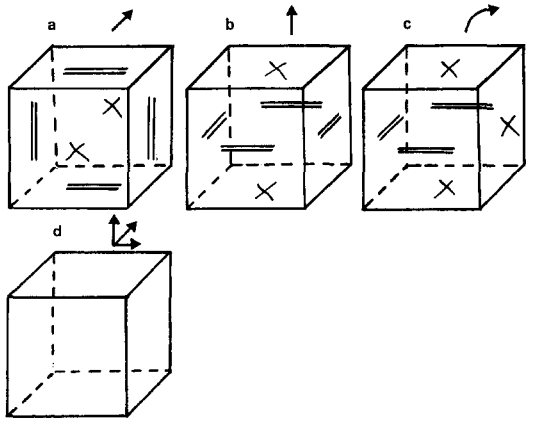


*Figure 1.* Notional cuboidal cells with CMTs whose arrays are independently arranged on each wall facet. The arrays are indicated as being either parallel (||), random (×), or absent (unmarked). The cell grows in accordance with the orientation of the CMTs, in the direction(s) indicated by the arrows above the cell: (**a**) radially, (**b**) longitudinally, (**c**) differentially, e.g. faster on one side than on the opposite side, (**d**) isotropically.

EMTs may be their participation in constructing the pre-prophase band (PPB) of MTs (see Figure 2) which apparently infallibly predicts the site of attachment of a new cell wall formed at cytokinesis (Wick, 1991).

*Microtubules, root growth and root morphogenesis*

Once a root has become established as an independent organ as a result of a series of differentiation processes that occur within the early embryo (Barlow, 1996), it exhibits a continuing morphogenetic programme which runs in parallel with a developmental programme perpetuated within its apex. A number of processes within the root permit the execution of the morphogenetic programme at both the cell and organ levels of organization. At the cellular level, there are processes which (1) lead to the shape, differentiation, and specialised function of each type of cell within each of the root tissues. Well known examples are the differentiation, from ground parenchyma, of xylem and phloem elements, each with characteristic modifications to their walls. Then, at the organ level, there is (2) the shaping of the root apex which involves the differential growth of tissues and their boundaries so that the cortex, for example, widens with an accompanying increase in the number of its cell files. Later, there is (3) the initiation and establishment of new axes of root growth such as occur in (a) the development of cambium and its derivatives, and (b) the inception, in the pericycle and endodermis, of primordia for lateral roots and occasionally shoots. In all cases, these controlled modifications of growth are affected by the boundary sub-system of the root: at the cellular level, these modifications apply to the cell wall, whereas at the organ level, they apply both to the outer epidermis and cuticle and to the outer pericycle wall, the former being involved with cortical widening, the latter with the initiation of new organs (primordia).

It is fairly evident that, in the case of cellular morphogenesis, MTs are involved in the synthesis and patterning of the cell wall. In the case of the shaping or initiation of root organs, MTs may also play a role in regulating the plastic properties of the boundaries, but this is by no means certain. CMTs do, however, influence the orientation of cell growth once an incipient organ has been established (Barlow, 1994; Green and Selker, 1991), as in lateral root primordium initiation, for example. Possibly, the EMTs are involved in growth axis establishment by influencing both the construction of the PPB, and hence the plane of cell

division, as well as the subsequent initiation of CMTs in the daughter cells.

The interaction between MTs and growth orientation becomes more evident when MTs are depolymerised. Cells, at least in the cortex of the root, then tend to grow isotropically (Figure 1d), causing the root to swell radially at the expense of its expected longitudinal enlargement. But whether MTs influence the rate of cell growth is doubtful; it is more likely that this rate is responsive to materials which, having passed into the wall, affect its yield threshold (e.g. Edelman and Sievers, 1995), although it is possible that the number of CMT's could influence the rate at which such materials are attracted to sites beneath the walls. The orientation of the CMTs, because of its relationship with microfibril orientation, could influence the yield threshold of the walls through the biophysical properties of the cellulose network. When cell growth slows at the proximal limit of the extension zone of the root, the CMTs become longitudinally oriented, a feature that would preclude further growth in that direction. It is possible, however, that under certain circumstances this longitudinal orientation of CMTs could be deployed to bring about negative longitudinal growth of cells, as occurs in the root contraction phenomenon of geophytes (Smith-Huerta and Jernstedt, 1989). Self-cinching within the parallel arrays of longitudinal CMTs might lead to the shortening of the cortical cells.

## The origin and development of microtubules in meristematic cells of the root

One of the most important zones for root morphogenesis is the meristem. Here there are two classes of cell division, formative (longitudinal with respect to the cell file axis) and proliferative (transverse); the former class of divisions increases the number of cell files, the latter increases the number of cells in each file. It is important, therefore, to know how the cytoskeleton is deployed throughout the mitotic cell cycle because morphogenetic processes in the root depend on the dynamic properties of its MTs and their ability to bring about one or other class of cell division when conditions for each step of the morphogenetic programme are fulfilled. One key to understanding the controls which specify whether a transverse or a longitudinal division will occur resides in an ability to predict the orientation of the PPB. Unfortunately, the conditions that have to be fulfilled in order to orient the PPB in any given direction within the cell are unknown at present.

Microtubules are a major component in the construction of not only the MT cytoskeleton and PPB of interphase cells, but also the spindle and phragmoplast apparatuses upon which the segregation of chromosomes and the division of cells depend (Baskin and Cande, 1990; Cyr, 1994). Because of the dynamism of the various MT systems throughout the course of the cell cycle, the corresponding MT configurations could be regarded as a series of states that occur in a particular sequence during the course of one interdivisional period and which is then reiterated during the next period. In this sense, the entire MT system behaves like a deterministic intracellular automaton, its state (configuration) at time $t$ having been predicted by a former state at $t$-1. The same might hold true of chromatin structure which also shows predictable and sequential transformation of states during the course of interphase and mitosis, and which are reiterated during the next interdivisional period. The states of MTs and chromatin might even be interdependent, or at least interrelated.

It is easier to understand the various transformations of the MT system if the CMT arrays are considered separately from those of the EMTs. The justification for this is that the EMTs are, in all probability, the outcome of the activity of a centrosome-like apparatus (or cell body) which is associated with the nucleus (Mazia, 1987, 1993) and are therefore subject to a control which is separate from that of the CMTs. Whereas CMTs are concerned with cell wall synthesis and hence with cell growth and morphogenesis, EMTs have a more complex role in the life of meristematic cells, most evidently because of their association with the processes of nuclear and cell division (Baluška et al., 1996a). EMTs may possibly also assist in the construction of the CMT population (Figure 2). The idea behind this notion is as follows. Early in interphase, immediately following the reformation of the nuclear envelope around the telophase chromatin, there are no MTs anywhere in the cell. A little later, EMTs begin to radiate in all directions from the nucleus and to make contact with the plasma membrane (Figure 2a, 2b). The EMTs are assembled at MT-organizing sites on the outer surface of the nucleus (see Lambert, 1993; Stoppin et al., 1994), the material of these sites having perhaps accumulated on the nuclear surface following its synthesis within the early interphase nucleus. Given the continual synthesis and perinuclear accumulation of MT-organizing material during the interdivisional

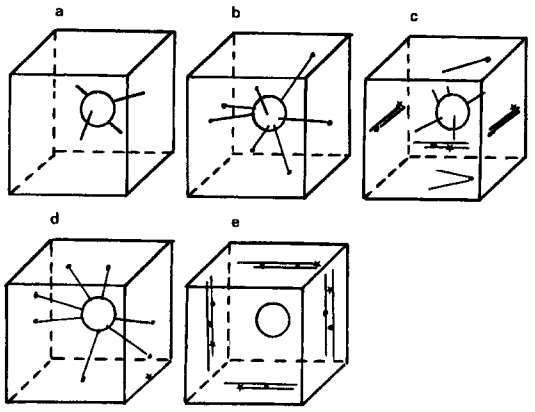


*Figure 2.* Schematic developmental relationship between EMTs and CMTs. (**a**) During early interphase, EMTs radiate out from the nuclear surface (ó) and (**b**) deposit MT-organizing material (•) on the inner surface of the plasma membrane. (**c**) This material is able to nucleate CMTs which then extend over the membrane surface becoming linked to it, and to other CMTs, by means of MT-associated proteins (*). (**d**) Later, during interphase, CMTs are dismantled and EMTs, which continue to radiate from the nucleus, 'seed' a new population of CMTs which becomes (**e**) the pre-prophase band that predicts the site of attachment of a new cell wall following mitosis.

period, it is possible that some of it is propelled along the endoplasmic MTs, either at their growing (+) ends or along the tubules themselves, and then becomes attached to the plasma membrane (Figure 2b). The MT-organizing material acts by lowering the threshold for the polymerization of tubulin in its vicinity. Thus, wherever this material is located, be it at the nuclear envelope, or *en route* from the nucleus to the cell cortex, or at its destination on the plasma membrane, MTs will assemble. Chance assemblage at organizing sites *en route* could account for the branching of EMTs within the cytoplasm (seen with confocal microscopy), the branch point being where a piece of organizing material, associated with an EMT, has facilitated the polymerisation of a new MT. At the plasma membrane itself, pieces of MT-organizing material, stabilised by additional links to the cell wall (Akashi and Shibaoka, 1991), serve as sites at which the CMTs can then begin to assemble (Figure 2c). Thus, each cortical site of CMT assemblage represents an earlier point of contact of an EMT with the plasma membrane and the concomitant successful deposition of MT-organizing material. This deposition occurs against both the transverse (anticlinal) and the longitudinal (radial and periclinal) walls in meristematic cells (Figure 2b).

The organization of the CMTs as arrays of various types (random, parallel, parallel bundles, etc.) within the various types of cells of the root apex, requires additional explanation (Figure 3). As mentioned, CMTs grow out from their organizing sites on the plasma membrane and extend parallel to it, perhaps being guided over its surface by proteinaceous linkages (microtubule-associated proteins, MAPs) between the CMTs and the plasma membrane, and being assisted by similar linkages between the CMTs themselves (Figures 3(2), 3(3)). The orientation of the CMTs - predominantly as transverse hoops around the longitudinal walls of the cell - might be favoured by these additional linkages between CMTs. This parallel orientation of MTs might, in turn, lead to a self-cinching phenomenon resulting in the further alignment of CMTs against all longitudinal walls (Figures 3(4), 3(5)). However, the potentially disruptive effect upon the protoplast of such a self-cinching phenomenon would need to be counteracted by osmotic pressure so that the plasma membrane is not pulled away from the wall by the tightening CMTs bundles. It is also possible that, in new-born early interphase meristematic cells, CMT alignments transverse to the cell axis are established as a result of an activation of already aligned but latent organizing sites (Figure 3(1)) that were present in the mother interphase cell. Thus, a memory of the direction of cell growth could be inherited by each of the two daughter cells of a mitosis as a consequence of the structure of the wall/plasma membrane/MAP complex that was present in the mother cell. This memory may be important for perpetuating the longitudinal growth and transverse divisions of proliferative meristematic cells. However, new axes of growth could only be established if this source of structural information was eradicated and the subsequent placement of CMT-organizing sites was solely determined in the way outlined earlier (see Figure 2). Absence of any cue for MT alignment, as would be expected at the surface of a newly formed transversal (anticlinal) wall, would account for the random arrangement of CMTs at such walls, though parallel arrangements, as a result of lateral interactions between CMTs, may form on these walls in later cell generations in response to new organogenetic conditions.

The tendency to form parallel arrays of CMTs may be enhanced by the predominant direction of wall expansion which, hence, sets up a reinforcement loop of these modes of MT (or MT-organizer) and growth orientation: parallel MTs → growth orientation → parallel MTs → ..., etc. This would conform to pathway c→d→c... in Figure 4. Such a loop of information may explain some of the difficulties in deciding whether CMT alignments are a cause or an effect of some of

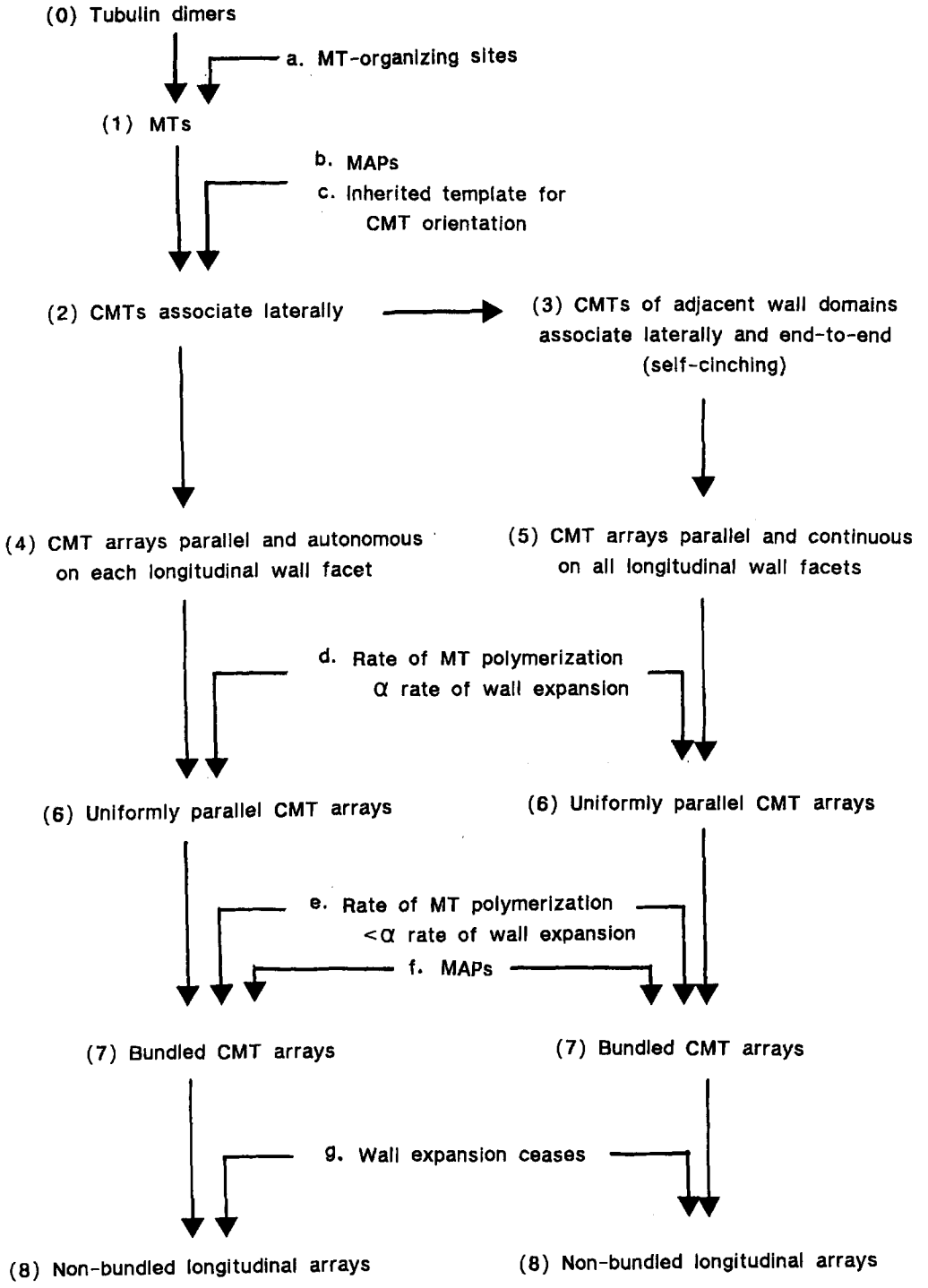


*Figure 3.* Scheme to show the evolution of various CMT arrays, or states, on longitudinal cell walls within a growing root apex, from their simplest components (0) to their final state (8). The development of the microtubular system here passes through nine states (0-8) governed by various pre-conditions (a-g), which are not exhaustively catalogued.

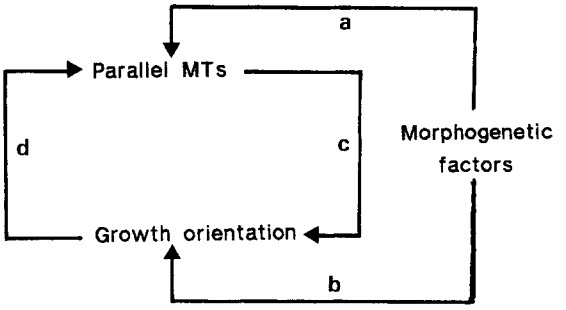


*Figure 4.* Scheme showing the possible interrelationships between morphogenetic conditions which influence CMT orientation and the predominant orientation of cell wall extension. Information determining the latter can be supplied along two alternative pathways, a→c or b→d→c.

the switches in CMT orientation that accompany morphogenetic events (Barlow, 1993a; see also Green and Selker, 1991). Usually, it is believed that pathway a→c of Figure 4 is utilized (which might then continue as c→d→c...), but it might also be possible that pathway b→d→c could be followed in certain circumstances. This latter pathway could be important in establishing new axes of growth, particularly if the information for MT orientation (at step d) which is usually handed-on from one cell generation to the next could be erased. Hormonal morphogenetic signals may be able to effect this erasure for a long enough period to enable the setting up of new EMT and CMT configurations.

Further states of the MT arrays (Figures 3(6), 3(7)) could occur as a result of altered relative rates of cell wall expansion and MT polymerization (a reflection of tubulin synthesis rate?) so that those CMTs which are already linked to one another in parallel arrays continue to remain together, while at the same time gaps appear between these arrays as the cell continues to elongate. This state may also be favoured by the synthesis of a particular class of MT-associated proteins necessary for MT-bundling, or by developmentally related changes to the metabolic state within the cell; suppression of rRNA synthesis, for example, was found to favour the formation of gaps in CMTs arrays (Baluška et al., 1995). Such gaps are a natural accompaniment of certain types of cellular morphogenesis in roots (e.g. the pit fields of xylem vessels) (Chaffey, submitted). The final state shown for the CMT population (Figure 3(8)) is where the CMTs are aligned, not perpendicular to the direction of growth, but parallel to it. Such a parallel co-orientation of a wall synthesising template with the growth direction was originally proposed by Green (1962); but it is found in root cells nearing the end of their elongation phase.

## Three studies of the involvement of MT in root morphogenesis

The ways in which MTs might be deployed to effect root morphogenesis can be studied by either interventionist (experimental) or non-interventionist (purely observational) methods. In the latter method, the pattern of MTs in undisturbed roots is examined and related to the morphogenetic programme. Any connection between the patterns of MTs and cell growth is only an inference, being based upon experience from other systems. In the former, experimental, approach MTs can be perturbed, either directly or indirectly, and the effects of this on root morphogenesis examined. Again, any conclusions as to the relationship between MTs and the processes which they are presumed to regulate are drawn by inferences from the undisturbed state. Another experimental approach, the converse of the previous one, is to try and disturb root morphogenesis more directly, or more locally, and see how this impinges on MT behaviour.

### *1. MTs and cell division pattern in tomato*

This study exemplifies the non-interventionist approach, making use of correlations between MT patterns and cellular patterns, the development of the latter being considered as a morphogenetic phenomenon (Barlow, 1995).

Roots of tomato (*Lycopersicon esculentum* L., cv. Moneymaker) were grown in modified White's medium using a weekly sub-culturing regime to maintain a population of proliferating meristems. Actively growing root apices were fixed on the 6th day following subculture, embedded in Steedman's low melting point wax (m.p. 37 °C) and sectioned longitudinally. After reacting the microtubules with an antibody raised in mouse to chick brain $\alpha$-tubulin, they were stained with FITC-conjugated antimouse IgG raised in goat (Baluška et al., 1992). All the cells seen in a median section were recorded for the construction of a cellular map of the apical portion of the meristem and the orientation of the CMTs within each cell was assessed. Notes were also made of the appearance of the EMTs which were usually clearly visible when CMTs were not, and vice versa (the CMTs being present in another section of the cell). By using median longitudinal

sections, it is the CMTs against only the longitudinal-radial, or near-radial, walls that can be seen. Tangential and anticlinal walls are cut perpendicularly in median section and hence the orientation of their CMTs cannot be seen. However, tangential walls are evident in non-median sections: the first longitudinal sections to pass through epidermis, for example, reveal only tangential walls and their CMTs. The CMTs against anticlinal walls are evident in transverse section (Barlow, 1995).

Median sections are especially useful because they reveal the radial and longitudinal arrangements of the cell files. Then, assuming steady-state development at the root apex, it is possible to infer regularities of cell production. It is evident, for example, that in the case of the cortex the files have a regular pattern of longitudinal branching by means of periclinal cell divisions. These divisions occur in quite precise positions along the innermost file of cortex (presumptive endodermis), and it is possible to predict which cells in this file are about to undergo a periclinal division (Barlow, 1993b, 1995). If located close to the root tip, both daughters of a cell that has just completed a longitudinal division may undergo another such division, or, if such cells are located more proximally, each will undergo one or more transverse divisions. Longitudinal cell divisions can be of two types: radial or periclinal. Only cells about to undergo the periclinal division can be unequivocally identified in longitudinal median section, though by using transverse sections the manner in which cell numbers in the concentric rings of cortical cells increase as a function of distance from the tip can also be used to predict where radial divisions occur.

The topic of interest in the tomato roots was whether cells in the inner cortex, particularly in the zone where longitudinal cell divisions occur, showed CMTs arrays different from those in cells of other cortical cell files where transverse divisions predominate. The results of our survey are presented in Table 2. It is immediately apparent that the innermost cortical file has many more cells with a randomized array of CMTs than do cortical files elsewhere (Barlow, 1995). EMTs mostly radiated into cytoplasmic space from organizing sites on the nuclear membrane. It was on the basis of their numbers and distribution that the scheme for MT dynamics in Figure 2 was proposed.

Clearly, cells of the innermost file are enlarging in both the radial and the circumferential directions simultaneously. This enlargement permits a regular sequence of periclinal and radial divisions. It is logical to assume that the more isotropic growth of these cells is a consequence of the more random orientation of the CMTs. However, as mentioned, there is the possibility that the pattern of cell enlargement may also influence the orientation of the CMTs, if information passes in such a way that pathway b→d→c of Figure 4 prevails. The occasional presence of cells in other files with random CMTs on the radial walls may be an indication that such cells are preparing to divide radially since there are cells in the central and outer cortex which enlarge in a circumferential direction as well as longitudinally, but which lack the radial growth component. It must remain an open question at present as to whether the pattern of growth and division is solely determined by the CMTs (via pathway a→c in Figure 4), in which case it is imperative to identify the file-specific and MT-specific morphogenetic signals, or whether an alternative pathway (via b→d→c of Figure 4) can contribute to cortical tissue morphogenesis. The fact that CMT orientation is sensitive to low amounts of auxin (Blancaflor and Hasenstein, 1995; Baluška et al., 1996b) and that auxin is tranported acropetally in the stele of roots, permits speculation that the stele/cortex junction near the root tip may be leaky to auxin and that this has consequences for the observed patterns of CMT and cellular behaviour and hence for root morphogenesis. Although auxin may favour isotropic growth, whether this is due to its effect on MT orientation (Baluska et al., 1996b) or to some more direct effect on cell wall plasticity remains to be elucidated.

*Table 2.* Orientations of cortical microtubule arrays (CMTs arrays may be: T, transverse; O, oblique; L, longitudinal; R, random) against the longitudinal radial walls of cells in successive rows across the cortex of tomato roots. Row numbers 1 and 6 are the innermost (endodermal) and outermost (sub-epidermal) cortical rows of cells, respectively

| | Percentage of CMTs in cells of row number | | |
|---|---|---|---|
| CMT array | 1 | 2-3 | 4-6 |
| T | 50 | 89 | 88 |
| O | 2 | 0 | 0 |
| L | 0 | 0 | 0 |
| R | 48 | 11 | 12 |

*2. MTs in maize roots and their response to removal of the root cap*

Microsurgical removal of the root cap also removes some of the information that specifies the normal course of maize root morphogenesis. Instead of the remaining portion of root continuing faithfully to execute its existing developmental and morphogenetic programmes, it reorganizes itself in such a way that a new cap is regenerated (Barlow, 1974). This regenerative process requires about 4 days to be completed, at least as judged by anatomical criteria. Renewal of the cap involves shifts in the pre-existing orientations of growth and division of the remaining cells at the root apex. How this reorientation is regulated is largely unknown, but presumably positional information is involved, the relevant signalling system making use of chemical (hormonal), biophysical and bioelectrical components. In the present context, the question is whether the rearrangements of the CMT populations, which occur in response to decapping are a necessary and sufficient determinant for cap regeneration.

As was done for tomato roots, so for maize a map of CMT orientations was prepared for all cells and tissues which comprised the apex of each intact root (including the cap). The generalized MT map, as well as the underlying cellular map to which the MT map relates, served as a reference against which the degree of root apex regeneration could be judged in the decapped samples (Figure 5).Throughout the undisturbed maize root apex, a high proportion of cells had CMTs with a transverse orientation with respect to the cell file axis (as did cells in the tomato apex), though a sizeable proportion of them, especially in the quiescent centre (QC), showed a random orientation. Longitudinal CMT arrays were generally absent. Six hours after removal of the cap, the orientation of CMTs was different. Longitudinal arrays had now become evident in an appreciable proportion of cortical cells and the number of cells with random CMTs increased also (Figure 5a). On the other hand, the original transverse CMT orientation tended to persist in the stele (Figure 5b). At this time (6 h), the cells of the QC began to display either longitudinal, random or transverse CMTs in roughly equal frequencies, but later their randomization increased (Figure 5c).

The frequencies of transverse and random CMT orientations within the various zones of the root seemed to fluctuate during the course of cap regeneration, as though there was a pulsation of some 'cytoskeletal organizer' to which the patterning of the CMTs was responsive. Even on the 4th day, when the cellular pattern at the apex was virtually normal, the CMTs did not show complete correspondence with the undisturbed, control pattern, but had a pattern which appeared to conform more to that anticipated of a slowly oscillating sequence of changes (e.g. Figure 5d).

Certain files of cortical cells showed a characteristic response to decapping. Here, many cells displayed longitudinal CMTs at about the second day of the recovery period (Figure 6). An expectation of this orientation of CMTs is that it should favour radial or tangential cell growth and so would be followed by longitudinal cell division. This was found. The significance of the longitudinal CMT orientation in such a file of cells in the cortex is that it co-locates with the boundary which regenerates between the original root and the new root cap. The outer daughter cells of the ensuing longitudinal divisions are presumed to retain the longitudinal CMT orientation which, as the apex continues to be remodelled by regeneration (epimorphosis), becomes a transverse orientation with respect to the growth axis of the new cap meristem, a tissue which the descendents of these cells then do indeed construct. The CMTs of the inner daughter cells, by contrast, revert to their original predominantly transversal orientation (which now would be perpendicular to the orientation of their sister cells) and the cells go on to develop a new epidermis. This apparently crucial step in cap regeneration would seem to be directly mediated by the CMTs. But how is the longitudinal CMT arrangement in this group of cells initiated by the loss of the root cap? All previous information available to these cells, which favoured their usual transverse CMT orientation and transverse cell division, is obviously no longer being supplied. It may be that, following decapping, the inherited intracellular template for CMT orientation (see Figure 3(1)c and pathway c→d→c in Figure 4) is erased and all new-born cells now respond solely to extracellular informational cues. Should morphogenesis of organs be conditioned by the boundary system as proposed by Barlow (1994) (see also Green and Selker, 1991, and Ishida and Katsumi, 1992), then it can be suggested that a new boundary between root and cap should be quickly reestablished for root apex morphogenesis to continue. Thus, CMTs may respond in accordance with pathway b→d→c in the scheme shown in Figure 4. In the cortex of the regenerating maize root apex, the stimulated radial extension of the previously anticlinal (transverse) and radial walls may force a reorientation of their CMTs into a parallel array.

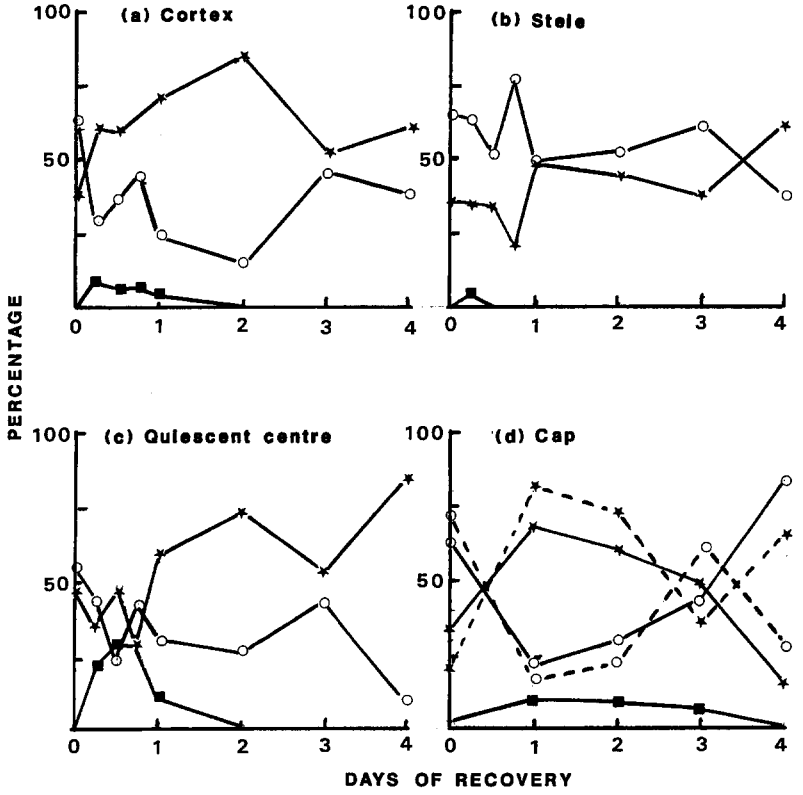


*Figure 5.* Proportion of cells in maize root apices with CMTs arrays which show the following orientations (with respect to the cell file axis): transverse (○), random (★), or longitudinal (■) in the cortex (**a**), stele (**b**), quiescent centre (**c**) and cap (**d**). In the cap, columella and flank regions are identified by solid and broken lines, respectively. The apex was decapped and allowed to regenerate a new cap over a 4-d period. Data from the undisturbed, intact tip are presented at time 0 d.

This may then go on to promote the radial direction as the predominant axis of cellular growth.

The QC of the maize root is stimulated to cell proliferation by decapping, but whether this is brought about by perturbation to the MTs was not established. Elsewhere it was suggested (Baluška and Barlow, 1993) that breakdown of the EMT population in the QC favoured the commencement of nuclear DNA synthesis and the loosening of chromatin. MTs may have to be absent only for a brief period for these responses to be initiated; so, unless roots are fixed at close intervals after decapping, this crucial period of MT absence could easily be missed, The QC is a zone where the original CMT pattern has definitely not been recovered by the 4th day of regeneration (Figure 5c) at which time the characteristic low proliferative rate of this zone is nevertheless restored (Barlow, 1974). Likewise, most of the new cap does not show a MT pattern similar to the pattern in the intact original cap, although this finding does not hold for the cap columella where the original CMT patterns are recovered (data not shown). Interestingly, both the central cap and the QC are zones where actin is poorly represented (Baluška et al., 1996c) suggesting that actin microfilaments might participate in reinforcing CMT orientation.

All the above findings tend to strengthen the notion implicit in Table 1 that, although CMTs may influence the shape of individual cells and the direction in which they grow and divide, it is by no means certain that MTs, in some collective manner, guide root morphogenesis. There is not much evidence that there are supracellular CMT domains responsible for the overall shape of the organ. On the other hand, it could be that there are some informational signals (bioelectricity, pattern of stresses and microfilament orientations) which do have an influence on CMT orientation at a supracellular level and which tend to attract the CMTs into a generally correct orientation for morphogenesis at a local cellular level within the root tissues. Even if there are no supracellular CMT domains, it is possible that the EMTs could form an extensive interconnected

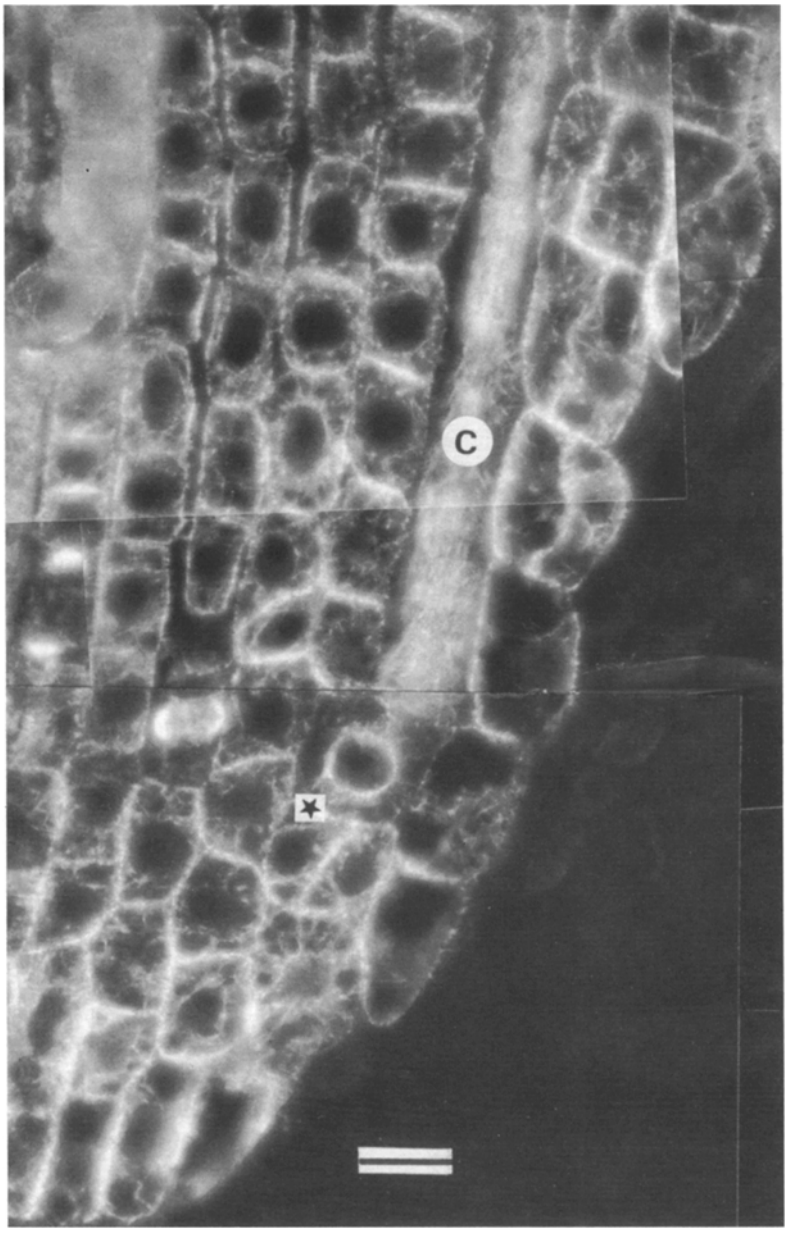


*Figure 6.* Longitudinal arrays of CMTs in a file of mid-cortical cells (C) close to the apex of maize roots as a response to decapping 18h earlier. Other cells (or cell-pairs) of the cortex appear to be wider than usual (some of these indicated by *). The stimulus to widening may have led to the longitudinal CMT orientation Scale *bar* = 25 $\mu$m.

network (Cyr, 1994), collating and transducing positional information in circumstances such as induced by decapping and thereby guiding the PPBs, when they form, into correct orientations for continued root morphogenesis.

### *3. MTs in maize roots and their response to inhibitors of DNA synthesis*

When inhibitors of nuclear DNA synthesis are presented to maize roots they have the paradoxical effect of inducing DNA synthesis in the quiescent centre. The reason for this is unknown, but we hypothesise that

information about the inhibition of DNA synthesis, which is widespread within the major part of the meristem, is communicated to the QC from its surrounding cells by MTs (either EMTs or CMTs) as though all MTs within the apex were connected and were part of some giant supracellular cytoskeletal lattice which also involved actin microfilaments.

Three inhibitors of DNA synthesis were employed: 5-aminouracil, 5-fluorodeoxyuridine and hydroxyurea. Each of the three inhibitors affect synthesis at a particular stage of the S phase: at the S/$G_2$ boundary, generally throughout S, and in early S, respectively. In no case is the block absolute, unless the inhibitor is exerting a toxic effect. The stage of DNA synthesis inhibition was also reflected in the MT configurations within the affected meristematic cells. Pre-prophase bands were abundant in S/G2-blocked cells of aminouracil-treated roots; arrays of CMTs and EMTs typical of cells in S phase were evident in the presence of the other two inhibitors.

Effects of fluorodeoxyuridine ($10^{-2}$ m$M$) on MT patterns were studied in some detail and related to the corresponding pattern of DNA synthesis in autoradiographs of root apices. Whereas DNA synthesis was depressed throughout the meristem following up to 24h of treatment, synthesis was maintained and even increased in the QC, as judged by both the intensity of nuclear labelling with $^3$H-thymidine and the number of S phase cells. This stimulation of DNA synthesis in the QC was accompanied by an increased fluorescence of the MT population in cells of this zone. A similar pattern of MT fluorescence was evident in the QC after treatments with aminouracil and hydroxyurea also. Striking, however, was the finding that CMTs in cells at the stele/cortex boundary showed a characteristic shift from a previously transverse orientation to one that was longitudinal (Figure 7). This group of cells also showed an elevated level of nuclear DNA labelling. Other work with maize roots (Barlow, unpublished) has shown that these same boundary cells were active in longitudinal divisions when the roots were decapped and grown in either hydroxyurea or aminouracil. A stimulation of DNA synthesis in aminouracil-treated pea roots was also recorded by Van't Hof and Lamm (1992). They conjectured that the additional synthesis occurred in the QC.

The present work indicates that under certain conditions a longitudinal CMT orientation will appear in selected cells of the root apex. It would seem that some hitherto unrecognized or unappreciated property of divisional control in the root apex is being highlighted by the inhibitor treatments and this becomes clearly evident when CMT orientation is examined. Moreover, these are groups of cells, important in establishing cellular patterns. For some unknown reason they are able to resist to inhibitor treatments and continue to maintain the ability to synthesise DNA and to assemble a population of functional CMTs.

## Conclusions

Although a good deal is known about the relationship between MTs, especially CMTs, and modifications of the walls of root cells, these modifications being part of the cellular differentiation process (morphogenesis at the cellular level of organization), relatively little is known about the relation of MTs to morphogenesis of the root organ as a whole. This is partly because organ morphogenesis depends upon supracellular properties and MTs may be only indirectly connected, but nevertheless responsive, to these. Whereas cellular morphogenesis depends on a well-established relationship between CMTs and cell wall synthesis, this latter can in turn influence the direction of cell growth upon which organ morphogenesis in part depends. However, additional information, especially information which overrides the more local effects of MTs such as physiologically or biophysically regulated changes to growth orientation, may also be important in this regard. Such information may be able to effect new orientations of the CMTs which only later are able to assume their usual regulatory function in cell growth orientation and differentiation. A second class of MTs within the cell, the EMTs, should not be overlooked in morphogenetic phenomena. Because of their connection with both plasma membrane and nucleus they could serve as important channels of communication between the extracellular environment and the genetic material. They are important in specifying the orientation of cell division through determining the orientation of the PPB and they may also be instrumental in constructing and orienting the CMT population of interphase cells.

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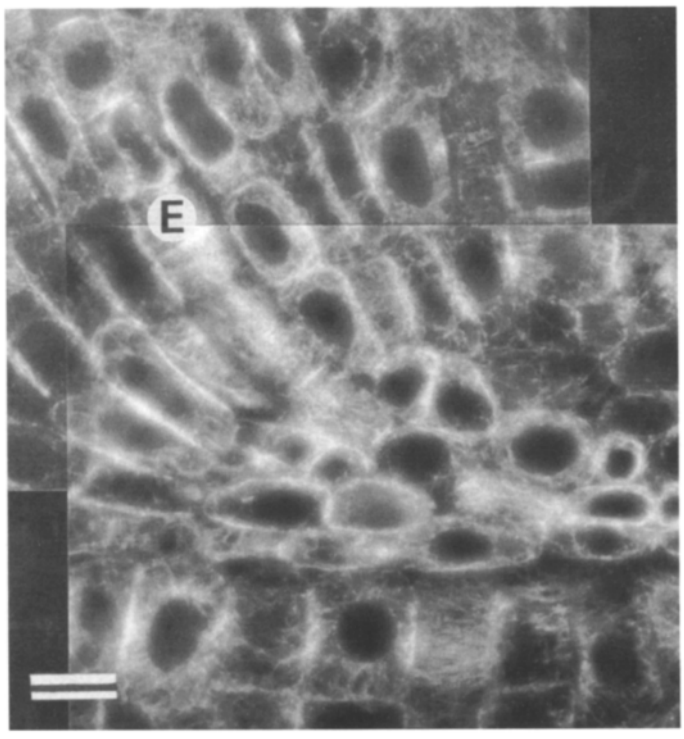


*Figure 7.* Longitudinal arrays of CMTs in a file of presumptive endodermal cells (E) close to the apex of a maize root exposed to 1 m*M* hydroxyurea for 24h. Scale *bar* = 25 $\mu$m.

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