Cytoskeletal Perspectives on Root Growth and Morphogenesis

Peter W. Barlow

IACR–Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS41 9AF, United Kingdom; e-mail: peter.barlow@bbsrc.ac.uk

František Baluška

Botanisches Institut, Rheinische Friedrich-Wilhelms-Universität Bonn, Kirschallee 1, D-53115 Bonn, Germany; e-mail: baluska@uni-bonn.de

Key Words   actin, microfilaments, microtubules, tubulin

■ **Abstract**   Growth and development of all plant cells and organs relies on a fully functional cytoskeleton comprised principally of microtubules and microfilaments. These two polymeric macromolecules, because of their location within the cell, confer structure upon, and convey information to, the peripheral regions of the cytoplasm where much of cellular growth is controlled and the formation of cellular identity takes place. Other ancillary molecules, such as motor proteins, are also important in assisting the cytoskeleton to participate in this front-line work of cellular development.

Roots provide not only a ready source of cells for fundamental analyses of the cytoskeleton, but the formative zone at their apices also provides a locale whereby experimental studies can be made of how the cytoskeleton permits cells to communicate between themselves and to cooperate with growth-regulating information supplied from the apoplasm.

CONTENTS

INTRODUCTION

Microtubules (MTs), polymers of the tubulin protein, are generally held to be responsible for the orientation of cellulose microfibrils within plant cell walls. The microfibrils both provide a scaffold for the assembly of other wall components and influence the orientation of cell growth. The latter process is driven by an internal hydrostatic pressure which shows no preferential direction in the application of its force upon the cell periphery. However, anisotropic expansion of cells is possible if different wall facets, or portions of a facet, have different yield thresholds to the internal pressure. Where they exist, such anisotropies come about because of differential depositions or modifications of wall materials. Again, this could be a consequence of an MT-directed process since MTs in the peripheral (cortical) zone of the cytoplasm can help shape the interior surface of the wall, thus bringing about the characteristic microanatomy of plant tissues and their cells (3, 105, 122, 174).

Given the dual function of MTs in individual cells—helping to define not only cell growth orientation but also cellular microanatomy—it is a challenge to understand how MTs participate in the more large-scale development of multicellular organs. Both aspects of MT function can be appreciated within multicellular callus systems (213). Often, what is lacking in callus is a signal for turning haphazard growth into orderly organogenetic growth. Usually such a cue is supplied by growth-regulating substances directed to target zones via the apoplasm or symplasm. Thus, MTs and actin microfilaments (MFs) might collectively be part of a sensory system for capturing and transducing information contained within the cellular milieu and then converting it into a coherent growth response which includes further cell differentiation (23, 175, 200, 222).

Microtubules and MFs are generally considered to be major components of an intracellular system, broadly known as the plant cytoskeleton (77). The limits of the cytoskeleton, in terms of what types of cytoplasmic structures are, or are not, part of it, are hard to define; the suffix skeleton might be regarded as being relevant only to a support structure. Nevertheless, it is fairly clear that MTs and MFs fulfil a cytoskeletal role in the sense that they confer structural order and stability on the interior of the cell and these, in turn, permit the orderly unfolding of cell growth and, consequently, organ growth.

Research into root biology continues to be central to plant sciences on account of the practical implications of the subject. Moreover, roots are a convenient source of tissues for fundamental studies of tissue differentiation and the physiological responses to environmental perturbations. Roots provide a ready source of cells for the examination of MTs and MFs in both the electron and fluorescence microscopes (for MTs see 22, 83; for MFs see 184, 187). In fact, the now classical relationship between the orientations of MTs in the cell cortex and the cellulose microfibrils in the cell wall, as well as the very existence of MTs in plants, were discovered in root meristems of *Juniperus chinensis* and *Phleum pratense* (133). In conjunction with fluorescence microscopy, where extensive use is made of fluorescent antibodies to cytoskeletal proteins (140), pharmacological agents, to which roots can easily be exposed, help dissect the relationship between cellular chemistry and cytoskeletal substructure (102, 171, 232).

This article draws upon the long history of various aspects of root research, as well as the notable advances in knowledge of the plant cytoskeleton, and combines them to appraise root development and morphogenesis from a cytoskeletal perspective. Although many other articles on roots have appeared in this series of Annual Reviews, the present one seems to be the first to examine their development from such a point of view. Observations from various systems other than roots are also mentioned because these give useful clues as to how the cytoskeleton–root development concept can be furthered. We would claim—if we may be permitted to paraphrase the dictum of the famous geneticist, Theodosius Dobzhansky—that many aspects of root growth and development make sense only in the light of cytoskeletal behavior, particularly in the way cytoskeletal MTs are deployed.

TYPES OF MTS IN ROOTS

Cortical MTs

The first observation of plant MTs in root meristem cells by Ledbetter & Porter (133) arose from the utilization of glutaraldehyde as a fixative for use in electron microscopy. Hitherto, the popular use of $KMnO_4$ as a fixative had rendered MTs unobservable (and many other structures as well). The MTs revealed in this study were of the type now known as cortical MTs due to their presence in the cell cortex underlying the cell periphery (56). Their conspicuous coalignment with the microfibrillar constituents of the adjacent cell wall at once made sense of Green's conjecture in the previous year (81) that cytoplasmic fibers or elements, similar in some of their properties to those of the mitotic spindle, lay within the cortical cytoplasm from where they somehow directed cell wall biosynthesis in a manner which influenced the orientation of cell growth.

Three of Ledbetter & Porter's initial observations (133) on cortical microtubules in root meristems are still relevant today, and are still without satisfactory explanation: (*a*) Adjacent, parallel MTs were never less than 35 nm apart (center to center).

This agrees with numerically more detailed observations (212) on radish roots that cortical MTs were mostly about 90 nm apart. Here, it is worth recalling observations on insect ovarian cells which had been caused to express two different mammalian MT-associated proteins, MAP2 and tau (46). When the cells expressed MAP2, the distance (as seen in cross-sections) between MTs in the induced cytoplasmic processes was ca 65 nm, but when tau was expressed the inter-MT distance was 20 nm. Deep-etch microscopy corroborated these findings and revealed molecules of correspondingly larger or smaller size linking the MTs. The two sets of inter-MT distances were within the range found in dendrites (60–70 nm) and axons (20–30 nm) of rat neuronal tissue. Thus, where different arrangements of MTs are associated with root tissue differentiation, they may have been defined by different complements of MT-associated proteins. (*b*) The zone of the meristematic cell cortex inhabited by MTs was enriched with ribosomes, so much so that Ledbetter & Porter thought this suggested an interrelationship between the two structures. More recently, preparations of pea roots were reported to show an association between polysomes and actin which cosedimented with the cytoskeleton fraction (236). Actin MFs are also components of the cell cortex (49, 55, 138, 149, 183), and it may be because of their presence (often undetectable in the electron microscope) that ribosomes are intermingled with the cortical MTs. Recent summaries of views about the plant cell cortex (23a, 98) indicates a complex set of structural and functional interactions between membranes, MFs, MTs, and even genes (31, 154). (*c*) MTs were aligned in as many as three layers beneath the plasma membrane. This observation is intriguing since current ideas suggest that the outermost layer of MTs is associated with wall biosynthesis; so, do MTs in the other two inner layers have a function? Or do the different layers of MTs reflect a sequence of recruitment from a more internal zone of cytoplasm, where they are assembled, to the outer zone, where the MTs are putatively active? The fact that the MT arrays in the innermost layer were less well ordered (i.e. nonparallel MTs) than those of the outer layers (which showed parallel MTs) suggests this might be the case; but an opposite sequence, of MT return to the inner cytoplasm, is also possible. Studies of the sequence of MT reorganization following treatment of tobacco BY-2 cells with the anti-MT agent, propyzamide, showed that nonparallel arrangements of cortical MTs reappeared a few minutes before parallel arrays (89). As for the lengths of cortical MTs in meristematic cells, these were estimated as being mostly between 2–4 μm long (86). The values were estimated from the cortices underlying longitudinal walls of interphase cells of *Azolla* and maize roots; cortical MT lengths in *Impatiens* roots were 4–6 μm long. In radish roots, mean cortical MT lengths were less in meristematic cells (means for individual root varying between 0.9–1.3 μm) than in expanded cells of the root hair zone (2.6–6.7 μm) (212). Here, however, most cortical MTs were short; the difference between the mean lengths in the two cell types was due to an increased proportion of longer (upto 14 μm) MTs in the expanded cells. In all cases, MT lengths corresponded to the cross-sectional width of a cell wall facet, suggesting the possibility for local cytoskeletal control of the wall properties between neighboring cells.

It has often been speculated that the cortical MTs have some connection with the so-called rosettes embedded in the internal face of the plasma membrane and the terminal globules in the exterior face. These rosettes and globules seem to be two halves of a common structure associated with cellulose microfibril synthesis at the inner surface of the cell wall (172). The cortical MTs do not lie directly beneath the rosettes, but are located to one side of them: in developing xylem cells of cress roots lateral connections were found between MTs and rosettes (99), and evidence from freeze-fractured plasma membranes of the alga, *Closterium*, suggested this too (76). Unidentified proteins link the MTs to the plasma membrane (1, 156; see 114 for a review), but whether any of these proteins are part of the rosette protein is not known. A further question is whether the rosettes determine the above-mentioned ∼90 nm spacing between parallel, cortical MTs (212), and whether they account for the images of bridges between these MTs seen in the electron microscope.

Concerning MT-associated proteins and the order which they might confer upon MT arrays (101), one needs to distinguish between associations that bring about bridging between MTs, and which would thus favor MT bundling, from associations that connect the MTs to the plasma membrane, as well as from those which anneal the free ends of MTs. Another category consists of the MT-organizing proteins which facilitate MT polymerization (195, 218), the main sites for this being the nuclear envelope (208), the cell cortex including the preprophase band, the spindle and phragmoplast (90), and the centromeres of mitotic chromosomes (32). And in this regard, attention should be paid to the 120-kDa protein isolated by Chan et al (45) and to γ-tubulin (137, 155), especially since this last-mentioned protein has significance for MT organization in animal (238) and fungal (150) cells. As far as MT–MT bridging is concerned, a 65-kDa protein with this property was extracted from tobacco BY-2 cells (117), and a 76-kDa protein isolated from suspension-cultured carrot cells also caused bundling of MTs (58). The MTs assayed in this last-mentioned work, and also in another study (217) in which an 83-kDa protein was isolated from maize suspension culture cells, were from animal brains, though a similar bundling response was shown when the 83-kDa protein was added to native plant MTs. The bundled MTs showed a center-to-center spacing of <350 nm. In view of the much closer spacing of cortical MTs in fixed cells (mentioned earlier), the question is whether these observations have relevance for MT bundling in the cell. A MT-annealing-type protein has been isolated which increased the rate of MT elongation (109). Concomitant bundling of MTs was also noticed. Thus, MT conformation may play a part in regulating MT dynamics, or vice versa. Also significant for modulation of plant MT arrays is elongation factor-1α (Ef-1α). This ubiquitous, ribosome-bound protein not only serves as a protein translation factor in eukaryotic cells, but also interacts with MTs. It, too, encourages MT-bundling, this property being negatively regulated by calcium and calmodulin (68). Ef-1α may also help re-establish the perinuclear MT complex following cytokinesis (130). Both these effects may involve interactions with actin and, hence, could be responsible for the stability of complexes between

MTs, actin MFs, and ribosomes. In this way, Ef-1α could assist in the intracellular compartmentation of protein synthesis (53).

Specialized bands of cortical MTs which help shape the secondary walls of plant cells are well known (174) even though the conditions which bring about these MT distributions are obscure; some perhaps, could involve self-organizing processes dependent upon reaction-diffusion mechanisms (208a). Less well known, however, are the small rings of MTs (2–3 μm diameter) which develop at peripheral sites of primary and secondary vascular cells. The MTs rings are responsible for defining pit fields, simple pits, and bordered pits (44, 103). They begin to form following a clearing of MTs from sites (4 μm diameter) in the cytoplasmic cortex. How this comes about is not known—it may relate to local changes in the plasma membrane—but such areas have also been recorded following various experimental treatments (9, 16, 33). Given the stiffness of MTs, the rings probably consist of short MTs linked together in some way, perhaps with the participation of actin. Even larger MT rings are features of the end walls of developing vessels (44). They involve nearly the whole perimeter of these walls and are thought to participate in their removal, thus allowing vessel–vessel continuity. Interestingly, a ring of MTs with a similar function is responsible for fashioning the lid of cyst cells of the alga *Acetabularia* (161). Whether the nucleus participates in forming the MT ring of vessels, as it does in *Acetabularia,* is not known.

A variant of the cortical class of MTs is the preprophase band (PPB), a transient example of MT bundling (163). The PPB begins to develop in cells which have reached late interphase of the mitotic cycle. It is recognizable at this stage partly because all the other cortical MTs are becoming disassembled in preparation for redeployment in the mitotic spindle. Those MTs which remain, i.e. those of the incipient PPB become crowded together in the cell cortex and hence appear particularly bright in the fluorescence microscope. With the passage from early to late prophase, the cortical MTs of the PPB of onion root cells become more numerous (50 MTs in cross-section, rising to 250 MTs), are arranged in more layers (3 layers, rising to 10 layers), and come closer together (center-to-center distances decreasing from 40 nm to <30 nm) (179). Treatment with the protein synthesis inhibitor, cycloheximide (36 μM for 2 h), diminished all these trends so that the PPB remained broader than usual (on average, the PPB was 4.5 μm wide in contrast to 3.2 μm in untreated roots) and contained 70% fewer MTs.

These observations on the microtubular PPB have to be considered in the light of the participation of actin MFs in its structure (65). The actin-disrupter, cytochalasin D, like cycloheximide, also prevented the narrowing of the PPB in prophase cells of onion roots (69, 167). Therefore, it is reasonable to suggest that the synthesis of a prophase-specific protein, or proteins, is required for the cell cycle-linked evolution of PPB structure. Such a protein may also protect the PPB MTs from whatever conditions cause the cortical MTs elsewhere in the cell to disassemble. One protein suggested for this role is the p34[cdc2] homologue from maize, known to be associated with the PPB (54). The corresponding maize cdc2 antibody, however, recognized only about 10% of PPBs, these being late, not early, PPBs (162). On the basis of results using the conserved PSTAIR sequence of p34[cdc2], it was suggested

that some nonstaining of PPBs was a technical problem rather than being of any biological significance (164). Probably, many other proteins associated with the PPB (reviewed in 163) could be considered as possible regulators of its function.

Even more intriguing is the significance that the PPB has for cell division and morphogenesis because the position at which the cell plate is inserted into the wall of a dividing cell is intimately linked with the position of the PPB during the preceding late interphase. On the basis of electron microscope evidence from onion roots, it was suggested (181) that the PPB continues to support incorporation of precursors into the underlying cell wall. The absence of cortical MTs elsewhere in the dividing cell would make this the only site of wall synthesis at this stage of the cell cycle. The PPB might therefore prepare a site at which wall precursor material contained within the expanding cell plate can adhere. The cell plate is also attracted to the location of the former PPB by long-range mechanisms, as centrifugation experiments have shown (75). This attraction is unlikely to involve the actin MFs associated with the PPB since the MF structures at this site disperse during prophase (182). Some kind of "negative" imprinting at the PPB site has also been suggested (51), but of what this might consist is unclear.

Circumstantial evidence about whether or not the parental wall plays an active role in cell plate insertion also comes from regenerating protoplasts and suggests that a minimal external wall, as well as a minimal cortical MT network, are required for cell division (85, 196, 202). Unfortunately, in these studies, no observations were made to determine the presence or absence of PPBs, only about whether or not there were division walls. Nor do the observations indicate why certain protoplasts lack a PPB (202), a finding which could be relevant for explaining how some higher plant cell types, such as cambium fusiform initials, lack a PPB (72) yet divide satisfactorily. It may be that such elongated cambial cells (cf. 42) simply lack sufficient numbers of MTs and tubulin gene transcripts to form a PPB.

If wall deposition does occur at the site of the PPB (181), this could help explain how some type of division wall growth can occur even in the absence of a phragmoplast. For instance, following caffeine treatment, stubs of what might normally have been part of the new division wall were found attached to parental cell walls (118, 193). Such wall stubs are not uncommon in other circumstances: they have been found, for example, in nematode-induced syncytia of *Impatiens* roots (119). These observations suggest that cytokinesis consists of two processes: centrifugal growth and maturation of the cell plate within the phragmoplast, and centripetal growth of the new division wall from the PPB site. Since the latter process is slow relative to the former, centripetal wall growth is not usually appreciated unless phragmoplast and cell plate are destroyed.

Endoplasmic MTs and Mitotic Spindle

Although Ledbetter & Porter (133) did not demonstrate MTs in the interior of interphase cells, they expected that MTs would be found there, even if identifiable only with difficulty. Fortunately, the anticipated difficulty disappeared with the advent of the immunofluorescence marking of MTs (231); hence, endoplasmic MT arrays

were identified (129). However, the frequent failure to appreciate endoplasmic MTs in squashes of root cells is understandable as they are easily masked by numerous over- and underlying cortical MTs. Laser scanning confocal microscopy removes this constraint to their identification (71, 84, 166). Tissue sections also provide an excellent means of revealing endoplasmic MTs (15) and for examining the relationships between their conformation and the differentiation status of the cell and, in the case of meristematic cells, the phase of the cell cycle (JS Parker & PW Barlow, unpublished data).

Endoplasmic MTs do not form the clustered associations that are characteristic of cortical MTs. They are more usually visualized as sparse populations of single or branched tubules traversing the cytoplasmic space, though a superabundance of endoplasmic MTs form in onion and maize root cells following exposure to cycloheximide (9, 166). Close observation has often suggested that one end of these endoplasmic MTs is attached to the nuclear surface and the other reaches into the cell cortex, a configuration which suggests the potential for communication between these two zones of the cell (21, 28).

The plant mitotic spindle may be regarded as a transformed set of endoplasmic MTs (10). In general, the mitotic spindle is a conservative structure and the behavior of its MTs has been reviewed (30). Endoplasmic and spindle MTs are also related through their common property of being organized upon the nuclear envelope. A good deal of evidence suggests that the nucleus itself is the site of synthesis of MT-organizing material, and that this material continually emerges from the nucleus to overlay the surface of its external membrane (21). Whereas during most of the interphase of the cell cycle endoplasmic MTs radiate from all over the nuclear surface, late in interphase putative motor proteins (4, 170) bring about the segregation of the MT-organizing material into two groups on opposite sides of the nuclear surface. Also segregated at this time is γ-tubulin (155). All the while, the organizing material continues to assemble endoplasmic MTs, some of them making contact with the PPB (179). When fully segregated, the two opposite groups of organizing material serve as the sole foci for MT assembly. At pro-metaphase, fine endoplasmic-like MTs radiate from each of the two half-spindle cones toward the end-walls and side-walls of the mitotic cell (84). These associations may ensure a suitable position for the nucleus and spindle prior to, and during, mitosis. When the nuclear envelope breaks down and the chromosomes condense, these foci (which may consist of a small number of subfoci) serve as the two poles of the mitotic spindle. At the same time, MT-organizing material, such as the 49-kDa protein of Hasezawa & Nagata (90), latches onto the centromeric regions of the prophase chromosomes, enabling their association with spindle MTs.

An essential feature of mitotically cycling cells is the sequential transformation of their MT arrays, the timing of which has been estimated in onion root meristems (216). Spindles exist only briefly, for about 1.5 h within a total cycle of 34 h (at 15°C); PPBs and phragmoplasts persist for 2.3 h and 2.0 h, respectively. These transformations are based on the continually changing equilibrium between free tubulin and MTs (153, 233) and the preferential activation of MT-organizing centres. How the timescale of these events is determined remains an open question.

Phragmoplast

Root meristem cells are usually devoid of any large vacuole, so there is no need of a phragmosome to support either the dividing nucleus or the forming phragmoplast and cell plate—unless one regards the whole of the cell interior as a type of phragmosome! But this does not mean that some of the cytoskeletal components and properties characteristic of phragmosomes, such as the tension that exists within cytoskeletal filaments (78, 80), are absent from meristematic cells. For example, actin filaments may radiate out from the edge of the phragmoplast and secure the attachment of the expanding cell plate with the parental wall (141) even if, in meristematic cells, there is no definite phragmosome structure within which this could occur. Indeed, disruption, by latrunculin A, of actin in meristematic cells leads to twisted phragmoplasts (17).

The density of MTs within the phragmoplast suggests the presence of many MT-organizing sites, as well as proteins which link MTs laterally and confer dynamic movements upon them (5). The rapidity with which the phragmoplast grows at telophase, and the coincidence of its formation with spindle disassembly, suggest a movement of tubulin dimers from one structure to the other. MT-organizing materials may be similarly redeployed at this time, materials formerly at the poles of the spindle and at the centromeres being relocated to the zone between the two telophase sister-nuclei (10). Possibly, the reformation of the pair of nuclear envelopes, together with an affinity of motor-proteins for the free ends of the MTs (6) which are polymerized on the nuclear surfaces, assist in relocating MT-organizing material toward the mid-zone. Besides MTs, actin MFs also provide an important component of the phragmoplast (197). Moreover, once cytokinesis is complete, actin remains within the plasmodesmata which traverse the new division wall (227), as well as heavily decorating this region of the cell periphery (17, 18). Myosin is also associated with the plasmodesmata (190, 191).

The role of the phragmoplast is to attract into itself membrane-bound vesicles bearing precursors for the cell plate. Studies with low doses of colchicine have shown that this process principally requires the participation of phragmoplast MTs (131). Also necessary are motor proteins such as the dynamin-like protein, phrag-moplastin (82), and the filamentous protein, centrin, which colocates with the phragmoplast vesicles (62). When the component molecules become assembled into a cell plate, the MT-organizing material of the phragmoplast is displaced to-ward its edge. What permits the cell plate to expand as a flat disc and not as a spheroid in the mid-zone [as it does in the *pilz* mutants of *Arabidopsis* (157)] is not firmly established. It seems that as material is added to the edges of the grow-ing cell plate by the phragmoplast, so these edges are pushed toward sites on the parental cell wall already prepared by the PPB to accept them. Whether this is a form of self-assembly (cell-plate crystallization), in much the same way that cellulose-forming rosettes are thought to be pushed forward by the crystallization of the new cellulose fibrils, is not known. Actin and vinculin filaments radiating from the edges of each sister nucleus may provide this pushing force (70). These molecules, as well as centrin (62), may also be responsible for straightening out the

undulations in the new division wall (165) which, at this stage, is rich in callose. Further details of cell plate formation are mentioned in the final section.

MT Cables

A little-understood fourth type of microtubular structure comprises the fluorescent MT cables seen following immunostaining with antitubulin. Long-lived tissues, such as the xylem ray parenchyma cells of roots of *Aesculus* and shoots of *Populus* (NJ Chaffey & PW Barlow, in preparation) clearly show such MT cables. Generally, they seem to exist in cells which have completed their growth and, hence, may be considered as mature and fully functional. The cables consist of 3–4 MTs (in cross-section) and are similar in this respect to the MT bundles found in cortical cells in mature regions of hyacinth roots (57, 136). We speculate that such cables are concerned with intra- and intercellular transport processes and with the general polarity of tissues, properties not so strongly expressed in association with younger cells with their more usual arrays of transverse or reticulate MTs. The cables are presumably not a degenerate type of MT because the final stages of cell differentiation in root and other tissues are usually marked by bright fluorescent spots of tubulin which supercede the MT population and which have no apparent order within the cell (88).

CYTOSKELETAL PROTEIN VARIABILITY AND ITS RELATION TO ROOT TISSUE IDENTITY

Tubulin Genes and Isotypes

Whatever their role in plant cell development, the MTs associated with the four types of array mentioned previously are all constructed of α- and β-tubulin dimers. The correlation between MTs and the developmental program of root cells suggests that genes for α- and β-tubulins, but with different coding sequences, are functional in different cell types. This leads to the identification of tubulin isotypes; the isotypes can, however, also be the result of posttranslational and postpolymerizational modification of one given type of tubulin protein (37, 77, 144, 145). It is not known to what extent the isotypes are interchangeable. In human HeLa cells, for example, four different β-tubulin isotypes can all be found within interphase and spindle MT arrays (134). But there is other, firm evidence that isotype substitution leads to developmental abnormalities if it occurs in a cell which does not normally support that isotype (106). Isotype interchangeability cannot therefore be generally acceptable, perhaps because of the different proteins with which the MTs associate and the consequences this has for cell differentiation (46). A second hypothesis is that tubulin isotypes differentially regulate the turnover of the MTs which contain them and that this variation of MT half-life might have some adaptive significance. Plant organisms, with a range of tissues, as well as a range of environmental conditions to contend with, may increase the options for the regulation of MT dynamics by possessing multiple isotypes. However, examination of

root tissue in relation to tubulin isotypes suggests that they also participate in specialized activities of cell differentiation. It seems, therefore, that there is a default system of tubulin deployment which operates in a standard, or optimal, growth environment, whereas another system, involving alternative tubulin isotypes, is evoked when growth is challenged by nonstandard environments.

c-DNA prepared from different tissues of maize revealed at least six α-tubulin sequences (221), some of which were similar to those found in the dicot *Arabidopsis thaliana* (126, 146). The $\alpha2$ isotype of maize was identified as a product of the tub$\alpha5$ gene (121). Specifically examining root tips, four α- and four β-tubulins were identified in *Phaseolus vulgaris* (111), and six β-tubulins were identified in both carrot (112) and maize (121). Although seven different β-tubulin genes were expressed in *Arabidopsis* roots (207), only three of them (TUB1, TUB6, and TUB8) showed notable amounts of transcript. Later work with Arabidopsis root (48) showed that the TUB1 gene product (as identified by GUS transgene reaction) was localized in the epidermis and cortex tissues, whereas TUB8 was confined to endodermis and phloem. In both cases, a strong GUS reaction was found in the zone of rapid elongation but none was evident in meristem or root cap. Of the β-tubulin isotypes described for carrot, the most strongly expressed was $\beta1$; some differences were encountered between seedling and mature tap roots with respect to the expression of isotypes $\beta5$ and $\beta6$. Among the c-DNA sequences identified from maize were those of the tub$\alpha1$, tub$\alpha2$, and tub$\alpha3$ genes (169). In maize roots, these genes showed a tissue-dependent pattern of expression (121, 214); tub$\alpha1$ was specifically expressed in roots (and pollen), but not in other organs (169, 192). In situ mRNA hybridization to root tissue sections revealed tub$\alpha1$ to be strongly expressed in the meristematic cells of the root cortex and root cap, but to a lesser degree in the vascular cylinder and quiescent center. Similar results were obtained when the promoter of tub$\alpha1$ was inserted into the genome of tobacco. Here, root meristem cells, but not the quiescent center, showed promoter expression (192). One difficulty in interpreting such observations in terms of MT function and of MT gene-switching is that different regions within root tissues have different concentrations of RNA in their cells (24). Differences in the level of tubα expression may therefore be a function of more general regional differences of RNA metabolism. Another problem is that it is by no means certain that all the tubulin RNA is translated into tubulin protein. Thus, on the basis of in situ hybridization, it may go beyond the evidence to imply (214) a link between the distribution of tub$\alpha1$ and the variously oriented divisions associated with the generation of cell files (formative divisions) within the root apex, although in such a zone of the meristem a relatively rapid deployment of tubulin might be expected to associated with the high frequency of cell division. The gene tub$\alpha3$ was not so strongly expressed in meristematic regions; the amount of tub$\alpha3$ mRNA was about 100-fold less than tub$\alpha1$ (168). Nevertheless, in situ hybridization with the tub$\alpha3$ probe gave a strong signal in the meristem, except for cortical cells which seemed to be relatively weakly labeled (214). The pattern of tub$\alpha2$ is especially interesting since it was expressed only in epidermal cells, whereas this tissue was not particularly marked by tub$\alpha1$ (214)—which is unexpected if the product of

tubα1 is associated with cell division. Epidermis was also marked by tubα3, but only in the older regions of root.

By separating the maize root into a tip portion and a mature portion 1–2 cm from the tip, and then further dissecting the mature portion into vascular cylinder and cortex, the α1 and α4 isotypes were seen to predominate in the tip while mature tissue expressed an abundance of α2 and α3 isotypes (121). β4 and β5 isotypes were features of vascular tissue, whereas β1 and β2 isotypes were abundant in the cortex; β1 isotype was absent from the root tip. When the transcripts of the six tubα genes were considered individually (121), those of tubα4 were particularly evident in vascular tissue (see also 66), suggesting that this gene is active when MTs are required to regulate secondary wall synthesis in either xylem or phloem (or both). The general situation for the maize root (see also 71a) has similarities with tubulin isotype distributions in barley leaves (92). Along a developmental gradient, from 0 mm to 35 mm from the leaf base, the α- and β-isotypes changed in frequency. This was paralleled by a change from random to bundled MTs in the mesophyll cells, just as occurs to the MT arrays in maturing maize root cells (15).

An evolutionary aspect of tubulin diversity is indicated by the discovery of a species biotype (R biotype) of the grass, *Eleusine indica,* which is resistant to anti-MT chemicals, such as dinitroaniline, and at the same time displays sensitivity to the MT-stabilizing compound, taxol (220). The R biotype possesses a novel β-tubulin isotype and its tubulin can polymerize in vitro in the presence of oryzalin (219). Although these results from roots of R-biotype *Eleusine* could not be confirmed by Waldin et al (224), it is possible that there was a change in the tubulin could have occurred which was undetectable by electrophoresis. Further investigation (233a) showed the R biotype to possess missense mutations in the TUA1 gene for α-tubulin.

MTs in roots of freeze-tolerant rye usually disassemble following exposure to freezing (0°C or less) temperatures, but this effect can be offset by a short period of acclimation at a slightly warmer temperature (4°C). In this rye-root test system, during the acclimation period, the freezing-sensitive population of MTs is exchanged for one that is more resistant (124). It was also found that, as a consequence of acclimation, two α- and one β-tubulin isotypes disappeared, while one new β-isotype appeared (125). Taxol rendered the rye root MTs more resistant to cold (47), tending to confirm that the cold-induced pattern of tubulin isotypes had increased their stability. Taxol treatment also stabilized actin MFs in the root cells toward cold, whereas MT disruption by amiprophos-methyl increased the cold-susceptibility of the MFs. These results suggest a structural link between MTs and MFs. Treatment of rye roots with hypertonic solutions of sorbitol simulated some of the physiological effects of chilling, but did not induce such a complete disintegration of the cortical MTs (188). As plant meristems often have to withstand long periods of cold during the winter months, and utilize their dormancy mechanism to do so, it would be of interest to compare isotypes from dormant and nondormant apices, with and without chilling. Dormant vascular cambium in *Aesculus* roots showed conspicuous cortical MTs, even when taken for fixation from frozen surroundings (43).

Another environmental challenge to maize roots is infection by arbuscular mycorrhizal fungi. Following infection with *Glomus versiforme* or *Gigaspora margarita,* tubα3 expression increased in the cortical cells containing the fungal arbuscules (36). Similarly, in ectomycorrhizal formation (short roots) induced by *Suillus bovinus* in Scots pine, new α-tubulin isotypes were detected, whereas there were no alterations to β-tubulin patterns (178). However, no particular changes in MT organization were noted which might correlate with the modified α-tubulin complement (177). Increased α-tubulin transcripts were also detected in the developing ectomycorrhizal root system of *Eucalyptus glomus* (41), but this may have been due simply to increased numbers of highly proliferative cells contained in lateral root primordia.

Posttranslational Modifications of Tubulin

Posttranslational modifications of α- or β-tubulins in plant cell are now becoming better understood as a means to regulate MT dynamics. Although a large number of modifications are possible, the best known are phosphorylation, acetylation, tyrosination, and polyglycylation (145), and all these, with the exception of polyglycylation, have been found in plants (113). Different modifications may exist even along a single microtubule, giving tremendous scope for MT polymorphism. The β-tubulin of tobacco (204, 205) was modified by polyglutamylation, whereas several other types of posttranslational modifications were found for the α-tubulins, all of which could be detected by immunofluorescence in the MTs of interphase and dividing root cells (76a, 76b, 204, 226).

Actin Genes and Isotypes

Actin genes are more diverse than tubulin genes (158, 159), many dozens having been discovered in *Petunia,* for example (8). The findings concerning actin isotypes are similar to those of tubulin. Although many of the actin isotypes are common to certain plant organs, some are specific to roots (115). In soybean roots, tissue-specific patterns have been found (152). Antibodies were raised to peptides of κ- and λ-actin and conjugated with gold particles. Particular cells of the cap flank, as well as some older statocyte cells, were marked by the λ-actin antibody, whereas the κ-actin antibody showed neglible reactivity toward the cap. Application of chicken anti-actin antibody, N350, also failed to react with soybean root cap. A lack of reaction of maize root caps toward another chick actin antibody (14, 18) is in keeping with this negative result. By contrast, using GUS constructs, two actin genes of *Arabidopsis, ACT1* and *ACT2,* were both found to be active in the *Arabidopsis* root cap and root meristem (2).

Four actin isotypes were identified in rice roots and the expression of their respective RNAs followed over a 35-day period following germination (151). The amounts of mRNA of two isotypes (Rac2 and Rac3) declined by approximately 80%, whereas Rac7 isotype remained constant; Rac1 transcripts showed a slight decrease. The significance of these findings remains obscure until the respective proteins are colocalized to the cellular structure of the roots. In roots of *Phaseolus*

vulgaris, where two main actin isotypes have been found (186), only one was present in nodules induced by *Rhizobium.* Whether this was a new isotype or one of the original isotype complement of uninfected roots, the second one being suppressed, was not mentioned. Complex rearrangements of actin MFs and MTs occurred during development of the *Bradyrhizobium*-induced nodules of soybean roots (229). The actin MFs formed an unusual honeycomb pattern, the significance of which may be to establish and maintain the distribution of symbiosomes in the infected cells. They may also take part in delivering vesicles for the elaboration of additional membranous structures.

EARLIEST DEVELOPMENT OF ROOTS, THEIR MT ARRAYS, AND HOW MTS HELP GENERATE CELL FILES

The first root of most dicot plants differentiates in the early embryo. In the case of *Arabidopsis thaliana,* root differentiation begins after the first 5 or 6 cell divisions have established a proembryo (123). Microtubules have been examined during the early embryogenic stages of a few species, including *Arabidopsis* (107, 225, 235). Their behavior shares many of the features which continue to be seen in the subsequent phases of organogenesis. Thus, early embryos, just like adult meristems, have MT-based mechanisms that establish the planes of cell division and subsequent cell growth.

A comprehensive description of MTs in early *Arabidopsis* embryos is due to Webb & Gunning (225). Cortical MTs appeared before the first division of the zygote and were aligned perpendicular to the direction of cell elongation. A more dense, transverse array of MTs occurred at the distal end of the zygote and may mark a zone of localized wall extension, although an alternative idea is that the closely packed MTs restrict lateral expansion and hence reinforce the cell periphery (235). Later, another transverse band of MTs, which corresponded to the PPB, made its appearance. The first-mentioned distal array is of interest because similar rings of cortical MTs, which look like (and could be mistaken for) PPBs, have been seen in other systems (79, 173, 223) and also in growing root hairs of the fern, *Azolla* (AL Cleary, cited in Reference 225). Wherever these MT rings have been reported, the cells are free of lateral contact with other cells. However, the correlation between cortical MTs and localized growth rate of the wall is not clear. In the giant internodal cells of the alga, *Nitella flexilis*, for example, bands of growth and nongrowth alternate along the cell wall, yet no corresponding variation in cortical MT patterns could be found (128).

Following the first division of the proembryo, cortical MTs became oriented randomly and growth entered an isotropic phase (225). Endoplasmic MTs were prominent and closely associated with the nuclei and probably position them in the center of the cells. During the isotropic growth phase, nuclei divided in each of the three available orthogonal planes. The ability to divide successively in three planes is probably set not by chance, but is due to a property of the MT-organizing material located on the nuclear surface. This material partitions into two equal

groups which repel each other (see 59). When repulsion is maximal, the material lies in two sites on opposite sides of the nucleus where it serves as organizers for the two poles of the forthcoming mitotic spindle (21). How the nucleus, following mitosis, senses in which plane to set up the future spindle is not known. It may be that some imprint on the nuclear surface survives mitosis; or each pole of the spindle may mark out a domain at the cell periphery which then does not permit a spindle pole of the next division to form in proximity to it. This last-mentioned system would require communication between cell periphery and spindle, a feature which, in animal cells, is mediated by dynactin (38).

Eventually, both the sequence of early orthogonal divisions and the symmetry of globular growth are broken. This may have to do with the fact that after three zygotic divisions there are now four internal division walls. Opportunities arise for cortical MTs to form transverse arrays around the perimeter of these cells and their growth can become polarized; for this to occur, a specification of an apical-basal axis is required (e.g. a reference point, such as the suspensor, may mark the basal end). This would be the prelude for cell file formation parallel to the embryonic axis, and hence would establish a bipolar, torpedo-shaped embryo containing domains where root and shoot structures can form. Meanwhile, a group of structural initial cells (26) establishes a series of divisions within the new root apex.

The later stages of embryogenesis see the completion of the primary root meristems in terms of the number of dividing cells. The ensuing dormant period seems to be accompanied by the loss of the microtubular cytoskeleton in the embryonic primary root meristem—at least within the radicle of tomato seeds. In this system, three β-tubulin isotypes become strongly evident at the time of the first wave of DNA synthesis during germination (61). In seasonally dormant, two-year-old taproots of horse chestnut, cortical MTs persisted in the presumptive vascular cambial cells (43). Here, all the MTs adopted a helical mode, whereas MTs in active cambium were more randomly arranged.

ROOT GROWTH AND THE CYTOSKELETON

Rectilinear Root Growth

The reactivation of cell growth and division during germination builds up the primary root meristem to a maximal length. Later, the meristem shortens and root growth rate slows. At the same time, the number of cell files may be reduced and the root becomes thinner (194). Germination also enables cells to enter a phase of rapid growth, a growth step which was missing in the embryonic radicle. This rapid mode of growth is assumed to depend on the development of the vacuolar compartment in cells immediately behind the meristem, but whether it is entirely driven by turgor is an open question in view of the fact that retardation of root growth follows on from treatments with cytochalasins (189, 209) or latrunculin (17; F Baluška & D Volkmann, submitted) and the consequent disassembly of actin. Likewise, whether the level of tubulin gene transcripts and cortical MT numbers are directly related to the rate of cell or root growth—as seems to be

the case in some shoot tissues, for example (39, 160)—is not known. Increased numbers of cortical MTs and rates of their interpolation within root cells of *Azolla pinnata* occurred during their transition from meristematic to elongation growth and differentiation (87). That these increases in MTs were related to increased rates of secondary wall deposition is clear, and it may follow that they were also related to changing cellular growth rates. The dramatic disappearance of *Tub B1* gene transcripts which occurs in soybean roots older than 6 days postgermination (120) was, unfortunately, not considered in relation to any root growth characteristics.

Unlike the cells of the meristem, where cortical MTs have a variable but mainly transverse orientation, cells of the elongation zone of maize roots have strictly transverse MTs (15). This new orientation, which can be accompanied by a bundling of MTs in the cortical cells, develops in the transition zone at the base of the meristem (19). A similar maintenance of transverse orientation was noted for radish roots, though, as the cells elongated, the angle of the cortical MTs with respect to the cell axis became more variable (212). The MT-switch in the transition zone might depend upon the activity of a new set of tubulin genes or the utilization of a new population of MT-associated proteins, notably those which favor MT bundling. Presumably, these bundles must not be fixed in their position; they need to equalize their association with the longitudinal walls in order to produce a uniform thickness of secondary wall. However, some locational stability of MTs along the longitudinal walls must also occur because small areas from which cortical MTs are excluded correspond to regions where pit fields will form (33). These areas need to remain free of cortical MTs long enough to allow this wall feature to develop.

A similar sequence of cellular development—meristem, elongation, maturation—also exists in the root cap. The corresponding arrays of cortical MTs have been examined in root caps of maize (15, 28), radish (63), and cress (93). In the first two species, cortical MTs rearranged from transverse to random as the cells progressed from meristem to the cap flanks. By the time the cap cells detached, their MTs were either random or absent. No longitudinal cortical MTs were found in mature cap cells and in this respect MT reorganization differed from that occurring in derivatives of the proximal root meristem. Equally noteworthy is the contrast between the cells of the cap and of the root proper with respect to the disposition of actin MFs (18). Conspicuous MFs were largely absent from the cap, whereas cable-like MFs were abundant in root cells, especially within those of the vascular cylinder. In the central cap cells, actin is thought to exist as fine filaments whose structure is modified to assist in the gravisensing role of these cells (discussed later). The difficulty of observing these filaments by immunofluorescence may, in part, because the process involves chemical fixation. Freeze-substituted tobacco root caps sectioned for electron microscopy (64) revealed various categories of MFs. Both techniques, however, showed that actin MFs were absent in the quiescent center (14, 18, 64).

The rearrangement of cortical MTs along the growth zone of roots has been mapped in maize, radish, and pea roots. For pea, Hogetsu & Oshima (104) noted that the zone where the MTs switched from transversal to oblique orientation was located in a 1-mm zone where growth had recently ceased; it was estimated that

the reorientation required 2 h to complete. Often the cortical MTs reoriented longitudinally and then made a meandering course between each end of the cell. The transverse-to-longitudinal orientation is paralleled in other systems: it occurs in the single cells of cotton fibers as they grow (198), and also in files of isolated BY-2 cells growing and maturing in culture (88). It is as though MT reorientation is an inherent feature of a cellular growth cycle, irrespective of whether division occurs or not. Because MTs are sensitive to electrical stimuli (110), it is tempting to speculate that the changing pattern of electrical flux along the length of growing roots (210) has some relationship with the reorientations of their MTs. Similar transverse-to-oblique reorientations of MTs were also found in onion root meristem cells exposed to inhibitors of RNA synthesis (215), suggesting a metabolic basis for this effect.

In the maturing cortex of both pea (104) and hyacinth roots (136), it was noticed that the cortical MTs existed in a criss-cross arrangement against the longitudinal walls of neighboring cells. A more detailed study of cortical and epidermal cells of maize and *Arabidopsis* roots revealed that the cortical MTs had a particular chirality (135). By observing MTs against the radial longitudinal walls, arrays of MTs in an S helix were observed toward the end of the elongation zone. Just beyond the elongation zone, however, cortical MT arrays displayed a Z helix arrangement. In the intervening zone, the cortical MTs were longitudinal. The S → Z helix transition is most simply achieved by a rotation of the MT array in a clockwise direction. The consistency of the S → Z helix transition (over 100 maize roots and 13 *Arabidopsis* roots were examined) raises the question, at least for *Arabidopsis,* whether this microtubular chirality has any relationship with the natural chirality of root growth (203). Not only can there be consistent handedness of the MT arrays, but cortical MT bundles in adjacent cells sometimes also appear to have co-alignment (e.g. 230), as though the MTs had been aligned by a common factor. If so, this would be an indication that some type of intercellular communication influences the cytoskeleton.

Root Contraction

If root cells were able to redirect their growth in the transverse plane and also to shrink their length, root contraction would result. The longitudinal hoops of cortical MTs found in the elongated, mature zone of cortical tissue of hyacinth roots (206) could assist this natural shrinkage process, especially if helped by some type of cytoskeletal contraction mechanism. Longitudinal MTs could also bring about the expansion of the previously unwidened transversal end walls. Tubulin levels more than doubled in the contracting zone of the hyacinth root and, in the electron microscope, the cortical MTs were surrounded by additional amounts of electron-dense material which was suggested to be MT-associated proteins (57).

In the root cortex of hyacinth and other species, the shift in cortical MT orientation commenced at different distances from the tip, with the outer cortical cell files showing MT reorientation before the inner files (116). Usually, the more internal cell files, including pericycle (which belongs to the stele) remain juvenile, with random MTs, longer than outer cortical files (15). Moreover, cortical MTs of the

inner root cortex of maize showed increased instability toward ethylene (11). This probably accounts for the ethylene-induced swelling of these roots, a process that enables them to overcome mechanical impedence.

Curvilinear Root Growth

The curvilinear growth of roots, which is associated with tropisms, results from differential extension rates of cells on opposite sides of the root in the distal part of the zone of rapid elongation. The question is whether there is a contribution from the cytoskeleton to the growth differential. In the case of gravitropic coleoptiles, alterations to their cortical MTs (100) and wall microfibrils (74) provide an affirmative answer. But in roots the answer seems to be negative, if only because of the simple observation that gravitropism occurs in roots which had been exposed to oryzalin or colchicine, and hence lacked MTs, before they were gravistimulated (13). Nevertheless, altered arrangements of cortical MTs have been seen, and complex rearrangements of growth did occur, within gravireacting maize roots. However, the stresses and strains associated with the bending (237) and the gravity-induced alterations in auxin levels could have had rapid effects on cortical MTs levels (20, 34). Accordingly, it is not easy to disentangle which are the primary physiological and biophysical perturbations to the cytoskeleton resulting from the root graviresponse, and which are secondary effects induced by the bending reaction.

The positive graviresponse involves alterations to the timing of cellular development in upper and lower portions of the root. Thus, when longitudinal arrays of MTs have been seen on the upper side of a horizontal root tip 2 h into its gravireaction (13), it could be an indication that cell maturation has been advanced during this period, especially since a transverse-to-longitudinal MT reorientation normally accompanies the maturation process (15, 104). Other criteria also suggest premature cell maturation on the upper side of gravireacting roots (27, 60). The longitudinal orientation of MTs, whether found on the upper or lower sides of the root, would have the effect of diminishing the contribution of the affected cells to forward elongation growth and, hence, would initiate the required growth differential.

Careful examination of F-actin networks in gravibending maize roots did not reveal any difference between the cells on the upper and the lower sides (35). However, it is timely to reconsider this observation in the light of the proposal that actin plays an active role in cell elongation (209). The critical location for a contribution from actin may be in the transition zone, at the changeover between meristematic and rapid elongation growth. If an actin-triggered switch to rapid elongation were advanced in the upper portion of the root relative to the lower side, a gravireaction could be initiated without any dramatic change in actin configuration.

Cytoskeleton, Cell Structure, and Graviperception

A more certain area where the cytoskeleton impinges on root gravitropism is in graviperception. There are three aspects to consider: (*a*) the structure of the graviperceptive cells, generally held to be the statocytes of the central root cap, (*b*) the capture of information relating to root orientation with respect to the gravity

vector, and (*c*) the subsequent transduction of graviperception into a signal for a graviresponse.

A crucial element in relation to the role of statocyte cells in graviperception is their asymmetry. In the cap cells of vertical roots of cress or lentil, where ultrastructural organization has been examined in some detail (95, 96, 185), endoplasmic reticulum (ER) and amyloplasts gather at the distal end of the cell, whereas the nucleus is found at the proximal end. This asymmetry is developed during germination and involves actin-dependent movement of the ER (96). Observations on roots exposed to cytochalasin (95, 143) or to colchicine (96) suggest that the position of the nucleus in the statocytes is regulated by actin MFs, whereas positioning of the distal ER is mediated by cortical MTs. Interestingly, MTs associated with the ER tend to be more sensitive to dissassembly by colchicine than are the MTs elsewhere in the same statocyte (94).

Comparison of results of experiments performed in the microgravity (1×10^{-4} **g**) of spaceflights with those done on Earth (at 1 **g**) reveals that statocyte asymmetry is, in part, regulated by the cytoskeleton (142). A similar conclusion was also reached from simulated microgravity experiments performed with the clinostat (142). In microgravity, the nucleus is displaced distally from its usual position and the amyloplasts move basally. Thus, these two organelles normally (i.e. in 1 **g**) assume a position within the cell that is the result of the restraints imposed upon them by the cytoskeleton and by their tendency to displacement due to their mass.

The relationship between the amyloplasts and the cytoskeleton is becoming clearer. Schemes have been proposed whereby the amyloplasts are supported by, or impinge upon, delicate transcellular filaments of actin (12). Evidence for this comes from an experiment which showed that, when the actin was dissassembled by cytochalasin, amyloplast sedimentation was at least three times quicker than usual (201). Less significant was the alteration of sedimentation rate following dissassembly of the MTs (14). Unfortunately, it has been difficult to visualize the putative actin strands in the statocytes by immunofluorescence (14, 97, 127, 228, but see 64). It is probable that the actin turns over rapidly and that it exists only as short filamentous elements, or even as G actin. The actin filaments within the statocytes probably attach to receptors in the plasma membrane and/or ER. During graviperception they trigger the asymmetric outflow of information from the cells which is crucial for initiating the graviresponse (12). Mutation at the gene locus *ARG1* (Altered Response to Gravity) in *Arabidopsis* brings about a slower rate of root gravitropic bending. The protein encoded by *ARG1* is a DnaJ-like protein (199) and it is possible that it transduces gravity signals in the statocytes by its interaction with the actin cytoskeleton.

MUTATIONS AFFECTING THE CYTOSKELETON AND ROOT DEVELOPMENT

Although mutations affecting root systems have been know for a long time, it is only recently that those affecting the cellular behavior of roots have come to the

fore. There are at least two types of mutation which affect the cytoskeleton and, hence, the morphogenesis of the root apex. They involve: (*a*) impairment to the orientation of cortical MTs, and (*b*) the formation of the cell plate.

Screens of mutagenized *Arabidopsis* seedlings revealed stunted individuals with depolarized cell growth in their roots (211). Meristematic cells of the *ton1* and *ton2* mutants were characterized by random orientations of their cortical MTs. Significantly, PPBs were consistently absent, although mitotic spindles and phragmoplasts were present and normal. The *fass* mutant presented a similar phenotype to *ton* and may be allelic to it. Electron microcopy showed the cortical MTs to be more sparsely spaced along the plasma membranes and to have a more haphazard orientation (148). One possible basis of the cytoskeletal lesion is the failure to regulate endogenonous hormonal levels. *fass* seedlings contained 2.6-times more free auxin (IAA) than did wild-type, though these levels varied considerably between samples (73). The elevated auxin may be responsible for a two- to threefold rise in ethylene production which, in turn, could have had an impact upon the cortical MT arrays: earlier we mentioned that ethylene and auxin tend to disturb cortical MT orientation (11, 20).

More subtle effects on cortical MTs were associated with altered levels of gibberellins. The respective *d5* and *gib-1* mutants of maize and tomato, with impaired gibberellin biosynthesis, had slightly thicker roots and, in the meristematic cells, the cortical MTs also tended to deviate from the usual transverse orientation (16). This, in turn, led to altered division patterns in the formative zone of the root cortex (23, 25). These effects on the MTs can be phenocopied in wild-type roots by exposing them to the gibberellin biosynthesis-inhibitor, paclobutrazol, and corrected by addition of gibberellic acid. Although there is a large conceptual gap between hormones and MT orientation, one factor which could provide a link is the posttranslational modification of tubulin isotypes. Internodal epidermal cells of a pea mutant, dwarfed as a result of the *le* gene which depresses gibberellin levels, had cortical MTs which tended to be longitudinally oriented (67). Within 2 h of its application, gibberellic acid had promoted transverse MTs. This alteration to the MTs was associated with an inability of the α-1 tubulin isotype to react with YL1/2 antibody which specifically probes tyrosinated tubulin. Since the α-1 isotype continued to be present, the implication is that the α-1 tubulin of the *le* mutant was detyrosinated when gibberellin levels were high, and that this led to MT reorientation. In another system (protoplasts from maize cell suspension), addition of gibberellic acid resulted in a stimulation of α-tubulin acetylation (as judged by affinity for 6-11B-1 antibody), more organized cortical MTs, and greater resistance to freezing temperatures (108).

A contrasting situation is found in the roots of certain conditional mutants of *Arabidopsis* (91). The mutations *cobra, quill,* and some others, showed altered polarity of cell growth, but it is uncertain whether this was due to altered cortical MT orientation. Presumably, PPBs were normal since there was no mention of aberrant cell divisions. A deeper analysis of cell growth seems in prospect, given the numerous mutations that influence cell shape in the root (29, 91). The *tangled*

mutant of maize, for example, is a good candidate for further study, given its effect on MT orientation in leaves (52). In fact, root tissues of the *pygmy* mutant of maize (40), which is cognate with *tangled,* also show disturbed cortical MT arrangements (PW Barlow & JS Parker, unpublished data), and these almost certainly account for the stunted root growth and irregular cell files (40). Nevertheless, it is possible that such mutants have their basis in cytoskeletal components other than the MTs. Involvement of actin filaments is a possibility.

A second class of mutants, again in *Arabidopsis*, affected in the division process, yield information about the molecules involved in phragmoplast structure and function. The *knolle* mutant presents embryos with both large and small cells with incomplete division walls (147). It was said that endomitotic cycles were present, but the evidence suggests that the nuclei in question are mitotic and polyploid as a result of nuclear fusion following incomplete cytokinesis. Further characterization revealed (132) that the *KNOLLE* gene encodes for a syntaxin protein involved in the fusion of the vesicles from which the cell plate is assembled at late telophase. The defect is not totally effective since walls do form, though a fraction of them are incomplete in their central portion. Wall stubs are often present. As mentioned earlier, these stubs may develop by a complementary pathway which becomes apparent only when cell plate formation is defective. The incompleteness of the walls can also result in a failure to develop cell layers. Only when such layers have been constructed can the positional information inherent within the developing embryo be interpreted correctly. Thus, in *knolle*, anthocyanin accumulates in epidermal cells instead of subepidermal cells, an error which probably occurs because the walls separating these two layers are incomplete. The *keule* mutant also shows incomplete division walls due to defective cytokinesis (7), but these are seen mainly in meristematic rather than mature cells. It may be that the defective division wall is eventually completed by continued growth of the wall stubs. Another cytokinesis-defective mutant of *Arabidopsis, cyt-1,* also shows defective division walls (wall stubs were present) (176). This mutant seems to be affected in a way distinct from *keulle* or *knolle* since it also showed an altered pattern of callose deposition not shared by the other mutants. Callose is a component of the cell plate and early division wall, so it is possible that it is not deployed correctly within the phragmoplast, and it is this that causes the cell plate to fail.

Mutations in the *PILZ* group of genes of *Arabidopsis* appear to abolish the functional assembly of all classes of MTs (157). Although some aberrant cell divisions can nevertheless occur (again with poorly differentiated wall stubs), no root organ forms in such mutant embryos.

A mutant, *cyd,* discovered in pea, has some similarities with *keulle* and *knolle* of *Arabidopsis* in that multinucleate cells occur (139). Such cells were more frequent in embryonic cotyledons (73%) than they were in roots (28%). However, it is not clear where in the root tip the abnormal cells were produced since meristematic cells were not multinucleate. This led to the presumption that they had been formed in cells outside the meristem. This unsatisfactory conclusion may indicate that the gene shows penetrance only during the early stages of embryo axis development

and that multinucleate cells were formed at this time, but not later on. This would agree with the finding that seedlings derived from cultured embryos had a lower frequency of multinucleate cells than did the embryos. It could be that the mutation has a penetrance regulated by the type of meristem or the stage of its development. Penetrance effects are clearly seen in the multinucleate *MUN* mutants of *Arabidopsis:* defects of cytokinesis are found only in the roots and not elsewhere (180). By contrast, the *cyd1* mutation results in aberrant cytokineses in all dividing cells except those of the root meristems (234). Is it possible that such differential effects are regulated by organ-specific isoforms of cytoskeletal proteins?

CONCLUDING REMARKS

The identification of mutations which impair cell division and disturb the usual orientation and arrangement of cell growth in roots will continue to unravel the molecular mechanism by which cells in general reproduce and attain identities within tissues. The role of the cytoskeleton in differentiation could also be revealed in a more specific way if the affected cells—i.e. those which utilize particular cytoskeletal arrays for their development—could be identified in mutagenized plant populations. Close analysis of tissue differentiation, with the concept of positional information as a context for interpretation, might reveal the role of tissue compartmentation (resulting from cytokinesis) in the differentiation process. It might even be possible to test whether there are local genetic controls over cytokinesis, invoking specific orientations of cell division at precise locations within the root meristem.

ACKNOWLEDGMENTS

Visit the Annual Reviews home page at www.AnnualReviews.org

LITERATURE CITED

1. Akashi T, Shibaoka H. 1991. Involvement of transmembrane proteins in the association of cortical microtubules with the plasma membrane in tobacco BY-2 cells. *J. Cell Sci.* 98:169–74

2. An Y-Q, Huang S, McDowell JM, Mc-Kinney EC, Meagher RB. 1996. Conserved expression of the *Arabidopsis* ACT1 and ACT3 actin subclass in organ primordia and mature pollen. *Plant Cell* 8:15–30

3. Apostolakos P, Galatis B, Panteris E. 1991. Microtubules in cell morphogenesis and

intercellular formation in *Zea mays* leaf mesophyll and *Pilea cadierei* epithem. *J. Plant Physiol.* 137:591–601

4. Asada T, Collings D. 1997. Molecular motors in higher plants. *Trends Plant Sci.* 2:29–37

5. Asada T, Kuriyama R, Shibaoka H. 1997. TKRP125, a kinesis-related protein involved in the centrosome-independent organization of the cytokinetic apparatus in tobacco BY-2 cells. *J. Cell Sci.* 110:179–89

6. Asada T, Shibaoka H. 1994. Isolation of polypeptides with microtubule-translocating activity from phragmoplasts of tobacco BY-2 cells. *J. Cell Sci.* 107:2249–57

7. Assaad FF, Mayer U, Wanner G, Jürgens G. 1996. The *KEULE* gene is involved in cytokinesis in *Arabidopsis. Mol. Gen. Genet.* 253:267–77

8. Baird WV, Meagher RB. 1987. A complex gene superfamily encodes actin in petunia. *EMBO J.* 6:3223–31

9. Baluška F, Barlow PW, Hauskrecht M, Kubica Š, Parker JS, Volkmann D. 1995. Microtubule arrays in maize root cells. Interplay between the cytoskeleton, nuclear organization and post-mitotic cellular growth patterns. *New Phytol.* 130:177–92

10. Baluška F, Barlow PW, Lichtscheidl IK, Volkmann D. 1998. The plant cell body: a cytoskeletal tool for cellular development and morphogenesis. *Protoplasma* 202:1–10

11. Baluška F, Brailsford RW, Hauskrecht M, Jackson MB, Barlow PW. 1993. Cellular dimorphism in the maize root cortex: involvement of microtubules, ethylene and gibberellin in the differentiation of cellular behaviour in postmitotic gowth zones. *Bot. Acta* 106:394–403

12. Baluška F, Hasenstein KH. 1997. Root cytoskeleton: its role in perception of and response to gravity. *Planta* 303:S69–S78

13. Baluška F, Hauskrecht M, Barlow PW, Sievers A. 1996. Gravitropism of the primary root of maize: a complex pattern of differential cellular growth in the cortex independent of the microtubular cytoskeleton. *Planta* 198:310–18

14. Baluška F, Kreibaum A, Vitha S, Parker JS, Barlow PW, Sievers A. 1997. Central root cap cells are depleted of endoplasmic microtubules and actin microfilament bundles: implications for their role as gravity-sensing statocytes. *Protoplasma* 196:212–23

15. Baluška F, Parker JS, Barlow PW. 1992. Specific patterns of cortical and endoplasmic microtubules associated with cell growth and tissue differentiation in roots of maize (*Zea mays* L.). *J. Cell Sci.* 103:191–200

16. Baluška F, Parker JS, Barlow PW. 1993. A role for gibberellic acid in orienting microtubules and cell growth polarity in the maize root cortex. *Planta* 191:149–57

17. Baluška F, Šamaj J, Kendrick-Jones J, Barlow PW, Staiger CJ, Volkmann. 1997. Tissue- and domain-specific distributions and re-distributions of actin microfilaments, myosins, and profilin isoforms in cells of root apices. *Cell Biol. Int.* 21:852–54

18. Baluška F, Vitha S, Barlow PW, Volkmann D. 1997. Rearrangements of F-actin in growing cells of intact maize root tissue: A major developmental switch occurs in the postmitotic transition region. *Eur. J. Cell Biol.* 72:113–21

19. Baluška F, Volkmann D, Barlow PW. 1996. Specialized zones of development in roots: view from the cellular level. *Plant Physiol.* 112:3–4

20. Baluška F, Volkmann D, Barlow PW. 1996. Complete disintegration of the microtubular cytoskeleton precedes auxin-mediated reconstruction in post-mitotic maize root cells. *Plant Cell Physiol.* 37:1013–21

21. Baluška F, Volkmann D, Barlow PW. 1997. Nuclear components with microtubule-organizing properties in multicellular eukaryotes: functional and evolutionary considerations. *Int. Rev. Cytol.* 175:91–135

22. Baluška F, Volkmann D, Barlow PW. 1998. Tissue- and development-specific distributions of cytoskeletal elements in growing cells of the maize root apex. *Plant Biosyst.* 132:251–65

23. Baluška F, Volkmann D, Barlow PW. 1999. Hormone-cytoskeleton interactions in plant cells. In *Biochemistry and Molecular Biology of Plant Hormones*, ed. PJJ Hooykaas, MA Hall, KR Libbenga, pp. 363–90. Amsterdam: Elsevier

23a. Baluška F, Volkmann D, Barlow PW. 2000. Actin-based cell cortex domains and their association with polarized 'plant-cell bodies' in higher plants. *Plant. Biol.* In press

24. Barlow PW. 1971. Properties of cells in the root apex. *Rev. Fac. Agron. La Plata* 47:275–301

25. Barlow PW. 1995. The cytoskeleton and its role in determining the cellular architecture of roots. *G. Bot. Ital.* 129:863–72

26. Barlow PW. 1997. Stem cells and founder zones in plants, particularly their roots. In *Stem Cells*, ed. CS Potten, pp. 29–57. London: Academic

27. Barlow PW, Hofer R-M. 1982. Mitotic activity and cell elongation in geostimulated roots of *Zea mays. Physiol. Plant.* 54:137–41

28. Barlow PW, Parker JS. 1996. Microtubular cytoskeleton and root morphogenesis. *Plant Soil* 187:23–36

29. Baskin TI, Betzner AS, Hoggart R, Cork A, Williamson RE. 1992. Root morphology mutants in *Arabidopsis thaliana. Aust. J. Plant Physiol.* 10:427–37

30. Baskin TI, Cande WZ. 1990. The structure and function of the mitotic spindle in flowering plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41:277–315

31. Bassell G, Singer RH. 1997. mRNA and cytoskeletal filaments. *Curr. Opin. Cell Biol.* 9:109–15

32. Binarova P, Rennie P, Fowke L. 1994. Probing microtubule organizing centres with MPM-2 in dividing cells of higher plants using immunofluorescence and immunogold techniques. *Protoplasma* 180: 106–17

33. Blancaflor EB, Hasenstein KH. 1993. Organization of cortical microtubules in graviresponding maize roots. *Planta* 191: 231–37

34. Blancaflor EB, Hasenstein KH. 1995. Time course and auxin sensitivity of cortical microtubule reorientation in maize roots. *Protoplasma* 185:72–82

35. Blancaflor EB, Hasenstein KH. 1997. The organization of the actin cytosksleton in vertical and graviresponding primary roots of maize. *Plant Physiol.* 113:1447–55

36. Bonfante P, Bergero R, Uribe X, Romera C, Rigau J, Puigdomènech P. 1996. Transcriptional activation of maize *α*-tubulin gene in mycorrhizal maize and transgenic tobacco plants. *Plant J.* 9:737–43

37. Bulinski JC, Gundersen GG. 1991. Stabilization and post-translational modification of microtubules during cellular morphogenesis. *BioEssays* 13:285–93

38. Busson S, Dujardin D, Moreau A, Dompierre J, De May JR. 1998. Dynein and dynactin are localized to astral microtubules and at cortical sites in mitotic epithelial cells. *Curr. Biol.* 8:541–44

39. Bustos MM, Guiltinan MF, Cyr RJ, Ahdoot D, Fosket DE. 1989. Light regulation of *β*-tubulin gene expression during internode development in soybean (*Glycine max* [L.] Merr.). *Plant Physiol.* 91:1157–61

40. Byer M. 1957–58. Cytohistological abnormalities associated with the gene "Pigmy" (Py-1) in the primary root tip of corn (*Zea mays* L.). *Proc. Minn. Acad. Sci.* 25/26:1–10

41. Carnero Diaz E, Martin F, Tagu D. 1996. Eucalypt *α*-tubulin: cDNA cloning and increased level of transcript in ectomycorrhizal root system. *Plant Mol. Biol.* 31:905–10

42. Chaffey NJ, Barlow PW, Barnett JR. 1997.

Cortical microtubules rearrange during differentiation of vascular cambial derivatives, microfilaments do not. *Trees* 11:333–41

43. Chaffey NJ, Barlow PW, Barnett JR. 1998. A seasonal cycle of cell wall structure is accompanied by a cyclical rearrangement of cortical microtubules in fusiform cambial cells within taproots of *Aesculus hippocastanum* (Hippocastanaceae). *New Phytol.* 139:623–35

44. Chaffey NJ, Barnett JR, Barlow PW. 1999. A cytoskeletal basis for wood formation in angiosperm trees: the involvement of cortical microtubules. *Planta* 208:19–30

45. Chan J, Rutten T, Lloyd C. 1996. Isolation of microtubule-associated proteins from carrot cytoskeletons: A 120 kDa MAP decorates all four microtubule arrays and the nucleus. *Plant J.* 10:251–59

46. Chen J, Kanai Y, Cowan NJ, Hirokawa N. 1992. Projection domains of MAP2 and tau determine spacing between microtubules in dendrites and axons. *Nature* 360:674–77

47. Chu B, Kerr GP, Carter JV. 1993. Stabilizing microtubules with taxol increases microfilament stability during freezing of rye root tips. *Plant Cell Environ.* 16:883–89

48. Chu B, Wilson TJ, McCune-Zierath C, Snustad DP, Carter JV. 1998. Two β-tubulin genes, TUB1 and TUB8, of *Arabidopsis* exhibit largely nonoverlapping patterns of expression. *Plant Mol. Biol.* 37:785–90

49. Clayton L, Lloyd CW. 1985. Actin organization during the cell cycle in meristematic plant cells. *Exp. Cell* Res. 156:231–38

50. Deleted in proof

51. Cleary AL, Gunning BES, Wasteneys GO, Hepler PK. 1992. Microtubules and F-actin dynamics at the division site in living *Tradescantia* stamen hair cells. *J. Cell Sci.* 103:977–88

52. Cleary AL, Smith LC. 1998. The *Tangled1* gene is required for spatial control of cytoskeletal arrays associated with cell division during maize leaf development. *Plant Cell* 10:1875–88

53. Clore AM, Dannehafer JM, Larkins BM. 1996. Ef-1α is associated with a cytoskeletal network surrounding protein bodies in maize endosperm cells. *Plant Cell* 8:2003–14

54. Colasanti J, Cho S-O, Wick S, Sundaresan V. 1993. Localization of the functional p34^{cdc2} homolog of maize in root tip and stomatal complex cells: association with predicted division sites. *Plant Cell* 5:1101–11

55. Collings DA, Asada T, Allen NS, Shibaoka H. 1998. Plasma membrane-associated actin in Bright Yellow 2 tobacco cells. Evidence for interaction with microtubules. *Plant Physiol.* 118:917–28

56. Cyr RJ. 1994. Microtubules in plant morphogenesis. Role of the cortical array. *Annu. Rev. Cell Biol.* 10:153–80

57. Cyr RJ, Lin B-L, Jernstedt JA. 1988. Root contraction in hyacinth. II. Changes in tubulin levels, microtubule number and orientation associated with differential cell expansion. *Planta* 174:446–52

58. Cyr RJ, Palevitz BA. 1989. Microtubule-binding proteins from carrot I. Initial characterization and microtubule binding. *Planta* 177:245–60

59. Czihak G, Kojima M, Linhart J, Vogel H. 1991. Multipolar mitosis in procaine-treated polyspermic sea urchin eggs and in eggs fertilized with UV-irradiated spermatozoa with a computer model to simulate the positioning of centrosomes. *Eur. J. Cell Biol.* 55:255–61

60. Darbelley N, Perbal G. 1984. Gravité et différentiation des cellules corticales dans la racine de lentille. *Biol. Cell* 50:93–98

61. De Castro R, Zheng X, Bergervoet JHW, De Vos CHR, Bino RJ. 1995. β-tubulin accumulation and DNA replication in

imbibing tomato seeds. *Plant Physiol.* 109:499–504

62. Del Vecchio AJ, Harper JDI, Vaughn KC, Baron AT, Salisbury JL, Overall RL. 1997. Centrin homologues in higher plants are prominently associated with the developing cell plate. *Protoplasma* 196:224–34

63. Derksen J, Jeucken G, Traas JA, Van Lammeren AAM. 1986. The microtubular cytoskeleton in differentiating root tips of *Raphanus sativus* L. *Acta Bot. Neerl.* 35:223–31

64. Ding B, Turgeon R, Parthasarathy MV. 1991. Microfilament organization and distribution in freeze substituted tobacco plant tissues. *Protoplasma* 165:96–105

65. Ding B, Turgeon R, Parthasarathy MV. 1991. Microfilaments in the preprophase band of freeze substituted tobacco root cells. *Protoplasma* 165:209–11

66. Dolfini S, Consonni G, Mereghetti M, Tonelli C. 1993. Antiparallel expression of the sense and antisense transcripts of maize α-tubulin genes. *Mol. Gen. Genet.* 241:161–69

67. Duckett CM, Lloyd, CW. 1994. Gibberellic acid-induced microtubule reorientation in dwarf peas is accompanied by rapid modification of an α-tubulin isotype. *Plant J.* 5:363–72

68. Durso NA, Cyr RJ. 1994. Beyond translation: elongation factor-1α and the cytoskeleton. *Protoplasma* 180:99–105

69. Eleftheriou EP, Palevitz BA. 1992. The effect of cytochalsin D on preprophase band organization in root tip cells of *Allium. J. Cell Sci.* 103:989–98

70. Endlé M-C, Stoppin V, Lambert A-M, Schmit A-C. 1998. The growing cell plate of higher plants is a site of both actin assembly and vinculin-like antigen recruitment. *Eur. J. Cell Biol.* 77:10–18

71. Ericson ME, Carter JV. 1996. Immunolabeled microtubules and microfilaments are visible in multiple cell layers of rye root tip sections. *Protoplasma* 191:215–19

71a. Eun S-O, Wick SM. 1998. Tubulin isoform usage in maize microtubules. *Protoplasma* 204:235–44

72. Farrar JJ, Evert RF. 1997. Ultrastructure of cell division in the fusiform cells of the vascular cambium of *Robinia pseudoacacia. Trees* 11:203–15

73. Fisher RH, Barton MK, Cohen JD, Cooke TJ. 1996. Hormonal studies of *fass,* an *Arabidopsis* mutant that is altered in organ elongation. *Plant Physiol.* 110:1109–21

74. Folsom DB, Brown RM Jr. 1987. Changes in cellulose microfibril orientation during differential growth in oat coleoptiles. In *Physiology of Cell Expansion during Plant Growth*, ed. DJ Cosgrove, DP Knievel, pp. 58–73. Rockville: Am. Soc. Plant Physiol.

75. Galatis B, Apostolakos P, Katsaros C. 1984. Experimental studies on the function of the cortical cytoplasmic zone of the preprophase microtubule band. *Protoplasma* 122:11–26

76. Giddings TH Jr, Staehelin LA. 1988. Spatial relationship between microtubules and plasma-membrane rosettes during the deposition of primary wall microfibrils in *Closterium* sp. *Planta* 173:22–30

76a. Glimer S, Clay P, MacRae TH, Fowke LC. 1999. Acetylated tubulin is found in all microtubule arrays of two species of pine. *Protoplasma* 207:174–85

76b. Glimer S, Clay P, MacRae TH, Fowke LC. 1999. Tyrosinated, but not detyrosinated, α-tubulin is present in root tip cells. *Protoplasma* 210:92–98

77. Goddard RH, Wick SM, Silflow CD, Snustad DP. 1994. Microtubule components of the plant cytoskeleton. *Plant Physiol.* 104:1–6

78. Goodbody KC, Venverloo CJ, Lloyd CW. 1991. Laser microsurgery demonstrates that cytoplasmic strands anchoring the nucleus across the vacuole of

premitotic plant cells are under tension. Implications for division plane alignment. *Development* 113:931–39

79. Goode JA, Alfano F, Stead AD, Duckett JG. 1993. The formation of aplastidic abscission (tmema) cells and protonemal disruption in the moss *Bryum tenuisetum* Limpr. is associated with transverse arrays of microtubules and microfilaments. *Protoplasma* 174:158–72

80. Grabski S, Xie XG, Holland JF, Schindler M. 1994. Lipids trigger changes in the elasticity of the cytoskeleton in plant cells: a cell optical displacement assay for live cell measurements. *J. Cell Biol.* 126:713–26

81. Green PB. 1962. Mechanism for plant cellular morphogenesis. *Science* 138:1404–5

82. Gu X, Verma DPS. 1997. Dynamics of phragmoplastin in living cells during cell plate formation and microscopy of elongation from the plane of cell division. *Plant Cell* 9:157–69

83. Gunning BES. 1982. The root of the water fern *Azolla:* cellular basis of development and multiple roles for cortical microtubules. In *Developmental Order: Its Origin and Regulation,* ed. S Subtelny, pp. 379–421. New York: Liss

84. Gunning BES. 1992. Use of confocal microscopy to examine transitions between successive microtubule arrays in the plant cell division cycle. In *Proc. Int. Jpn. Prize Symp. Cell. Basis Growth Dev. Plants*, 7th, ed. H Shibaoka, pp. 145–55. Osaka: Osaka Univ. Press

85. Hahne G, Hoffmann F. 1985. Cortical microtubular lattices: absent from mature mesophyll and necessary for division? *Planta* 166:309–13

86. Hardham AR, Gunning BES. 1978. Structure of cortical microtubule arrays in plant cells. *J. Cell Biol.* 77:14–34

87. Hardham AR, Gunning BES. 1979. Interpolation of microtubules into cortical arrays during cell elongation and differentiation in roots of *Azolla pinnata. J. Cell Sci.* 37:411–42

88. Hasezawa S, Hogetsu T, Syono K. 1988. Rearrangement of cortical microtubules in elongating cells derived from tobacco protoplasts—a time-course observation by immunofluorescence microscopy. *J. Plant Physiol.* 133:46–51

89. Hasezawa S, Kumagai F, Nagata T. 1997. Sites of microtubule reorganization in tobacco BY-2 cells during cell-cycle progression. *Protoplasma* 198:202–9

90. Hasezawa S, Nagata T. 1993. Microtubule organizing centers in plant cells: localization of a 49 kDa protein that is immunologically cross-reactive to a 51 kDa protein from sea urchin centrosomes in synchronized tobacco BY-2 cells. *Protoplasma* 176:64–74

91. Hauser M-T, Morikami A, Benfy PN. 1995. Conditional root expansion mutants of *Arabidopsis. Development* 121:1237–52

92. Hellmann A, Wernicke W. 1998. Changes in tubulin protein expression accompanying reorganization of microtubular arrays during cell shaping in barley leaves. *Planta* 204:220–25

93. Hensel W. 1984. Microtubules in statocytes from roots of cress (*Lepidium sativum* L.). *Protoplasma* 119:121–34

94. Hensel W. 1984. A role for microtubules in the polarity of statocytes from roots of *Lepidium sativum* L. *Planta* 162:404–14

95. Hensel W. 1985. Cytochalasin B affects the structural polarity of statocytes from cress roots (*Lepidium sativum* L.). *Protoplasma* 129:178–87

96. Hensel W. 1986. Cytodifferentiation of polar plant cells. Use of anti-microtubular agents during differentiation of statocytes from cress roots (*Lepidium sativum* L.). *Planta* 169:293–303

97. Hensel W. 1989. Tissue slices from living root caps as a model system in which to study cytodifferentiation of polar cells. *Planta* 177:296–303

98. Hepler PK, Palevitz BA, Lancelle SA, McCauley MM, Lichtscheidl IK. 1990.

Cortical endoplasm in plants. *J.Cell Sci.* 96:355–73

99. Herth W. 1985. Plasma-membrane rosettes involved in localized wall thickening during xylem vessel formation of *Lepidium sativum* L. *Planta* 164:12–21

100. Himmelspach R, Wymer CL, Lloyd CW, Nick P. 1999. Gravity-induced reorientation of cortical microtubules observed in vivo. *Plant J.* 18:449–53

101. Hirokawa N. 1994. Microtubule organization and dynamics dependent on microtubule-associated proteins. *Curr. Opin. Cell Biol.* 6:74–81

102. Hoffmann JC, Vaughn KC. 1994. Mitotic disrupter herbicides act by a single mechanism but vary in efficacy. *Protoplasma* 179:16–25

103. Hogetsu T. 1990. Mechanism for formation of the secondary wall thickening in tracheary elements: microtubules and microfibrils of tracheary elements of *Pisum sativum* L. and *Commelina communis* L. and the effects of amiprophos-methyl. *Planta* 185:190–200

104. Hogetsu T, Oshima Y. 1986. Immunofluorescence microscopy of microtubule arrangement in root cells of *Pisum sativum* L. var Alaska. *Plant Cell Physiol.* 27:939–45

105. Hoss S, Wernicke W. 1995. Microtubules and the establishment of apparent cell wall invaginations in mesophyll cells of *Pinus silvestris* L. *J. Plant Physiol.* 147:474–76

106. Hoyle HD, Raff EC. 1990. Two *Drosophila* beta tubulin isoforms are not functionally equivalent. *J. Cell Biol.* 111:1009–26

107. Huang B-Q, Ye X-L, Yeung EC, Zee SY. 1998. Embryology of *Cymbidium sinense:* the microtubule organization of early embryos. *Ann. Bot.* 81:741–50

108. Huang RF, Lloyd CW. 1999. Gibberellic acid stabilizes microtubules in maize suspension cells to cold and stimulates acety-

lation of α-tubulin. *FEBS Letts.* 443:317–20

109. Hugdahl JD, Bokros CL, Morejohn LC. 1995. End-to-end annealing of plant microtubules by the p86 subunit of eukaryotic initiation factor–(iso)4F. *Plant Cell* 7:2129–38

110. Hush JM, Overall RL. 1991. Electrical and mechanical fields orient cortical microtubules in higher plant tissues. *Cell Biol. Int. Rep.* 15:551–60

111. Hussey PJ, Gull K. 1985. Multiple isotypes of alpha-tubulin and beta-tubulin in the plant *Phaseolus vulgaris. FEBS Letts.* 181:113–18

112. Hussey PJ, Lloyd CW, Gull K. 1988. Differential and developmental expression of β-tubulins in a higher plant. *J. Biol. Chem.* 263:5474–79

113. Hussey PJ, Snustad DP, Silflow CD. 1991. Tubulin gene expression in higher plants. See Ref. 140a, pp. 15–27

113a. Hyams JS, Lloyd CW, eds. 1994. *Microtubules.* New York: Liss

114. Isenberg G, Niggli V. 1998. Interaction of cytoskeletal proteins with membrane lipids. *Int. Rev. Cytol.* 178:73–125

115. Janssen M, Hunte C, Schulz M, Schnabl H. 1996. Tissue specification and intracellular distribution of actin isoforms in *Vicia faba* L. *Protoplasma* 191:158–63

116. Jernstedt JA. 1984. Root contraction in Hyacinth. I. Effects of IAA on differential cell expansion. *Am. J. Bot.* 71:1080–89

117. Jiang C-L, Sonobe S. 1993. Identification and preliminary characterization of a 65 kDa higher-plant microtubule-associated protein. *J. Cell Sci.* 105:891–901

118. Jones MGK, Payne HL. 1977. Cytokinesis in *Impatiens balsamina* and the effect of caffeine. *Cytobios* 20:79–91

119. Jones MGK, Payne HL. 1977. Early stages of nematode-induced giant-cell formation in roots of *Impatiens*

balsamina. J. Nematol. 10:70–84

120. Jongewaard I, Colon A, Fosket DE, 1994. Distribution of transcripts of the *tub B1* β-tubulin gene in developing soybean (*Glycine max* [L.] Merr.) seedling organs. *Protoplasma* 183:77–85

121. Joyce CM, Villemur R, Snustad DP, Silflow CD. 1992. Tubulin gene expression in maize (*Zea mays* L.). Change in isotype expression along the developmental axis of seedling root. *J. Mol. Biol.* 227:97–107

122. Jung G, Wernicke W. 1990. Cell shaping and microtubules in developing mesophyll of wheat (*Triticum aestivum* L.). *Protoplasma* 153:141–48

123. Jürgens G, Mayer U. 1994. Arabidopsis. In *Embryos. A Colour Atlas of Development*, ed. J Bard, pp. 7–21. London: Wolfe

124. Kerr GP, Carter JV. 1990. Relationship between freezing tolerance of root-tip cells and cold stability of microtubules in rye (*Secale cereale* L. cv Puma). *Plant Physiol.* 93:77–82

125. Kerr GP, Carter JV. 1990. Tubulin isotypes in rye roots are altered during cold acclimation. *Plant Physiol.* 93:83–88

126. Kopczak SD, Haas NA, Hussey PJ, Silflow CD, Snustad DP. 1992. The small genome of *Arabidopsis* contains at least six expressed α-tubulin genes. *Plant Cell* 4:539–47

127. Koropp K, Volkmann D. 1994. Monoclonal antibody CRA against a fraction of actin from cress roots recognizes its antigen in different plant species. *Eur. J. Cell Biol.* 64:116–26

128. Kropf DL, Williamson RE, Wasteneys GO. 1997. Microtubule orientation and dynamics in elongating characean internodal cells following cytosolic acidification, induction of pH bands, or premature growth arrest. *Protoplasma* 197:188–98

129. Kubiak JZ, Tarkowska JA. 1987. Evidence for two sets of cytoplasmic microtubules in interphase and prophase cells of onion root. An immunofluorescence

study. *Cytologia* 52:781–86

130. Kumagai F, Hasezawa S, Takahashi Y, Nagata T. 1995. The involvement of protein synthesis elongation factor 1α in the organization of microtubules on the perinuclear region during the cell cycle transition from M phase to G_1 phase in tobacco BY-2 cells. *Bot. Acta* 108:467–73

131. Lasselain M-J, Deysson G. 1979. Maturation et cheminement des vésicules de cytodiérèse: étude cytochimique sur les phragmoplastes incomplets. *C. R. Acad. Sci. Paris, Ser. D* 288:879–81

132. Lauber M, Waizenegger I, Steinmann T, Schwarz H, Mayer U, et al. 1997. The *Arabidopsis KNOLLE* protein is a cytokinesis-specific syntaxin. *J. Cell Biol.* 139:1485–93

133. Ledbetter MC, Porter KR. 1963. A "microtubule" in plant cell fine structure. *J. Cell Biol.* 19:239–50

134. Lewis SA, Gu W, Cowan NJ. 1987. Free intermingling of mammalian β-tubulin isotypes among functionally distinct microtubules. *Cell* 49:539–48

135. Liang BM, Dennings AM, Sharp RE, Baskin TI. 1996. Consistent handedness of microtubule helical arrays in maize and *Arabidopsis* primary roots. *Protoplasma* 190:8–15

136. Lin B-L, Jernstedt JA. 1987. Microtubule organization in root cortical cells of *Hyacinthus orientalis*. *Protoplasma* 141:13–23

137. Liu B, Marc J, Joshi HC, Palevitz BA. 1993. A γ-tubulin-related protein associated with the microtubule arrays of higher plants in a cell cycle-dependent manner. *J. Cell Sci.* 104:1217–28

138. Liu B, Palevitz BA. 1992. Organization of cortical microfilaments in dividing root cells. *Cell Motil. Cytoskel.* 23:252–64

139. Liu C-M, Johnson S, Wang TL. 1995. *cyd*, a mutant of pea that alters embryo morphology is defective in cytokinesis. *Dev. Genet.* 16:321–31

140. Lloyd CW. 1987. The plant cytoskeleton:

the impact of fluorescence microscopy. *Annu. Rev. Plant Physiol.* 38:119–39

140a. Lloyd CW, ed. 1991. *The Cytoskeletal Basis of Plant Growth and Form.* London: Academic

141. Lloyd CW, Traas JA. 1988. The role of F-actin in determining the division plane in carrot suspension cells. Drug studies. *Development* 102:211–21

142. Lorenzi G, Perbal G. 1990. Root growth and statocyte polarity in lentil seedling roots grown in microgravity or on a slowly rotating clinostat. *Physiol. Plant.* 78:532–37

143. Lorenzi G, Perbal G. 1990. Actin filaments responsible for the location of the nucleus in the lentil statocyte are sensitive to gravity. *Biol. Cell* 68:259–63

144. Ludueña RF. 1993. Are tubulin isotypes functionally significant? *Mol. Biol. Cell* 4:445–57

145. Ludueña RF. 1998. Multiple forms of tubulin: different gene products and covalent modifications. *Int. Rev. Cytol.* 178:207–75

146. Ludwig SR, Oppenheimer DG, Silflow CD, Snustad DP. 1987. Characterization of the α-tubulin gene family in *Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA* 84:5833–37

147. Lukowitz W, Mayer U, Jürgens G. 1996. Cytokinesis in the *Arabidopsis* embryo involves the syntaxin-related *KNOLLE* gene product. *Cell* 84:61–71

148. McClinton RS, Sung ZR. 1997. Organization of cortical microtubules at the plasma membrane in *Arabidopsis. Planta* 201:252–60

149. McCurdy, DW, Sammut M, Gunning BES. 1988. Immunofluorescent visualization of arrays of transverse cortical actin microfilaments in wheat root-tip cells. *Protoplasma* 147:204–6

150. McDaniel DP, Roberson RW. 1998. γ-tubulin is a component of the Spitzenkörper and centrosomes in hyphal-tip cells of *Allomyces macrogynus. Proto-*

plasma 203:118–23

151. McElroy D, Rothenberg M, Wu R. 1990. Structural characterization of a rice actin gene. *Plant Mol. Biol.* 14:163–77

152. McLean BG, Eubanks S, Meagher RB. 1990. Tissue-specific expression of divergent actins in soybean root. *Plant Cell* 2:335–44

153. McNally FJ. 1996. Modulation of microtubule dynamics during the cell cycle. *Curr. Opin. Cell Biol.* 8:23–29

154. Maniotis AJ, Chen CS, Ingber DE. 1997. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc. Natl. Acad. Sci. USA* 94:849–54

155. Marc J. 1997. Microtubule-organizing centres in plants. *Trends Plant Sci.* 2:223–30

156. Marc J, Sharkey DE, Durso NA, Zhang M, Cyr RJ. 1996. Isolation of a 90-kD microtubule-associated protein from tobacco membranes. *Plant Cell* 8:2127–38

157. Mayer U, Herzog U, Berger F, Inzé D, Jürgens G. 1999. Mutations in the *PILZ* group genes disrupt the microtubule cytoskeleton and uncouple cell cycle progression from cell division in *Arabidopsis* embryo and endosperm. *Eur. J. Cell Biol.* 78:100–8

158. Meagher RB. 1991. Divergence and differential expression of actin gene families in higher plants. *Int. Rev. Cytol.* 125:139–163

159. Meagher RB, McKinney EC, Kandasamy MK. 1999. Isovariant dynamics expand and buffer the responses of complex systems: the diverse plant actin gene family. *Plant Cell* 11:995–1005

160. Mendu N, Silflow CD. 1993. Elevated levels of tubulin transcripts accompany the GA3-induced elongation of oat internode segments. *Plant Cell Physiol.* 34:973–83

161. Menzel D, Elsner-Menzel C. 1990. The microtubule cytoskeleton in developing

cysts of the green alga *Acetabularia:* involvement in cell wall differentiation. *Protoplasma* 157:52–63

162. Mews M, Sek FJ, Moore R, Volkmann D, Gunning BES, John PCL. 1997. Mitotic cyclin distribution during maize cell division: implications for the sequence diversity and function of cyclins in plants. *Protoplasma* 200:128–45

163. Mineyuki Y. 1999. The preprophase band of microtubules: its function as a cytokinetic apparatus in higher plants. *Int. Rev. Cytol.* 187:1–49

164. Mineyuki Y, Aioi H, Yamashita M, Nagahama Y. 1996. A comparative study of stainability of preprophase bands by the PSTAIR antibody. *J. Plant Res.* 109:185–92

165. Mineyuki Y, Gunning BES. 1990. A role for preprophase bands of microtubules in maturation of new cell walls, and a general proposal on the function of preprophase band sites in cell division in higher plants. *J. Cell Sci.* 97:527–37

166. Mineyuki Y, Iida H, Anraku Y. 1994. Loss of microtubules in the interphase cells of onion (*Allium cepa* L.) root tips from the cell cortex and their appearance in the cytoplasm after treatement with cycloheximide. *Plant Physiol.* 104:281–84

167. Mineyuki Y, Palevitz BA. 1990. Relationship between preprophase band organization, F-actin and the division site in *Allium.* Fluorescence and morphometric studies on cytochalasin-treated cells. *J. Cell Sci.* 97:283–95

168. Montoliu Ll, Puigdomènech P, Rigau J. 1990. The *Tubα3* gene from *Zea mays*: structure and expression in dividing plant tissues. *Gene* 94:201–7

169. Montoliu Ll, Rigau J, Puigdomènech P. 1989. A tandem of α-tubulin genes preferentially expressed in radicular tissue from *Zea mays*. *Plant Mol. Biol.* 14:1–15

170. Moore JD, Endow SA. 1996. Kinesin proteins: a phylum of motors for microtubule-based motility. *BioEssays* 18:207–19

171. Morejohn LC. 1991. The molecular pharmacology of plant tubulin and microtubules. See Ref. 140a, pp. 29–43

172. Mueller SC, Brown RM Jr. 1980. Evidence for an intramembrane component associated with a cellulose microfibril-synthesizing complex in higher plants. *J. Cell Biol.* 84:315–26

173. Murata T, Kadota A, Hogetsu T, Wada M. 1987. Circular arrangement of cortical microtubules around the subapical part of a tip-growing fern protonema. *Protoplasma* 141:135–38

174. Newcomb EH. 1969. Plant microtubules. *Annu. Rev. Plant Physiol.* 20:253–88

175. Nick P. 1998. Signalling to the microtubular cytoskeleton in plants. *Int. Rev. Cytol.* 184:33–80

176. Nickle TC, Meinke DW. 1998. A cytokinesis-defective mutant of *Arabidopsis (cyt1)* characterized by embryonic lethality, incomplete cell walls, and excessive callose accumulation. *Plant J.* 15:321–32

177. Niini S, Raudaskoski M. 1998. Growth patterns in non-mycorrhizal and mycorrhizal short shoots of *Pinus sylvestris*. *Symbiosis* 25:101–14

178. Niini SS, Tarkka MT, Raudaskoski M. 1996. Tubulin and actin protein patterns in Scots pine (*Pinus sylvestris*) roots and developing ectomycorrhiza with *Suillus bovinus*. *Physiol. Plant.* 96:186–92

179. Nogami A, Suzaki T, Shigenaka Y, Nagahama Y, Mineyuki Y. 1996. Effects of cycloheximide on preprophase bands and prophase spindles in onion (*Allium cepa* L.) root tip cells. *Protoplasma* 192:109–21

180. Oveka M, iamporová M, Hauser M-T. 1996. Root specific mutation of *Arabidopsis thaliana* affecting cytokinesis: production and fate of multinucleate cells. *J. Comput.-Assist. Microsc.* 8:277–78

181. Packard MJ, Stack SM. 1976. The pre-prophase band: possible involvement in the formation of the cell wall. *J. Cell Sci.* 22:403–11

182. Palevitz BA. 1987. Actin in the pre-prophase band of *Allium cepa*. *J. Cell Biol.* 104:1515–19

183. Panteris E, Apostolakos P, Galatis B. 1992. The organization of F-actin in root tip cells of *Adiantum capillus veneris* throughout the cell cycle. A double label fluorescence microscopy study. *Protoplasma* 170:128–37

184. Parthasarathy MV, Pesacreta TC. 1980. Microfilaments in plant vascular cells. *Can. J. Bot.* 58:807–15

185. Perbal G. 1978. The mechanism of geoperception in lentil roots. *J. Exp. Bot.* 29:631–38.

186. Pérez HE, Sánchez N, Vidali L, Hernández JM, Lara M, Sánchez F. 1994. Actin isoforms in non-infected roots and symbiotic root nodules of *Phaseolus vulgaris* L. *Planta* 193:51–56

187. Pesacreta TC, Carley WW, Webb WW, Parthasarathy MV. 1982. F-actin in conifer roots. *Proc. Natl. Acad. Sci. USA* 79:2898–901

188. Pihakaski-Maunsbach K, Puhakainen T. 1995. Effect of cold exposure on cortical microtubules of rye (*Secale cereale*) as observed by immunocytochemistry. *Physiol. Plant.* 93:563–71

189. Pope DG, Thorpe JR, Al-Azzawi MJ, Hall JL. 1979. The effect of cytochalasin B on the rate of growth and ultrastructure in wheat coleoptiles and maize roots. *Planta* 144:373–83

190. Radford JE, White RG. 1998. Localization of a myosin-like protein to plasmodesmata. *Plant J.* 14:743–50

191. Reichelt S, Knight AE, Hodge TP, Baluška F, Šamaj J, et al. 1999. Characterization of the unconventional myosin VIII in plant cells and its localisation at the post-cytokinetic wall. *Plant J.* 19:555–69

192. Rigau J, Capellades M, Montoliu Ll, Torres MA, Romera C, et al. 1993. An analysis of a maize α-tubulin gene promoter by transient expression and in transgenic tobacco plants. *Plant J.* 4:1043–50

193. Röper W, Röper S. 1977. Centripetal wall formation in roots of *Vicia faba* after caffeine treatment. *Protoplasma* 93:89–100

194. Rost TL, Baum S. 1988. On the correlation of primary root length, meristem size and protoxylem tracheary element position in pea seedlings. *Am. J. Bot.* 75:414–24

195. Schellenbaum P, Vantard M, Lambert A-M. 1992. Higher plant microtubule-associated proteins (MAPs): a survey. *Biol. Cell* 76:359–64

196. Schilde-Rentschler L. 1977. Role of the cell wall in the ability of tobacco protoplasts to form callus. *Planta* 135:177–81

197. Schmit A-C, Lambert A-M. 1990. Microinjected fluorescent phalloidin in vivo reveals the F-actin dynamics and assembly in higher plant mitotic cells. *Plant Cell* 2:129–38

198. Seagull RW. 1992. A quantitative electron microscopic study of changes in microtubule arrays and wall microfibril orientation during in vitro cotton fiber development. *J. Cell Sci.* 101:561–77

199. Sedbrook JC, Chen R, Masson PH. 1999. *ARG1* (Altered Response to Gravity) encodes a DnaJ-like protein that potentially interacts with the cytoskeleton. *Proc. Natl. Acad. Sci. USA* 96:1140–45

200. Shibaoka H. 1994. Plant hormone-induced changes in the orientation of cortical microtubules: alterations in the cross-linking between microtubules and the plasma membrane. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45:527–44

201. Sievers A, Kruse S, Kuo-Huang L.-L., Wendt M. 1989. Statoliths and microfilaments in plants. *Planta* 179:275–78

202. Simmonds DH. 1992. Plant cell wall removal. Cause for microtubular instability and division abnormalities in protoplast cultures. *Physiol. Plant.* 85:387–90

203. Simmons C, Söll D, Migliaccio F. 1995. Circumnutation and gravitropism cause root waving in *Arabidopsis thaliana*. *J. Exp. Bot.* 46:143–50

204. Smertenko A, Blume Y, Viklický V, Opatrný Z, Dráber P. 1997. Posttranslational modifications and multiple tubulin isoforms in *Nicotiana tabacum* L. *cells. Planta* 201:349–58

205. Smertenko AP, Lawrence SL, Hussey PJ. 1998. Immunological homologues of the *Arabidopsis thaliana* β1 tubulin are polyglutamylated in *Nicotiana tabacum*. *Protoplasma* 203:138–43

206. Smith-Huerta NL, Jernstedt JA. 1989. Root contraction in hyacinth III. Orientation of cortical microtubules visualized by immunofluorescence microscopy. *Protoplasma* 151:1–10

207. Snustad DP, Haas NA, Kopczak SD, Silflow CD. 1992. The small genome of *Arabidopsis* contains at least nine expressed β-tubulin genes. *Plant Cell* 4:549–56

208. Stoppin V, Lambert A-M, Vantard M. 1996. Plant microtubule-associated proteins (MAPs) affect microtubule nucleation and growth at plant nuclei and mammalian centrosomes. *Eur. J. Cell Sci.* 69:11–23

208a. Tabony J. 1996. Self-organization in a simple biological system through chemically dissipative processes. *Nanobiology* 4:117–37

209. Thimann KV, Reese K, Nachmias VT. 1992. Actin and the elongation of plant cells. *Protoplasma* 171:153–61

210. Toko K, Iiyama S, Tanaka C, Hayashi K, Yamafuji K, Yamafuji K. 1987. Relation of growth process to spatial patterns of electric potential and enzyme activity in bean roots. *Biophys. Chem.* 27:39–58

211. Traas J, Bellini C, Nacry P, Kronenberger J, Bouchez D, Caboche M. 1995. Normal differentiation patterns in plants lacking microtubular preprophase bands. *Nature* 375:676–77

212. Traas JA, Braat P, Derksen JW. 1984. Changes in microtubule arrays during the differentiation of cortical root cells of *Raphanus sativus*. *Eur. J. Cell Biol.* 34:229–38

213. Traas JA, Renaudin JP, Teyssendier de la Serve B. 1990. Changes in microtubular organization mark the transition to organized growth during organogenesis in *Petunia* hybrid. *Plant Sci.* 68:249–56

214. Uribe X, Torres MA, Capellades M, Puigdomènech P, Rigau J. 1998. Maize α-tubulin genes are expressed according to specific patterns of cell differentiation. *Plant Mol. Biol.* 37:1069–78

215. Utrilla L, De la Torre C. 1991. Loss of microtubular orientation and impaired development of prophase bands upon inhibition of RNA synthesis in root meristems. *Plant Cell Rep.* 9:492–95

216. Utrilla L, Giménez-Abián MI, De la Torre C. 1993. Timing the phases of microtubule cycles involved in cytoplasmic and nuclear division in cells of undisturbed onion root meristems. *Biol. Cell* 78:235–41

217. Vantard M, Schellenbaum P, Fellous A, Lambert A-M. 1991. Characterization of maize microtubule-associated proteins, one of which is immunologically related to tau. *Biochemistry* 38:9334–46

218. Vaughn KC, Harper JDI. 1998. Microtubule-organizing centers and nucleating sites in land plants. *Int. Rev. Cytol.* 181:75–149

219. Vaughn KC, Vaughan MA. 1990. Structural and biochemical characterization of dinitroaniline-resistant *Eleusine*. In *Managing Resistance to Agrochemicals. From Fundamental Research to Practical Strategies*, ed. MB Gree, HM LeBaron, WK Moberg, Am. Chem. Soc. Symp. Ser. 421:364–75. Washington, DC: Am. Chem. Soc.

220. Vaughn KC, Vaughan MA. 1991. Dinitroaniline resistance in *Eleusine indica* may be due to hyperstabilized

microtubules. In *Herbicide Resistance in Weeds and Crops*, ed. JC Caseley, GW Cussans, RK Atkin, pp. 177–86. Oxford: Butterworth/Heinemann

221. Villemur R, Joyce CM, Haas NA, Goddard RH, Kopczak SD, et al. 1992. α-Tubulin gene family of maize (*Zea mays* L.). Evidence for two ancient α-tubulin genes in plants. *J. Mol. Biol.* 227:81–96

222. Volkmann D, Baluška F. 1999. The actin cytoskeleton in plants: from transport networks to signaling networks. *Microsc. Res. Tech.* 47:135–54

223. Wada M, Nozue K, Kadota A. 1998. Cytoskeletal pattern change during branch formation in a centrifuged *Adiantum* protonema. *J. Plant Res.* 111:53–58

224. Waldin TR, Ellis JR, Hussey PJ. 1992. Tubulin-isotype analysis of two grass species resistant to dinitroaniline herbicides. *Planta* 188:258–64

225. Webb MC, Gunning BES. 1991. The microtubular cytoskeleton during development of the zygote, proembryo and free nuclear endosperm in *Arabidopsis thaliana* (L.) Heynh. *Planta* 184:187–95

226. Wehland J, Schroeder M, Weber K. 1984. Organization of microtubules in stabilized meristematic plant cells revealed by a rat monclonal antibody reacting only with the tyrosinated form of α-tubulin. *Cell Biol. Int. Rep.* 8:147–50

227. White RG, Badelt K, Overall RL, Vesk M. 1994. Actin associated with plasmodesmata. *Protoplasma* 180:169–84

228. White RG, Sack FD. 1990. Actin microfilaments in presumptive statocytes of root caps and coleoptiles. *Am. J. Bot.* 77:17–26

229. Whitehead LF, Day DA, Hardham AR. 1998. Cytoskeletal arrays in the cells of soybean root nodules: the role of actin microfilaments in the organization of symbiosomes. *Protoplasma* 203:194–205

230. Wick SM. 1985. Immunofluorescence microscopy of tubulin and microtubule arrays in plant cells. III. Transitions between mitotic/cytokinetic and interphase microtubule arrays. *Cell Biol. Int. Rep.* 9:357–71

231. Wick SM, Seagull RM, Osborn M, Weber K, Gunning BES. 1981. Immunofluorescence microscopy of organized microtubule arrays in structurally-stabilized meristematic plant cells. *J. Cell Biol.* 89:685–90

232. Wilson L, Jordan MA. 1994. Pharmacological probes of microtubule function. See Ref. 113a, pp. 59–83

233. Wordeman L, Mitchison TJ. 1994. Dynamics of microtubule assembly in vivo. See Ref. 113a, pp. 287–301

233a. Yamamoto E, Zeng L, Baird WV. 1998. α-Tubulin missense mutations correlate with antimicrotubule drug resistance in *Eleusine indica*. *Plant Cell* 10:297–308

234. Yang M, Nadeau JA, Zhao L, Sack FD. 1999. Characterization of a *cytokinesis defective (cyd1)* mutant of *Arabidopsis*. *J. Exp. Bot.* 50:1437–46

235. Ye XL, Zee SY, Yeung EC. 1997. Suspensor development in the nun orchid, *Phaius tankervilliae*. *Int. J. Plant Sci.* 158:704–12

236. You W, Abe S, Davies E. 1992. Cosedimentation of pea root polysomes with the cytoskeleton. *Cell Biol. Int. Rep.* 16:663–73

237. Zandomeni K, Schopfer P. 1994. Mechanosensory microtubule reorientation in the epidermis of maize coleoptiles subjected to bending stress. *Protoplasma* 182:96–101

238. Zheng Y, Jung MK, Oakley BR. 1991. γ-tubulin is present in *Drosophila melanogaster* and *Homo sapiens* and is associated with the centrosome. *Cell* 65:817–23