Signaling to the Microtubular Cytoskeleton in Plants

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Specific aspects of the microtubular cytoskeleton in plants are described with special focus on signal-triggered responses. The control of cell shape by environmental signals plays a pivotal role for plant development, and the plant cytoskeleton has evolved specialized structures to fulfill this function. Different components of the microtubular cytoskeleton are discussed in structure and function on both the cell biological and the molecular levels concentrating on those arrays that are not found in animal systems. The role of these plant-specific microtubular structures in the spatial control of cell division and cell expansion is discussed. The microtubular responses to environmental signals, such as light, gravity, cold, and internal signals, such as plant hormones or development, are extensively described, with emphasis on the developmental significance of these responses. There exist several sites where signal chains interact with microtubular dynamics. The role of phosphorylation cascades, calcium/calmodulin, tubulin isotypes and modifications, and microtubule-associated proteins for signaling to the microtubular cytoskeleton are discussed. The review introduces current approaches to the problem of microtubular signaling.

KEY WORDS: Microtubules, Plant development, Signal transduction, Plant hormones.

I. Introduction

A. Open versus Closed Morphogenesis

The typical animal *bauplan* is characterized by a maximum of internal surfaces. In contrast, plants as photosynthetic organisms are designed for a maximum external surfaces. As a consequence, plants are sessile organisms. These basic principles govern most aspects of plant development.

In animals, the general organization of the organism, *bauplan,* is laid down very early in development. Often maternal influences complement the inherited DNA in a kind of morphogenetic inheritance. Anterior–posterior polarity of the *Drosophila* embryo, for instance, is determined by a gradient of maternal, untranslated mRNA for the transcription factors BICOID and OSKAR during oogenesis (St. Johnston and Nüsslein-Volhard, 1992). Dorsiventral polarity in the same organism, in contrast, is based on the differential activity of the membrane protein TOLL. After fertilization, a signal cascade is initiated by this protein that culminates in the transport of the transcription factor DORSAL into the nuclei of the ventral side (Rushlow *et al.*, 1989). Dorsiventral polarity in frog eggs is established by autocatalytic feedback of a polarizing signal (gravity) to an inherited pattern of morphogenetic movements involving transport and translation of maternal mRNA coding for cytoskeletal proteins and polar determinants (Spemann, 1936; Elinson and Rowning, 1988; Melton, 1991). These examples demonstrate that *bauplan* is determined early, often before cellularization. In this type of *closed morphogenesis* the differentiation initiates at the level of the whole organism and proceeds down to the cellular level.

In contrast, plant shape is typically not determined genetically but depends on the given environment (*open morphogenesis*). Growth is not confined to early development but rather continues throughout the entire life cycle. The ability to change growth in response to environmental stimuli plays a central role in the adaptation of individual plants to their habitat. Plant development is further characterized by an increase of external surfaces causing special requirements for effective stabilization of architecture that stimulated the evolution of rigid cell walls. As a consequence, plant cells rarely change position. Cell movement, a central topic in animal development, does not play a role for plant morphogenesis. The basic morphogenetic unit of plant development is the individual cell. Differentiation initiates from the cellular level and subsequently proceeds up to the level of the whole organism. This fact is emphasized by the possibility to regenerate entire plants from almost any plant cell (*totipotency*). In animals, only the egg cell and occasionally its immediate descendants are endowed with a comparative developmental potential (Spemann, 1936).

Thus, the principal difference of animal and plant morphogenesis can be condensed into the following statement: In animals, the organism produces cells, and in plants, cells produce an organism. Any attempt to understand plant morphogenesis must focus on the morphogenesis of the plant cell.

B. Signal Control of Cell Shape

In animals, the typical adaptive response to unfavorable environmental conditions is locomotion. Plants, in contrast, survive because they can tune

development and morphogenesis with signals perceived from the environment. This developmental plasticity is mirrored on the cellular level—plant cells can change shape in response to exogenous and endogenous signals. This control of cell shape depends on two processes, namely, cell division and cell expansion, and both processes can be controlled by environmental stimuli.

During mitosis, both the axis of division and the symmetry of division can respond to the environment (Fig. 1A). In fern protonemata that are cultivated in darkness or under red light, the division axis of the apical cell is laid down parallel to the axis of the protonema. Upon transfer to blue light this axis is tilted by 90° resulting in two-dimensional growth and the formation of a prothallium (Mohr, 1956; Wada and Furuya, 1970). Upon transfer back to the red light, the division axis returns to the original state.

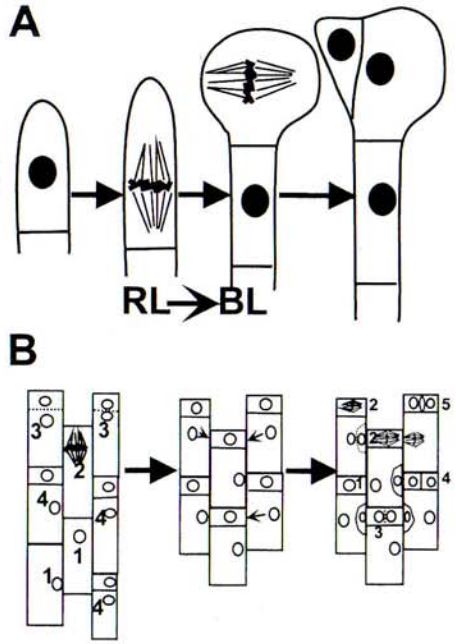


FIG. 1 Response of cell division to environmental and developmental signals in plants. (A) Switch of division axis in fern protonemata in response to a transfer from red light (RL) to blue light (BL). (B) Switch of division axis and symmetry during stomata formation in epidermal cells of monocotyledonous plants. The numbers designate successive stages of the response: The nucleus moves to the prospective division site (left, 1), and the guard cell mother cell is separated by asymmetric division (left, 2 and 3). Then the nuclei of the neighboring cells migrate to the poles of the guard mother cell (middle). The guard cell mother cell subsequently divides in a perpendicular direction (right, 2–4) in parallel with an asymmetric division of the neighboring cells in the same (tilted) axis.

The wound response of higher plants involves axis realignments of the surrounding cells such that the divisions occur perpendicularly to the wound surface (Hush *et al.*, 1990).

Cell differentiation is often linked to asymmetric divisions, sometimes in addition to a switch of division axis. Stomata formation is the most prominent example of this phenomenon (Fig. 1B). The triggering signals are not known, but the switch of axis and symmetry seems to depend on unknown factors that are emitted from neighboring stomata (Bünning, 1965), and mutants have been isolated in which the response to these signals is disturbed (Yang and Sack, 1995). A switch of division axis and symmetry is also characteristic for the formation of water-storing hyalin cells in the peat moss *Sphagnum* (Zepf, 1952). In many spores and zygotes of lower plants, the first asymmetric cell division separating the prospective thallus from the prospective rhizoid is oriented by environmental stimuli such as blue light (Haupt, 1957; Jaffe, 1958), electrical fields, gravity (Edwards and Roux, 1994), or ion gradients (Quatrano, 1978; Weisenseel, 1979). By treatment with antimicrotubular drugs these divisions can be rendered symmetric, resulting in the formation of two thalli (Vogelmann *et al.*, 1981). It should be mentioned in this context that the first zygotic division in higher plants is asymmetric as well. In the *gnom* mutant, in which the first zygotic division is symmetric, the developmental fate of the descendant cells is dramatically altered, resulting in embryos with defect apicobasal polarity (Mayer *et al.*, 1993). These examples suggest that signal-dependent control of division symmetry and axiality plays a pivotal role in development and cell differentiation in plants.

The response of cell division to environmental stimuli is relatively slow and usually requires several hours to become detectable. Stimulus-triggered growth responses occur more rapidly than that—gravi- or phototropic bending, for instance, becomes detectable within a few minutes (Iino and Baskin, 1984)—and the growth response of individual cells is even faster (Nick and Furuya, 1996). These fast growth responses are achieved by changes in the amplitude and proportionality of cell expansion. The most prominent signal-triggered growth response is probably the deetiolation of higher plant seedlings. In darkness, stem elongation dominates seedling development. Within a few minutes after illumination, this elongation response is halted and very often a certain degree of stem thickening is observed. In those cases in which it was investigated, this light response of stem elongation can be ascribed perfectly to a light-induced block of cell elongation (Lockhart, 1960; Toyomasu *et al.*, 1994; Waller and Nick, 1997). A similar response of cell expansion is observed in the ethylene-induced barreer response of pea epicotyls (Lang *et al.*, 1982), in which growth is redistributed entirely from longitudinal toward lateral growth.

These examples illustrate that both morphogenetic responses of the plant cell—spatial control of cell division and spatial control of cell expansion—can be controlled by external and internal stimuli. Both morphogenetic responses are intimately linked to the cytoskeleton. Signaling to the cytoskeleton is therefore a central topic in plant morphogenesis as a whole. This special role of the cytoskeleton for plant morphogenesis is mirrored in specialized cytoskeletal arrays that are exclusively found in plant cells. This review will therefore begin with a cell biological and molecular description of these unique cytoskeletal components. Section III will discuss the function of these components for the spatial control of cell division and cell expansion. Section IV will review the multitude of signal-induced responses observed for plant microtubules (MTs), and Section V will present recent approaches to the problem.

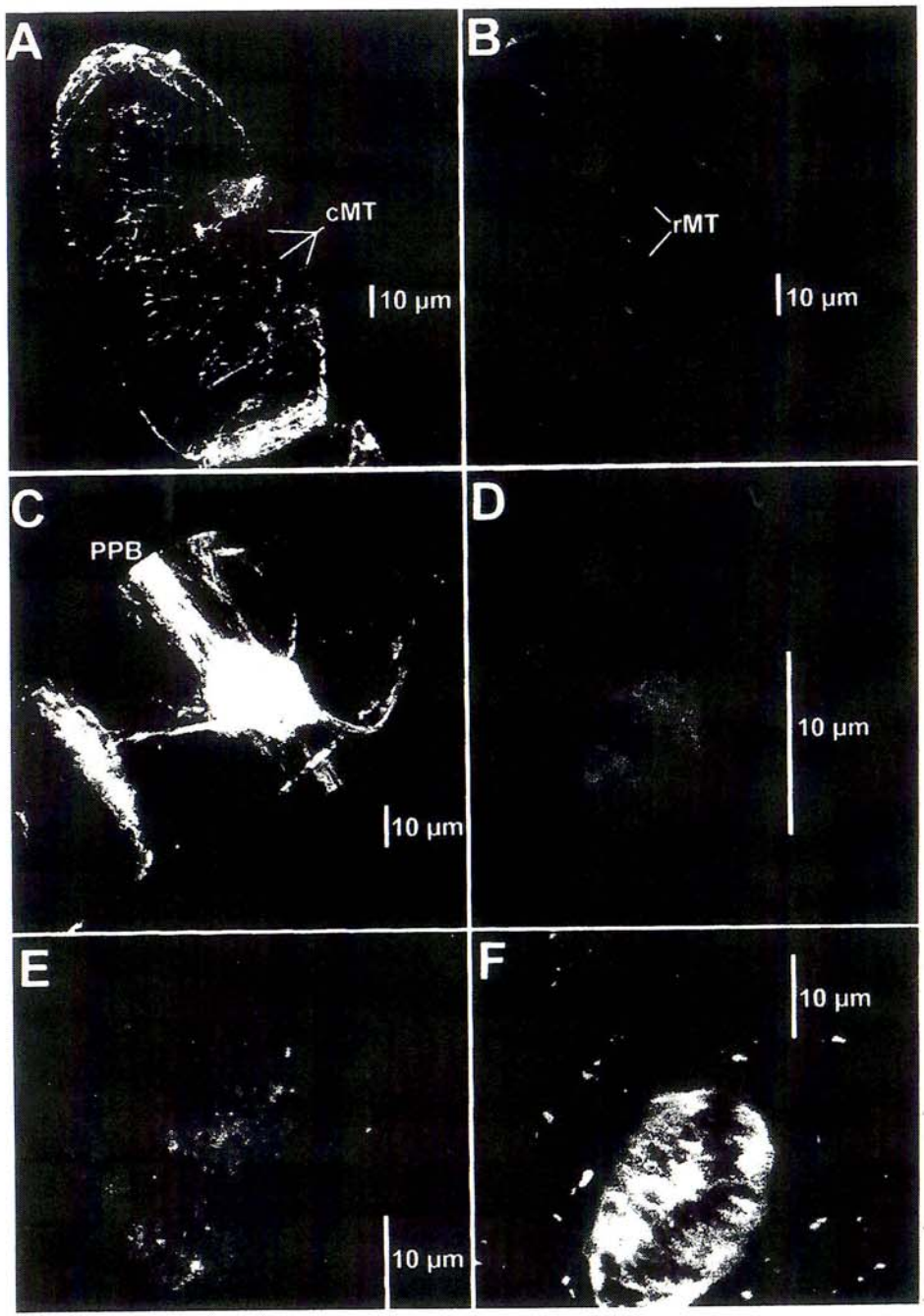
II. Components of the Microtubular Cytoskeleton in Plants

A. Microtubular Arrays of Higher Plants: Cortical Microtubules, Radial Microtubules, Preprophase Band, Spindle, and Phragmoplast

During the evolution of higher plants several specialized populations of MTs have emerged that are not found in animal cells. These specialized populations are intimately linked to the spatial control of cell expansion and cell division and thus to the problem of signal-triggered cell shape control.

Interphase cells are characterized by arrays of *cortical MTs* adjacent to the plasma membrane. These cortical MTs are usually arranged parallel to each other and in most cases perpendicular to the axis of preferential cell expansion (Fig. 2A). They are believed to control the direction of cellulose deposition and thus to participate in the reinforcement of axial cell growth (Green, 1980; Williamson, 1991). One feature of cortical MTs is especially relevant to the problem of signal-controlled cell shape: They can change orientation in response to various stimuli as will be discussed in more detail.

As a first indication of ensuing cell divisions, the nucleus migrates to the cell center, i.e., to the site where the prospective cell plate will be formed. This movement of the nucleus is linked to the phragmosome, a specialized array of the actin cytoskeleton (Katsuta and Shibaoka, 1988; Lloyd, 1991). At the same time, the microtubular cytoskeleton becomes reorganized in a dramatic way: Cortical MTs are gradually replaced by a new network of *radial MTs* that emanate from the nuclear surface and often merge with the cortical cytoskeleton (Fig. 2B). The function of this radial network



is not understood, but it is exclusively found in cells that are prone to undergo mitosis.

In the next step, both cortical MTs and the radial network disappear and almost simultaneously a band of MTs emerges around the cell equator (Fig. 2C). This so-called *preprophase band* is organized by the nucleus and marks the site where the new cell plate will be formed after the completion of mitosis. Experiments in fern protonemata in which the formation of the preprophase band could be manipulated by centrifugation of the nucleus to a new location (Murata and Wada, 1991a) provide evidence for a causal relationship between preprophase band and cell plate formation. The nature of this relationship, however, is not understood—the preprophase band disappears simultaneously with the formation of the division spindle. Nevertheless, it must leave a trace that persists during cell division and that is able to organize the cell plate during telophase. In those cells in which the axis or symmetry of cell division changes, these changes are always heralded by corresponding changes in the formation of the preprophase band (Hush *et al.*, 1990; Wick, 1991; Mineyuki *et al.*, 1988).

The *division spindle* (Fig. 2D) is initially organized perpendicular to the preprophase band even in those cases in which it later becomes oblique due to space restrictions as for instance during the formation of stomata (Mineyuki *et al.*, 1988). The spindle equator is always identical to the position of the preprophase band. Among the microtubular arrays found in plant cells, the division spindle is the only array that is known from animal cells. However, even the spindle shows some characteristic differences, especially in the organization of the spindle poles. Whereas the spindle of animal cells appears pointed and clearly bipolar, the pole region is observed to be broader in plant cells (Fig. 2D) and to emanate from several poles (Smirnova and Bajer, 1992).

Subsequent to the separation of chromosomes, the site of the ensuing cell plate becomes marked by a new array of MTs, the *phragmoplast* (Figs. 2E and 2F). This microtubular structure is involved in the transport of vesicles to the periphery of the growing cell plate. Electron microscopical

FIG. 2 Microtubular arrays observed during the cell cycle of higher plant cells. Microtubules have been visualized by immunofluorescence microscopy in axially dividing tobacco VBI-O cells. (A) Interphase cell with cortical microtubules (cMT). (B) Cell preparing for mitosis. The nucleus has migrated into the center toward the prospective site of cell plate formation. It is tethered to the cell periphery by radial microtubules (rMT). (C) Preprophase band (PPB) that is formed around the equator of cells prone to undergo mitosis. (D) Division spindle. (E, F) Phragmoplast as seen in a direction parallel to the division axis (E) or as seen from the side (F).

evidence supports a model in which MTs pull at tubular–vesicular outgrowths that emanate from the endoplasmatic reticulum (Samuels *et al.*, 1995). The phragmoplast appears as a double ring of interdigitating MTs that is growing in diameter with increasing size of the cell plate. Along the edge of the growing phragmoplast new MTs are organized (Vantard *et al.*, 1990).

These principal peculiarities of the plant cytoskeleton raise the question of their functional significance. Certainly, some of these structures have evolved due to the special requirement imposed upon cell differentiation by the invention of rigid cell walls. The high degree of dynamics, however, suggests that these structures might play a role for the developmental plasticity that is characteristic for plant cells.

B. Molecular Components of the Microtubular Cytoskeleton in Plants

One might expect that these fundamental differences in organization and function of the microtubular cytoskeleton in plants are mirrored by dramatic differences of molecular composition. However, regarding the major component, tubulin, a surprising degree of similarity is observed between plants and animals. Tubulins are relatively conservative proteins with respect to sequence and function. Tubulins from plants, animals, and even fungi are able to copolymerize *in vitro* and *in vivo* (Zhang *et al.*, 1990; Vantard *et al.*, 1990; Yuan *et al.*, 1994), and the sequence homology is fairly high. Angiosperm and vertebrate tubulin, for instance, exhibit 79% sequence identity (Fosket and Morejohn, 1992). There exist some differences between plant and animal tubulin, though, especially with respect to the sensitivity to tubulin polymerization blockers such as colchicin (Morejohn, 1991). The genes coding for α- and β-tubulin exist in several copies coding for proteins with subtle differences in charge and possibly function. These so-called tubulin isotypes are expressed differentially with respect to tissue and developmental state (Silflow *et al.*, 1987; Hussey *et al.*, 1990; Montoliu *et al.*, 1990; Jongewaard *et al.*, 1994; Rogers *et al.*, 1993). The functional significance of this complexity has remained obscure. A solution to this problem is further hampered by the occurrence of MTs that are composed of several isotypes (Hussey *et al.*, 1987).

The complexity of tubulin genes is complemented by complex patterns of posttranslational modifications. In animals, tubulins can be detyrosinated by a carboxypeptidase acting preferentially on polymerized MTs, they are tyrosinated by a tyrosin ligase preferentially interacting with tubulin dimers, they can be acetylated by a tubulin acetyl transferase, and they have been observed to be phosphorylated, polyglutamylated, and polyglycylated

(McRae, 1997). Except for the acetylation, all modifying reactions occur at the C terminus of tubulin. Some of these modifications, such as detyrosination, have been correlated with MT stability although the detyrosination is probably the consequence rather than the cause of increased MT stability (Khawaja *et al.*, 1988; Webster *et al.*, 1990). Immunological evidence suggests that at least some of these modifications exist in plants in specific MT arrays (Duckett and Lloyd, 1994; Smertenko *et al.*, 1997).

Neurotubulin can coassemble with plant tubulin *in vitro* and *in vivo* and participates in the dynamic reactions of the host cytoskeleton (Vantard *et al.*, 1990; Zhang *et al.*, 1990; Yuan *et al.*, 1994). These observations suggest that the factors responsible for the specific organization of the plant cytoskeleton are extrinsic to tubulin itself. Tubulin assembly *in vitro* depends on temperature, GTP, and magnesium. *In vivo*, the nucleation of new MTs is strictly regulated and occurs on the surface of specialized organelles, the centrosomes. One fascinating problem of cell biology is the fact that higher plants do not possess centrosomes. They do, however, possess functional analogs, so-called MT-organizing centers (MTOCs). In dividing cells the nuclear surface seems to be an important MTOC (Lambert, 1993), and it has been shown to induce the formation of new MTs (Stoppin *et al.*, 1994). In noncycling cells, however, there exist cortical MTOCs, in which new MTs are formed during the recovery from depolymerization induced by drugs, cold, or high pressure (Marc and Palevitz, 1990; Cleary and Hardham, 1990). The molecular composition of these MTOCs is unknown, but it seems that epitopes that are present in centrosomes such as γ-tubulin can be detected in plant MTOCs (Liu *et al.*, 1994). It is generally believed that these MTOCs must contain MT-associated proteins that are able to induce nucleation and elongation of MTs.

Microtubule-associated proteins (MAPs) are known from neural tissue, in which they have been copurified along with tubulin. They are conventionally classified into two classes:

1. The MT motors kinesin and dynein have ATPase function and they are able to move along MTs either in the direction of MT polarity or in the opposite sense (Hyman and Mitchison, 1991). These motors are involved in mutual sliding of MTs or in the directional transport of proteins along MTs. During oogenesis in *Drosophila*, for instance, the determinants of the posterior pole are localized by means of a MT-dependent kinesin-driven transport system (Clark *et al.*, 1994). Dynein can be coupled to the dynactin complex and thus allows a sliding of MTs along the actin system (Allan, 1994). Proteins that are immunologically related to kinesin have been detected in pollen tubes (Tiezzi *et al.*, 1992) and kinesin-homologous sequences have been reported in *Arabidopsis* (Mitsui *et al.*, 1993).

2. The second group of MAPs is heterogenous in both function and molecular properties and is generally designated as structural MAPs. Struc-

tural MAPs usually lower the critical concentration of tubulin necessary for MT assembly (Matus, 1990) and have been described to increase MT stability (Barlow *et al.*, 1994), although there exist MAPs that can induce severing of MTs (McNally and Vale, 1993). Our knowledge about structural plant MAPs is still very rudimentary. Several potential MAPs have been described during the past few years (Maekawa *et al.*, 1990; Yasuhara *et al.*, 1992; Chang-Jie and Sonobe, 1993; Jablonsky *et al.*, 1993; Vantard *et al.*, 1994; Mizuno, 1995; Nick *et al.*, 1995; Marc *et al.*, 1996) but only two plant MT-associated proteins have been cloned, both of them being factors required for protein translation. One of these factors, EF-1α (Durso and Cyr, 1994), has been described to cause bundling of MTs *in vitro* (Cyr and Palevitz, 1989), although it is not clear whether it has this function *in vivo*. The other MT-associated protein, IF-(iso)4F, has been shown to induce end-to-end annealing of MT *in vitro* (Hugdahl *et al.*, 1995). Again, the function of this protein *in vivo* is still unknown.

Despite the relatively high conservation in the molecular properties of tubulin, there seems to be little sequence similarity between plant and animal MAPs. The most straightforward approach, namely, to screen with heterologous probes for plant homologs of animal MAPs, has not lead to the cloning of a plant MAP. This indicates that plant MAPs might provide the molecular key for the fundamental differences in the organization of the plant cytoskeleton.

C. Targets for Signaling: Microtubule Movement versus Microtubule Reassembly

The microtubular cytoskeleton in plant cells is characterized by an extremely high dynamics throughout the cell cycle. This dynamic behavior must be the target where signal transduction chains are linked with the morphogenetic plasticity that is typical for plant cells. There exist principally two ways that MTs could reorganize that may even act cooperatively. Microtubules either could move as entities and be transported to their new location or they could be disassembled and reassembled in a new direction and/or a new location.

The plant cytoskeleton in its three-dimensional organization became accessible for investigation with the development of immunofluorescence analysis (Lloyd *et al.*, 1980). Pioneering studies revealed that, in elongating cells, the MTs are arranged in helicoidal arrays along the periphery of the cell. This observation stimulated the first model for MT reorientation (Lloyd and Seagull, 1985): The helicoidal arrays were perceived as dynamic springs with a variable pitch. If the MTs comprising this helix slide in such a way

that the helix is shortened, this will result in a steep pitch and in longitudinal MTs (Fig. 3A). If they slide in the opposite direction, the spring will relax, resulting in an almost transverse pitch. According to this model, the molecular mechanism of reorientation is expected to involve MT motors such as kinesin or dynein.

This model is in fact very attractive because of its simplicity and elegance. However, increasing information brings into question the validity of the dynamic-spring model. This evidence can be summarized as follows:

1. According to the dynamic-spring model, the cortical MTs are mechanically coupled and comprise a physical entity. However, in epidermal tissues, the reorientation of cortical MTs is confined to the MTs adjacent to the outer wall (Bergfeld *et al.*, 1988; Nick *et al.*, 1990; Nick and Furuya, 1996;

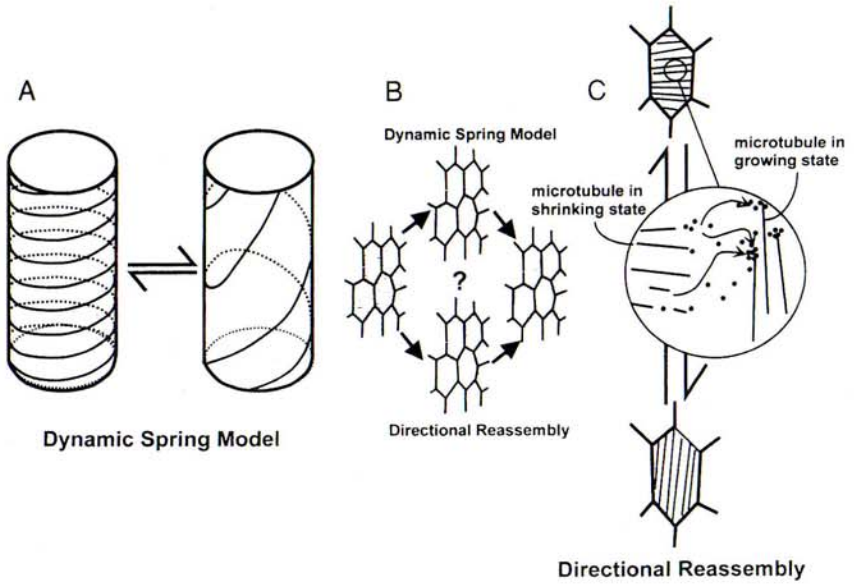


FIG. 3 Alternative models for MT orientation in interphase cells. (A) Original dynamic spring model by Lloyd and Seagull (1985). Cortical MTs are organized in a spring with variable pitch. The pitch is changed by mutual sliding of MTs, resulting in relaxation of the spring (transverse MTs) or in a contraction of the spring (oblique or longitudinal MTs). (B) Transitions between transverse and longitudinal MTs as they are predicted by the dynamic spring model in contrast to the directional reassembly model. (C) Directional reassembly model. The equilibrium between assembly and disassembly depends on the direction of a given MT. If the direction dependency of the equilibriums shifts, this will result in a net elimination of MTs in the "old" direction and increased numbers of MTs in the "new" direction. The transitions between transverse and longitudinal MTs are characterized by cells, where patches of MTs in different orientations coexist.

Wymer and Lloyd, 1996) leading to a situation in which transverse MTs are at the inner wall and longitudinal MTs are at the outer wall. This difference in orientation is difficult to reconcile with the concept of a mechanically coupled spring.

2. The transitions between transverse and longitudinal arrays of MTs should involve situations in which MTs are homogeneously oblique and then gradually increase their pitch until the longitudinal array is reached. Although oblique MTs can be observed, they seem to occur as a late step rather than as a transitional state (Gunning and Hardham, 1982; Hush *et al.*, 1990). Early phases of reorientation, triggered by strong stimuli or incomplete reorientation in response to suboptimal stimulation, are characterized by a different type of transition (Nick *et al.*, 1990, 1992). In contrast to the situation predicted by the dynamic-spring model, a patchwork of transverse and longitudinal MTs is observed with transverse and longitudinal MTs being interspersed even within the same cell (Fig. 3B).

3. If MT depolymerization is suppressed by the addition of taxol, an inhibition of MT reorientation has been observed in several cases (Falconer and Seagull, 1985; Nick *et al.*, 1997a) indicating that MT depolymerization is required for reorientation. This is not expected from the dynamic-spring model. It should be mentioned, however, that this inhibition of taxol was not found during MT ordering during wall regeneration of tobacco protoplasts (Wymer *et al.*, 1996). A second reorganization step that requires MT disassembly seems to be the formation of the phragmoplast during late cell division (Yasuhara *et al.*, 1993).

4. Cortical MTs were originally thought to be relatively inert lattices. However, recent experiments involving microinjection of fluorescent-labeled neurotubulin into living epidermal cells (Yuan *et al.*, 1994; Wymer and Lloyd, 1996) revealed (i) that the injected tubulin was incorporated extremely rapidly into the preexisting cortical network and (ii) that upon bleaching of fluorescence by a laser beam the fluorescence of the bleached spot recovered within a few minutes, indicating an extremely high turnover of tubulin monomers. This high dynamics of tubulin assembly and disassembly contrasts with the concept of a mechanically coupled cytoskeletal helix.

5. The observation of MT reorientation *in vivo* in epidermal cells that were microinjected with fluorescent-labeled neurotubulin (Yuan *et al.*, 1994; Wymer and Lloyd, 1996) demonstrates local phase transitions from transverse to longitudinal arrays as first steps of reorientation resulting in stochastically arranged patches of transverse and longitudinal MTs. These patches subsequently extend, eventually merging into a homogeneously longitudinal array.

These observations suggest an alternative mechanism of MT reorientation: direction-dependent assembly and disassembly (Fig. 3C). Dependent on the orientation of a given MT, it might be in a growing state (assembly dominating over disassembly) or in a shrinking state (so-called catastrophe with disassembly dominating over assembly). If this model is correct, the target for signals interfering with MT orientation has to be sought among those factors that control assembly and disassembly of MTs.

III. Functions of the Microtubular Cytoskeleton in Plants

A. Spatial Control of Cell Division: Symmetry and Axis, Nuclear Migration, and Organization of the Cell Plate

In plants, the symmetry and the axis of cell division is subject to strict spatial control. Cell division is heralded by a nuclear migration toward the site of the prospective cell plate. This nuclear migration seems to be driven by actin microfilaments because treatment with the actin inhibitor cytochalasin B was found to block this movement (Katsuta and Shibaoka, 1988). In addition, the tethering of the nucleus to a fixed position in the cell was interrupted, and it could be displaced by mild centrifugation. These observations suggest that the phragmosome, a specialized actin array emanating from the premitotic nucleus in vacuolated cells (Lloyd, 1991), is responsible for premitotic migration of the nucleus to the site of the prospective cell plate. In parallel to the formation of the phragmosome, a new microtubular array evolves that is not observed in interphase cells. These radial MTs (Fig. 2B) emerge from the nuclear envelope and seem to be interconnected with the cortical cytoskeleton. The functional significance of this microtubular array is not thoroughly understood, but the tethering of the nucleus to its new site can be disturbed by antimicrotubular drugs such as colchicin or propyzamide (Thomas *et al.*, 1977; Katsuta and Shibaoka, 1988), suggesting that MTs participate in nuclear positioning.

In the next step, the premitotic nucleus will organize the preprophase band, a band of MTs around the nuclear equator (Fig. 2C). The formation of the preprophase band is initiated at the end of the S phase and is continued throughout the G2 phase (Gunning and Sammut, 1990). The preprophase band predicts symmetry and axis of the ensuing cell division: The spindle axis will be laid down perpendicular with the *preprophase band* and the new cell plate will always form in the site predicted by the preprophase band. This is especially impressive with cell divisions that are asymmetric, such as those observed during the formation of stomata or trichomes (Wick, 1991).

The preprophase band is not just a true indicator for the spatial aspects of cell division. In fact, two lines of evidence demonstrate a causal link between preprophase band and division axis and symmetry: (i) In the *Arabidopsis* mutant *ton* the preprophase band is absent (Traas *et al.*, 1995). The ordered pattern of cell division observed during the development of wild-type seedlings appears to be completely randomized in this mutant; and (ii) in apical cells of fern protonemata, the formation of the preprophase band can be manipulated by cold treatment (causing depolymerization of MTs) and/or by centrifugation of the nucleus toward the basal end of the cell (Murata and Wada, 1991a,b). If these manipulations were performed just prior to the formation of the preprophase band, a new preprophase band was established in the cell base and, subsequently, the new cell plate formed there. If the nucleus was centrifuged at a later stage (when a preprophase band had already been formed in the cell apex), a second preprophase band was found in the cell base, and the new cell plate developed randomly with respect to orientation and symmetry. These experiments demonstrate that (i) the nucleus induces and guides the formation of the preprophase band and (ii) that the correct formation of the preprophase band is a prerequisite for the spatial control of cell division.

The division spindle is always established perpendicular to the preprophase band, although it may be tilted and distorted to a more oblique array due to space restrictions. This is observed in elongated cells, in which the division axis becomes switched by 90° in the course of cell differentiation as, for instance, during the formation of stomata (Mineyuki *et al.*, 1988). Interestingly, this does not result in the formation of an oblique phragmoplast or an oblique cell plate. It seems that the division spindle is uncoupled of the morphogenetic processes responsible for cell plate formation. This is surprising because the preprophase band disappears in the same moment when the spindle appears. It remains to be elucidated, how it can nevertheless guide the formation of the phragmoplast. It seems that a specialized actin array, the phragmosome, persists during mitosis and somehow participates in the establishment of the phragmoplast during telophase (Lloyd *et al.*, 1991). The molecular relationship between phragmoplast and preprophase band remains enigmatic, however. The *ton* mutant (Traas *et al.*, 1995) and the centrifugation experiments with fern protonemata (Murata and Wada, 1991a,b) indicate, however, that the phragmosome must interact with the preprophase band to become competent for phragmoplast organization.

This evidence emphasizes the importance of the preprophase band in the spatial control of cell division. The observation that cell cycle-dependent protein kinases are localized to the preprophase band (Colasanti *et al.*, 1993) indicates that this step is the target, in which signal transduction

chains triggered by external stimuli interconnect with the spatial control of cell division and thus with cellular morphogenesis.

B. Spatial Control of Cell Expansion: Microtubule–Microfibril Syndrome and Wall Thickening

The expansion of plant cells is subjected to spatial control. The driving force for this increase in volume is a gradient of water potential, with a more negative water potential in cytoplasm and vacuole compared to the apoplast (Kutschera, 1991). This difference in water potential causes pressure. This pressure by itself is not directional, and the cell is therefore expected to grow isotropically. This is indeed observed in protoplasts, emphasizing the importance of the cell wall in the spatial control of cell expansion. It is the yielding of the cell wall that limits growth and the extensibility of the cell wall is actively controlled, for instance, by growth-promoting factors such as auxin (Kutschera *et al.*, 1987).

Most plant cells derive directly or indirectly from isodiametric meristematic cells. Nevertheless, most differentiated cells in expanding tissues, such as hypocotyls, internodes, petioles, or coleoptiles, are characterized by an approximately cylindrical shape. This cylindrical shape is usually lost upon removal of the cell wall—protoplasts are spherical with very few exceptions. These simple observations demonstrate the importance of the cell wall for the control of cell shape.

In cylindrical cells, cell expansion is expected to occur preferentially in a lateral direction, which should gradually corrobate the axiality of these cells. This means, however, that cylindrical cells must provide some kind of *reinforcement mechanism* to maintain their original axiality (Green, 1980). This reinforcement mechanism seems to reside in the cell wall and was first described in the long internodal cells of the alga *Nitella* (Green and King, 1966). In these elongate cells, the cellulose microfibrils were demonstrated by electron microscopy to be arranged in transverse rings, especially in the newly deposited inner layers of the wall. It should be mentioned that an anisotropic arrangement of cellulose had already been inferred from polarization microscopy much earlier and that birefringency of the cell wall had been related to growth (Ziegenspeck, 1948). It is evident that the transverse arrangement of microfibrils can account for the reinforcement mechanism that maintains the longitudinal growth axis in cylindrical cells. Such a correlation between transverse microfibrils and cell elongation has been demonstrated in numerous cases (Robinson and Quader, 1982; Kristen, 1985; Giddings and Staehelin, 1991).

Moreover, reorientation of the growth axis is often accompanied by a loss or a reorientation in the anisotropy of cellulose deposition (Lang *et al.*,

1982; Green and Lang, 1981; Hush *et al.*, 1991). Therefore, the correlation between guided cellulose deposition and cell growth seems to be very tight. The so-called terminal complexes synthetizing cellulose are usually organized in rosette-like hexagonal arrays (Giddings and Staehelin, 1991). It is generally believed that these rosettes slide within the membrane, leaving behind bundles of crystallizing cellulose fibers, the microfibrils (Fig. 4A). Cortical MTs seem to be responsible for the guided movement of the terminal complexes and thus for the axiality of cell expansion. The evidence for this statement can be summarized as follows:

1. Cortical MTs are closely associated with the plasma membrane, and in plasmolyzing cells a direct contact between cortical MTs and newly formed cellulose microfibrils has been detected by electron microscopy (Robinson and Quader, 1982; Kristen, 1985; Giddings and Staehelin, 1991).

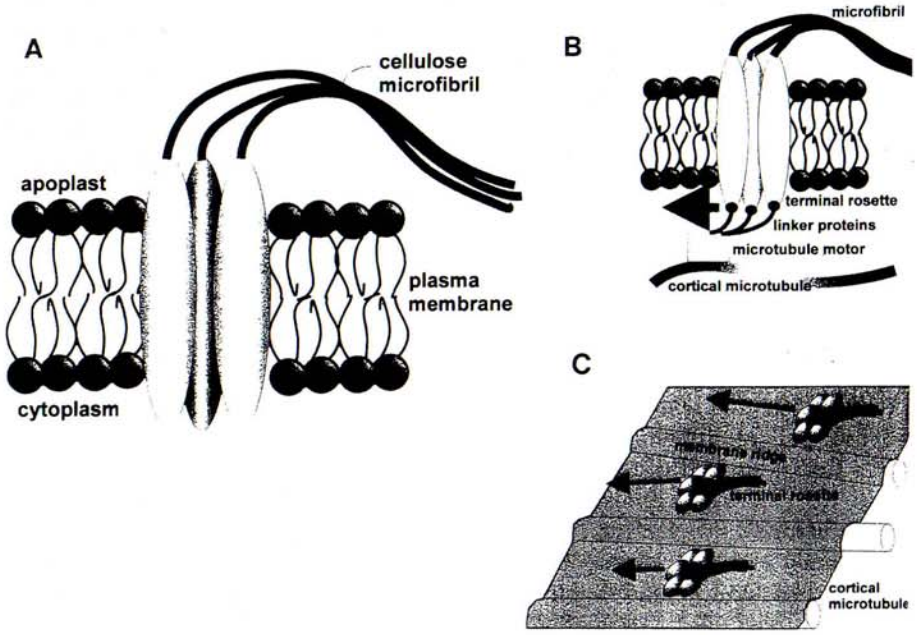


FIG. 4 Role of cortical MTs in the guided deposition of cellulose microfibrils. (A) Schematic view of the terminal complexes responsible for cellulose synthesis. (B) Original model by Heath (1974) assuming that cortical MTs are coupled to the terminal complexes by linker proteins that are bound to microtubular motors such as dynein. According to this model, cortical MTs are responsible for both the direction of the microfibril and the movement of the terminal complexes. (C) Alternative model by Giddings and Staehelin (1991) assuming that cortical MTs induce membrane ridges that guide the movement of the terminal complexes. According to this model, the driving force for the movement originates from the crystallization of cellulose itself; MTs are only responsible for the direction of the movement.

2. The prospective sites where secondary wall thickenings will form are marked by parallel thick bundles of cortical MTs (Fukuda and Kobayashi, 1989; Jung and Wernicke, 1990).

3. In those cases in which changes of the preferential axis of cellulose deposition (and, concomitantly, the axis of cell growth) occur, this reorientation is heralded by a reorientation of cortical MTs (Lang *et al.*, 1982, for the ethylene response; Bergfeld *et al.*, 1988, for the auxin response; Toyomasu *et al.*, 1994, for the gibberellin response; Abe *et al.*, 1995, for wood formation).

4. Elimination of cortical MTs by antimicrotubular drugs results in a gradual loss of growth anisotropy and a block of cell elongation leading, in extreme cases, to lateral swelling (Hogetsu and Shibaoka, 1978; Robinson and Quader, 1981; Kataoka, 1982; Vaughan and Vaughn, 1988; Bergfeld *et al.*, 1988; Baskin and Bivens, 1995; Nick *et al.*, 1994).

The exact mechanism by which MTs drive and guide cellulose deposition has been under debate since the discovery of cortical MTs by Ledbetter and Porter (1963), and many different hypotheses have been proposed (Robinson and Quader, 1982; Giddings and Staehelin, 1991). The principal debate can be summarized into two alternative models:

1. According to the original model by Heath (1974), cortical MTs are physically linked to the terminal complexes and the linking molecule(s) can be pulled along the MTs by dynein-like motor proteins. Thus, the whole complex will be moved in a direction parallel to the adjacent MTs (Fig. 4B). It has been observed in several cases that removal of the cell wall during formation of protoplasts causes a dramatic restructuring of cortical MTs (Jung *et al.*, 1993) and makes them susceptible to cold (Akashi *et al.*, 1990). These observations demonstrate a stabilization of cortical MTs by the cell wall and indicate a physical link between MTs and microfibrils across the plasma membrane.

2. The alternative model is based on the observation that, in some cases, the terminal complexes have been observed in the interspaces outlined by the MTs rather than being directly attached to them (Giddings and Staehelin, 1991). The guiding of rosette movement, according to this model, is not caused by a physical link of the terminal complexes to MT motors. Microtubules are rather supposed to induce membrane channels that impede lateral deviations of rosette movement (Fig. 4C). The driving force for the movement would be cellulose crystallization itself, propelling the terminal rosette through the MT-dependent membrane channels.

Currently, it is difficult to decide between which is the best model. Moreover, neither of them seems to be complete and able to accommodate all

observations. It is necessary to understand, on the molecular level, the interaction between MTs and the plasma membrane and the potential role of motor proteins for guided cellulose deposition. In this context, the recent discovery of a 90-kDa membrane-associated MT-binding protein is interesting. Such proteins might mediate the association of MTs with the plasma membrane (Marc *et al.*, 1996) and might be candidates for proteins that interact through the membrane with the terminal complexes. The exact mechanism by which cortical MTs guide the deposition of cellulose and thus define the axis of cellular growth remains to be elucidated. Nevertheless, the close relation between MT orientation and the direction of growth suggests that the main function of cortical MTs has to be sought in the control of cell shape by external and internal signals.

C. Other Functions: Mechano- and Gravisensing and Intracellular Transport

In addition to the spatial control of cell division and cell expansion, there seem to exist other functions of MTs that are often overlooked. Microtubules are endowed with axiality and even polarity and this makes them ideal mediators for all processes that require directionality. Among such processes, two will be discussed; (i) The axiality of MTs and their high flexural rigidity (Gittes *et al.*, 1993) could be used to amplify weak mechanic stresses and thus to transduce mechanosensing, and (ii) the polarity of MTs and the existence of MT motors that are driven along or against this polarity is an ideal prerequisite for intracellular transport.

A number of observations indicate that MTs are not only involved in the spatial control of cell expansion but also in the transduction of mechanic or gravitational stimuli:

1. Tubulin polymerization and depolymerization is one of the few biochemical events that respond directly to mechanical stress, leading to an alignment of MTs parallel to the gravity vector even during tubulin assembly *in vitro* (Tabony and Job, 1992).
2. A stretch-activated calcium channel that has been described for onion cells is irreversibly inhibited by the antimicrotubular drug ethyl-*N*-phenylcarbamate (Ding and Pickard, 1993).
3. Gravitropism, triggered by the pressure of sedimenting amyloplasts upon mechanosensitive ion channels (Kuznetsov and Hasenstein, 1996), has been observed in several cases to be blocked by antimicrotubular compounds at concentrations that leave phototropism and/or growth essentially unaltered (Friedrich and Hertel, 1973, for the *Chara* rhizoid; Schwuchow *et al.*, 1990; and Walker and Sack, 1990, for moss protonemata; Nick *et al.*, 1991, for maize coleoptiles; Nick *et al.*, 1997, for rice coleoptiles).
4. Mutants with reduced microtubular dynamics or wild-type coleoptiles that have been treated with taxol, a drug that stabilizes MTs, exhibit a conspicuous delay of the gravitropic response (Nick *et al.*, 1997a).
5. Reorientation of cortical MTs that can be observed in gravity-sensing cells (Blancaflor and Hasenstein, 1993) is blocked by taxol (Nick *et al.*, 1997a).
6. The application of mechanic load to maize coleoptiles causes a reorientation of cortical MTs in the epidermis (Zandomeni and Schopfer, 1994).

Microtubule motors seem to exist not only in animals but also in plants (Tiezzi *et al.*, 1992; Mitsui *et al.*, 1993), although their function is far from understood. From purified phragmoplasts a MT-binding protein has been isolated that binds to MTs dependent on ATP (Yasuhara *et al.*, 1992). It is likely that this protein is involved in the guided transport of vesicles toward the growing cell plate. Indirect evidence for a role of MTs in guided transport comes from investigation of the transmission of plant viruses that seem to usurpate endogenous systems of cellular trafficking. The movement protein of the tobacco mosaic virus travels along MTs (Heinlein *et al.*, 1995), and the aphid transmission factor of the cauliflower mosaic virus has been shown to form a stable complex with MTs *in vivo* and *in vitro* (Blanc *et al.*, 1996). Several organelles, such as the nucleus (Edwards and Roux, 1997), the chloroplasts (Serlin and Ferrell, 1989), and the amyloplasts (Sievers and Hejnowicz, 1994; Nick *et al.*, 1997a), seem to be attached to a microtubular lattice and their movement appears to be guided and/or limited by the microtubular cytoskeleton. The role of cytoskeleton-guided intracellular transport for cellular morphogenesis is still a relatively new topic in plant biology. However, the exciting observation that untranslated mRNA is transported in a polar manner during the polarization of *Fucus* zygotes (Bouget *et al.*, 1995) indicates that guided transport of morphogenetic determinants along the cytoskeleton is characteristic for the establishment of not only the animal *Bauplan* (Pokrywka and Stephenson, 1991) but also for plant morphogenesis.

IV. Signal-Controlled Responses of MTs

A. Light: Control of Cell Elongation, Phototropism, and Polarity Induction

The response of etiolated plants to light is very dramatic. Elongation comes to a halt almost immediately and growth is redistributed to lateral growth. This response is caused entirely by according changes of cell expansion (Lockhart, 1960; Furuya *et al.*, 1969; Waller and Nick, 1997). The role of

MTs in this response has been analyzed in detail for the Graminean coleoptile. The coleoptile is a specialized ephemeric organ that sheaths the primary leaves in germinating grass seedlings and protects them until they reach the soil surface. Upon illumination by daylight, the coleoptile terminates growth and the primary leaves subsequently pierce the coleoptile tip. The coleoptile has been a favorite object of plant physiology since Darwin and Darwin (1881) described the swift phototropic response of this organ. Cholodny and Went demonstrated independently for gravitropism (Cholodny, 1927) and for phototropism (Went, 1928) that tropistic stimuli cause a shift of a growth-promoting factor across the coleoptile. This factor was then identified as the first plant hormone, auxin (Went and Thimann, 1937).

In coleoptiles that grow straight, the target for the action of auxin seems to be the epidermis. The expansion of the inner tissues is constrained by the relatively low extensibility of the outer epidermis, and auxin appears to act by releasing this constraint (Kutschera *et al.*, 1987). In coleoptiles that undergo rapid elongation, cortical MTs are found to be transverse both in the cells of the inner tissue and in the epidermis (Nick *et al.*, 1990; Toyomasu *et al.*, 1994). Consistently, cellulose microfibrils are deposited in transverse direction reinforcing the elongation of the cell (Bergfeld *et al.*, 1988; Toyomasu *et al.*, 1994). If the coleoptile tip (the major source of auxin) is excised and the cells are depleted from endogenous auxin by incubation of coleoptile segments in water, MTs change their orientation from transverse to longitudinal and the cellulose microfibrils are deposited in a longitudinal direction (Bergfeld *et al.*, 1988). This results in a loss of growth reinforcement and, consequently, in a block of coleoptile elongation. This process can be reversed by addition of exogenous indole acetic acid (Bergfeld *et al.*, 1988; Nick *et al.*, 1990, 1992). The time course of this phenomenon reveals that MTs respond within 10–15 min to the addition of indole acetic acid (Nick *et al.*, 1990, 1992) and complete their reorientation within 1 h (Fig. 5). Interestingly, this reorientation response is restricted to the outer epidermal wall (Nick *et al.*, 1990; Nick and Furuya, 1996), the target of auxin-dependent growth control (Kutschera *et al.*, 1987).

Phototropic stimulation of intact coleoptiles induces a reorientation of cortical MTs in the lighted flank, whereas the MTs in the shaded flank reenforce their transverse orientation (Nick *et al.*, 1990). This gradient of MT orientation is correlated with a gradient of growth (inhibition of growth in the flank, where MTs are longitudinal, and stimulation of growth in the flank, where MTs are transverse) resulting in tropistic bending. The time course of phototropically induced MT reorientation argues against the possibility that reorientation is a consequence of asymmetric growth: MT reorientation becomes detectable 10 min after stimulation and is complete within 1 h, whereas phototropic curvature becomes detectable 20–30 min

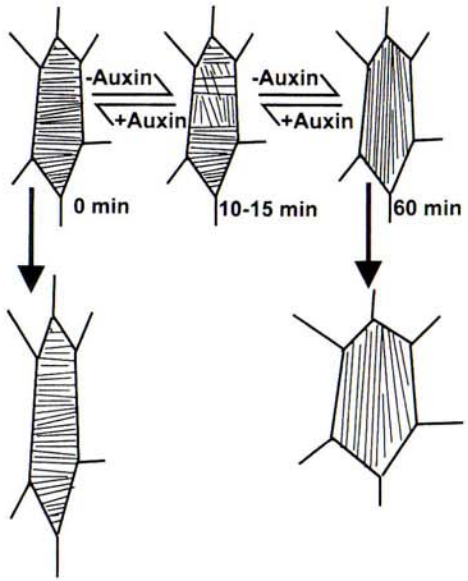


FIG. 5 Reorientation of cortical MTs in epidermal cells of Graminean coleoptiles in response to auxin. Depletion from endogenous auxin causes a reorientation of cortical MTs from transverse to longitudinal within 1 h and a block of cell elongation. Addition of exogenous auxin (indole acetic acid) can reverse this process with the same time course and can stimulate cell elongation.

after stimulation and reaches a maximum at 2 h after stimulation (Nick and Schäfer, 1988).

A detailed analysis of the relation between the gradient of MT orientation and bending (Nick *et al.*, 1991b) revealed that it is possible to separate both phenomena. This means that the functional significance of phototropically induced MT reorientation has to be sought elsewhere.

The bending response to blue light is transient, reaching a maximum at 2 h after induction and disappearing subsequently (Nick and Schäfer, 1988). However, if gravitropic stimulation is rendered symmetrical by rotation on a horizontal clinostat, a stable bending toward the inducing pulse is observed. This demonstrates the existence of a *transverse polarity* that had been induced by the stimulus and that persisted even during the time of gravitropic straightening. This transverse polarity can thus be separated from bending itself. This polarity was probed for stability against opposing counterstimulation and was found to become irreversibly fixed 2 h after irradiation (Nick and Schäfer, 1988). Microtubule orientation was tightly correlated to the establishment and fixation of this polarity (Nick and

Schäfer, 1994). The orientation of MTs became irreversibly fixed at the same time as when the transverse polarity became irreversibly fixed (Nick and Schäfer, 1988, 1994). This fixation of MT orientation induced by blue light blocked the reorientation response to auxin as well. This blue light effect on MT stability could not be mimicked by a depletion of endogenous auxin or by gradients of auxin, demonstrating that it was not transduced by auxin (in contrast to phototropic bending). Experiments involving partial irradiation showed that the site of perception for phototropism is situated in the coleoptile tip, whereas the site of perception for polarity induction and MT fixation is situated in the base of the coleoptile (Nick and Schäfer, 1994).

These observations suggest that the reorientation of MTs is the cellular expression of phototropically induced transverse polarity, and that the fixation of MT orientation is the cellular correlate to the irreversible fixation of this polarity. A similar blue light-induced reorientation of epidermal MTs could be observed in pea stems. In this system, the microtubular response was found to be slower than the light-induced decline of growth rate, providing further evidence against a role of MT reorientation for fast growth responses (Laskowski, 1990).

Microtubule reorientation can be elicited not only by blue light but also by other light qualities. The transverse MT array becomes more frequent upon short-term irradiation with red light in maize coleoptiles (Nick *et al.*, 1990; Zandomeni and Schopfer, 1993) along with a stimulation of growth. This response could be shown to be induced by the plant photoreceptor phytochrome (Zandomeni and Schopfer, 1993). In contrast, in rice coleoptiles, in which red light inhibits elongation, activation of the phytochrome system is observed to promote the formation of longitudinal arrays (Nick and Furuya, 1993; Toyomasu *et al.*, 1994). The light effect is not necessarily transduced by changes of auxin level: For the mesocotyl of rice that is inhibited by red light, the light effect on growth and MT orientation is not dependent on auxin (in contrast to the coleoptile) but seems to involve light-dependent changes in sensitivity and responsiveness to gibberellins (Nick and Furuya, 1993; Toyomasu *et al.*, 1994). Although the light signal is perceived by the same photoreceptor (phytochrome) and results in the same response (reorientation of cortical MTs from the transverse to the longitudinal array), this response can be transduced in different cells of the same plant by different events (auxin level in the coleoptile and gibberellin responsiveness in the mesocotyl).

The phenomenological analysis of light-induced MT reorientation indicates that there exist several links between signal transduction and microtubular dynamics.

B. Gravity: Gravitropism and Gravimorphosis

Gravity is possibly the environmental factor with the largest impact on plant architecture. The evolution of land plants was to a large extent driven by the need to overcome the mechanic constraints of gravity that was no longer compensated by buoyancy. It is not astonishing, therefore, that gravity-triggered signal transduction is linked to morphogenesis in plants. This becomes manifest in two basic phenomena: (i) When the orientation of a plant is changed with respect to gravity it will respond by a very sensitive bending response that restores the original orientation (*gravitropism*) and (ii) the formation and orientation of new organs is often adjusted with respect to gravity (*gravimorphosis*). Signaling to the cytoskeleton seems to be a common aspect in both phenomena.

It is generally believed that the trigger for gravitropism is a change in the direction of amyloplast sedimentation or amyloplast pressure upon unknown gravity-susceptible structures. Numerous observations (see Section III,C) suggest a role for MTs and/or microfilaments during sensing or early transduction of gravity. Here, the MTs will be discussed not primarily as sensors but with respect to their response to gravity. In epidermal cells of gravitropically stimulated maize coleoptiles or sunflower hypocotyls, cortical MTs were observed to respond by reorientation from transverse to longitudinal in the upper flank of the stimulated organ, whereas they maintained or even reinforced their original transverse orientation in the lower flank (Nick *et al.*, 1990). The time course of this response was consistent with a model in which gravitropic stimulation induced a lateral shift of auxin transport toward the lower organ flank and, consequently, a depletion of auxin in the upper flank. Epidermal MTs would then respond primarily to this decrease in auxin rather than to gravity itself. A similar reorientation response from transverse to longitudinal can be observed in cortical cells of maize roots (Blancaflor and Hasenstein, 1993). These MT responses seem to be connected more to the growth responses triggered by gravity than to gravity directly. There exist, however, observations about MTs in the gravity-sensing cells. In moss protonemata, a redistribution toward the lower cell flank has been observed for MTs that are adjacent to the amyloplasts in response to gravitropic stimulation (Schwuchow *et al.*, 1990). In rice coleoptiles, MTs in the gravity-sensing bundle-sheath cells are found to reorient from transverse to longitudinal: If this reorientation is blocked by taxol, the gravitropic response becomes delayed, and if the reorientation is promoted by pretreatment with the actin-inhibitor cytochalasin D, a precocious gravitropic response is observed (Nick *et al.*, 1997a). The functional role of this reorientation remains to be elucidated, but these examples demonstrate that MTs are capable of a direct response to gravity.

The simple observation that roots are formed at the basal pole may suffice to illustrate the importance of gravimorphosis. Although a considerable amount of phenomenological work had been dedicated to this problem at the turn of the century (Sachs, 1880; Vöchting, 1878; Goebel, 1908) the responsible mechanisms have remained obscure. One reason for this problem was certainly the use of adult organs for regeneration experiments, i.e., structures in which polarity had already been fixed and was therefore hard to be inverted. In the past few years, new systems have been introduced that may be more appropriate for the study of gravimorphosis. Germinating fern spores initiate development with an asymmetric division that separates, similar to the first zygotic division in higher plants, a larger, more vacuolated basal cell from a smaller, apical cell that is filled by a more dense cytoplasm. This first cell division seems to be formative in nature; by treatment with antimicrotubular drugs (Vogelmann *et al.*, 1981) it can be made symmetric, resulting in the formation of two thalli and abortion of rhizoid development. The axis of this first division is aligned with gravity; if the spore is tilted after this cell division, the rhizoid grows in the wrong way and is not able to correct the direction of growth (Edwards and Roux, 1994), demonstrating that gravitropism does not play a role in this system. Prior to division, at the time when the spore is competent to the aligning influence of gravity, a vivid migration of the nucleus toward the lower pole of the spore is observed. The movement is not steady but displays rhythmic oscillations in velocity, nor is it a simple sedimentation because it is sometimes actively inverted and seems to be guided by tethering of the nucleus to the cell wall (Edwards and Roux, 1997). The action of antimicrotubular compounds strongly suggests that this guiding mechanism is based on MTs that must then realign with respect to the gravity vector. It should be mentioned that a similar mechanism of gravimorphosis has been detected in the determination of the dorsiventral axis of the frog egg (Gerhart *et al.*, 1981). There, the axis is determined by an interplay of gravity-dependent sedimentation of yolk particles, sperm-induced nucleation of MTs, and self-amplifying alignment of newly formed MTs driving the cortical rotation (Elinson and Rowning, 1988).

C. Mechanic Stimuli: Barreer Response, Wounding, Primordia Initiation, Mechanic Load, and Vessel Regeneration

The response to mechanic stimulation is probably related to the problem of gravity sensing because mechanosensitive ion channels might be important for both signal chains. However, in most cases the response to mechanic stimulation is not limited to the activation of mechanosensitive channels but rather accompanied by hormonal responses as well.

When seedlings of higher plants encounter mechanical obstacles, they display a characteristic barreer response, i.e., a block of elongation accompanied by induction of stem thickening. The trigger for this response seems to be the plant hormone ethylene (Nee *et al.*, 1978), which is constantly formed by the growing stem and accumulates in front of physical obstacles. By electron microscopy in pea epicotyls, cortical MTs were observed to reorient from their original transverse array into steeply oblique or even longitudinal arrays. This reorientation is followed by a shift of cellulose deposition from transverse to longitudinal synthesis and subsequently a thickening of the stem (Lang *et al.*, 1982).

Even in mature plant tissue, the polarity of cells can change in response to mechanic injury. Usually, cortical cells are induced to reenter the mitotic cycle and to align cell division such that the new cell walls are laid down parallel to the wound surface. In addition, the new axis of elongation is oriented perpendicular to the wound surface. This behavior ensures rapid closing of the wound (Hush *et al.*, 1990). In wounded pea roots, these changes in the axis of growth and division were observed to be preceded by a reorientation of cortical MTs, with MTs being aligned parallel to the wound surface (Hush *et al.*, 1990). In the same cells, a few hours later the formation of preprophase bands was initiated in parallel to the direction of cortical MTs. This was especially impressive in elongated cells, in which the preprophase band was then parallel to the long axis of the cell. The inducing signal is not known, but the authors argue that MTs are aligned in the form of an arc around the wound and follow the lines of mechanical stresses produced by the removal of tissue during wounding.

Orientation of MTs accompanies the formation of new leaf primordia (Hardham *et al.*, 1980) and it has been discussed whether the alignment of MTs over rows of several cells is caused by mechanic stresses that are patterned by the preexisting primordia (Green, 1992).

In addition to these circumstantial observations it is also possible to demonstrate mechanically induced reorientation of MTs directly. Application of mechanical fields (Hush and Overall, 1991), high pressure (Cleary and Hardham, 1993), or bending and mechanic load (Zandomeni and Schopfer, 1994) can induce a reorientation of cortical MTs from an originally transverse to a longitudinal array. It should be kept in mind, however, that the treatments used during these experiments were quite drastic and far beyond physiological levels. It is therefore difficult to know to what extent the observed effects are caused by the release of stress hormones that are well-known triggers of MT reorientation. In woody plants, however, there exists an unequivocal correlation between the orientation of MTs and

cellulose fibers and the pattern of mechanic stresses during the formation of so-called tension wood (Prodhan *et al.*, 1995).

Cortical MTs are clearly stabilized by the cell wall (Akashi *et al.*, 1990), indicating the existence of transmembrane proteins that link MTs to cellulose microfibrils. Moreover, a close interaction between microfibrils and MTs is a central element of MT–microfibril parallelity (Heath, 1974). Changed patterns of mechanic stress might be transferred on MTs via such hypothetical transmembrane proteins and might result in direction-dependent stability of MTs (Williamson, 1991). This would allow MTs to sense changes in cell growth via changes in the pattern of wall strains and to align themselves with those changes in a stabilizing feedback loop. This model has even been used to reduce the multitude of microtubular reorientation responses to a simple mechanosensory model (Fischer and Schopfer, 1997). As interesting as this model may be, it is certainly oversimplified: Growth responses and MT reorientation are often correlated, but they have been separated in a range of systems and a range of conditions (Nick *et al.*, 1991b; Baluška *et al.*, 1992; Sauter *et al.*, 1993; Kaneta *et al.*, 1993; Sakiyama-Sogo and Shibaoka, 1993; Nick and Schäfer, 1994; Mayumi and Shibaoka, 1996; Baskin, 1997).

D. Cold: Induction of Chilling Resistance

Animal MTs depolymerize in the cold and they reassemble in the warm. This property has been used extensively to purify tubulin by repeated temperature cycles (Shelanski *et al.*, 1973). In plants, however, many MTs are found to be cold stable and to exhibit dramatic differences in the degree of cold stability. These differences of cold stability have been correlated to cold hardiness:

1. Cold resistance of MTs is barely pronounced in chilling-sensitive species such as tomato or cucumber but becomes remarkable in chilling-resistant species such as winter wheat (Jian *et al.*, 1989).
2. Cold acclimation of rye roots results in increased frost stability and osmotic tolerance of cortical MTs (Pihakaski-Maunsbach and Puhakainen, 1995).
3. Antimicrotubular drugs increase chilling injury (Rikin *et al.*, 1980).
4. Abscisic acid, which increases frost hardiness in many species, can induce increased cold resistance of cortical MTs (Sakiyama and Shibaoka, 1990).

The exact mechanism of cold adaptation of MTs is far from being understood. There are several indications that the cold-induced depolymerization of MTs as well as the development of microtubular cold resistance involve active signaling:

1. If the phosphoinositide pathway was blocked by addition of lithium, the cold-induced depolymerization of MTs was inhibited in spinach (Bartolo and Carter, 1992). Cold signaling in plants has been shown to involve cellular pools of calcium (Knight *et al.*, 1996) and lithium is probably acting by interference with this pathway. The link between this cold-triggered calcium pathway and MT depolymerization is likely to involve calmodulin (Fisher *et al.*, 1996) and possibly interaction of calmodulin with MT-associated proteins such as the elongation factor EF-1α (Durso and Cyr, 1994).
2. Cold stability of MTs is observed in stationary cell cultures of tobacco but not in freshly subcultivated cultures. Culture medium from stationary cultures can induce microtubular cold stability as well as protein kinase inhibitors such as 6-dimethylaminopurine or staurosporin (Mizuno, 1992). This means that cold lability is actively maintained by a pathway that involves protein kinases. The target for this pathway is not known, but it might involve changes in the pattern of tubulin isotypes. In rye roots, the pattern of tubulin isotypes has been described to respond relatively rapidly to cold acclimation (Kerr and Carter, 1990). In *Arabidopsis*, the expression of several tubulin genes changes in response to cold acclimation. Some isotypes disappear (such as *TUB2*, *TUB3*, *TUB6*, and *TUB8*), but one isotype (*TUB9*) is upregulated (Chu *et al.*, 1993). Interestingly, a certain degree of tubulin depolymerization is required for acquired cold resistance. If MT disassembly is suppressed by taxol, chilling resistance becomes markedly reduced (Bartolo and Carter, 1991). This indicates that, in fact, existing MTs have to be replaced by new MTs with changed isotype composition. Whether these isotypes confer higher cold stability *per se* or whether they interact with a different set of associated proteins that confer the increased cold stability remains to be elucidated. It has been shown that an extensin-dependent microtubular interaction with the cell wall can confer increased cold stability (Akashi *et al.*, 1990), indicating that cold acclimation might involve several processes in parallel.

E. Hormones: Auxin, Gibberellins, Brassinolide, Cytokinins, Ethylene, and Abscisic Acid

Many of the signal responses mentioned previously are accompanied by changes in the level of endogenous hormones and it is even possible to mimick certain aspects of these signal responses by application of exogenous hormones. For instance, the reorientation of MTs in response to phototropic

or gravitropic stimulation can be mimicked by auxin or auxin gradients (Nick *et al.*, 1990; Nick and Schäfer, 1994), the induction of longitudinal MTs during the barreer response of pea shoots can be mimicked by induction of ethylen (Lang *et al.*, 1982), and the effect of red light on the elongation of rice mesocotyls can be mimicked by an inhibition of gibberellin synthesis (Nick and Furuya, 1993). These correlations do not prove that the hormones are actually the transducers for these different signals, but they illustrate the strong response of the microtubular cytoskeleton to plant hormones. For almost all plant hormones, a response of cortical MTs has been described. These responses have been reviewed (Shibaoka, 1994) and therefore will be only briefly summarized.

Auxin produces transverse MTs in shoots and coleoptiles (Sakoda *et al.*, 1992, radish hypocotyl; Bergfeld *et al.*, 1988; Nick *et al.*, 1990, maize coleoptile) and auxin depletion can mimick the blue light effect of phototropic stimulation on MT orientation (Nick and Schäfer, 1994) but not the blue light effect on MT fixation. In roots, in which auxin acts inhibitory on cell elongation, it is found to cause a reorientation in the opposite sense, i.e., from transverse to longitudinal (Blancaflor and Hasenstein, 1995).

Gibberellins usually stimulate cell elongation in roots (Baluška *et al.*, 1993) and shoots (Shibaoka, 1993), and these effects are accompanied by increased frequencies of transverse MTs. Whereas in most cases gibberellin can actually induce a reorientation of MTs from a longitudinal to a transverse array, it is not able to do so in the internode of deep water rice. In those internodes, gibberellin is only able to stabilize MTs against age-dependent factors that favor oblique or longitudinal arrays (Sauter *et al.*, 1993). This indicates that even one hormone (gibberellin) can interact with MTs at different sites of actions. It should be mentioned that the induction of transverse MTs by gibberellins is not always accompanied by a stimulation of growth (Sakiyama-Sogo and Shibaoka, 1993). Gibberellins can counteract the inhibition of mesocotyl elongation by red light in rice (Toyomasu *et al.*, 1994) and cause a transverse orientation of cortical MTs in the epidermis. Interestingly, they can induce a similar orientation from oblique to transverse in cortical cells of the rice coleoptile (Nick *et al.*, 1994) without any effect on growth rate (Toyomasu *et al.*, 1994).

Brassinolides stimulate stem elongation and increase the frequency of transverse MTs in azuki beans (Mayumi and Shibaoka, 1995). This system is very interesting because two hormones, auxin and gibberellin, have to interact to increase the frequency of transverse MTs. Brassinolide, in contrast, can induce transverse MTs by itself, without cooperation with auxin or gibberellin, again indicating several sites of interaction between signaling and MT reorientation (Mayumi and Shibaoka, 1995).

Cytokinins typically suppress stem elongation and induce stem thickening and they induce longitudinal arrays of MTs (Shibaoka, 1974; Volfová *et al.*, 1977). The same is true for ethylen (Lang *et al.*, 1982) and abscisic acid (Sakiyama and Shibaoka, 1990; Sakiyama-Sogo and Shibaoka, 1993).

The phenomenology of hormone action on MT orientation suggests that there exist multiple sites of interaction (Mizuno, 1994; Mayumi and Shibaoka, 1996). This is confirmed by pharmacological evidence. If azuki bean stem sections were treated with protein kinase inhibitors, such as 6-dimethylaminopurine, an increase of longitudinal MTs was observed even in the presence of gibberellin. This increase could be reverted by subsequent addition of auxin. This indicates that the action of gibberellins (suppression of MT reorientation from a transverse into a longitudinal array) seems to depend on protein kinases, whereas the action of auxin (stimulation of MT reorientation from a longitudinal into a transverse array) does not.

These rapid effects, possibly related to phosphorylation cascades, are then followed by alterations in the pattern of tubulin isotypes and tubulin modifications. In dwarf peas, in which elongation can be triggered by exogenous gibberellic acid, a detyrosination of a specific α-tubulin isotype can be observed from as early as 2 h after induction (Duckett and Lloyd, 1994). Whether this posttranslational modification of tubulin is the cause of MT reorientation (from longitudinal to transverse arrays) in this system or whether it mirrors an increased lifetime of transverse MTs (allowing for prolonged action of modifying enzymes) remains to be elucidated. Interestingly, in the experiments with azuki bean segments discussed previously, treatment with protein kinase inhibitors not only prevented the action of gibberellin on MT orientation but also suppressed the modification of an α-tubulin isotype that was observed in the controls (Mizuno, 1994). This raises the possibility that gibberellins can trigger a protein kinase cascade that might increase the lifetime of transverse MTs. This might produce an increased degree of posttranslational modifications that possibly reinforces the stabilization of transverse MTs. In contrast, the signal chain triggered by auxin seems to proceed independently and does not result in a change in the pattern of tubulin modifications (Mizuno, 1994).

F. Development: Phyllotaxis, Differentiation, Tuberization, and Stomata Formation

Microtubules also respond to developmental signals. Some of these signals may be identical to those discussed previously, but this is still speculative in most cases. Therefore, developmental responses of MTs are discussed in a separate section.

The formation of new leaf primordia in the apical meristem involves a shift in the axis of growth and division. One of the first indications of primordia initiation is a reorientation of MTs. Microtubules reorient in a

ring of cells around the margins of the prospective primordium that are otherwise undistinguishable from their neighbors with respect to dimension or growth axis (Hardham *et al.*, 1980). In the beginning the orientation of MTs can change quite dramatically from cell to cell. With time, an alignment of MTs is observed leading to smoother transitions in MT orientation between neighboring cells. Again, the primary signal is not known, but it is possible to predict the site where this orientation will occur by calculating the positions of minimal energy based on the pattern of mechanic stress that is produced by the preexisting primordia (Green, 1992). The gradual alignment also might be based on the ability of MTs to sense and to respond to wall stresses.

The orientation of cortical MTs can also change when cells differentiate or become older. This phenomenon is impressively illustrated by immunofluorescence studies of root tissue in which the course of cell differentiation can be conveniently followed by comparing cells at different distances from the root tip (Baluška *et al.*, 1992). These studies demonstrate that even neighboring cells that follow a different path of differentiation can be dramatically different with respect to their microtubular system. During the differentiation of conifer tracheids cortical MTs change their orientation and this is followed by a reorientation of cellulose deposition and, eventually, corresponding changes in cell lumen (Abe *et al.*, 1995). In most shoots, there is a marked tissue specificity of MT responses. Reorientation is usually confined to the cells of the outer epidermis (Iwata and Hogetsu, 1988; Nick *et al.*, 1990; Mizuno, 1994). In coleoptiles, it is the epidermis that is the most sensitive to aging, with cortical MTs assuming increasingly oblique and longitudinal arrays at a time when the inner tissues still maintain transverse MTs. This is consistent with a special role of the epidermis in the control of shoot elongation (Kutschera *et al.*, 1987). The MT response to cell differentiation is not restricted to reorientation. In wheat leaves, in which differentiation can be observed in a linear gradient from leaf base to leaf tip, the number of MTs was found to decrease with proceeding differentiation. This decrease was not caused by increased disassembly but seemed to involve tubulin proteolysis (Jung *et al.*, 1993). In tracheary elements that are characterized by secondary wall thickening, cell differentiation is accompanied by a bundling of cortical MTs adjacent to those sites where new wall thickenings will form (Fukuda and Kobayashi, 1989).

The formation of tubers or bulbs is accompanied by a shift of growth axis from elongation toward lateral growth. There seem to exist several pathways that can culminate in tuberization. A tuber-inducing factor was isolated from potato leaves from plants that had been made competent for tuberization by cultivation during short days (Koda *et al.*, 1988). This factor was later identified as a glucoside of jasmonic acid (Yoshihara *et al.*, 1989). In tobacco cells, jasmonic acid was found to cause the disruption of cortical MTs and thus to disturb the reinforcement of cell elongation. This might induce lateral swelling as a first step of tuber formation. However, in the same species, tuber formation from axillary buds was found to be suppressed by gibberellins that maintained MTs in a transverse orientation (Sanz *et al.*, 1996). If the buds were cultivated in the absence of gibberellin, they switched from shoot to tuber formation accompanied by a switch in the direction of MTs from transverse to longitudinal. This means that tuberization can be induced either by depolymerization of MTs (possibly transduced by an increase in the activity of jasmonic acid) or by a reorientation of MTs from transverse arrays supporting cell elongation to longitudinal arrays allowing for lateral growth (possibly transduced by decreased activity of gibberellins).

Stomata formation is a classic example of pattern formation since it involves a switch in the axis and symmetry of cell division. The first visible sign of stomata formation in monocotyledonous plants is an asymmetric division of epidermal cells (Fig. 6A). The choice of cells that undergo this process seems to be programmed during early development, when these cells pass a zone of patterning at the leaf base (Boetsch *et al.*, 1995). The

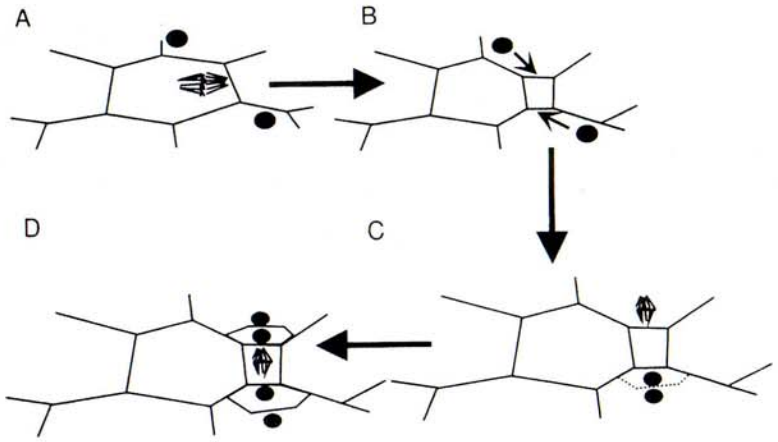


FIG. 6 Stages in the reorientation of cell division in response to developmental signals during stomata formation in the monocotyledonous plant *Tradescantia*. (A) An epidermal cell determined for stomata formation divides asymmetrically accompanied by nuclear migration, asymmetric formation of a preprophase band, and the formation of an asymmetric division spindle. (B) The nuclei of the neighboring cells migrate toward the smaller daughter cell (the guard cell mother cell). (C) They induce the formation of asymmetric preprophase bands with a tilted orientation and asymmetric division spindles with a tilted orientation. (D) The guard cell mother cell divides in the same direction as the neighboring cells (i.e., tilted by 90° with respect to the original axis) but symmetrically. This behavior is heralded by the formation of a tilted preprophase band in the guard cell mother cell.

cells have to pass through a defined period of the cell cycle to be competent for the reception of this determining signal (Chin *et al.*, 1995), although the response to this signal (the initiation of stomata formation) occurs later in development. In the next step a peculiar migration of nuclei (Bünning, 1965) toward the smaller daughter cell (the guard cell progenitor) is observed in the neighboring cells (Fig. 6B). These neighboring cells now divide asymmetrically but in a direction that is perpendicular to the original axis, resulting in two small accompanying cells (Fig. 6C). Simultaneously, the guard cell progenitor divides symmetrically but in a direction that is perpendicular to the original axis, giving rise to the two stoma cells (Fig. 6D). The whole process can be suppressed by signals from preexisting stomata just prior to nuclear migration (Boetsch *et al.*, 1995). The nature of this signal is unknown, but it must interfere with nuclear migration (defining the symmetry of division) and with the direction of the preprophase band (defining the axis of division). There must be two pathways of signaling: The guard cell mother cell can induce nuclear migration in the neighbors, and preexisting stomata can suppress further development of the guard cell. Although this inhibitory signal might involve mechanosensing, this is certainly not true for the signal that is transmitted from the guard cell mother cell.

G. Biotic Factors: Fungal Attack and Viral Movement

Plant life is endangered not only by abiotic stresses, such as cold, wind, or drought, but also by biotic stresses. Again, the microtubular cytoskeleton is involved in the adaptive response of plants to biotic stress. The response of MTs during wound healing (e.g., as a consequence of herbivore attack) has already been discussed. When plants are attacked by pathogens such as fungi, there exist additional responses that enable the plants to cope with this pathogen attack.

The attempt of fungal hyphae to penetrate plant cells usually triggers the production of secondary plant metabolites that can suppress fungal growth. Almost immediately after penetration, the nucleus and the cytoplasm of the host cell move toward the penetration site (Gross *et al.*, 1993). This movement was dependent on actin filaments and was accompanied by a local depolymerization of cortical MTs around the penetration site. This response seems to be essential for a successful defense against fungal attack as has been shown in barley coleoptiles (Kobayashi *et al.*, 1997). If microfilaments and/or MTs are eliminated by anticytoskeletal drugs, the nuclear movement is inhibited, and fungi that normally are unable to infect the host cells become pathogenic. The functional consequence of this dynamic reorganization of MTs is not clearly understood, but the observation that callose formation around the penetration site seems to be impaired (Kobayashi *et al.*, 1997) after treatment with anticytoskeletal drugs suggests that a redistribution of vesicle transport toward the penetration site might be essential for successful inhibition of fungal penetration. The initial trigger for these responses seems to be cell wall fragments of fungal origin—so-called elicitors (Gross *et al.*, 1993). The question whether a fungus is a pathogen or a nonpathogen with respect to a given plant species or cultivar might thus be related to the problem of whether the fungal cell wall contains elicitors that induce signaling culminating in microtubular depolymerization.

Microtubules play a positive role in the response to fungal attack. Plant viruses, in contrast, invented strategies to usurpate MTs for their own purpose. To spread from the infection site through the whole plant, viruses such as the tobacco mosaic virus or the cauliflower mosaic virus produce special proteins that are required for successful transmission and migration. The movement protein from tobacco mosaic virus was fused to the green fluorescent protein from jellyfish and a virus harboring this construct was used to infect tobacco leaf discs (Heinlein *et al.*, 1995). The fusion protein became aligned in long filaments shortly after infection. These filaments were shown to be MTs by double-immunofluorescence microscopy and by treatment with antimicrotubular drugs. A similar colocalization was found for the aphid transmission factor of cauliflower mosaic virus (Blanc *et al.*, 1996). This viral protein behaved as a MT-associated protein *in vitro*, i.e., it formed stable sedimentable complexes with preformed, taxol-stabilized MTs. These observations suggest that viruses use the microtubular cytoskeleton for targeting and transporting across the cell toward the plasmodesmata (Heinlein *et al.*, 1995). For viral attack, signaling to the MTs is not evident at first glance. Nevertheless, it must involve the ability of the viral movement protein to interact and to utilize MT-driven transport.

V. Approaches

A. Signal-Related Cytoskeletal Mutants

Knowledge of the signals that control the dynamic behavior of plant MTs is still relatively preliminary. This severely hampers the design of molecular approaches. A mutant approach is often appropriate if one does not know very much about mechanisms. Although cytoskeletal mutants have been obtained in plants, they have been rarely characterized with respect to signaling. One problem may be that cytoskeletal mutants are expected to disturb development dramatically, causing sterility or even lethality. To

obtain cytoskeletal mutants, plants are usually selected for resistance to antimicrotubular drugs or herbicides (Lee and Huang, 1990; Vaughn *et al.*, 1987; Goldman *et al.*, 1993; Strashnyuk *et al.*, 1993). Further candidates might be mutants in which cell shape is altered: The *tonneau* mutant of *Arabidopsis* lacks preprophase bands and exhibits disordered patterns of cell division resulting in a swollen phenotype (Traas *et al.*, 1995). In *Arabidopsis* roots, several mutants exist in which cell expansion becomes tilted in response to strong growth (Benfey *et al.*, 1993) or ethylene (Aeschbacher *et al.*, 1995).

To screen directly for mutants affected in signal-dependent MT responses, rice coleoptiles were chosen because growth is entirely based on auxin-triggered cell elongation (Furuya *et al.*, 1969). More than 7000 individual γ-ray mutant lines of rice were screened for resistance of coleoptile elongation against ethyl-*N*-phenylcarbamate (EPC). The screen was designed as a lethal mutant screen in case the mutation results in sterility or lethality (Nick *et al.*, 1994). Treatment with EPC inhibits the polymerization of MTs and causes their elimination depending on their rate of turnover (Mizuno and Suzaki, 1990). This blocked elongation of coleoptiles and roots and caused distorted growth due to a block of coleoptile gravitropism (Nick *et al.*, 1991a, 1997). Resistant mutants were raised to maturity and their offspring tested for inheritance of the trait over two generations of selfing. As expected, some of these mutations (presumably affecting the cytoskeleton) had dramatic effects on development, such as a lack of shoots, duplications of the entire seedling axis, or degeneration of female flowers.

Among these mutants, the line *EPC-resistant 31* (*ER31*) was found to be lethal in the homozygous state, whereas heterozygotes remained viable and displayed a changed pattern of growth (Nick *et al.*, 1994): In the absence of the drug, cell elongation in the mesocotyl was enhanced (accompanied by a more transverse orientation of MTs compared to the wild type), whereas it was conspicuously reduced in the mutant coleoptile (with MTs being longitudinal in contrast to transverse MTs in the wild type). A similar situation was observed in the leaves: the leaf sheath was longer and the leaf blade was shorter than those in the wild type, which were related to changes in the direction of MTs. The resistance to the drug was found to be related to a reduced turnover of cortical MTs and reduced the response to taxol (a blocker of MT depolymerization) (Nick *et al.*, 1994). To investigate the cause for the longitudinal orientation of MTs in the mutant coleoptiles, segments were treated with the auxin indole acetic acid, which can reorient MTs by 90° within 1 h in the wild type. In the mutant, however, auxin failed to induce any reorientation of MTs, although other auxin responses such as callus induction were found to be normal (Nick *et al.*, 1994). Interestingly, MT reorientation in *ER31* could be induced by other stimuli in the same way as in the wild type: Gibberellin can cause a reorientation into transverse arrays, whereas red light can induce longitudinal MTs (Nick *et al.*, 1994). Reorientation is also caused by gravity, although it is delayed by 2 h compared to the wild type. This delayed reorientation is correlated to a delay of gravitropic bending by the same time interval (Nick *et al.*, 1997). These data suggest that in *ER31*, the link between auxin-triggered signal transduction and microtubular dynamics, is impaired, although auxin signaling *per se* as well as MT reorientation *per se* are functional.

It should be possible to find similar mutants in other systems. They can be used, in the long term, for two approaches: (i) Cloning and identification of the mutated gene products should allow insight into the components that are involved in signaling to the microtubular cytoskeleton and (ii) these mutants can be used to test the functional role of potential signaling events or components.

B. Signal-Related Microtubule-Associated Proteins

Any signal that can cause microtubular reorientation must interact with those factors that control the assembly or disassembly of MTs as discussed previously. Although microtubular motors are likely to be involved, one major target for signaling is expected to be the structural MAPs. Despite the limited knowledge of these molecules in plants there is evidence for links of plant MAPs to signal transduction: (i) A protein, $p34^{cdc2}$, which is involved in the regulation of the cell cycle, has been found to be colocalized with the preprophase band in maize root tips and cells of the stomatal guard cells (Colasanti *et al.*, 1993), and (ii) the translation factor EF-1α, which has been identified as a MT-associated protein in cultured carrot cells, was reported to interact with MTs dependent on calmodulin (Durso and Cyr, 1994), and the function of this MAP might change from MT bundling to MT severing depending on the interaction with the calcium/calmodulin pathway (Fisher *et al.*, 1996).

To isolate plant MAPs that are regulated by environmental signals, extracts from noncycling, nonelongating maize coleoptiles were compared to extracts from coleoptiles, in which cell elongation had been triggered by activation of the plant photoreceptor phytochrome (Nick *et al.*, 1995). Both types of extracts were assayed for the presence of proteins that were able to coassemble with endogenous tubulin into MTs after lowering the critical tubulin concentration necessary for assembly by taxol. From nonelongating tissue, a 100-kDa protein could be isolated that was immunologically related to the neural MAP τ. This protein disappeared upon activation of the phytochrome system and became replaced, along with the induction of cell elongation, by a 50-kDa protein that was also immunologically related to

τ (Nick *et al.*, 1995). The 100-kDa protein was found to be associated with nuclei, whereas the 50-kDa protein was enriched in plasma membrane preparations and colocalized with ordered MT bundles that are formed during the onset of cell elongation but not with the fine, nonaligned MT that are characteristic of young coleoptiles prior to elongation (Nick *et al.*, 1995). Both proteins were purified by thermostable extraction in combination with anion-exchange chromatography and partial peptide sequences could be obtained. Whereas the 100-kDa protein could be identified as a member of the Hsp90 group of molecular chaperones (Nick *et al.*, 1997b), the 50-kDa protein seems to belong to a novel class of proteins.

In the near future, more plant MAPs will be cloned and identified. This type of work is expected to deepen our understanding of signaling to the cytoskeleton for the following reasons: (i) Different MAPs accomplish different functions with respect to MTs such as nucleation of new MTs, elongation of existing MTs, bundling of MTs, severing of MTs, and connection of MTs to microfilaments, the plasma membrane, and organelles. The regulation pattern of these different MAPs with respect to different signals might indicate the target process for signaling; (ii) the genes of these MAPs can be fused to the gene encoding the green fluorescent protein and the behavior of these proteins can then be followed *in vivo*; and (iii) these MAPs can be used to fish for upstream elements in the signal transduction.

C. Signal-Related Responses of Tubulin Isotypes and Tubulin Modifications

The complexity of tubulin isotypes and modifications is far from being understood, but they would provide an ideal target for signaling. The requirement for specificity and for flexibility was the driving force for the evolution of branched and interconnected signal transduction chains. This review attempted to show that complex signaling is also characteristic for the plant cytoskeleton. Tubulin isotypes are therefore ideal candidates if one were to design a system that allows for multiple interaction sites.

Changes of isotype patterns and modifications have been found during the response to cold acclimation (Kerr and Carter, 1990; Chu *et al.*, 1993) and during the response to gibberellins (Duckett and Lloyd, 1994; Mizuno, 1994). Further signal-triggered changes are expected to be discovered in the future. The interesting question regards the functional significance of these changes. Although MTs can be composed of various isotypes (Hussey *et al.*, 1987), there seem to exist microtubular arrays of varying composition (Smertenko *et al.*, 1997). Are these differences the cause or the consequence of differences in microtubular dynamics? In animal cells, at least for tyrosination, microtubular modification seems to be caused by changes in the lifetime of MTs (Khawaja *et al.*, 1988; Webster *et al.*, 1990).

Despite our complete ignorance concerning the functional role of tubulin isotypes and modifications, there exists the intriguing possibility that these target signaling to specific subsets of the microtubular cytoskeleton that are then recognized by specific MAPs conferring differences in microtubular dynamics. To test this hypothesis, two questions have to be investigated: (i) Does the isotype composition of MTs change in response to signaling? and (ii) Does the interaction between MTs and MAPs depend on the composition of tubulin isotypes?

D. Toward an *in Vivo* Assay for Signal-Triggered Microtubule Responses

Cytoskeletal mutants, for MT-associated proteins, and for tubulin isotypes will provide molecular tools for a molecular analysis of microtubular signaling. However, to develop functional approaches for this problem, *in vitro* assays such as MT-binding assays (Vantard *et al.*, 1994) or MT-bundling approaches (Cyr and Palevitz, 1989) are not sufficient. On the other hand, transgenic approaches based on the overexpression of MAPs or tubulin isotypes and/or the transformation with the respective antisense constructs are expected to produce either phenotypes that are characterized by extreme pleiotropy or, even worse, phenotypes that are completely hidden (in the case of mutual replacement of signal chains). To circumvent this drawback of a transgenic approach, an *in vivo* assay for MT function has to be developed that meets the following requirements:

1. It has to work in the natural tissue context to allow for intercellular signaling.
2. It should be confined to alterations of individual cells to minimize pleiotropic effects on development.
3. It can be manipulated by exogenous signals.
4. It can be observed and analyzed over the time that is typical for the signal response, i.e., up to 1 or 2 h.
5. It can be performed without extensive wounding in order to avoid artifacts caused by stress responses.
6. It should allow for simultaneous observation of at least two cytoskeletal components.

This type of *in vivo* assay is not yet available. Nevertheless, important steps toward such an assay have been accomplished. It seems that microinjection into intact plant tissue is the method of choice because it does not require the production of protoplasts (the removal of the cell wall alters

the behavior of the cytoskeleton completely, and protoplasts are therefore inappropriate models for the intact plant) and because it allows experiments in the context of the whole organ. Microinjection of fluorescent-labeled animal tubulin has been successfully employed for the study of microtubular dynamics *in vivo* in dividing (Zhang *et al.*, 1990; Vantard *et al.*, 1990) as well as in elongating cells (Wasteneys *et al.*, 1993; Yuan *et al.*, 1994). Upon microinjection the labeled neurotubulin is inserted into the microtubular system of the host cell with an astonishing velocity and it seems to participate in the dynamic behavior of the host cytoskeleton. In epidermal cells, for instance, the reorientation of cortical MTs could be visualized by this system (Yuan *et al.*, 1994; Wymer and Lloyd, 1996). To obtain an *in vivo* assay to study signaling to the microtubular cytoskeleton, this microinjection approach should be extended in the following way:

1. The reorientation response must become inducible by external triggers such as hormones, light, gravity, or electrical fields. Although a reorientation of MTs has been observed using microinjected neurotubulin (Yuan *et al.*, 1994), the analysis of this event is hampered by the problem that the trigger for this reorientation was neither known nor was it active in a reasonable proportion of the injected cells. This review intended to demonstrate that this is a technical and not a principle problem of the microinjection approach.

2. The microinjection approach will detect mainly those MTs that are characterized by high turnover. However, there seems to exist, even within a single cell, a great variation in the lifetime of individual MTs (Wasteneys *et al.*, 1993). This means that stable MTs that may account for a minor but important fraction of the microtubular cytoskeleton are overlooked. It should be possible, however, to circumvent this drawback if cells are coinjected by components that also interact with stable MTs. Such components might be fluorescent-labeled MAPs or fusions between MAPs and the green fluorescent protein. Such constructs have been utilized successfully to demonstrate the dynamics of MTs in living animal cells (Kaech *et al.*, 1996). In addition, such approaches can be used to follow the dynamic interaction between MAPs and MTs or among MAPs themselves.

VI. Concluding Remarks

Developmental plasticity is a central topic of plant morphogenesis and involves the ability to tune the direction of cell division and cell expansion with signals that are perceived from the environment. Signaling to the microtubular cytoskeleton plays a pivotal role. The major targets for signaling to the MTs are the preprophase band controlling axis and symmetry of cell division and the cortical MTs that define the direction of cellulose deposition and thus the axis of cell expansion. Much remains to be learned about the plant cytoskeleton, but it has become evident that it is organized and governed by different principles than MTs in animal cells. The preprophase band, the phragmoplast, and the cortical MTs are specific for plants. These differences might be related to the problem of signaling. The phenomenology of signaling demonstrates that the different signals do not merge into one chain that controls microtubular organization at one site of interaction. Even for one triggering signal (e.g., gibberellin) there exist multiple sites of interaction. To date, phosphorylation cascades, the calcium–calmodulin pathway, tubulin isotypes and modifications, and MT-associated proteins have been determined to be related to microtubular signaling. A molecular approach to this phenomenon is only gradually emerging and lags behind the work done in animal systems (a frustrating but common theme in plant biology). Nevertheless, new tools are gradually emerging. These tools involve cytoskeletal mutants, MT-associated proteins, the genes for different tubulin isotypes, and approaches to follow microtubular dynamics *in vivo* by microinjection of fluorescent-labeled tubulin. These developments justify the hope that the near future will bring real advances in our understanding of the plant cytoskeleton–advances that are interesting for biology as a whole.

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