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Control of Cell Axis

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Abstract Cell movement constitutes a basic mechanism in animal development, for instance during gastrulation or during the development of neural systems. Plant cells with their rigid cell walls cannot move and therefore had to evolve alternative mechanisms to organize their *Bauplan*. In plants, morphogenesis is controlled by the initiation of a cell axis during cell division and by the expression of this axis during subsequent cell expansion. Axiality of both division and expansion is intimately linked with specific microtubular arrays such as the radial array of endoplasmic microtubules, the preprophase band, the phragmoplast, and the cortical cytoskeleton. This chapter will review the role of microtubules in the control of cell axis, and attempt a synthesis of classical research with recent developments in the field. During the last few years, our understanding of two central enigmas of plant microtubule organization has been advanced substantially.

It had been observed for a long time that the spatial configuration of the phragmoplast was guided by events that take place prior to mitosis. However, the premitotic microtubular arrays disappear at the time when the spindle appears. It was therefore unclear how they could define the formation of a phragmoplast. The deposition of an endosomic belt adjacent to the phragmoplast, in combination with highly dynamic exploratory microtubules nucleated at the spindle poles, provides a conceptual framework for understanding these key events of cell axiality.

The microtubule–microfibril concept, which is central to understanding the axiality of cell expansion, has been enriched by molecular candidates and elaborate feedback controls between the cell wall and cytoskeleton. Special attention is paid to the impact of signalling to cortical microtubules, and to the mechanisms of microtubule reorientation. By means of live-cell imaging it has become possible to follow the behaviour of individual microtubules and thus to assess the roles of treadmilling and mutual sliding in the organization of microtubular arrays. Direction-dependent microtubule lifetimes, spatial patterns of post-translational modifications, and new mutants with deviating orientation of microtubules shed light on a complexity that is still far from being understood, but reveals a network of highly dynamic, nonlinear interactions that are endowed with pattern-generating properties. The chapter concludes with potential approaches to manipulation of the cell axis either through cell division or through cell expansion.

1
Cell Axis and Plant Development

During the growth of any organism, volume increases with the third power of the radius. Surface extension, however, increases only with the second power and thus progressively lags behind. In order to balance these two processes, the surface has to be enlarged substantially, either by internal or external exten-

sions. Due to their photosynthetic lifestyle, plants must increase their surface in an outward direction. As a consequence, plant architecture must be able to cope with a considerable degree of mechanical load. In aquatic plants, this is partially relieved by buoyancy, allowing considerable body sizes even on the base of fairly simple architectures. The transition to terrestrial habitats, however, required the development of a flexible and simultaneously robust mechanical lattice, the vessel system. The evolutionary importance of the vessel is emphasized by a large body of evidence. For instance, the so-called telome theory (Zimmermann 1965) had been quite successfully employed to describe the evolution of higher land plants in terms of a modular complexity based on load-bearing elements (the telomes) that are organized around such vessels.

The architectural response of plant evolution to the challenges of mechanical load had a second consequence, namely, a completely sessile lifestyle. This immobility, in turn, determined plant development with respect to its dependence on the environment. During animal development, body shape is mostly independent of the environment. In contrast, plants have to tune their *Bauplan* to a large degree to the conditions of their habitat. Morphogenetic plasticity thus has been the major evolutionary strategy of plants to cope with environmental changes, and fitness seems to be intimately linked to plant shape (Fig. 1).

Mechanical load shapes plant architecture, reaching down to the cellular level. Plant cells are endowed with a rigid cell wall and this affects plant development very specifically and fundamentally. The morphogenetic plasticity of a plant is therefore mirrored by a plastic response of both cell division and cell expansion with respect to axiality. In this response, cell division has to be placed upstream of cell expansion because it defines the original axis of a cell and thus the framework in which expansion can proceed. The deposition of the new cell plate determines the patterns of mechanical strain that, during subsequent cell expansion, will guide the complex interplay between protoplast expansion. This is mainly driven by the swelling vacuole, with the

Fig. 1 Adaptive response of morphogenesis in a tendril of *Vicia faba*. In response to the mechanical stimulus, upon contact with the support, cell elongation becomes arrested in the flank facing the support, whereas it continues at the opposite flank. The resulting growth differential causes a bending response towards the support and will, eventually, result in spiral growth of the tendril around the support. The time-course of the figure covers 24 h

cell wall as a limiting and guiding counterforce. It is even possible to describe the shape of individual cells in a plant tissue as a manifestation of minimal mechanical tension (Thompson 1959), emphasizing the strong influence of mechanical load on plant development.

When plants are challenged by mechanical load, they respond by changes in architecture that will allocate load-bearing elements (vessels and fibres on the organ level, cellulose microfibrils and lignin incrustations on the cellular level) in such a way that mechanical strains are balanced in an optimal fashion at minimal investment of energy and biomatter. This response of architecture is fundamental and involves changes on different levels of organization, from the spatial arrangement of macromolecules up to the allocation of biomatter to different organs.

Mechanical load affects architecture and the composition of the cell wall during cell elongation and subsequent cell differentiation. For instance, mechanical compression leads to a suppression of certain layers of the cell wall (the so-called S_3-layer) in conifer tracheids (Timell 1986; Yoshizawa 1987). Conversely, mechanical tension causes a shift in orientation of cellulose in the gelatinous layer of the challenged wood fibres in such a way that the mechanical strain is optimally buffered (Prodhan et al. 1995).

However, the effect of mechanical load by far exceeds these responses on the subcellular level. Plant cells can respond to a mechanical challenge by acute changes of cell axiality. It is even possible to demonstrate this directly: When protoplasts are embedded into agarose and the agarose block is subsequently subjected to controlled mechanical load (Lynch and Lintilhac 1997), the division planes of the embedded cells will then be aligned either perpendicular or parallel to the principle stress tensors (Fig. 2).

On the level of whole-plant physiology, mechanical stress can cause so-called thigmomorphogenesis, i.e. alterations of growth that result in adaptive changes of shape. For instance, unidirectional stem flexure of young pines (as produced, for instance, by exposure to wind) induced a larger biomass allocation to the roots parallel to the plane of flexing, which in turn resulted in an increased mechanical resistance within the plane of bending stress (Mickovski and Ennon 2003). In other words, the mechanical stimulus altered root architecture in an adaptive way to ensure optimal resistance to the triggering mechanical stress. The losses in yield that are caused by wind are conspicuous – estimates range between 20 and 50% for Graminean crops and reach up to 80% for certain apple varieties (Grace 1977). In addition to the allocation of lateral roots, it is the the angle between the primary root and the branch roots that defines the uprooting resistance of a root system to wind stress (Stokes et al. 1996).

The economic impact of thigmomorphogenesis is tremendous, but very often overlooked. Repetitive mechanical stimulation, e.g. by wind, will cause a redistribution of growth towards lateral expansion. Again, this thigmomorphogenetic response is clearly of adaptive quality. The resistance of a plant to

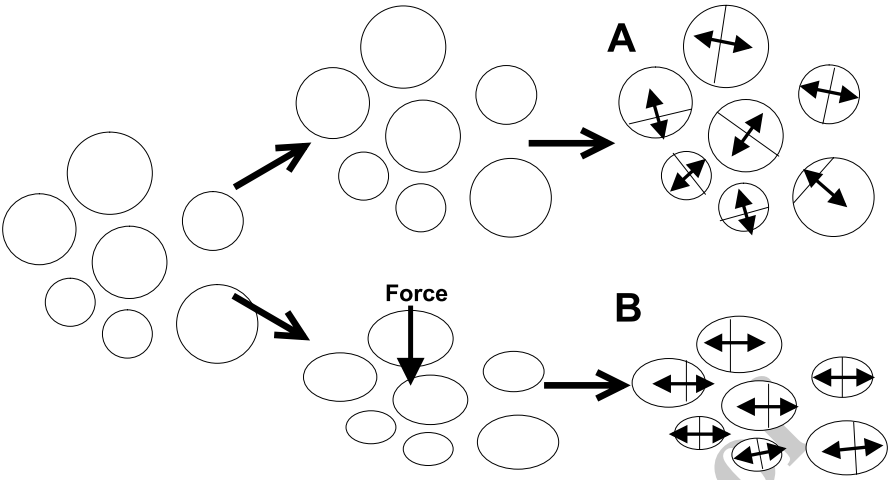


Fig. 2 Alignment of cell division in response to mechanical tension. Protoplasts that are embedded into agarose will divide randomly upon regeneration of the cell wall (**A**). However, when they subjected to mechanical tension, the direction of the subsequent division will be aligned (**B**)

windbreak and lodging is inversely related to plant height (Oda et al. 1966):

$$L_R = \frac{W \cdot M}{L^2\, w},$$

with W = fresh weight, M = bending momentum at breaking, L = shoot length and w = dry weight of the shoot. Thus, lodging resistance will increase parabolically with decreasing plant weight, and a repartitioning of growth from elongation to thickening is a very efficient strategy for increasing lodging resistance, because fresh weight W is kept constant, while the reduction of the shoot length by a given factor will contribute with the second power of this factor.

Lodging is of enormous importance for agriculture and accounts for yield losses up to 10–50% in wheat (Laude and Pauli 1956; Weibel and Pendleton 1961), up to 60% in barley (Schott and Lang 1977; Knittel et al. 1983) and 20–40% in rice (Basak 1962; Kwon and Yim 1986; Nishiyama 1986). The increase of lodging resistance therefore has been a traditional target for agricultural technology over several decades, especially in Graminean crops. This includes genetic approaches, where dwarfing genes are introduced into high-yield cultivars (Borner et al. 1996; Makela et al. 1996; Mcleod and Payne 1996), as well as the application of growth regulators such as chlormequat chloride or ethephone (Schott and Lang 1977; Schreiner and Reed 1908; Tolbert 1960).

The success of these strategies is limited by the specific environment generated by modern agriculture, such as high nutrient influx and high canopy densities. These conditions stimulate internode elongation and thus increase

the susceptibility of the crops to lodging and windbreak (Luib and Schott 1990). Most crop plants are typical sun plants, i.e. they exhibit a pronounced shade-avoidance response when grown in dense canopies (Smith 1981). They are able to sense their neighbours through subtle changes in the ratio between red and far-red light utilizing the photoreversible plant photoreceptor phytochrome. They respond to this change in red/far-red ratio by enhanced stem and petiole elongation. The shade-avoidance response is supposed to protect these plants against overgrowth by neighbouring plants. Indeed, this has been confirmed in field trials, where photoreceptor mutants of *Arabidopsis thaliana* that were not able to trigger shade avoidance were monitored under field conditions and found to be less competitive as compared to the respective wild type (Ballaré and Scopel 1997). As useful as this response may be for the survival of a weed like thale cress in a canopy, it is undesired for a crop plant. In the dense canopy of a wheat field, for example, shade avoidance will increase the risk of lodging. In fact, field trials with tobacco plants that overexpress phytochrome and are thus incapable of sensing the reflected light from their neighbours demonstrated that the suppression of shade avoidance allows for increased yield (Robson et al. 1996).

A classical example of thigmomorphogenesis is the barrier response of young seedlings. Upon contact with a mechanical barrier, the major axis of growth tilts from elongation towards stem thickening. This barrier response is triggered by the ethylene that is constantly released from growing stems and accumulates in front of physical obstacles (Nee et al. 1978). The increase in diameter improves the mechanical properties of the seedling, for instance the flexural rigidity, and thus allows the seedling to remove the barrier.

These examples may suffice to illustrate the impact of cell axis on growth, architecture and eventually on the performance of the plant under challenge by the environment. There are basically two mechanisms that define and contribute to the axis of a plant cell: first, the basic geometry of a cell is defined by the axis of cell division; and second, the manifestation of this geometry depends on the axis of subsequent cell expansion. The next two sections will therefore survey the mechanisms that control the axiality of division and expansion.

2
Control of Cell Division

The spatial control of cell division employs specialized populations of microtubules that are unique to plant cells: cortical microtubules, preprophase band (PPB) and phragmoplast (Fig. 3). The cortical microtubules prevailing in interphase cells are usually arranged in parallel bundles perpendicular to the main axis of cell expansion (Fig. 3a). They are involved in the directional control of cellulose deposition and thus in the axiality of cell growth and will

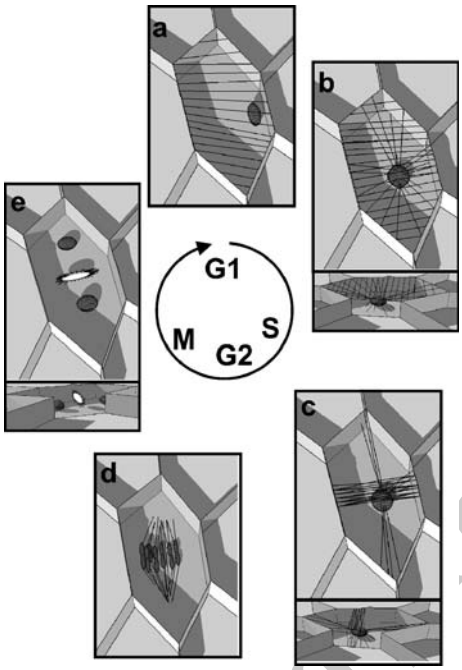


Fig. 3 Microtubular arrays during the cell cycle of higher plants. **a** Elongating interphase cell with corticale microtubules. The nucleus is situated in the periphery of the cell. **b** Cell preparing for mitosis seen from above and from the side. The nucleus has moved towards the cell centre and is tethered by radial microtubules emanating from the nuclear envelope. **c** Preprophase band of microtubules. **d** Mitosis and division spindle. **e** Cell in telophase with phragmoplast that organizes the new cell plate extending in centrifugal direction

be discussed in more detail in Sect. 3. When a plant cell prepares for mitosis, this is heralded by a migration of the nucleus to the site, where the prospective cell plate will form. The nucleus is surrounded by a specialized array of actin microfilaments, the phragmosome (for review see Lloyd 1991; Sano et al. 2005). This phragmosome is, in fact, responsible for the correct positioning of the nucleus (Katsuta and Shibaoka 1988). At the same time, the cortical microtubules are progressively replaced by a new structure, the radial or endoplasmic microtubules that emanate from the nuclear envelope and often merge with the cortical cytoskeleton (Fig. 3b).

Concomitantly with the eclipse of cortical microtubules a band of microtubules emerges at the cell equator. This preprophase band (Fig. 3c) is laid down in parallel to the direction of cortical microtubules and is connected with the nucleus by the radial microtubules and by the phragmosome. The preprophase band (PPB) marks the site and orientation of the prospective cell plate. However, it disappears with the formation of the division spindle that is usually organized in an axis perpendicular to the PPB, whereby the

spindle equator is situated in the plane heralded by the PPB (Fig. 3d). Once the daughter chromosomes have separated, a new array of microtubules, the phragmoplast, emerges at the site of the ensuing cell plate (Fig. 3e). The phragmoplast targets vesicle transport to the periphery of the expanding cell plate. Microtubules seem to pull at tubular-vesicular protrusions emanating from the endoplasmatic reticulum (Samuels et al. 1995). The phragmoplast consists of a double ring of interdigitating microtubules that grows in diameter with progressive extension of the cell plate. New microtubules are organized along the outer edge of the expanding phragmoplast (Vantard et al. 1990).

These observations assign to nuclear migration a central role in the control of division symmetry. Nuclear migration can be blocked by actin inhibitors such as cytochalasin B (Katsuta and Shibaoka 1988), suggesting that the phragmosome forming the characteristic "Maltesian cross" seen in premitotic vacuolated plant cells is, in fact, moving and tethering the nucleus and thus ultimately defines the site where the new cell plate is formed. However, microtubules also seem to be involved in nuclear positioning, since antimicrotubular compounds such as colchicine (Thomas et al. 1977) or pronamide (Katsuta and Shibaoka 1988) have been found to loosen the nucleus such that it can be displaced by mild centrifugation.

At the end of the S-phase, formation of the PPB begins (Gunning and Sammut 1990), which faithfully predicts the symmetry and axis of the ensuing cell division. This is impressively illustrated by asymmetric divisions, for instance during the formation of guard cells (Wick 1991) or in the response of root tissue to wounding (Hush et al. 1990). It has been under debate whether the PPB is more than just a true indicator for the spatial organization of mitosis.

In classical studies, Murata and Wada analysed the functions of the nucleus and PPB in the formation of the ensuing cell plate by means of centrifugation at different time points prior to mitosis (Murata and Wada 1991). As experimental system, they used protonemata of the fern *Adiantum* and elegantly exploited the advantages of these cells. Since they are very long, it is possible to displace the nucleus over a considerable distance leading to clear outcomes. To avoid migration of the displaced nucleus back to its original position (due to the tethering cytoskeletal network), they first induced a phototropic bending and subsequently centrifuged the nucleus into the curved part of the protonemata such that it was prevented from shifting back to the apex. Upon centrifugation prior to the formation of the PPB, the nucleus induced a new PPB in the new (basal) position, where later the new cell plate was formed, suggesting that it is the nucleus that defines the position of the PPB. When the nucleus was displaced from the apex somewhat later (when a PPB had already been laid down), the nucleus induced a second, somewhat smaller, PPB in its new position in the base of the cell. If the centrifugation occurred even later, the nucleus had already lost the ability to induce a second PPB, leading to a situation where an isolated PPB was observed near the apex, whereas the nucleus was found void of a PPB in the cell base. This situ-

ation allowed logical discrimination of the functions of nucleus and PPB in the orientation of cell division. In those cells, the new cell plate was established at the site of the nucleus (i.e. in the cell base) and not at the site of the PPB (i.e. in the cell apex) demonstrating unequivocally that it is the nucleus and not the PPB that determines the position of the ensuing cell plate. However, the cell plate in those cells was laid down randomly with respect to its orientation. Thus, the PPB is responsible for the correct orientation of the ensuing cell plate.

This guiding function of the PPB is supported by evidence from *Arabidopsis* mutants, where the PPB has been reported to be absent. In these so-called *tonneau* or *fass* mutants, the ordered pattern of cell divisions that characterizes the development of the wild type is replaced by a completely randomized pattern of cross walls (Traas et al. 1995; McClinton and Sung 1997). It should be mentioned, however, that, during meiosis, the division plane can be controlled in the absence of a PPB (Brown and Lemmon 1991), suggesting that there exist additional mechanisms of spatial control.

The organization of the PPB is accompanied by a phosphorylation of proteins. Some of these phosphorylated proteins reside in the nucleus (Young et al. 1994), whereas the cell-cycle-dependent protein kinase $p34^{cdc2}$ localizes to the PPB (Colasanti et al. 1993). The formation of the radial array of endoplasmic microtubules can be triggered in interphase cells by cycloheximide, a blocker of protein synthesis (Mineyuki et al. 1994). This suggests that the radial array represents a kind of default state, whereas the cortical microtubules have to be actively maintained by the synthesis of proteins with a relatively short lifetime. Interestingly, the formation of the PPB was not inhibited by cycloheximide, indicating that it is independent from these rapidly cycling proteins.

An intriguing question has been how the PPB can guide the formation of the phragmoplast, since it disappears completely at the time when the nuclear envelope breaks down. Recently, this mystery was at least partially unveiled by in-vivo microscopy. In a beautiful study, Dhonukshe et al. (2005) followed the behaviour of individual microtubules during mitosis of tobacco BY-2 cells. Using the plus-end marker EB1, they observed that the radial microtubules that emanate from the premitotic nucleus are indeed oriented with their plus-ends pointing outwards. They observed further that during the formation of the PPB, a belt composed of endosomes is laid down adjacent to the PPB probably produced by joint action of microtubule- and actin-driven transport. This belt persists during mitosis. Upon completed separation of the chromosome, a new set of microtubules emerges from the spindle poles and "explores" the cell periphery in different directions. Hereby the lifetime of microtubules that hit the endosomal belt is enhanced over that of microtubules that fail to interact with the endosomes and are therefore prone to undergo catastrophic decay. As a consequence, microtubules will be enriched at the site where the PPB was located prior to mitosis. In principle, the individual

"exploratory" microtubules are bound to compete for a limited pool of soluble dimers and thus are linked by mutual inhibition. This system is nothing other than a realization of a reaction-diffusion system, in the Turing sense (1952), combining self-amplification with lateral inhibition. Such systems are capable of self-organization and will rapidly produce a clear output pattern even in a situation of variable and noisy inputs.

Thus, the persistent "trace" that is laid down by nucleus, radial, endoplasmic microtubules and the PPB seems to be an endosomal belt. This "trace" is "read" by "exploratory" microtubules after mitosis, employing their self-organizing properties. As a consequence, the phragmoplast will be formed at the site heralded by the PPB. Thus, the PPB represents the earliest manifestation of the division axis known so far. The spindle is always established strictly in a direction perpendicular to the PPB. However, in small cells (e.g. precursors of the guard cells), it can become secondarily tilted or distorted to oblique orientations as a consequence of limited space (Mineyuki et al. 1988). This does not result in the formation of an oblique phragmoplast or an oblique cell plate, though, indicating that the formation of the spindle must be seen as a bypass of the morphogenetic processes that link nuclear migration, the formation of the PPB and the induction of the phragmoplast.

The PPB decides over the division plane. For the symmetry of division, however, it is nuclear migration and the nuclear envelope that are the decisive factors. They define where the radial microtubule network and the PPB is organized, they define the position of the spindle, and they mark the site where phragmoplast and cell plate will develop. The decisive questions remain to be solved – how is the nuclear movement directed towards the prospective plane of division? How is the nuclear surface differentiated into an equatorial region that can organize a PPB and two polar domains that seem to lack this ability?

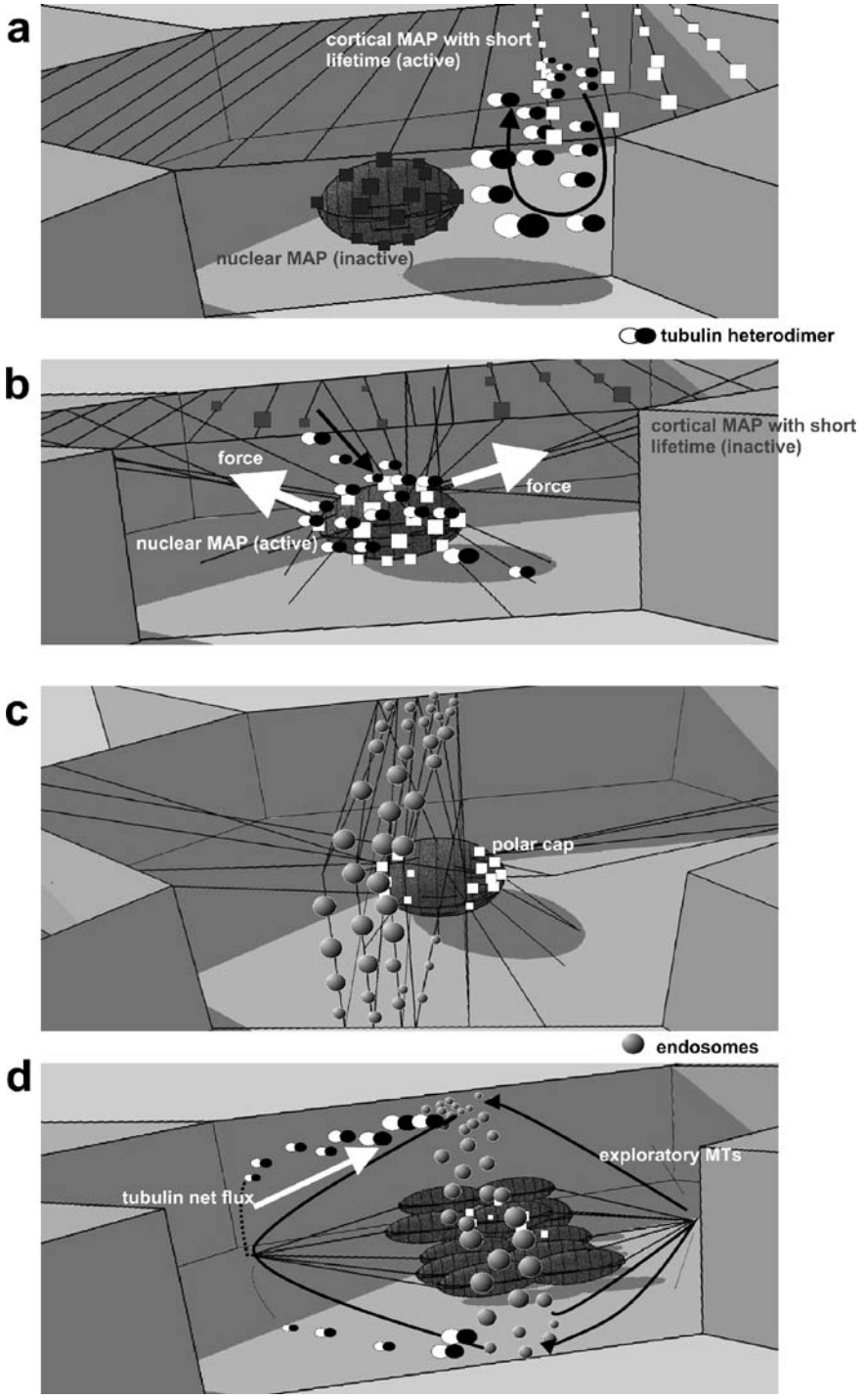
The function of the nucleus as the ultimate organizer of division symmetry is supported by its ability to nucleate microtubules. Whereas spindle microtubules are nucleated from centrosomes in animal and algal cells (Wiese and Zheng 1999), they emerge from rather diffuse microtubule-organizing centres (MTOCs) in the acentriolar cells of higher plants (Baskin and Cande 1990; Shimamura et al. 2004). However, the major MTOC of higher plants seems to be the nuclear envelope (for review see Lambert 1993). In addition, the kinetochores of both animal and plant cells are endowed with a microtubule-nucleating activity (Cande 1990). The nucleating activity of plant MTOCs is mirrored by their molecular composition. For instance, γ-tubulin, a minus-end nucleator of microtubule assembly, is found in centrosomes as well as in MTOCs (Pereira and Schiebel 1997; Stoppin-Mellet et al. 2000), and is also enriched in the nuclear envelope (Liu et al. 1994). The same holds true for CCT, a chaperone that specifically folds nascent tubulin (Himmelspach et al. 1997; Nick et al. 2000). Even during the G_2 phase, i.e. prior to the disintegration of the nuclear envelope, γ-tubulin is imported into the nucleus (Binarová et al. 2000). Interestingly, the breakdown of the nuclear envelope coincides with the

formation of the spindle, suggesting that microtubule-nucleating components of the nuclear envelope might be used to organize spindle microtubules (for review see Nick 1998). In fact, RanGAP1, an accessory protein of the small GTPase Ran involved in nuclear transport, not only localizes to the nuclear envelope, but also decorates spindle microtubules (Pay et al. 2002). The same protein can co-assemble with tubulin into microtubules, but only if the interaction takes place in extracts from cycling (not from non-cycling) cells. The specific role of the nuclear envelope is possibly linked with the presence of proteins or protein domains that are specific for plants. For instance, the plant homologues of RanGAP1 share an N-terminal extension that is absent from their animal counterparts. Conversely, the nuclear-rim protein MAF1 (present at the site where the microtubules of the preprophase band are nucleated) is not found in animals at all (Patel et al. 2004).

Although many of the molecular components organizing cell division in time and space are unknown, it is possible to build first models on the sequence of events (Fig. 4):

1. The cortical array of microtubules is actively maintained in interphase cells by proteins that have to be synthesized continuously (Mineyuki et al. 1994). If the activity of these proteins decreases, this will result in a rapid deterioration of the cortical array. The efficiency of this transition will depend on the lifetimes of individual microtubules. These have been assessed either by microinjection of fluorescent tubulin (e.g. Yuan et al. 1994; Himmelspach et al. 1999) or by expression of GFP-fusions of plant tubulins (e.g. Shaw et al. 2003) and found to be in the range of 30–60 s. Under these conditions, cortical arrays are expected to deteriorate within minutes if their active maintenance becomes arrested.
2. The nuclear envelope contains proteins that are able to nucleate new microtubules (Liu et al. 1994; Stoppin et al. 1994; Himmelspach et al. 1997), and it seems that this nucleating function is actively suppressed during

Fig. 4 Possible mechanisms for the control of division axis and symmetry. During interphase, tubulin dimers are partitioned into cortical microtubule arrays (**a**) due to the activity of a rapidly cycling cortical MAP, whereas the nucleation activity of the nuclear envelope is low. In premitotic cells, the nucleus is moved to a central position (**b**), and the MAPs at the nuclear envelope are activated or unmasked. The activity and/or synthesis of the cortical MAP is reduced such that a net flux of tubulin towards radial microtubules occurs that interacts with the force-generating system (probably actomyosin) that drives and tethers the nucleus. The formation of the preprophase band is accompanied by an endosomal belt in the cell equator (**c**). The microtubule-organizing activity of the nuclear envelope is spatially organized into different domains such as the polar caps. From late anaphase, the endosomal belt is "read" by exploratory microtubules (**d**) that emanate from the spindle poles and differ in lifetime depending on their contact with the endosomal belt, resulting in a net flux from incorrectly oriented microtubules towards those microtubules that are correctly oriented ►


a
cortical MAP with short lifetime (active)
nuclear MAP (inactive)
tubulin heterodimer
b
force
force
cortical MAP with short lifetime (inactive)
nuclear MAP (active)
c
polar cap
endosomes
d
exploratory MTs
tubulin net flux

interphase. In the simplest case, the inhibition of microtubule nucleation at the nuclear envelope might be the direct consequence of elevated nucleation activity in the cortical plasma if both sites compete for a limited number of free tubulin dimers (Fig. 4a).

3. At the onset of G_2, this suppression is released (possibly by weakening the active maintenance of nucleation in the cortical cytoplasm leading to an increase of tubulin dimers available for nucleation elsewhere). New microtubules will form spontaneously at the nuclear envelope with their growing ends pointing outwards (Fig. 4b; Dhonukshe et al. 2005).
4. These microtubules, probably in joint action with the microfilaments of the phragmosome, organize the PPB along with a belt of endosomal vesicles in the symmetry plane of the prospective division (Dhonukshe et al. 2005). The detection of cell-cycle regulators such as $p34^{cdc2}$ in the PPB (Colasanti et al. 1993) suggests that these events involve the activity of associated proteins that are under cell-cycle control. An important aspect that is often ignored is the partitioning of the nuclear envelope into different domains (Fig. 4c). Confocal sectioning of the nucleus in *Arabidopsis* cells that express GFP-tagged RanGAP1 reveals that the nuclear surface is not labelled uniformly, but in large patches (Pay et al. 2002). It might be possible that similar types of partitioning could define different regions that differ in their nucleating activity and thus contribute to a regionalization of the nuclear envelope, contributing to the definition of a division plane.
5. The spindle seems to be established independently of the PPB and represents a bypass to the causal chain between radial, endoplasmic microtubules, endosomal belt, PPB and phragmoplast. This is evident from situations where the spindle is secondarily tilted or distorted with respect to the orientation of the PPB due to space limitations (e.g. during the formation of guard cells), but nevertheless the cell plate is deposited correctly, parallel to the PPB (Mineyuki et al. 1988). Moreover, when, in wheat roots, the dissolution of the PPB was blocked by treatment with taxol, an inhibitor of microtubule disassembly, a spindle was formed although the PPB persisted (Panteris et al. 1995). This spindle, although being multipolar and aberrant, demonstrated clearly that it can be formed independently of the PPB.
6. Following the separation of chromosomes, highly dynamic microtubules emanate from the spindle poles in various directions (Fig. 4d). Those that touch the endosomal belt deposited prior to mitosis are stabilized such that a net redistribution towards this belt is achieved (Dhonukshe et al. 2005). This self-organization requires a high dynamics of microtubules, because misoriented microtubules have to disassemble in order to reach this net redistribution. Consequently, taxol should block the formation of a phragmoplast such that microtubules will be trapped in the spindle. This has indeed been shown for tobacco BY-2 cells (Yasuhara et al. 1993).

7. The phragmoplast will then organize the cell plate by means of motor proteins that are able to bind and transport vesicles containing cell wall material. Phragmoplasts could be purified from synchronized tobacco BY-2 cells and yielded a microtubule-associated protein that binds microtubules dependent on ATP (Yasuhara et al. 1992). A dynamin-like protein, termed phragmoplastin, binds to the newly formed cell plate and is supposed to recruit exocytotic vesicles to the growing cell plate (Gu and Verma 1995). Additional candidates for microtubule-bound cargo have been identified from genetic screens, for instance the KNOLLE protein, a syntaxin that decorates the phragmoplast.

The control of cell axis during cell division is a central element of plant morphogenesis. During the past few years our understanding of this process has advanced quite a bit, although many molecular components still remain to be identified. However, the underlying mechanisms are beginning to emerge. It has become clear that the mother cell does not transmit cell axis in form of a fixed structure. It rather transmits surprisingly vague spatial cues that will guide the self-assembly of microtubular arrays on the background of a high level of stochastic noise. The "exploratory" microtubules, for instance, which emanate from the spindle poles and eventually establish the phragmoplast, grow initially in various directions. Their final orientation is brought about by mutual competition of these highly dynamic microtubules for free tubulin heterodimers. Those microtubules that *by chance* hit the endosomal belt laid down prior to mitosis are stabilized over other microtubules that are misoriented. In a recent conceptual review, the classical view of the cell as a complex type of clockwork was confronted with the findings from live-imaging. This leads to a more dynamic and flexible view of the cell (Kurakin 2005) and the conclusion that cells are not organized in a "Watchmaker" fashion, but mainly by self organization. The way that the cell axis emerges during the division of plant cells provides an excellent illustration of this view. The ultimate tool for this self-organization is the nonlinear nature of microtubules, which can switch rapidly between growth and catastrophe and mutually compete for free dimers.

3
Control of Cell Expansion

Organisms grow by increasing the number of cells (division growth) or the volume of individual cells (expansion growth). In plants, division growth is confined to specific tissues or developmental states, e.g. to embryogenesis or the apical meristems (Steeves and Sussex 1989). During most of their life-cycle, plants grow predominantly by cell expansion. In some organs, such as hypocotyls (Lockhart 1960) or coleoptiles (Furuya et al. 1969; Nick et al. 1994), the growth response is even carried by cell expansion alone.

Plant cells expand by increasing the volume of the vacuole, which accounts for more than 90% of total cell volume in most differentiated cells. The driving force for this volume increment is a gradient of water potential from the apoplast towards the cytoplasm and vacuole, where the potentials are more negative (Kutschera et al. 1987). The expansion of the vacuole would eventually result in infinite swelling and a burst of the cell were it not limited by rigid cell walls. The importance of the cell wall for the integrity of plant cells can be impressively demonstrated when protoplasts are placed in a hypotonic medium (Fig. 5a).

Most plant cells derive from isodiametric meristematic cells, but assume approximately cylindrical shapes during differentiation, especially pronounced in expanding tissues such as hypocotyls, internode, petioles or coleoptiles. This cylindrical shape is usually lost upon removal of the cell wall;

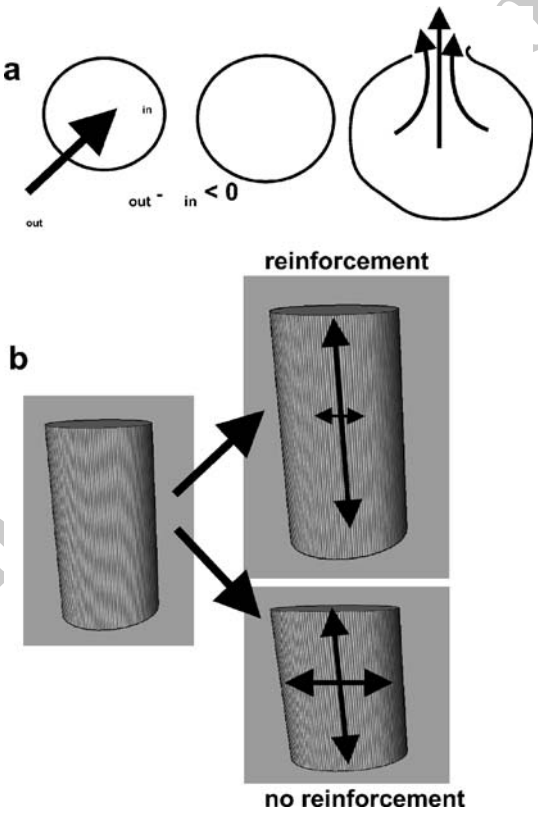


Fig. 5 Role of the cell wall for the axis of cell expansion. **a** Swelling and burst of protoplasts in the absence of a cell wall due to a gradient in water potential between the environment and cell interior. **b** Corroboration of cell axiality (*upper cell*) when expansion is not actively maintained anisotropic by a reinforcement mechanism (*lower cell*)

protoplasts, with very few exceptions, are spherical. This simple fact already illustrates 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 progressively corroborate the axiality of these cells (Fig. 5b). This means, on the other hand, that cylindrical cells must be endowed with some kind of reinforcement mechanism to maintain their original axiality during expansion (Green 1980). This reinforcement mechanism seems to reside in the cell wall and was first described for the long internodal cells of the green 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, much earlier, the birefringency of the cell wall had been discovered by polarization microscopy in growing tissue and interpreted in terms of an anisotropic arrangement of cellulose (Ziegenspeck 1948). However, the functional significance of this observation had not been recognized at that time. It is evident that the transverse arrangement of microfibrils can account for the reinforcement mechanism that maintains longitudinal expansion in cylindrical cells (Fig. 5c). The tight correlation between transverse microfibrils and cell elongation has been confirmed in numerous studies and has been discussed in several reviews (Robinson and Quader 1982; Kristen 1985; Giddings and Staehelin 1991; Smith 2005). As expected, reorientations in the axis of growth are accompanied either by a loss or by a switch in the anisotropy of cellulose deposition (Green and Lang 1981; Hardham et al. 1981; Lang et al. 1982; Hush et al. 1990).

In intact organs, the control of growth axiality is not necessarily maintained actively by each cell individually, but is sometimes confined to specific tissues. These tissues, the epidermis in most cases, are responsible for growth control of the entire organ. This can be demonstrated by a very simple experiment in which stem sections are split and subsequently allowed to grow in water. They will then curl inside out because the inner tissues expand faster than the epidermis. If growth-promoting agents such as auxins are added, the sections begin to curl outside inwards, because now it is the epidermis that exceeds the inner tissues in growth. This curling response is so sensitive that it had been used as a classical biotest for auxin (Schlenker 1937). Biophysical measurements confirmed later that, in fact, auxin stimulates the elongation of maize coleoptiles by increasing the extensibility of the epidermis such that its constraint upon the elongation of the compressed inner tissues is released (Kutschera et al. 1987).

As outlined above, the preferential axis of cell expansion is linked with a preferential orientation of cellulose microfibrils. The cell wall in those cells is formed by apposition of cellulose to the inner surface of the cell wall. Specialized cells such as root hairs or pollen tubes, in contrast, grow by intussusception of cell wall material into the cell poles and follow different mechanisms, which have been reviewed elsewhere (Taylor and Hepler

1997; Geitmann and Emons 2000) and will not be considered here. Cellulose is synthesized by specialized enzyme complexes that, in freeze-fracture preparations, appear as rosettes of six subunits of about 25–30 nm diameter surrounding a central pore (e.g. Kimura et al. 1999). These so-called terminal rosettes are integrated into the membrane of exocytic vesicles and, upon fusion of the vesicle, are then inserted into the plasma membrane. UDP-glucose is transported towards the central pore and polymerized in a β-1,4 configuration. Each subunit has been inferred to produce around six cellulose chains that will be integrated by hydrogen bonds yielding a long and fairly stiff cellulose microfibril. These enzyme complexes are thought to move within the fluid membrane and leave a "trace" of crystallizing cellulose behind them. This movement will thus decide the orientation of cellulose microfibrils and thus the anisotropy of the cell wall. It is at this point that the microtubules come into the play.

Even before they were actually discovered microscopically by Ledbetter and Porter (1963), cortical microtubules were predicted to exist and to guide cellulose deposition (Green 1962). During subsequent years, an intimate link between cortical microtubules and the preferential axis of growth has been proposed by a number of studies:

1. Cortical microtubules are closely associated with the plasma membrane, and upon plasmolysis a direct contact between cortical microtubules and newly formed cellulose microfibrils could be demonstrated by electron microscopy (for review see Giddings and Staehelin 1991; Smith 2005).
2. Parallel bundles of thick microtubules mark the prospective sites of cell wall thickening in differentiating cells (Fukuda and Kobayashi 1989; Jung and Wernicke 1990).
3. Changes in the preferential axis of cell expansion are accompanied by a switch in the preferential axis of cellulose deposition, and are preceded by a corresponding reorientation of cortical microtubules (ethylene response, Lang et al. 1982; auxin response, Bergfeld et al. 1988; gibberellin response, Toyomasu et al. 1994; wood formation, Abe et al. 1995; for review see Nick 1998).
4. When cortical microtubules are eliminated by antimicrotubular compounds, this results in a progressive loss of ordered cellulose texture and the axiality of cell expansion, leading, in extreme cases, to lateral swelling and bulbous growth. This effect was first discovered in the green alga *Nitella* (Green 1962), but was later observed in higher plants as well (Hogetsu and Shibaoka 1978; Robinson and Quader 1981; Kataoka 1982; Bergfeld et al. 1988; Vaughan and Vaughn 1988; Nick et al. 1994; Baskin and Bivens 1995; Hasenstein et al. 1999). This phenomenon is even of importance for application, since the mode of action of some herbicides, such as the phenyl carbamates or the dinitroanilines, is based on the elimination of cortical microtubules and the subsequent inhibition of

elongation growth. Especially impressive are the effects of colchicine on differentiating xylem elements, where the characteristic cell wall thickenings do not form at all in presence of the drug (Pickett-Heaps 1967; Robert and Baba 1968; Barlow 1969; Hepler and Fosket 1971; Hardham and Gunning 1980).

The striking parallelity between cortical microtubules and newly deposited cellulose microfibrils has stimulated the proposal of two alternative models:

The original model postulated that cortical microtubules adjacent to the plasma membrane guide the movement of the cellulose-synthesizing enzyme complexes and thus generate a pattern of microfibrils that parallels the orientation of microtubules (Heath 1974). The differences in length between microtubules and microfibrils would be explained by an overlap of individual microtubules that are organized in bundles. The driving force for the movement of cellulose synthases in this "monorail" model would be active transport through microtubule motors (Fig. 6a).

Alternatively, the interaction between microtubules and cellulose-synthases could be more indirect, whereby the microtubules act as "guard rails" that induce small folds of the plasma membrane that confine the movement of the enzyme complexes (Herth 1980; Giddings and Staehelin 1991). The driving force for the movement would result from the crystallization of cellulose. The solidifying microfibril would thus push the enzyme complex through the fluid plasma membrane and the role of microtubules would be limited to delineate the direction of this movement (Fig. 6b).

The practical discrimination between these two models is not trivial because experimental evidence was mostly based on electron microscopical

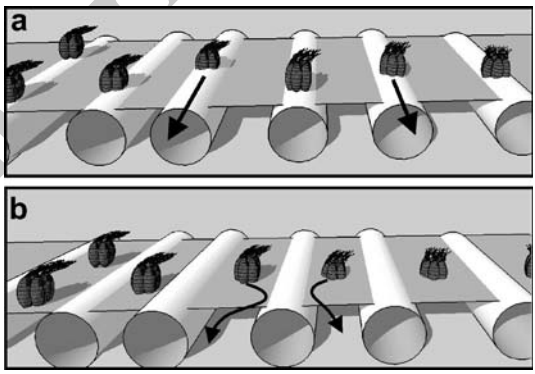


Fig. 6 Models on the guidance of cellulose synthesis by cortical microtubules. **a** Monorail model, where the cellulose-synthesizing complexes are moved along microtubules driven by a microtubule-dependent motor. **b** Guardrail model, where the cellulose-synthesizing complexes are moved by the force from the crystallizing cellulose, but are confined to the troughs between individual microtubules

observation and thus was prone to fixation artifacts, and great luck was required to locate the right section. For instance, the newly synthesized cellulose microfibrils formed after a treatment with taxol were found to be directly adjacent to individual microtubules in tobacco BY-2 cells (Hasezawa and Nozaki 1999), favouring the monorail model. On the other hand, the cellulose synthase complexes were observed "in gap" between adjacent microtubules in the alga *Closterium* (Giddings and Staehelin 1988), which was difficult to reconcile with a monorail mechanism.

The situation is further complicated by situations where the orientation of microtubules and cellulose microfibrils differ (for instance Emons and Mulder 1998; Himmelspach et al. 2003; for review see Baskin 2001; Wasteneys 2004). Some of these inconsistencies may depend on the choice of the system – for instance, the root hair of *Equisetum hyemale* with its helicoidal wall texture deviating from the orientation of cortical microtubules (Emons et al. 1992) is a cell endowed with tip growth and differs from a tissue cell that is expanding in a diffuse manner and is subject to considerable tissue tensions. In addition, the orientation of cellulose microfibrils is shifted and distorted when the wall lamella gradually shift from the plasma membrane to the periphery of the apoplast during the apposition of the subsequent lamellae. The contribution of these older lamellae to the reinforcement of growth vanishes progressively. It had been estimated for *Nitella* that only the innermost fifth of the wall is responsible for the majority of reinforcement (Green and King 1966). It is not trivial to determine the cellulose texture of the innermost lamellae of a cell wall (Robinson and Quader 1982; Kristen 1985). Moreover, the orientation of microtubules as well as the orientation of cellulose can change rhythmically (Zandomeni and Schopfer 1993; Mayumi et al. 1996; Hejnowicz 2005) leading to transitional situations where the microtubules have already assumed a new orientation and the time elapsed since this transition has not been sufficient to deposit a significant number of microfibrils in the new direction.

Despite these caveats in the interpretation of apparent differences between microtubule and microfibril orientation, they have led to a debate on the role of microtubules in the guidance of cellulose synthesis. This debate stimulated a key experiment exploiting the potential of live-cell imaging in *Arabidopsis thaliana* (Paredez et al. 2006). A component of the terminal rosette, the cellulose synthase subunit A6 (CESA6), was expressed as fusion with the yellow fluorescent protein under the native promotor in the background of a *cesa6* null mutant, such that overexpression artifacts could be excluded. The resulting punctate signal was observed to be localized adjacent to the plasma membrane and to move along parallel pathways that resembled cortical microtubules. By crossing this line into a background, where one of the α-tubulins was expressed as fusion with a blue fluorescent protein, it became possible to follow this movement under simultaneous visualization of CESA6 and microtubules. This dual-image approach demonstrated very

clearly that CESA6 was moving along individual microtubule bundles. Moreover, in a very recent publication, a central problem of the monorail model, i.e. the existence of polylamellate walls with layers of differing microfibril orientation, could be plausibly explained by a rotary movement of groups of microtubules (Chan et al. 2007).

The original monorail model postulated a microtubule motor that pulls the cellulose synthase complex along the microtubules. If this motor were defective, a situation would result where microtubules were arranged in the usual transverse arrays, whereas cellulose microfibrils were deposited deviantly. A screen for reduced mechanical resistance in *Arabidopsis thaliana* yielded a series of so-called *fragile fiber* mutants (Burk et al. 2001; Burk and Ye 2002) that were shown to be completely normal in terms of cell wall thickness or cell wall composition, but were affected in wall texture. One of these mutants, *fragile fiber 2*, allelic to the mutant *botero* (Bichet et al. 2001), was affected in the microtubule-severing protein katanin, leading to swollen cells and increased lateral expansion. A second mutant, *fragile fiber 1*, was mutated in a kinesin-related protein belonging to the KIF4 family of microtubule motors. As expected, the array of cortical microtubules was completely normal; however, the helicoidal arrangement of cellulose microfibrils was messed up in these mutants. This suggests that this KIF4 motor is involved in the guidance of cellulose synthesis and might be a component of the monorail complex.

Thus, the original monorail model for the microtubule guidance of the terminal rosettes (Heath 1974) experienced a rehabilitation after more than three decades of dispute. However, the microtubule–microfibril model is still far from complete. In addition to the discordant orientations of microtubules and microfibrils discussed above, there are cell wall textures that are difficult to reconcile with a simple monorail model. For instance, cellulose microfibrils are often observed to be intertwined (for instance Preston 1981). This has stimulated views that claim that microtubules are more or less dispensable for the correct texture of microfibrils. The self-organization of cellulose synthesis would be sufficient to perpetuate the pattern because the geometrical constraints from microfibrils that are already laid down would act as templates for the synthesis of new microfibrils (Emons and Mulder 1998; for review see Mulder et al. 2004). This view ignores the fact that microtubules and microfibrils *are* parallel in most cases, at least if cells in a tissue context are analysed. It also ignores the disruption of microtubules either by inhibitors (see above) or by mutations that impair the formation of ordered microtubule arrays, causing a progressive loss of ordered cell wall texture and a loss of growth axiality (Burk et al. 2001; Bichet et al. 2001 for katanin; Whittington et al. 2001 for *mor1*).

However, the focus on the self-organizing properties of cellulose synthesis forces the original microtubule–microfibril model to be extended by a feedback control of microfibrils upon cortical microtubules. A mounting body of evidence shows that the cell wall acts to stabilize cortical microtubules. For instance, removal of the cell wall results in enhanced cold sensitivity of cortical

microtubules in tobacco cells (Akashi et al. 1990). When, in the same cells, the incorporation of UDP-glucose into the cell wall was blocked by the herbicide isoxaben (Fisher and Cyr 1998), this impaired the axiality of cell expansion resulting in isodiametric cells and disordered cortical arrays of microtubules. This suggests that the mechanical strains exerted by the cellulose microfibrils during axial expansion provide directional cues for the alignment of microtubules. The fact that microtubules are able to sense mechanical stimuli will be discussed in detail in Sect. 6.

At this point it should be pointed out that this mechanosensory function will close a feedback loop between cell wall and cytoskeleton. Since expansion is reinforced in a direction perpendicular to the orientation of microtubules and microfibrils, biophysical forces will be generated parallel to the major strain axis. These forces are then relayed back through the plasma membrane upon cortical microtubules that are aligned with relation to these strains. In other words, microtubules and microfibrils constitute a self-reinforcing regulatory circuit. Since individual microtubules mutually compete for a limited supply with tubulin-heterodimers, and since the number of microfibrils is limited by the quantity of cellulose synthase rosettes, this regulatory circuit should be capable of self-organization and patterning.

In fact, microtubule–microfibril patterns that transcend the borders of individual cells have been reported in early work on plant microtubules in apical meristems (Hardham et al. 1980). Here, the formation of new primordia is suppressed by the older primordia. The tissue tension present in an expanding meristem would yield considerable mechanical stresses resulting from buckling from the older primordia. In fact, models of stress–strain patterns could perfectly predict the position of incipient primordia (for review see Green 1980). One of the earliest events of primordial initiation is a reorientation of cortical microtubules that are perpendicular with respect to the microtubules of their non-committed neighbours. This difference is sharp, but later it is smoothed by a transitional zone of cells with oblique microtubules, such that eventually a gradual, progressive change in microtubular reorientation emerges over several rows of cells. A similar supracellular gradient of microtubule orientation was reported upon wounding of pea roots (Hush et al. 1990), heralding corresponding changes of cell axis and cell divisions that align such that the wound is efficiently closed. A curious case of microtubule patterning was discovered in the *Arabidopsis* mutants *spiral*, *lefty* and *tortifolia* (Furutani et al. 2000; Thitamadee et al. 2002; Buschmann et al. 2004). In these mutants, microtubules are obliquely aligned over many cells in the distal elongation zone of the root (*spiral* and *lefty*) or the petiole (*tortifolia*), accompanied by twisted growth. In contrast, in the temperature-sensitive mutant *radially swollen 6* (Bannigan et al. 2006) microtubule arrays of individual cells are ordered and parallel, but arrays between neighbouring cells deviate strongly, suggesting that this mutant is affected in the supracellular patterning of microtubule arrays.

The twisted growth phenotype of these mutants is conventionally explained on the base of uniformly oblique arrays of microtubules (and consequently microfibrils). In the *spiral*, *lefty* and *tortifolia* mutants it is the microtubular cytoskeleton that is affected by these mutations. Moreover, spiral growth can be phenocopied in the wild type by inhibitors of microtubule assembly (Furutani et al. 2000). As pointed out above, the microtubule–microfibril circuit is endowed with self-amplification linked to mutual inhibition. A typical systemic property of such a self-organizing morphogenetic system is an oscillating output (Gierer 1981). Any factor that alters the lifetime of microtubules will alter the relay times within this feedback circuit. Since neighbouring cells are mechanically coupled by tissue tension, even a weak coupling will result in a partial synchronization of the individual circuits (Campanoni et al. 2003). The degree of synchrony will depend on the velocity of the feedback circuit. Thus, mutations in an associated protein such as the *tortifolia* gene product (Buschmann et al. 2004), mutations in tubulin itself, as in case of *lefty* (Thitamadee et al. 2002), or treatment with microtubule inhibitors (for review see Hashimoto and Kato 2006) are expected to enhance synchrony leading to the observed oscillations of growth. Interestingly, the mutant *root swollen 6*, where microtubule arrays of individual cells are completely uncoupled, is reported to be endowed with increased resistance to microtubule inhibitors suggesting that microtubule lifetimes are increased in this mutant (Bannigan et al. 2006).

The spatial control of cell expansion is a central element of the developmental flexibility crucial for survival in organisms with a sessile lifestyle. The past few years have seen a surprising rehabilitation of the classical ideas on the mechanisms driving this control. However, the original straightforward model of microtubules as guiding tracks for cellulose synthesis has been extended by elaborate feedback controls from the microfibrils upon microtubules. This means that the self-organizing properties of microtubules are combined with the self-organizing properties of cellulose synthesis, constituting a patterning system that is composed of oscillators (the microtubule–microfibril circuits of individual cells) that are coupled through mechanical strains. Thus, in analogy to the spatial control of cell division, the nonlinear properties of microtubules are utilized to generate and maintain a flexible, but nevertheless defined, axis of cell expansion.

4 Signal-Triggered Reorientation of Microtubules

The previous two sections have described microtubules as central players in the definition of cell division and cell expansion. Both phenomena have to be flexibly tuned with the environment. This means that plant microtubules must be able to reorganize in response to signals.

In fact, this has been observed in numerous cases (for review see Nick 1998). A classical example is the ethylene response of growth: When angiosperm seedlings encounter mechanical obstacles, they display a characteristic barrier response that involves a shift of the growth axis from elongation towards stem thickening. The trigger for this response is ethylene (Nee et al. 1978), which is constantly released by the elongating shoot and accumulates in front of physical obstacles. It is, by the way, this ethylene-induced block of internode elongation accompanied by a thickening of the stem by which the growth regulator ethephone increases lodging resistance (Andersen 1979).

Using this ethylene-triggered switch of the growth axis, Lang et al. (1982) succeeded in demonstrating that environmental signals probably control growth through the microtubule–microfibril pathway. Electron microscopy in pea epicotyls showed that the cortical microtubules reorient from their original transverse orientation into steeply oblique or even longitudinal arrays. This reorientation is followed by a shift of cellulose deposition from transverse to longitudinal, and a thickening of the stem.

During subsequent years, similar correlations between growth, microfibril deposition and cortical microtubules could be shown for other hormones as well. In coleoptile segments of maize, where elongation is under the control of auxin and limited by the epidermal extensibility (Kutschera et al. 1987), microtubules and microfibrils were oriented longitudinally when the segments had been depleted of endogenous auxin (Bergfeld et al. 1988). However, they became transverse when exogenous auxin was added. In parallel, elongation growth was restored. Interestingly, this response is confined to the outer epidermal cell wall, and it is exactly this cell wall where auxin has been shown to stimulate growth by increasing the extensibility of cell walls.

With the adaptation of immunofluorescence to plant cells (Lloyd et al. 1980) it became possible to follow the dynamics of reorientation and to investigate the factors that trigger a reorientation of microtubules. These studies identified various plant hormones such as auxin (Bergfeld et al. 1988; Nick et al. 1990, 1992; Nick and Schäfer 1994), gibberellins (Mita and Katsumi 1986; Nick and Furuya 1993; Sakiyama-Sogo and Shibaoka 1993; Shibaoka 1993; Toyomasu et al. 1994) and abscisic acid (Sakiyama-Sogo and Shibaoka 1993) as triggers of microtubule reorientation, but also physical factors such as blue light (Nick et al. 1990; Laskowski 1990; Zandomeni and Schopfer 1993), red light (Nick et al. 1990; Nick and Furuya 1993; Zandomeni and Schopfer 1993; Toyomasu et al. 1994), gravity (Nick et al. 1990; Godbolé et al. 2000; Blancaflor and Hasenstein 1993; Himmelspach et al. 1999; Himmelspach and Nick 2001), high pressure (Cleary and Hardham 1993), mechanical stress (Zandomeni and Schopfer 1994), wounding (Hush et al. 1990) or electrical fields (Hush and Overall 1991).

However, only in a few cases has the dynamics of microtubule reorientation been analysed in direct comparison with signal-induced changes of growth. In maize coleoptiles, microtubules were observed to reorient rapidly from

transverse to longitudinal upon phototropic stimulation (Nick et al. 1990). This reorientation was confined to the lighted flank of the coleoptile and clearly preceded the onset of phototropic curvature. The time-course for the auxin-dependent reorientation in the same organ supported a model (Fig. 7)

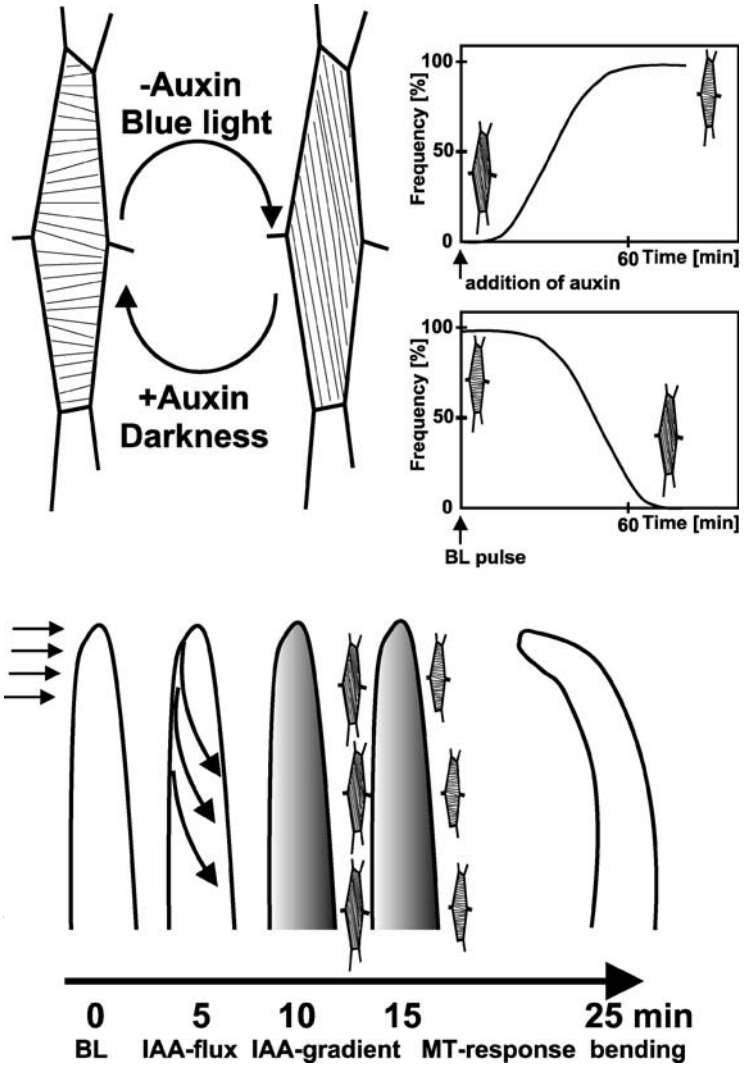


Fig. 7 Behaviour of cortical microtubules during phototropic curvature of maize coleoptiles (Nick et al. 1990). Microtubules reorient from transverse to longitudinal in response to auxin depletion or in response to phototropic stimulation. The reorientation induced by phototropic stimulation is confined to the lighted flank of the coleoptile and initiates subsequent to the auxin displacement across the coleoptile, but prior to the onset of the phototropic curvature

where photo- or gravitropic stimulation induced a shift of auxin transport from the lighted towards the shaded flank of the coleoptile. The depletion of auxin in the lighted flank subsequently stimulated a reorientation of cortical microtubules into longitudinal arrays (Nick et al. 1990), and, in parallel, a longitudinal deposition of cellulose microfibrils (Bergfeld et al. 1988). Conversely, microtubules, as well as cellulose microfibrils, remain transverse in the auxin-enriched shaded flank. The gradient of microfibril orientation would then result in a decreased longitudinal extensibility of epidermal cell walls in the lighted flank, and, as a consequence, a decrease in asymmetric growth leading to phototropic curvature towards the light stimulus.

A more detailed investigation of the phenomenon revealed, however, a more complex reality (Nick et al. 1992; Nick and Schäfer 1994; Nick and Furuya 1996). It is possible, by rotating the seedlings on a clinostat in the absence of tropistic stimulation, to generate a so-called nastic bending. This nastic response is not preceded or accompanied by a reorientation of microtubules and thus occurs without a corresponding gradient of orientation across the coleoptile cross-section (Nick et al. 1991). On the other hand, the gradient of microtubule orientation established in response to a light pulse persists, whereas the curvature vanishes due to gravitropic straightening (Nick et al. 1991). In parallel to phototropic curvature, a phototropic stimulus can induce a stable transverse polarization of the coleoptile that persists over several days. This polarity can mediate stable changes in growth rate (Nick and Schäfer 1988, 1991, 1994) and can even control morphogenetic events such as the emergence of crown-roots manifest several days after the inducing stimulus had been administered (Nick 1997). These stable changes in growth are closely related to a stabilization of microtubule orientation (Nick and Schäfer 1994) because 2 h after the inducing light stimulus, cortical microtubules had lost their ability to reorient in response to a counter-directed light pulse. At the same time, the transverse polarity manifest as stable change in growth becomes persistent. Interestingly, the microtubules lose their ability to respond to auxin as well, indicating that it is not sensory adaptation of phototropic perception that is responsible for the block of the reorientation response (Nick and Schäfer 1994). The stabilization of microtubule orientation 2 h after an inducing light pulse requires blue light, and this light effect cannot be mimicked by a mere depletion of auxin nor by gradients of auxin depletion.

These studies suggest that the microtubule–microfibril pathway is responsible for persistent changes of growth. They also suggest, however, that a second pathway can control fast growth responses independently. In most cases, both pathways seem to act in concert; it required detailed time-course studies to detect discrepancies between growth and microtubule reorientation. In this context it should be mentioned that in some cases the microtubule response has been found to be somewhat slower than the signal response of growth, for instance in the blue light-induced inhibition of growth in pea stems (Laskowski 1990) or root gravitropism in maize (Blancaflor and

Hasenstein 1993). Here, a microtubule-independent mechanism seems to be at work. The microtubule–microfibril pathway is designed for persistent changes of growth, since it requires a certain time until enough cellulose microfibrils are deposited in a new direction (Lang et al. 1982) before a corresponding change of growth can occur. When the two growth patterns have been analysed in parallel (e.g. Nick and Schäfer 1994), they were observed to act in parallel and to play complementary roles. However, it seems to be the microtubule–microfibril pathway that is crucial for the morphogenetic flexibility essential for plant survival. Thus, to understand developmental flexibility and its link to signal transduction, it is necessary to understand, how cortical microtubules reorient.

5
How Do Microtubules Change Direction?

Before the mechanism of microtubule reorientation could be seriously investigated it was necessary to visualize the plant cytoskeleton in its three-dimensional organization. Thus, our understanding of microtubules was shaped by the methodology that was available. Originally, microtubule orientation could only be inferred from the shape of the cross-sections in stacks of ultrathin sections viewed by electron microscopy, which was very cumbersome and at the edge of the impossible. The first breakthrough was therefore the combination of fluorescence microscopy with immunolabelling, which allowed for the first time observation of the microtubular cytoskeleton as an entity (Lloyd et al. 1980). When this approach was later complemented by confocal microscopy, it became possible to view microtubules in different layers of an intact tissue. However, for immunofluorescence, microtubules have to be fixed by aldehydes to preserve their structure during the preparation process. This means that the dynamics of microtubules could not be observed by this approach, and the term "cytoskeleton" evoking a more or less rigid structure was inspired by the structural appearance of fixed microtubules seen in electron micrographs and later immunofluorescence images. It was a big surprise when microtubules could be visualized in living cells, first by microinjection of fluorescent tubulin (Yuan et al. 1994), and later by the use of GFP-tagged markers such as the microtubule-binding domain of MAP4 (Marc et al. 1998) or tubulins themselves (Kumagai et al. 2001). Our understanding of microtubule reorientation represents a classical example for the interdependence of biological concepts and experimental approach.

When microtubule arrays could be visualized for the first time as an entity, it was discovered that, in elongating cells, they are arranged in helicoidal arrays along the cell periphery. This stimulated the first model for microtubule reorientation (Lloyd and Seagull 1985). This very elegant and beautiful model perceived cortical microtubules as a mechanically coupled entity that cor-

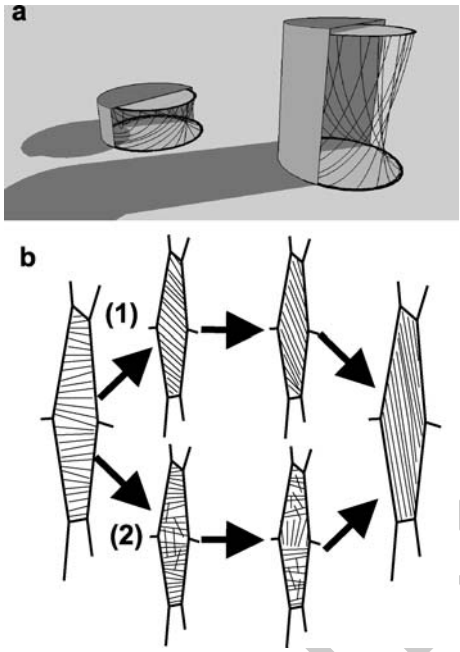


Fig. 8 Potential mechanisms for the reorientation of cortical microtubules. **a** Dynamic spring model: microtubules are organized into a mechanically coupled helicoidal array. By mutual sliding of microtubules the helix can change from a relaxed state with almost transverse pitch (*left*) to a tightened state with almost longitudinal microtubules (*right*). **b** Directional reassembly model: the equilibrium between assembly and disassembly of a given microtubule depends on its orientation with respect to the cell axis. A switch in the direction of preferential stability will result in a net reorientation of microtubules. Whereas the final result is the same as for the dynamic spring model, the transitional states are different. In the dynamic spring model (1), the transition would consist of homogenously oblique microtubules. In the directional reassembly model (2), transverse and longitudinal microtubules coexist during a transitional phase. These are coaligned to patches that subsequently move and reorient as coupled entities until a homogenous new array is established

responds to a dynamic spring. By releasing or increasing the tension in this spring (caused by mutual sliding of the constituting microtubules), the pitch of this helix would change between transverse and longitudinal (Fig. 8a). According to this model, the molecular mechanism of reorientation is expected to involve microtubule motors.

However, it became evident during subsequent years that the dynamic-spring model failed to describe microtubule reorientation:

1. In epidermal tissues, the reorientation of cortical microtubules is confined to the microtubules adjacent to the outer cell wall, leading to a situation where microtubules were transverse at the inner wall, but longitudinal at

the outer wall (Bergfeld et al. 1988; Nick et al. 1990; for review Wymer and Lloyd 1996). This difference in orientation within a single cell was difficult to reconcile with the concept of a mechanically coupled spring.

2. The transitions between transverse and longitudinal arrays of microtubules should involve situations where microtubules are homogenously oblique and then gradually change pitch until the longitudinal array is established. Although oblique microtubules can be observed, they seem to occur as a final rather than as a transitional situation (Gunning and Hardham 1982; Hush et al. 1990). In contrast, early phases of reorientation in response to strong stimuli, or incomplete reorientation in response to a suboptimal stimulation, tend to look different (Nick et al. 1990, 1992). Here, a patchwork of transverse and microtubules is observed, where transverse and longitudinal microtubules can coexist even within the very same cell (Fig. 8b).
3. Taxol inhibits microtubule disassembly and was found to suppress microtubule reorientation (Falconer and Seagull 1985; Nick et al. 1997), indicating that microtubule disassembly is required for reorientation, contrasting with the dynamic-spring model. Taxol did not inhibit, however, the coalignment of initially disordered microtubules into the parallel arrays that are observed in regenerating protoplasts (Wymer et al. 1996) suggesting that a disassembly-independent mechanism contributes to the organization of cortical microtubules.
4. Cortical microtubules were initially thought to be relatively inert lattices. However, when microtubules were visualized in living plant cells by microinjecting fluorescent tubulin, the lifetime of individual microtubules was found to be extremely short (Yuan et al. 1994; Wymer and Lloyd 1996, Himmelspach et al. 1999). The injected tubulin was incorporated extremely rapidly into the preexisting cortical network. Upon bleaching the fluorescence by a laser beam, the fluorescence of the bleached spot recovered within a few minutes, indicating an extremely high turnover of tubulin dimers. This dynamics of tubulin assembly and disassembly contrasts with the concept of a mechanically coupled microtubular helix.
5. By using lines of *Arabidopsis thaliana* expressing a fusion of an α-tubulin with GFP it became possible to analyse and quantify the dynamic behaviour of individual microtubules in living epidermal cells (Shaw et al. 2003). Microtubules were found to move through the cortex by a treadmilling mechanism. Interestingly, both ends of the microtubule contributed to a net motility in the direction of the plus-end. When parts of these microtubules were bleached in fluorescence-recovery after photobleaching (FRAP) experiments, the bleached region did not move, suggesting that translocation of assembled microtubules did not occur. Thus, it was the assembly and disassembly of microtubules that was responsible for the net movement of microtubules. This conclusion is supported by experiments where the be-

haviour of the plus-end marker EB1 was followed by means of a GFP-tag. Here, a conspicuous, bidirectional movement of the plus-ends was observed in interphase arrays of microtubules (Dhonukshe et al. 2003; Chan et al. 2003). In contrast to the situation in animal cells, where catastrophic disassembly is fast but relatively rare, treadmilling in *Arabidopsis thaliana* was found to be quite common but moderate, possibly through tight regulation of the minus-ends.

6. When microtubule reorientation was followed in vivo upon microinjection of fluorescent neurotubulin (Yuan et al. 1994; Wymer and Lloyd 1996, Himmelspach et al. 1999), the reorientation was observed to proceed in two distinct stages. The first stage local phase transitions from transverse to longitudinal arrays lead to individual discordant microtubules that herald the prospective orientation of the array. These discordant "exploratory" microtubules subsequently become more frequent, leading to a patchy situation where longitudinal and transverse microtubules coexist in the very same cell. Only during a second stage are microtubules coaligned into a new parallel array whose direction is defined by the original, "exploratory" microtubules. This coalignment is characterized by a distinct group behaviour of cortical microtubules, as demonstrated quite recently (Chan et al. 2007): By using spinning-disc microscopy in seedlings that expressed fluorescently tagged versions of tubulin or the tubulin plus-end marker EB1, it was possible to follow microtubule reorientation over longer timescales at high temporal resolution. This approach uncovered patches of microtubules that act in concert and are of equal polarity. These domains move around the cell until they collide with other patches. New microtubules are preferentially generated along tracks where other microtubules had been before.

These observations led to a revision of the original dynamic-spring model (for review see Lloyd 1994). The actual *reorientation* involves direction-dependent changes of microtubule lifetime. For instance, in an array that undergoes reorientation from a transverse into a longitudinal orientation, the "exploratory" longitudinal microtubules will acquire increased stability, whereas the transverse microtubules will be more labile. This reorientation in sensu strictu will then be followed by a phase of *coalignment* where the reoriented, but still disordered, microtubules are coupled into patches of identical polarity. These patches subsequently move around the cell and progressively align into a new parallel array. From the mechanistic point of view, the first phase would require changes in the activity of structural microtubule-associated proteins (MAPs) that control the lifetime of a given microtubule, whereas the second phase would be driven by microtubule motors.

A central prerequisite for the first phase (*reorientation*) would be differences in the lifetime of microtubules that are dependent on some kind of vector, i.e. orientation with respect to the cell axis. The direction of this

unknown vector would then change in response to the signal that triggers microtubular reorientation.

Stable microtubules have been observed in both animals and plants to consist of tubulin that is post-translationally modified (for review, see MacRae 1997). All α-tubulins, with the exception of one species (the slime mould *Physarum polycephalum*; Watts et al. 1987), carry a carboxy terminal tyrosine, which can be post-translationally cleaved off by a tubulin tyrosine carboxypeptidase. The carboxy terminal tyrosine can be restored by a tubulin tyrosine ligase. The biological role of this detyrosination process is not really understood but, in mammalian cells, microtubules consisting of detyrosinylated tubulin are less dynamic (Kreis 1987). The initial model assumed that the detyrosinylated tubulin was the cause of the increased stability. However, it turned out later that the tubulin tyrosine carboxypeptidase, responsible for this modification, preferentially binds to tubulin that is assembled in microtubules, whereas it shows less affinity for dimeric tubulin. Conversely, the tubulin tyrosine ligase acts predominantly on dimeric tubulin (Kumar and Flavin 1981). This would favour an alternative scenario where tubulin tyrosination would primarily depend on microtubule dynamics (Khawaja et al. 1988). In fact, the dynamics of microtubules assembled in vitro from tyrosinylated or detyrosinylated tubulin is indistinguishable (Skoufias and Wilson 1998). Detyrosination has been described for plant tubulin as well and can be triggered by signals that control growth (Duckett and Lloyd, 1994). Although it is not known whether detyrosination is cause or consequence of microtubule stability, it can be used as a marker for microtubules with increased lifetime. There exist a couple of well-characterized monoclonal antibodies that detect tyrosinylated tubulin (Kilmartin et al. 1982; Kreis 1987). Using such antibodies it should be possible to test whether signal-triggered microtubule re-orientation really involves direction-dependent differences of microtubule lifetime. Using maize coleoptiles as model, it could be shown that detyrosination can be controlled via auxin (Wiesler et al. 2002). In fact, the longitudinal microtubules produced in response to auxin depletion predominantly contained the detyrosinylated form of α-tubulin, indicating direction-dependent differences in microtubule stability.

The stability of microtubules is generally believed to depend on the activity of structural MAPs that decrease the frequency of microtubule catastrophe (Bin-Bing and Kirschner 1999; Caudron et al. 2000). In vitro, the assembly of microtubules is promoted by warm temperatures, by GTP, and by magnesium. In vivo, the nucleation of new microtubules occurs on the surface of specialized organelles, the centrosomes. Higher plants are unique in this respect because they do not possess centrosomes, and an entire chapter of the present volume (see Chapter "Microtubules and the Evolution of Mitosis" of this book) is dedicated to this topic. Higher plants organize microtubules on functional analogues, so-called microtubule-organizing centres (MTOCs). The major MTOC in dividing cells seems to be the nuclear sur-

face (Lambert 1993). In fact, it is possible to induce microtubule asters by addition of purified nuclei (Stoppin et al. 1994). In differentiated interphase cells, the activity of the nuclear envelope is masked by cortical MTOCs that become manifest during the recovery of microtubules from disassembly induced by drugs, low temperature or high pressure (Marc and Palevitz 1990; Cleary and Hardham 1993). These MTOCs contain microtubule-nucleating factors such as γ-tubulin (Liu et al. 1994; Pastuglia et al. 2006) or elements of the tubulin-chaperoning complex CCT (Himmelspach et al. 1997; Nick et al. 2000). A couple of so-called structural plant MAPs have been identified in the meantime that can regulate different aspects of microtubule dynamics such as nucleation, severing or bundling (for review see Lloyd et al. 2004).

However, the central problem of reorientation remains to be solved: how the stability of the discordant microtubules can be regulated differently from that of the bulk microtubules that are still oriented in the original orientation. It seems that there must be interactions with a vectorial field or lattice that are regulated. Membrane-bound MAPs that are regulated by signal-transduction chains might be the key players in this context. For instance, certain isoforms of phospholipase D have been isolated as microtubule-binding proteins (Marc et al. 1996) and participate in the interaction of cortical microtubules with the plasma membrane (Gardiner et al. 2001).

It is obvious that the direction-dependent stability of a given microtubule cannot be intrinsic to the microtubule itself, but is produced by the interaction of this microtubule with a directional lattice or vectorial field. This lattice or field could be a different component of the cytoskeleton, such as actin filaments (the interaction of microtubules and actin filaments is explored in the chapter "Crossed-wires: Interactions and cross-talk between the microtubule and microfilament networks in plants" of this book). It could be a physical field, such as mechanical strain or bound dipoles, or it could be an apoplastic lattice, such as the cellulose microfibrils that have been found to feed back on microtubules (see above). The MTOCs could be transported and organized along such a lattice (Fig. 9a). This would cause a dynamic shift in the nucleation activity. It would not explain, however, why microtubules are more stable when they grow into a certain, the "right", direction as compared to their fellow microtubules that grow into the "wrong" direction. It could be the cross-linking with proteins that stabilizes microtubules (e.g. through bundling or capping). If the distance between such microtubule stabilizers were dependent upon direction, it would provide a mechanism to favour certain microtubule orientations. A simple possibility for such a mechanism is depicted in Fig. 9b, where the hypothetical microtubule stabilizers are aligned on a longitudinal lattice that can change between a disperse and a bundled configuration. Experiments in which the orientation of microtubules changed after pharmacological manipulation of actin (Kobayashi et al. 1988; Seagull 1990; Nick et al. 1997) suggest that this lattice is related to actin microfilaments. In the disperse configuration of this lattice, the average minimal

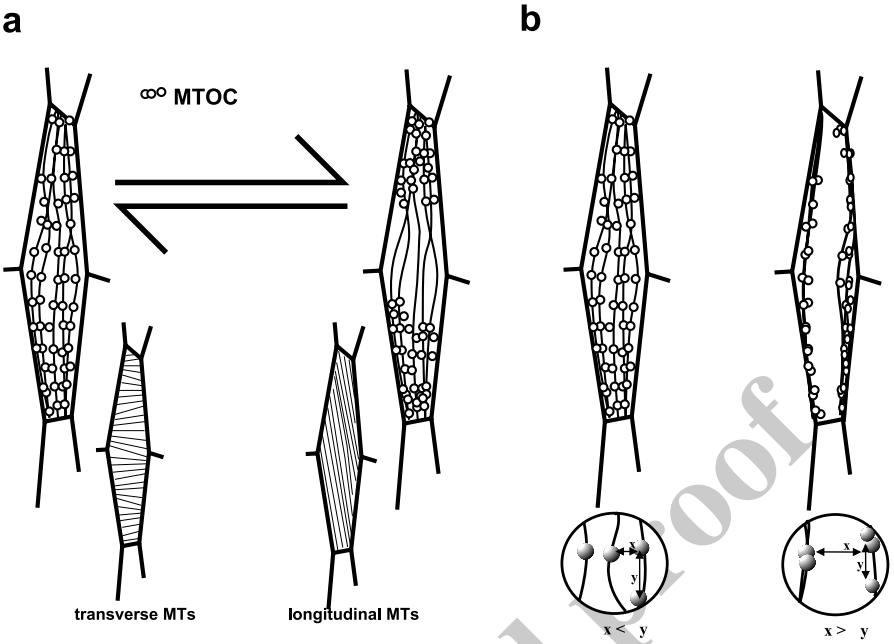


Fig. 9 Model for the directional reassembly of microtubules. **a** Microtubule-organizing centres moving along a directional matrix/lattice resulting in a redistribution of microtubule-nucleating sites. **b** Microtubule-organizing centres are tethered to a directional matrix/lattice. Upon bundling of this lattice, longitudinal microtubules will be favoured over transverse microtubules due to a lower minimal distance (Δy) in the longitudinal direction as compared to the minimal distance in the transverse direction (Δx)

distance between the microtubule stabilizers would be smaller in the transverse direction, favouring a transverse orientation of microtubules. In the bundled configuration, the average distance in the transverse direction would increase such that a longitudinal microtubule would become more stable than its transverse counterpart.

6 Mechanisms for the Control of Cell Axis

Plants can basically use two mechanisms of adjusting their axis in congruence with their environment. To respond rapidly, they can change the axis of cell expansion. To obtain more persistent changes, for instance in a situation where the environmental change is not transient, they can alter the axis of cell division. Microtubules are central players in both adaptive processes and, in addition, seem to be targets of numerous signalling cascades that control their organization and dynamics. In a nutshell, their function is twofold: they

act as devices that collect and process some signals, and they act as devices that transform the outcome of this signal processing into persistent changes of shape. In fact, they represent a versatile tool for linking signalling with morphogenesis.

The versatility of this tool is illustrated by the ample opportunities to control cell expansion. The control of cell expansion by environmental stimuli such as light or gravity includes: stimulus perception; signal-transduction cascades that are often organized as complex networks; response of the (still unknown) directional matrix that defines direction-dependent microtubule assembly and disassembly leading to a net reorientation of cortical microtubules; coalignment of a new, parallel array and reorientation of cellulose deposition, resulting in a directional switch in the anisotropy of the cell wall; and, eventually, a switch in the axis of preferential cell expansion. Each of these steps is regulated and can be used to control cell axis (both by the plant itself and by biotechnological manipulation):

1. Manipulation of stimulus perception. The dense canopies characteristic of industrial agriculture lead to a pronounced shade-avoidance response that is triggered by the plant photoreceptor phytochrome (Smith 1981). When stem elongation is stimulated in consequence of shade avoidance, this will render the plant prone to lodging and thus will reduce yield (Oda et al. 1966). The agronomical impact of this phenomenon was demonstrated by experiments in which phytochrome was overexpressed, resulting in reduced shade avoidance. This approach allowed higher yields (Robson et al. 1996). However, the consequences of this strategy are not confined to cell expansion, but affect all responses that are dependent on phytochrome including the composition of photosystems, branching, tropistic responses, hormonal balance and induction of flowering. This pleiotropy will cause, depending on species and crop type, undesired side effects that are difficult to foretell. The same type of argumentation is valid not only for shade avoidance, but in principle for any other signalling pathway that affects cell elongation, for instance, by temperature, nutrient uptake or abiotic or biotic factors.
2. Manipulation of signalling cascades. The classic approach for the suppression of lodging through dwarfing genes (Peng et al. 1999) or growth regulators such as CCC or ethephone belong to this approach (Andersen 1979). Since most signal cascades are not linear, but split and interwoven into complex networks, again a high degree of pleiotropy is expected. The experience with undesired side effects of ethephone such as incomplete grain filling (Makela et al. 1996) or reduced root development (Luib and Schott 1990) illustrate the drawbacks of this approach. In plants, where coordination is not brought about by a neuronal network, but basically by chemical signalling, it will be difficult to define signalling events that are confined to one specific target. Thus, a strategy aimed at events down-

stream of the various branching points of signal transduction is expected to be more localized.

3. Manipulation of the directional matrix. This would represent the most specific target because it is expected to affect cortical microtubules in the first place and thus mainly the axis of cell expansion. The nature of this matrix is to be identified, but experiments in which actin drugs have been observed to affect the organization of microtubules (Kobayashi et al. 1988; Seagull 1990; Nick et al. 1997) suggest that the interaction between microfilaments and microtubules (as covered in detail in the chapter "Crossed-wires: Interactions and cross-talk between the microtubule and microfilament networks in plants" of this book) might be crucial in this context.
4. Manipulation of microtubule disassembly and reassembly. At present, this is the target easiest to access. Disassembly of microtubules can be blocked by taxol (Parness and Horwitz 1981) or it can be enhanced by a broad panel of compounds including the alkaloids colchicine, vinblastine and colcemid, or by herbicides such as dinitroanilines or phenylcarbamates (for review see Morejohn 1991; Vaughn 2000). Disassembly of microtubules can also be triggered through the calcium–calmodulin pathway (Fisher et al. 1998) or through low temperature (see the chapter "Microtubules as Sensors for Abiotic Stimuli" of this book). In the meantime, a few microtubule-associated proteins have been identified that participate in the regulation of microtubule dynamics. These include the microtubule-severing katanins, bundling proteins such as EF-1α (Durso and Cyr 1994), MAP65 or MOR1. It should be mentioned that microtubule stability can also be regulated through signals that might act through altering the activity of such microtubule-associated proteins (for reviews see Nick 1998, 1999). These include hormones such as abscisic acid (Sakiyama and Shibaoka 1990; Wang and Nick 2001), auxin (Wiesler et al. 2002), but also exogenous factors such as blue light (Nick and Schäfer 1994) or temperature (Abdrakhamanova et al. 2003). These signals and molecules could be used as tools to control cell axis, especially if they are targeted to the still-unknown lattice that decides over the orientation-dependent differences in microtubule turnover.
5. Manipulation of post-translational modification. Tubulins are subject to elaborate post-translational modifications (for review see Ludueña 1998) that correlate with enhanced stability of microtubules. Whereas this increased stability was originally thought to be the consequence of the modification, it is now thought to be the cause (for review see Bulinski and Gundersen 1991; Erck et al. 2000): The modifying enzymes seem to prefer microtubules as substrates rather than soluble tubulin heterodimers (Kumar and Flavin 1981). Thus, a microtubule with an increased lifetime will be modified to a larger degree than a rapidly cycling microtubule. These modifications include phosphorylation of β-tubulin, acety-

lation, polyglutamylation and detyrosination/retyrosination of α-tubulin. Recently, even a nitration of α-tubulin has been discovered (Cappelletti et al. 2003) in neural cells. From these modifications, acetylation (Vaughn and Renzaglia 2006) and detyrosination (Duckett and Lloyd 1994; Wiesler et al. 2002) have been shown to occur in plants as well. The responsible enzymes have to be identified. However, the enzyme responsible for the retyrosination of α-tubulin has been cloned from pig brain (Ersfeld et al. 1993), and homologues of this enzyme are present in plants (Nick et al., unpublished results). A homologue of the retyrosination enzyme has been recently shown to be responsible for polyglutamylation in *Tetrahymena* and mouse (Janke et al. 2005). The post-translational modifications are thought to act as signals that regulate the interaction of microtubules with organelles or microtubule motors (Gurland und Gundersen 1995; Kreitzer et al. 1999) and thus differentiate different populations of microtubules that differ in function. It could be shown for auxin-dependent reorientation of cortical microtubules that the discordant microtubules that herald the new orientation of the array are detyrosinated, whereas the microtubules that still maintain the original orientation are not (Wiesler et al. 2002). The increased detyrosination of the discordant microtubules might enhance their interaction with kinesins (Kreitzer et al. 1999) such that they are preferentially moved, leading in the coalignment of the new, longitudinal array. By modification of the retyrosination enzyme it might be possible to suppress or to enhance the coalignment of microtubules and thus to control cell axis in a specific manner.

6. Manipulation of cellulose synthesis. As pointed out above, cortical microtubules and cellulose microfibrils are linked through a self-referring feedback control. Pharmacological or molecular interference with cellulose synthesis could be used to generate even non-intuitive consequences. However, a constitutive manipulation would simply result in a thinning of cell walls and a reduced mechanical stability (Edelmann et al. 1989). Conversely, the cell wall could be irreversibly stiffened by induction of peroxidase-triggered lignification (Fry 1979; Liszkay et al. 2004). If the interaction of microtubules and cellulose microfibrils were altered in a switchable way, this might provide an elegant tool for achieving specific alterations of cell axis.

Since length control represents a central topic of agriculture, microtubule-based rationales designed to regulate the axis of growth are expected to be a meaningful biotechnological strategy. The basic idea is to utilize the morphogenetic responses that occur naturally in a plant and to target these responses either to cells, where they usually do not occur, or to modify them minimally to obtain changes in the response amplitude. In the ideal case, a manipulation of plant axis and shape could be produced without the help

of heterologous genes and promotors, if the endogenous genes and promotors are recombined in an intelligent way. Even relatively subtle changes in assembly dynamics, microtubule bundling or post-translational modification should produce conspicuous and specific effects on plant shape.

References

Abdrakhamanova A, Wang QY, Khokhlova L, Nick P (2003). Is microtubule assembly a trigger for cold acclimation? Plant Cell Physiol 44:676–686

Abe H, Funada R, Imaizumi H, Ohtani J, Fukuzawa K (1995) Dynamic changes in the arrangement of cortical microtubules in conifer tracheids during differentiation. Planta 197:418–421

Akashi T, Kawasaki S, Shibaoka H (1990) Stabilization of cortical microtubules by the cell wall in cultured tobacco cells. Effect of extensin on the cold stability of cortical microtubules. Planta 182:363–369

Andersen AS (1979) Plant growth retardants: present and future use in food production. NATO ASI Ser USA 22:251–277

Ballaré CL, Scopel AL (1997) Phytochrome signalling in plant canopies: testing its population-level implications with photoreceptor mutants of *Arabidopsis*. Funct Ecol 11:441–450

Bannigan A, Wiedemeier AMD, Williamson RE, Overall RL, Baskin TI (2006) Cortical microtubule arrays lose uniform alignment between cells and are oryzalin resistant in the *Arabidopsis* mutant, *radially swollen 6*. Plant Cell Physiol 47:949–958

Barlow PW (1969) Differences in response to colchicine by differentiating xylem cells in roots of *Pisum*. Protoplasma 68:79–83

Basak MN (1962) Nutrient uptake by the rice plant and its effect on yield. Agron J 54:373–376

Baskin TI (2001) On the alignment of cellulose microfibrils by cortical microtubules: a review and a model. Protoplasma 215:150–171

Baskin TI, Bivens NJ (1995) Stimulation of radial expansion in *Arabidopsis* roots by inhibitors of actomyosin and vesicle secretion but not by various inhibitors of metabolism. Planta 197:514–521

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

Bergfeld R, Speth V, Schopfer P (1988) Reorientation of microfibrils and microtubules at the outer epidermal wall of maize coleoptiles during auxin-mediated growth. Bot Acta 101:57–67

Bichet A, Desnos T, Turner S, Grandjean O, Höfte H (2001) BOTERO1 is required for normal orientation of cortical microtubules and anisotropic cell expansion in *Arabidopsis*. Plant J 25:137–148

Binarová P, Cenklová V, Hause B, Kubátová E, Lysák M, Doležel J, Bogre L, Dráber P (2000) Nuclear gamma-tubulin during acentriolar plant mitosis. Plant Cell 12:433–442

Bin-Bing ZH, Kirschner MW (1999) Quantitative measurement of the catastrophe rate of dynamic microtubules. Cell Motil Cytoskeleton 43:43–51

Blancaflor EB, Hasenstein KH (1993) Organization of cortical microtubules in graviresponding maize roots. Planta 191:230–237

Borner A, Plaschke J, Korzun V, Worland AJ (1996) The relationship between the dwarfing genes of wheat and rye. Euphytica 89:69–75

Brown RC, Lemmon BE (1991) The cytokinetic apparatus in meiosis: control of division plane in the absence of a preprophase band of microtubules. In: Lloyd CW (ed) The cytoskeletal basis of plant growth and form. Academic, London, pp 259–273

Bulinski JC, Gundersen GG (1991) Stablilization and post-translational modification of microtubules during cellular morphogenesis. BioEssays 13:285–293

Burk DH, Liu B, Zhong R, Morrison WH, Ye ZH (2001), A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. Plant Cell 13:807–827

Burk DH, Ye ZH (2002) Alteration of oriented deposition of cellulose microfibrils by mutation of a katanin-like microtubule severing protein. Plant Cell 14:2145–2160

Buschmann H, Fabri CO, Hauptmann M, Hutzler P, Laux T, Lloyd CW, Schäffner AR (2004) Helical growth of the Arabidopsis mutant *tortifolia1* reveals a plant-specific microtubule-associated protein. Curr Biol 14:1515–1521

Campanoni P, Blasius B, Nick P (2003) Auxin transport synchronizes the pattern of cell division in a tobacco cell line. Plant Physiol 133:1251–1260

Cande WZ (1990) Centrosomes: composition and reproduction. Curr Opin Cell Biol 2:301–305

Cappelletti G, Maggioni MG, Tedeschi G, Macia R (2003) Protein tyrosine nitration is triggered by nerve growth factor during neuronal differentiation of PC12 cells. Exp Cell Res 288:9–20

Caudron N, Valiron O, Usson Y, Valiron P, Job D (2000) A reassessment of the factors affecting microtubule assembly and disassembly in vitro. J Mol Biol 297:211–220

Chan J, Calder G, Fox S, Lloyd C (2007) Cortical microtubule arrays undergo rotary movements in *Arabidopsis* hypocotyl epidermal cells. Nat Cell Biol 9:171–175

Chan J, Calder GM, Doonan JH, Lloyd CW (2003) EB1 reveals mobile microtubule nucleation sites in *Arabidopsis*. Nat Cell Biol 5:967–971

Cleary AL, Hardham AR (1993) Pressure-induced reorientation of cortical microtubules in epidermal cells of *Lolium rigidum* leaves. Plant Cell Physiol 34:1003–1008

Colasanti J, Cho SO, Wick S, Sundaresan V (1993) Localization of the functional $p34^{cdc2}$ homolog of maize in root tip and stomatal complex cells: association with predicted division sites. Plant 1101–1111

Dhonukshe P, Mathur J, Hülskamp M, Gadella TWJ (2005) Microtubule plus-ends reveal essential links between intracellular polarization and localized modulation of endocytosis during division-plane establishment in plant cells. BMC Biol 3:11–26

Duckett CM, Lloyd CW (1994) Gibberellic acid-induced microtubule orientation in dwarf peas is accompanied by rapid modification of an α-tubulin isotype. Plant J 5:363–372

Durso NA, Cyr RJ (1994) A calmodulin-sensitive interaction between microtubules and a higher plant homolog of elongation factor 1α. Plant Cell 6:893–905

Edelmann H, Bergfeld R, Schopfer P (1989) Role of cell-wall biogenesis in the initiation of auxin mediated growth in coleoptiles of *Zea mays* L. Planta 179:486–494

Eiserich JP, Estévez AG, Bamberg TV, Ye YZ, Chumley PH, Beckman JS, Freeman BA (1999) Microtubule dysfunction by posttranslational nitrotyrosination of alpha-tubulin: A nitric oxide-dependent mechanism of cellular injury. Proc Natl Acad Sci USA 96:6365–6370

Emons AM, Derksen JHM, Sassen MMA (1992): Do microtubules orient plant cell wall microfibrils? Physiol Plantarum 84:486–493

Emons AM, Mulder BM (1998) The making of the architecture of the plant cell wall: How cells exploit geometry. Proc Natl Acad Sci USA 95:7215–7219

Erck C, Frank R, Wehland J (2000) Tubulin–tyrosine ligase, a long-lasting enigma. Neurochem Res 25:5–10

Ersfeld K, Wehland J, Plessmann U, Dodemont H, Gerke V, Weber K (1993) Characterization of the tubulin–tyrosine ligase. J Cell Biol 120:725–732
Falconer MM, Seagull RW (1985) Xylogenesis in tissue culture: taxol effects on microtubule reorientation and lateral association in differentiating cells. Protoplasma 128:157–166
Fisher DD, Cyr RJ (1998) Extending the microtubule/microfibril paradigm – cellulose synthesis is required for normal cortical microtubule alignment in elongating cells. Plant Physiol 116:1043–1051
Fisher DD, Gilroy S, Cyr RJ (1998) Evidence for opposing effects of calmodulin on cortical microtubules. Plant Physiol 112:1079–1087
Fry SC (1979) Phenolic components of the primary cell wall and their possible role in the hormonal regulation of growth in spinach. Planta 146:343–351
Fukuda H, Kobayashi H (1989) Dynamic organization of the cytoskeleton during tracheary-element differentiation. Dev Growth Differ 31:9–16
Furutani I, Watanabe Y, Prieto R, Masukawa M, Suzuki K, Naoi K, Thitamadee S, Shikanai T, Hashimoto T (2000) The SPIRAL genes are required for directional control of cell plates elongation in *Arabidopsis thaliana*. Development 127:4443–4453
Furuya M, Pjon CJ, Fujii T, Ito M (1969) Phytochrome action in *Oryza sativa* L. III. The separation of photoperceptive site and growing zone in coleoptiles, and auxin transport as effector system. Dev Growth Differ 11:62–75
Gardiner JC, Harper JDI, Weerakon ND, Collings DA, Ritchie S, Gilroy S, Cyr RJ, Marc J (2001) A 90-kD Phospholipase D from tobacco binds to microtubules and the plasma membrane. Plant Cell 13:2143–2158
Geitmann A, Emons AMC (2000) The cytoskeleton in plant and fungal cell tip growth. J Microsc 198:218–245
Giddings TH, Staehelin A (1991) Microtubule-mediated control of microfibril deposition. A re-examination of the hypothesis. In: Lloyd CW (ed) The cytoskeletal basis of plant growth and form. Academic, London, pp 85–99
Giddings TH, Staehelin LA (1988) Spatial relationship between microtubules and plasma-membrane rosettes during the deposition of primary wall microfibrils in *Closterium* spec. Planta 173:22–30
Gierer A (1981) Generation of biological patterns and form: some physical, mathematical, and logical aspects. Progr Biophys Mol Biol 37:1–47
Godbolé R, Michalke W, Nick P, Hertel R (2000) Cytoskeletal drugs and gravity-induced lateral auxin transport in rice coleoptiles. Plant Biol 2:176–181
Grace J (1977) Plant response to wind. Academic, London, pp 121–142
Green PB (1962) Mechanism for plant cellular morphogenesis. Science 138:1404–1405
Green PB (1980) Organogenesis – a biophysical view. Annu Rev Plant Physiol 31:51–82
Green PB, King A (1966) A mechanism for the origin of specifically oriented textures with special reference to *Nitella* wall texture. Aust J Biol Sci 19:421–437
Green PB, Lang JM (1981) Towards a biophysical tehory of organogenesis: birefringence observations on regenerating leaves in the succulent *Graptopetalum paraguayense*. Planta 151:413–426
Gu XJ, Verma DPS (1995) Phragmoplastin, a dynamin-like protein associated with cell plate formation in plants. EMBO J 15:695–704
Gunning BES, Hardham AR (1982) Microtubules. Annu Rev Plant Physiol 33:651–698
Gunning BES, Sammut M (1990) Rearrangement of microtubules involved in establishing cell division planes start immediately after DNA synthesis and are completed just before mitosis. Plant Cell 2:1273–1282

Gurland G, Gundersen GG (1995) Stable, Detyrosinated microtubules function to localize vimentin intermediate filaments in fibroblasts. J Cell Biol 131:1275–1290
Hardham AR, Green PB, Lang JM (1980) Reorganization of cortical microtubules and cellulose deposition during leaf formation of *Graptopetalum paraguayense*. Planta 149:181–195
Hardham AR, Gunning BES (1980) Some effects of colchicine on microtubules and cell division in roots of Azolla pinnata. Protoplasma 102:31–51
Hasenstein KH, Blancaflor EB, Lee JS (1999) The microtubule cytoskeleton does not integrate auxin transport and gravitropism in maize roots. Physiol Plant 105:729–738
Hasezawa S, Nozaki H (1999) Role of cortical microtubules in the orientation of cellulose microfibril deposition in higher-plant cells. Protoplasma 209:98–104
Hashimoto T, Kato T (2006) Cortical control of plant microtubules. Curr Opin Plant Biol 9:5–11
Heath IB (1974) A unified hypothesis for the role of membrane bound enzyme complexes and microtubules in plant cell wall synthesis. J Theor Biol 48:445–449
Hejnowicz Z (2005) Autonomous changes in the orientation of cortical microtubules underlying the helicoidal cell wall of the sunflower hypocotyl epidermis: spatial variation translated into temporal changes. Protoplasma 225:243–256
Hepler PK, Fosket DE (1971) The role of microtubules in vessel member differentiation in *Coleus*. Protoplasma 72:213–236
Herth W (1980) Calcofluor white and Congo red inhibit chitin microfibril assembly of *Poterioochromonas*: Evidence for a gap between polymerization and microfibril formation. J Cell Biol 87:442–450
Himmelspach R, Nick P (2001) Gravitropic microtubule reorientation can be uncoupled from growth. Planta 212:184–189
Himmelspach R, Nick P, Schäfer E, Ehmann B (1997) Developmental and light-dependent changes of the cytosolic chaperonin containing TCP-1 (CCT) subunits in maize seedlings, and the localization in coleoptiles. Plant J 12:1299–1310
Himmelspach R, Williamson RE, Wasteneys GO (2003) Cellulose micro.bril alignment recovers from DCB-induced disruption despite microtubule disorganization. Plant J 36:565–575
Himmelspach R, Wymer CL, Lloyd CW, Nick P (1999) Gravity-induced reorientation of cortical microtubules observed in vivo. Plant J 18:449–453
Himmelspach R, Wymer CL, Lloyd CW, Nick P (1999) Gravity-induced re-orientation of cortical microtubules observed in vivo. Plant J 18:449–453
Hogetsu T, Shibaoka H (1978) Effects of colchicine on cell shape and microfibril arrangement in the cell wall of *Closterium acerosum*. Planta 140:15–18
Hush JM, Hawes CR, Overall RL (1990) Interphase microtubule re-orientation predicts a new cell polarity in wounded pea roots. J Cell Sci 96:47–61
Hush JM, Overall RL (1991) Electrical and mechanical fields orient cortical microtubules in higher plant tissues. Cell Biol Int Rev 15:551–560
Janke C, Rogowski K, Wloga D, Regnard C, Kajava AV, Strub JM, Temurak N, van Dijk J, Boucher D, van Dorsselaer A, Suryavanshi S, Gaertig J, Eddé B (2005) Tubulin polyglutamylase enzymes are members of the TTL domain protein family. Science 308:1758–1762
McLeod JG, Payne JF (1996) AC rifle winter rye. Can J Plant Sci 76:143–144
Jung G, Wernicke W (1990) Cell shaping and microtubules in developing mesophyll of wheat (*Triticum aestivum* L.). Protoplasma 153:141–148
Kataoka H (1982) Colchicine-induced expansion of *Vaucheria* cell apex. Alteration from isotropic to transversally anisotropic growth. Bot Mag Tokyo 95:317–330

Katsuta J, Shibaoka H (1988) The roles of the cytoskeleton and the cell wall in nuclear positioning in tobacco BY-2 cells. Plant Cell Physiol 29:403–413

Khawaja S, Gundersen GG, Bulinski JC (1988) Enhanced stability of microtubules enriched in de-tyrosinylated tubulin is not a direct function of de-tyrosination level. J Cell Biol 106:141–149

Kilmartin JV, Wright B, Milstein C (1982) Rat monoclonal antitubulin antibodies derived by using a new non-secreting rat cell line. J Cell Biol 93:576–582

Kimura S, Laosinchai W, Itoh T, Cui X, Linder CR, Brown RM (1999) Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant *Vigna angularis*. Plant Cell 11:2075–2086

Knittel H, Lang H, Schott PE, Höppner P (1983) Verbesserung der Standfestigkeit – auch bei Gerste und Roggen. BASF Mitt Landbau 2:1–31

Kobayashi H, Fukuda H, Shibaoka H (1988) Interrelation between the spatial disposition of actin filaments and microtubules during the differentiation of tracheary elements in cultured *Zinnia* cells. Protoplasma 143:29–37

Kreis TE (1987) Microtubules containing de-tyrosinylated tubulin are less dynamic. EMBO J 6:2597–2606

Kreitzer G, Liao G, Gundersen GG (1999) Detyrosination of tubulin regulates the interaction of intermediate filaments with microtubules in vivo via a kinesin-dependent mechanism. Mol Biol Cell 10:1105–1118

Kristen U (1985) The cell wall. Progr Bot 47:1–8

Kumagai F, Yoneda A, Tomida T, Sano T, Nagata T, Hasezawa S (2001) Fate of nascent microtubules organized at the M/G_1 interface, as visualized by synchronized tobacco BY-2 cells stably expressing GFP-tubulin: time-sequence observations of the reorganization of cortical microtubules in living plant cells. Plant Cell Physiol 42:723–732

Kumar N, Flavin M (1981) Preferential action of a brain detyrosinolating carboxypeptidase on polymerized tubulin. J Biol Chem 256:7678–7686

Kurakin A (2005) Self-organization versus Watchmaker: stochastic dynamics of cellular organization. Biol Chem 386:247–254

Kutschera U, Bergfeld R, Schopfer P (1987) Cooperation of epidermal and internal tissues in auxin mediated growth of maize coleoptiles. Planta 170:168–180

Kwon YW, Yim KO (1986) Paclobutrazol in rice. FFTC Book Ser Taiwan 35:130–137

Lambert AM (1993) Microtubule-organizing centers in higher plants. Curr Opin Cell Biol 5:116–122

Lang JM, Eisinger WR, Green PB (1982) Effects of ethylene on the orientation of microtubules and cellulose microfibrils of pea epicotyls with polylamellate cell walls. Protoplasma 110:5–14

Laskowski MJ (1990) Microtubule orientation in pea stem cells: a change in orientation follows the initiation of growth rate decline. Planta 181:44–52

Laude HH, Pauli AW (1956) Influence of lodging on yield and other characters in winter wheat. Agron J 148:453–455

Ledbetter MC, Porter KR (1963) A microtubule in plant cell fine structure. J Cell Biol 12:239–250

Liszkay A, van der Zalm E, Schopfer P (2004) Production of reactive oxygen intermediates (O_2^-), H_2O_2, and OH) by maize roots and their role in wall loosening and elongation growth. Plant Physiol 136:3114–3123

Liu B, Joshi HC, Wilson TJ, Silflow CD, Palevitz BA, Snustad DP (1994) γ-Tubulin in *Arabidopsis*: gene sequence, immunoblot, and immunofluorescence studies. Plant Cell 6:303–314

Lloyd CW (1991) Cytoskeletal elements of the phragmosome establish the division plane in vacuolated plant cells. In: Lloyd CW (ed) The cytoskeletal basis of plant growth and form. Academic, London, pp 245–257

Lloyd CW (1994) Why should stationary plant cells have such dynamic microtubules? Mol Biol Cell 5:1277–1280

Lloyd CW, Chan J, Hussey PI (2004) Microtubules and microtubule-associated proteins. In: Hussey PI (ed) The plant cytoskeleton in cell differentiation and development. CRC Blackwell, Oxford, pp 1–27

Lloyd CW, Seagull RW (1985) A spring for plant cell biology: microtubules as dynamic helices. Trends Biochem Sci 10:476–478

Lloyd CW, Slabas AR, Powell AJ, Lowe SB (1980) Microtubules, protoplasts and plant cell shape. An immunofluorescent study. Planta 147:500–506

Lockhart J (1960) Intracellular mechanisms of growth inhibition by radiant energy. Plant Physiol 35:129–135

Ludueña RF (1998) Multiple forms of tubulin: different gene products and covalent modifications. Int Rev Cytol 178:207–275

Luib M, Schott PE (1990) Einsatz von Bioregulatoren. In: Haug G, Schuhmann G, Fischbeck G (eds) Pflanzenproduktion im Wandel – Neue Aspekte in den Agrarwissenschaften. Verlag Chemie, Weinheim, pp 275–304

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

Lynch TM, Lintilhac PM (1997) Mechanical signals in plant development: a new method for single cell studies. Develop Biol 181:246–256

MacRae TH (1997) Tubulin post-translational modifications – enzymes and their mechanisms of action. Eur J Biochem 244:265–278

Makela P, Varaala L, Peltonensainio P (1996) Agronomic comparison of Minnesota-adapted dwarf oat with semi-dwarf, intermediate, and tall oat lines adapted to Northern growing conditions. Can J Plant Sci 76:727–734

Marc J, Granger CL, Brincat J, Fisher DD, Kao TH, McCubbin AG, Cyr RJ (1998) *GFP-MAP4* reporter gene for visualizing cortical microtubule rearrangements in living epidermal cells. Plant Cell 10:1927–1939

Marc J, Palevitz BA (1990) Regulation of the spatial order of cortical microtubules in developing guard cells in *Allium*. Planta 182:626–634

Marc J, Sharkey DE, Durso NA, Zhang M, Cyr RJ (1996) Isolation of a 90-kD microtubule-associated protein from tobacco membranes. Plant Cell 8:2127–2138

Mayumi K, Shibaoka H (1996) The cyclic reorientation of cortical microtubules on walls with crossed polyllamellate structure. Effects of plant hormones and an inhibitor of protein kinases on the progression of the cycle. Protoplasma 195:112–122

McClinton RS, Sung ZR (1997) Organization of cortical microtubules at the plasma membrane in *Arabidopsis*. Planta 201:252–260

Mickovski SB, Ennos AR (2003) The effect of unidirectional stem flexing on shoot and root morphology and architecture in young *Pinus sylvestris* trees. Can J Forest Res 33:2202–2209

Mineyuki Y, Iida H, Anraku Y (1994) Loss of microtubules in the interphase cells of onion (*Allium cepa* L.) root tips from the cell cortex and their appearance in the cytoplasm after treatment with cycloheximide. Plant Physiol 104:281–284

Mineyuki Y, Marc J, Palevitz BA (1988) Formation of the oblique spindle in dividing guard mother cells of *Allium*. Protoplasma 147:200–203

Mita T, Katsumi M (1986) Gibberellin control of microtubule arrangement in the mesocotyl epidermal cells of the d_5 mutant of *Zea mays* L. Plant Cell Physiol 27:651–659

Morejohn LC (1991) The molecular pharmacology of plant tubulin and microtubules. In: Lloyd CW (ed) The cytoskeletal basis of plant growth and form. Academic, London, pp 29–43
Mulder B, Schell J, Emons AM (2004) How the geometrical model for plant cell wall formation enables the production of a random texture. Cellulose 11:395–401
Murata T, Wada M (1991) Effects of centrifugation on preprophase-band formation in *Adiantum* protonemata. Planta 183:391–398
Nee M, Chiu L, Eisinger W (1978) Induction of swelling in pea internode tissue by ethylene. Plant Physiol 62:902–906
Nick P (1997) Phototropic induction can shift the gradient of crown-root emergence in maize. Bot Acta 110:291–297
Nick P (1998) Signalling to the microtubular cytoskeleton in plants. Int Rev Cytol 184:33–80
Nick P, Bergfeld R, Schäfer E, Schopfer P (1990) Unilateral reorientation of microtubules of the outer epidermal wall during photo- and gravitropic curvature of maize coleoptiles and sunflower hypocotyls. Planta 181:162–168
Nick P, Furuya M (1993) Phytochrome-dependent decrease of gibberellin sensitivity. Plant Growth Regul 12:195–206
Nick P, Furuya M (1996) Buder revisited – cell and organ polarity during phototropism. Plant Cell Environ 19:1179–1187
Nick P, Furuya M, Schäfer E (1991) Do microtubules control growth during tropism? Experiments with maize coleoptiles. Plant Cell Physiol 32:999–1006
Nick P, Furuya M, Schäfer E (1992) Auxin redistribution during first positive phototropism in corn coleoptiles – microtubule reorientation and the Cholodny–Went theory. Plant Physiol 99:1302–1308
Nick P, Godbolé R, Wang QY (1997) Probing rice gravitropism with cytoskeletal drugs and cytoskeletal mutants. Biol Bull 192:141–143
Nick P, Heuing A, Ehmann B (2000) Plant chaperonins: a role in microtubule-dependent wall-formation? Protoplasma 211:234–244
Nick P, Schäfer E (1988) Spatial memory during the tropism of maize (*Zea mays* L.) coleoptiles. Planta 175:380–388
Nick P, Schäfer E (1994) Polarity induction versus phototropism in maize: auxin cannot replace blue light. Planta 195:63–69
Nick P, Yatou O, Furuya M, Lambert AM (1994) Auxin-dependent microtubule responses and seedling development are affected in a rice mutant resistant to EPC. Plant J 6:651–663
Nick P (1999) Signals, motors, morphogenesis – the cytoskeleton in plant development. Plant Biol 1:169–179
Nishiyama I (1986) Lodging of rice plants and countermeasure. FFTC Book Ser Taiwan 34:152–163
Oda K, Suzuki M, Odagawa T (1966) Varietal analysis of physical characters in wheat and barley plants relating to lodging and lodging index. Bull Natl Inst Agric Sci Tokyo 15:55–91
Panteris E, Apostolakos P, Galatis B (1995) The effect of taxol on *Triticum* preprophase root cells – preprophase microtubule band organization seems to depend n new microtubule assembly. Protoplasma 186:72–78
Paredez AR, Somerville CR, Ehrhardt DW (2006) Visualization of cellulose synthase demonstrates functional association with microtubules. Science 312:1491–1495
Parness J, Horwitz SB (1981) Taxol binds to polymerized tubulin in vitro. J Cell Biol 91:479–487

Pastuglia M, Azimzadeh J, Goussot M, Camilleri C, Belcram K, Evrard LL, Schmit AC, Philippe-Guerche P, Bouchez D (2006) γ-Tubulin is essential for microtubule organization and development in *Arabidopsis*. Plant Cell 18:1412–1425

Patel SAR, Meulia T, Dixit R, Cyr R, Meier I (2004) Arabidopsis WPP-domain proteins are developmentally associated with the nuclear envelope and promote cell division. Plant Cell 16:3260–3273

Pay A, Resch K, Frohnmeyer H, Fejes E, Nagy F, Nick P (2002) Plant RanGAPs are localized at the nuclear envelope in interphase and associated with microtubules in mitotic cells. Plant J 30:699–709

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) Green revolution genes encode mutant gibberellin response modulators. Nature 400:256–261

Pereira G, Schiebel E (1997) Centrosome-microtubule nucleation. J Cell Sci 110:295–300

Pickett-Heaps JD (1967) The effects of colchicine on the ultrastructure of dividing plant cells, xylem wall differentiation and distribution of cytoplaamic microtubules. Develop Biol 15:206–236

Preston RD (1988) Cellulose-microfibril-orienting mechanisms in plant cell walls. Planta 174:67–74

Prodhan AKMA, Funada R, Ohtani J, Abe H, Fukuzawa K (1995) Orientation of microfibrils and microtubules in developing tension-wood fibres of Japanese ash (*Fraxinus mandshurica* var. *japonica*). Planta 196:577–585

Roberts LW, Baba S (1968) IAA-induced xylem differentiation in the presence of colchicine. Plant Cell Physiol 9:315–321

Robinson DG, Quader H (1981) Structure, synthesis, and orientation of microfibrils. IX: A freeze fracture investigation of the *Oocystis* plasma membrane after inhibitor treatments. Eur J Cell Biol 25:278–288

Robinson DG, Quader H (1981) Structure, synthesis, and orientation of microfibrils. IX: A freeze fracture investigation of the *Oocystis* plasma membrane after inhibitor treatments. Eur J Cell Biol 25:278–288

Robinson DG, Quader H (1982) The microtubule–microfibril syncrome. In: Lloyd CW (ed) The cytoskeleton in plant growth and development. Academic, London, pp 109–126

Robson PRH, Mccormac AC, Irvine AS, Smith H (1996) Genetic engineering of harvest index in tobacco through overexpression of a phytochrome gene. Nat Biotechnol 14:995–998

Sakiyama M, Shibaoka H (1990) Effects of abscisic acid on the orientation and cold stability of cortical microtubules in epicotyl cells of the dwarf pea. Protoplasma 157:165–171

Sakiyama-Sogo M, Shibaoka H (1993) Gibberellin A_3 and abscisic acid cause the reorientation of cortical microtubules in epicotyl cells of the decapitated dwarf pea. Plant Cell Physiol 34:431–437

Samuels AL, Giddings TH, Staehelin LA (1995) Cytokinesis in tobacco BY-2 and root tip cells – a new model of cell plate formation in higher plants. J Cell Biol 130:1345–1357

Sano T, Higaki T, Oda Y, Hayashi T, Hasezawa S (2005) Appearance of actin microfilament twin peaks in mitosis and their function in cell plate formation, as visualized in tobacco BY-2 cells expressing GFP-fimbrin. Plant J 44:595–605

Schlenker G (1937) Die Wuchsstoffe der Pflanzen. Lehmanns, München, pp 18–19

Schott PE, Lang H (1977) Mittel zur Regulierung des Pflanzenwachstums. BASF Patent No DE 275 594 0C2

Schreiner C, Reed HS (1908) The toxic action of certain organic plant constituents. Bot Gaz USA 45:73–102

Seagull RW (1990) The effects of microtubule and microfilament disrupting agents on cytoskeletal arrays and wall deposition in developing cotton fibers. Protoplasma 159:44–59

Shaw SL, Kamyar R, Ehrhardt DW (2003) Sustained microtubule treadmilling in *Arabidopsis* cortical arrays. Science 300:1715–1718

Shibaoka H (1993) Regulation by gibberellins of the orientation of cortical microtubules in plant cells. Austr J Plant Physiol 20:461–470

Shimamura M, Brown RC, Lemmon BE, Akashi T, Mizuno K, Nishihara N, Tomizawa KI, Yoshimoto K, Deguchi H, Hosoya H, Mineyuki Y (2004) Gamma-tubulin in basal land plants: characterization, localization, and implication in the evolution of acentriolar microtubule organizing centers. Plant Cell 16:45–59

Skoufias DA, Wilson L (1998) Assembly and colchicine binding characteristics of tubulin with maximally tyrosinylated and de-tyrosinylated alpha-tubulins. Arch Biochem Biophys 351:115–122

Smith H (1981) Adaptation to shade. In: Johnson CB (ed) Physiological processes limiting plant productivity. Butterworths, London, pp 159–173

Smith LG (2005) Spatial control of cell expansion by the plant cytoskeleton. Ann Rev Cell Develop Biol 21:271–295

Steeves TA, Sussex IM (1989) Patterns in plant development. Cambridge University Press, Cambridge

Stoppin V, Vantard M, Schmit AC, Lambert AM (1994) Isolated plant nuclei nucleate microtubule assembly: the nucleus surface in higher plants has centrosome-like activity. Plant Cell 6:1099–1106

Stoppin-Mellet V, Peter C, Lambert AM (2000) Distribution of gamma-tubulin in higher plant cells: cytosolic gamma-tubulin is part of high molecular weight complexes. Plant Biol 2:290–296

Taylor LP, Hepler PK (1997) Pollen germination and tube growth. Annu Rev Plant Physiol Plant Mol Biol 48:461–491

Thitamadee S, Tuchihara K, Hashimoto T (2002). Microtubule basis for left-handed helical growth in *Arabidopsis*. Nature 417:193–196

Thomas DDS, Dunn DM, Seagull RW (1977) Rapid cytoplasmic responses of oat coleoptiles to cytochalasin B, auxin, and colchicine. Can J Bot 55:1797–1800

Thompson DW (1959) On growth and form. University Press, Cambridge, pp 465–644

Timell TE (1986) Compression wood in gymnosperms. Springer, Berlin Heidelberg New York, pp 1–706

Tolbert NE (1960) (2-Chloroethyl)trimethyl-ammonium chloride and related compounds as plant growth substances. II. Effect on growth of wheat. Plant Physiol 35:380–385

Toyomasu T, Yamane H, Murofushi N, Nick P (1994) Phytochrome inhibits the effectiveness of gibberellins to induce cell elongation in rice. Planta 194:256–263

Toyomasu T, Yamane H, Murofushi N, Nick P (1994) Phytochrome inhibits the effectiveness of gibberellins to induce cell elongation in rice. Planta 194:256–263

Traas J, Bellini C, Nacry P, Kronenberger J, Bouchez D, Caboche M (1995) Normal differentiation patterns in plants lacking microtubular preprophase bands. Nature 375:676–677

Turing AM (1952) The chemical basis of morphogenesis. Phil Trans R Soc Lon, B Biol 237:37–72

Vantard M, Leviliiers N, Hill AM, Adoutte A, Lambert AM (1990) Incorporation of *Paramecium* axonemal tubulin into higher plant cells reveals functional sites of microtubue assembly. Proc Natl Acad Sci USA 87:8825–8829

Vaughan MA, Vaughn KC (1988) Carrot microtubules are dinitroaniline resistant. I. Cytological and cross-resistance studies. Weed Res 28:73–83
Vaughn KC (2000) Anticytoskeletal Herbicides. In: Nick P (ed) Plant microtubules – potential for biotechnology. Springer, Berlin Heidelberg New York, pp 193–205
Vaughn KC, Renzaglia A (2006) Structural and immunocytochemical characterization of the *Ginkgo biloba* L. sperm motility apparatus. Protoplasma 227:165–173
Wang QY, Nick P (2001) Cold acclimation can induce microtubular cold stability in a manner distinct from abscisic acid. Plant Cell Physiol 42:999–1005
Wasteneys GO (2004) Progress in understanding the role of microtubules in plant cells. Curr Opin Plant Biol 7:651–660
Watts DI, Monteiro MJ, Cox RA (1987) Identification of Eco-RV fragments spanning the n-alpha tubulin gene of *Physarum*. FEBS Lett 241:229–233
Weibel RO, Pendleton JW (1961) Effect of artificial lodging on winter wheat grain yield and quality. Agron J 56:187–188
Whittington AT, Vugrek O, Wei KJ, Hasenbein NG, Sugimoto K, Rashbrooke MC, Wasteneys GO (2001) MOR1 is essential for organizing cortical microtubules in plants. Nature 411:610–613
Wick SM (1991) The preprophase band. In: Lloyd CW (ed) The cytoskeletal basis of plant growth and form. Academic, London, pp 231–244
Wiese C, Zheng YX (1999) Gamma-tubulin complexes and their interaction with microtubule-organizing centers. Curr Opin Struct Biol 9:250–259
Wiesler B, Wang QY, Nick P (2002) The stability of cortical microtubules depends on their orientation. Plant J 32:1023–1032
Wymer CL, Lloyd CW (1996) Dynamic microtubules: Implications for cell wall patterns. Trends Plant Sci 1:222–227
Wymer CL, Fisher DD, Moore RC, Cyr RJ (1996) Elucidating the mechanism of cortical microtubule reorientation in plant cells. Cell Motil Cytoskeleton 35:162–173
Yasuhara H, Sonobe S, Shibaoka H (1992) ATP-sensitive binding to microtubules of polypeptides extracted from isolated phragmoplasts of tobacco BY-2 cells. Plant Cell Physiol 33:601–608
Yasuhara H, Sonobe S, Shibaoka S (1993) Effects of taxol on the development of the cell plate and of the phragmoplast in tobacco BY-2 cells. Plant Cell Physiol 34:21–29
Yoshizawa N (1987) Cambial responses to the stimulus of inclination and structural variation of compression wood tracheids in gymnosperms. Bull Utsunomiya Univ For 23:23–141
Young T, Hyams JS, Lloyd CW (1994) Increased cell-cycle-dependent staining of plant cells by the antibody MPM-2 correlates with preprophase band formation. Plant J 5:279–284
Yuan M, Shaw PJ, Warn RM, Lloyd CW (1994) Dynamic re-orientation of cortical microtubules from transverse to longitudinal, in living plant cells. Proc Natl Acad Sci USA 91:6050–6053
Zandomeni K, Schopfer P (1993) Reorientation of microtubules at the outer epidermal wall of maize coleoptiles by phytochrome, blue-light photoreceptor and auxin. Protoplasma 173:103–112
Zandomeni K, Schopfer P (1994) Mechanosensory microtubule reorientation in the epidermis of maize coleoptiles subjected to bending stress. Protoplasma 182:96–101
Ziegenspeck H (1948) Die Bedeutung des Feinbaus der pflanzlichen Zellwand für die physiologische Anatomie. Mikroskopie 3:72–85
Zimmermann W (1965) Die Telomtheorie. Gustav Fischer, Stuttgart