Signals, Motors, Morphogenesis – the Cytoskeleton in Plant Development[1]

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Abstract: Plant shape can adapt to a changing environment. This requires a structure that (1) must be highly dynamic, (2) can respond to a range of signals, and (3) can control cellular morphogenesis. The cytoskeleton, microtubules, actin microfilaments, and cytoskeletal motors meets these requirements, and plants have evolved specific cytoskeletal arrays consisting of both microtubules and microfilaments that can link signal transduction to cellular morphogenesis: cortical microtubules, preprophase band, phragmoplast on the microtubular side, transvacuolar microfilament bundles, and phragmosome on the actin side. These cytoskeletal arrays are reviewed with special focus on the signal responses of higher plants. The signal-triggered dynamic response of the cytoskeleton must be based on spatial cues that organize assembly and disassembly of tubulin and actin. In this context the great morphogenetic potential of cytoskeletal motors is discussed. The review closes with an outlook on new methodological approaches to the problem of signal-triggered morphogenesis.

Key words: Actin microfilaments, cytoskeletal motors, cytoskeleton, microtubules, morphogenesis, signal transduction.

How Plants Adapt: Signal Control of Cell Shape

Animals move, plants adapt – this simple fact governs most aspects of plant life. Plant cells move only rarely and thus cell movement, a central topic in animal development, does not play a role in plants. On the cellular level, plant morphogenesis is brought about by three phenomena: (1) spatiotemporal control of cell growth, (2) spatiotemporal control of cell division, and (3) spatiotemporal control of cell differentiation (which is not addressed in this review). Both, cell division and cell growth can be controlled by environmental stimuli.

The cell can align its axis as well as the symmetry of division in response to the environment. In fern protonemata, where the division of the apical cell is aligned with the axis of the protonema, this division can be tilted by 90° in response to blue light resulting in two-dimensional growth and the formation of a prothallium (Mohr, 1956[86]; Wada and Furuya, 1970[136]). In moss gametophytes, light in combination with cytokinins can shift the division axis even into the third dimension leading to the formation of buds (review in Reski, 1998[104]). The wound response of higher plants involves axis realignments of the surrounding tissue such that the cells divide perpendicularly to the wound surface (Hush et al., 1990[47]). When cells are committed to a new developmental pathway this is often accompanied by asymmetric divisions, as evident in formation of stomata or hyalin cells (Zepf, 1952[147]). In lower plants, the first cell division separating the prospective thallus from the prospective rhizoids is often asymmetric and can be oriented by environmental stimuli such as light (Haupt, 1957[43]; Jaffe, 1958[51]), electrical fields, gravity (Edwards and Roux, 1994[26]), or ion gradients (reviewed in Quatrano, 1978[100]; Weisenseel, 1979[141]). By treatment with antimicrotubular drugs these divisions can be rendered symmetric, resulting in the formation of two thalli (Vogelmann et al., 1981[133]). Recently, similar results have been obtained for the first asymmetric division of microspores (Twell et al., 1998[130]). The first zygotic division in higher plants is asymmetric as well. In the *gnom* mutant, where it is symmetric, the developmental fate of the descendant cells is dramatically altered resulting in embryos with defective apicobasal morphogenesis (Mayer et al., 1993[72]). These examples suggest that signal-dependent control of division symmetry and axiality play a pivotal role for development and cell differentiation in plants (Gunning, 1982[41]).

In addition to the relatively slow response of cell division, there exist stimulus-dependent responses of cell growth that can control cell shape much more rapidly – the bending of stems, roots or coleoptiles in response to a gravi- or phototropic stimulus, for instance, becomes detectable within a few minutes (Iino and Baskin, 1984[50]), and the growth response of individual cells is even faster (Nick and Furuya, 1996[95]). These fast growth responses are achieved by changes in amplitude and proportionality of cell expansion, most prominent in the deetiolation response. Whereas stem elongation is elevated in the dark, it is blocked immediately upon illumination. This light response of stem elongation can be ascribed perfectly to

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[1] This review is dedicated to the memory of Paul Green.

a light-induced block of cell elongation (Lockhart, 1960[67]; Toyomasu et al., 1994[130]; Waller and Nick, 1997[137]). A similar response of cell expansion is found in the ethylene-induced barrier response of pea epicotyls (Lang et al., 1982[62]), where growth is redistributed entirely from elongation towards thickening of the stem.

Both the spatial control of cell division as well as the spatial control of cell growth by signals are intimately linked to plant-specific arrays of the cytoskeleton. The next section will therefore discuss these arrays and focus on their function for the spatial control of cell division and cell expansion, and the third section will give a brief overview of the numerous signal responses of the cytoskeleton. A central question in these phenomena is the problem of directionality. The review will raise the issue whether directionality might be linked with cytoskeletal motor proteins. The outlook section will put a strong emphasis on approaches to monitor cytoskeletal dynamics *in vivo*, in single cells, in real time to obtain insight into this basic problem of plant morphogenesis.

The Players in the Game: Components of the Plant Cytoskeleton

Microtubular arrays of higher plants: cortical microtubules, radial microtubules, preprophase band, spindle, and phragmoplast

Interphase cells are characterized by arrays of *cortical microtubules* that are adjacent to the plasma membrane and usually arranged in parallel bundles in a direction that is perpendicular to the axis of preferential cell expansion (Fig. **1 A**). They are thought to control the direction of cellulose deposition and thus to participate in the reinforcement of axial cell growth (reviewed in Giddings and Staehelin, 1991[36]). For the problem of signal-triggered morphogenesis it is relevant that cortical microtubules can change orientation in response to various stimuli (refer to section III for details).

The ensuing mitosis is heralded by a displacement of the nucleus to the cell centre, i.e., to the site where the prospective cell plate will be formed. Simultaneously, radial microtubules emanate from the nuclear surface and merge with the cortical cytoskeleton (Fig. **1 B**), apparently tethering the nucleus to its new position. In the next step, the preprophase band is organized by the nucleus as a broad band of microtubules around the cell equator (Fig. **1 C**), marking the site where after completed mitosis the new cell plate will be formed. Experiments in fern protonemata where the formation of the preprophase band can be manipulated by centrifugation of the nucleus to a new location (Murata and Wada, 1991[87]) suggest a causal relationship between preprophase band and cell plate formation. Moreover, in cells, where the axis or symmetry of cell division changes, this change is always predicted by a corresponding localization of the preprophase band (reviewed in Wick, 1991[142]). The division spindle is always laid down perpendicular to the preprophase band with the spindle equator located in the plane of the preprophase band (Fig. **1 D**).

As soon as the chromosomes have separated, a new array of microtubules, the phragmoplast, appears at the site that had already been marked by the preprophase band (Fig. **1 E**). This microtubular structure is involved in the transport of vesicles

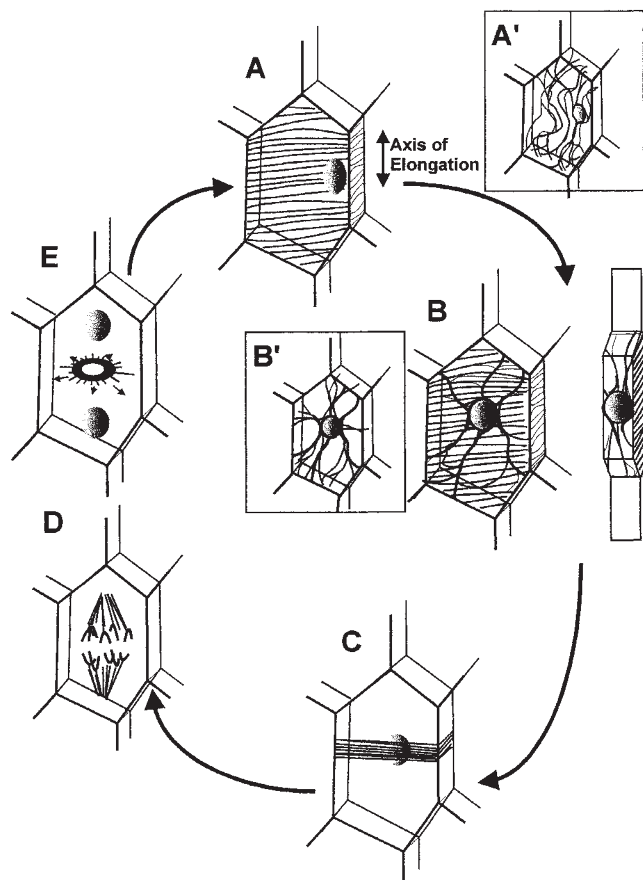

Fig. 1 Cytoskeletal arrays during the cell cycle of higher plants. (**A**) Elongating interphase cell with cortical microtubules. The nucleus is situated in the periphery of the cell. (**A'**) Transvacuolar actin bundles typical for elongating interphase cells. (**B**) Cell preparing for mitosis seen 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. (**B'**) Microfilaments establishing the phragmosome in a premitotic cell. (**C**) Preprophase band of microtubules. (**D**) Mitosis and division spindle. (**E**) Cell in telophase with phragmoplast that organizes the new cell plate and extends in a centrifugal direction (arrows).

to the periphery of the growing cell plate and consists of a double ring of interdigitating microtubules that increases in diameter with growing size of the cell plate. New microtubules are organized along the edge of the growing phragmoplast (Vantard et al., 1990[133]).

Actin microfilaments in higher plant cells: phragmosome, transvacuolar strands and cortical network

Similar to the microtubules, actin is organized into several distinct arrays with presumably different function. (1) In cells that prepare for mitosis, the phragmosome tethers the nucleus to its new position in the cell centre (Fig. **1 B'**) and, in contrast to the microtubular preprophase band, partially persists during meta- and anaphase. It seems to participate in the organization of new microtubules and the formation of the phragmoplast (reviewed in Lloyd, 1991[66]). (2) Longitudinal transvacuolar bundles (Fig. **1 A'**) of actin are characteristic for vacuolated interphase cells of higher plants (Parthasarathy,

1985[97]; Sonobe and Shibaoka, 1989[122]). The rigidity of these transvacuolar strands and the degree of their bundling is regulated by signals such as plant hormones (Grabski and Schindler, 1996[38]), kinase cascades (Grabski et al., 1998[39]) or light (Waller and Nick, 1997[137]). (3) In addition to the transvacuolar bundles, a fine network of highly dynamic microfilaments can be detected in the cortical cytoplasm of elongating cells. It can be rendered visible after pretreatment with protein cross-linkers (Sonobe and Shibaoka, 1989[122]) or upon very mild fixation (Waller and Nick, 1997[137]). This cortical meshwork might support auxin-triggered cell elongation (Thimann et al., 1992[127]; Wang and Nick, 1998[138]), possibly in combination with directional vesicle transport (Baskin and Bivens, 1995[8]). Such a link between actin and acropetal vesicle transport is well established for cells with pronounced tip growth such as pollen tubes or root hairs (reviewed in Staiger and Schliwa, 1987[123]).

Binding proteins for microtubules and microfilaments

One might expect that the fundamental differences in organization and function of the plant cytoskeleton are mirrored in dramatic differences in molecular composition. However, as far as the major components tubulin and actin are concerned, a surprising degree of similarity is observed between plants and animals (reviewed in Fosket and Morejohn, 1992[32], for tubulins, and in Meagher and McLean, 1990[77], for actins). For both protein families numerous isotypes are observed that are expressed differentially with respect to tissue and developmental specifity (reviewed in Meagher, 1991[76], for actin, and in Silflow et al., 1987[119], for tubulin). The functional significance of this complexity has remained obscure so far.

Neurotubulin can co-assemble with plant tubulin *in vitro* and *in vivo* and participates in the dynamic reactions of the host cytoskeleton (Vantard et al., 1990[133]; Zhang et al., 1990[148]; Yuan et al., 1994[145]). These observations suggest that the factors responsible for the specific organization of the plant cytoskeleton are extrinsic to tubulin itself. *In vivo*, the nucleation of new microtubules is strictly regulated and occurs on the surface of specialized organelles, the centrosomes containing a ring complex built up of a specialized tubulin, γ-tubulin (Reff et al., 1993[102]). Surprisingly, higher plants do not possess such centrosomes. They do possess, however, functional analogues, the so called microtubule-organizing centres (MTOCs) (reviewed in Lambert, 1993[61]). The molecular composition of these MTOCs is unknown, but it seems that they contain microtubule-associated proteins (MAPs) that lower the critical concentration of tubulin necessary for microtubule assembly. Plants do possess γ-tubulin as well but it is not confined to the MTOC, but associated with all microtubule arrays (Liu et al., 1994[64]), posing the question, whether it has the same function as γ-tubulin in animal cells. Although several potential plant MAPs have been described during recent years (Chang-Jie and Sonobe, 1993[15]; Mizuno, 1995[84]; Nick et al., 1995[94]; Marc et al., 1996[71]; Rutten et al., 1997[107]), only two plant microtubule-associated proteins have been cloned so far, both of them being factors required for protein translation (Durso and Cyr, 1994[25]; Hugdahl et al., 1995[46]). Although they are discussed as bundling or annealing MAPs, their function *in vivo* is not understood.

Actin-binding proteins have also been identified in plants (Collings et al., 1994[21]; Jiang et al., 1997[53]) that seem to fulfill different functions. The balance between actin monomers and filaments is controlled by the actin-depolymerizing factor ADF (Jiang et al., 1997[53]), cofilin (Lopez et al., 1996[68]), and profilin (reviewed in Staiger et al., 1997[124]), and ADF has been recently shown to be the target of calcium-dependent kinase cascades (Smertenko et al., 1998[120]). Other proteins, such as EF-1α (Collings et al., 1994[21]), are supposed to bundle actin microfilaments – since this protein has also been isolated as a microtubule-bundling protein (Durso and Cyr, 1994[25]) it might cross-link microtubules to the actin lattice.

Cytoskeletal motors

The microtubule motors kinesin and dynein embody an ATPase function and they are able to move along microtubules in a strict dependence on microtubule polarity (Hyman and Mitchison, 1991[49]). These motors are involved in the mutual sliding of microtubules or for the directional transport of proteins along microtubules. Dynein can be coupled to the dynactin complex and thus allows sliding of microtubules along the actin system (Allan, 1994[3]). Proteins that are immunologically related to kinesin have been detected in pollen tubes (Tiezzi et al., 1992[128]) and kinesin-homologous sequences have been reported in *Arabidopsis* (Mitsui et al., 1993[82]). Recently, a kinesin-like calmodulin-binding protein (KCBP) has been isolated from *Arabidopsis* (Reddy et al., 1996[101]). So far, it is the only microtubular motor in plants where an actual motor activity has been demonstrated. The bacterially over-expressed motor protein was attached to glass slides and was shown to move microtubules across the glass surface (Song et al., 1997[121]). Interestingly, the protein moves towards the minus end of microtubules (i.e., in the opposite sense to classic kinesin), and the binding to microtubules is inhibited by calcium–calmodulin.

The actin-based myosins share a conserved motor domain responsible for binding to actin (Goodson and Spudich, 1993[37]), and diverse tail domains that probably convey interactions with different partners. Although myosins have been found in plants as well (Moepps et al., 1993[85]; Kinkema et al., 1994[59]), they seem to differ from animal myosins and were therefore placed into separate classes. Little more than the sequence is known about these proteins. They do possess potential calmoldulin binding (Chapman et al., 1991[16]; Mercer et al., 1991[78]) and dimerization sites (Lupas et al., 1991[70]) that are typical for myosins and, additionally, a tail of unknown function. Although epitopes that are immunologically related to myosin have been detected in higher plant cells (Tirlapur et al., 1995[129]; Miller et al., 1995[80]), it is far from clear whether the antibodies actually recognize the respective plant myosins.

Caught in Action: Signal Responses of the Plant Cytoskeleton

Response to light

The light responses of microtubules and actin microfilaments have been analyzed in detail for the Graminean coleoptile, where cell elongation is blocked in the light. Cortical microtubules are found to be transverse in coleoptiles that elongate rapidly. They respond by a fast reorientation into longitudinal

arrays in response to light qualities that inhibit cell elongation (Nick et al., 1990[90]; Toyomasu et al., 1994[130]), and similar responses have been observed in dicotyledonous seedlings (Nick et al., 1990[90]; Laskowski, 1990[63]). A phototropic stimulation by a pulse of blue light induces a reorientation of cortical microtubules in the lighted flank of the coleoptile, whereas the microtubules in the shaded flank reinforce their transverse orientation (Nick et al., 1990[90]). This microtubule reorientation precedes the corresponding growth response by 10–20 min. Two hours after stimulation the orientation of microtubules is irreversibly fixed, which becomes manifest on the physiological level as a stable directional "memory" of stimulus direction (Nick and Schäfer, 1988[89]; Nick and Schäfer, 1994[93]). Reorientation from longitudinal into transverse arrays can be elicited by short-term irradiation with red light (Nick et al., 1990[90]; Zandomeni and Schopfer, 1993[146]) along with a stimulation of growth triggered by the plant photoreceptor phytochrome (Zandomeni and Schopfer, 1993[146]). In contrast, in rice coleoptiles where red light inhibits elongation, the activation of the phytochrome system is observed to promote the formation of longitudinal arrays (Nick and Furuya, 1993[92]; Toyomasu et al., 1994[130]). Despite this correlation between microtubule orientation and growth, it is possible to separate both phenomena transiently (Laskowski, 1990[63]; Nick et al., 1991[91]; Nick and Schäfer, 1994[93]).

Light responses of actin microfilaments have been described for lower plants. The phototropic response of moss protonemata to red light is accompanied by a reorganization of the microfilament system (Meske et al., 1995[79]), and the light-induced movement of chloroplasts in fern protonemata is accompanied by the formation of specific circular actin arrays (Kadota and Wada, 1989[56]). In maize coleoptiles the stimulation of cell elongation by the plant photoreceptor phytochrome is accompanied by a loosening of the transvacuolar actin strands, whereas the light inhibition of growth in the mesocotyl is correlated with a rapid bundling of actin (Waller and Nick, 1997[137]).

Response to gravity and mechanostimulation

Gravity can induce fast bending responses (gravitropism) and slower morphogenetic responses that tune plant architecture with gravity (gravimorphosis). In the upper flank of coleoptiles and hypocotyls, microtubules reorient into longitudinal arrays, whereas they remain transverse in the lower flank (Nick et al., 1990[90]). Interestingly, this orientation gradient is reversed in roots (Blancaflor and Hasenstein, 1993[12]). These microtubule responses seem to be connected to auxin redistribution triggered by gravity rather than to gravity directly. However, gravity-dependent microtubular responses can be observed in the sensing cells as well. Microtubules that are adjacent to the amyloplast of moss protonemata redistribute (Schwuchow et al., 1990[113]), and microtubules in the coleoptile bundle sheath reorient (Nick et al., 1997[96]). When these responses are manipulated by cytoskeletal drugs, this causes corresponding changes of the gravitropic response.

A beautiful example for gravimorphosis is the alignment of the first division in the spore of the fern *Ceratopteris*. When the spore is tilted after this asymmetric division, the rhizoid grows in the wrong way and is not able to correct this situation by gravitropic bending (Edwards and Roux, 1994[26]). A vivid, microtubule-driven migration of the nucleus towards the lower pole of the spore has been found to be essential for gravity sensing during the period of competence (Edwards and Roux, 1997[27]). It should be mentioned that a role for microtubules in gravimorphosis has been reported for animal development as well (Gerhart et al., 1981[35]). The dorsiventral axis of frog eggs is determined by an interplay of gravity-dependent sedimentation of yolk particles, sperm-induced nucleation of microtubules and self-amplifying alignment of newly formed microtubules driving the cortical rotation (Elinson and Rowning, 1988[28]).

Gravity sensing is probably related to mechanical stimulation because mechanosensitive ion channels might be important for both signal chains. However, it is not simple to separate the response to mechanic stimulation from accompanying hormonal responses. The barrier response of seedlings, for instance, is triggered by the plant hormone ethylene rather than by a mechanic stimulus (Nee et al., 1978[88]). This hormone, that is constantly formed by the growing stem, accumulates in front of physical obstacles and can induce a reorientation of cortical microtubules into longitudinal arrays leading to a corresponding shift in the direction of cellulose deposition favouring stem thickening (Lang et al., 1982[62]). In contrast, the reorientation of cortical microtubules in response to wounding has been shown to be independent of ethylene (Geitmann et al., 1997[33]), but might be caused by a changed pattern of mechanical stress aligning the cortical microtubules (and subsequently the preprophase band) with the surface of the wound. This set-up accelerates wound healing because the cells grow and divide in a direction that is perpendicular to the wound surface. Patterns of mechanical stress have been suggested to induce reorientation of cortical microtubules during the formation of new leaf primordia (Hardham et al., 1980[42]), and the formation of tension wood (Prodhan et al., 1995[99]). It is possible to induce microtubule reorientation by application of mechanical fields (Hush and Overall, 1991[48]), high pressure (Cleary and Hardham, 1993[19]) or tissue deformation (Fischer and Schopfer, 1998[30]). However, the treatments used during these experiments were quite drastic and far from being physiological, and in most cases it has not been clarified to what extent the observed effects were caused by the release of stress hormones that are well-known triggers of microtubule reorientation.

Cortical microtubules are clearly stabilized by the cell wall (Akashi et al., 1990[2]) indicating the existence of transmembrane proteins that link microtubules to cellulose microfibrils. Moreover, a close interaction between microfibrils and microtubules is a central element of microtubule–microfibril parallelity (Heath, 1974[44]). Changed patterns of mechanic stress might be transferred on microtubules via such transmembrane proteins and result in direction-dependent stability of microtubules (Williamson, 1991[143]). This would allow microtubules to sense changes in cell growth via changes in the pattern of wall strains and to align with those changes in a stabilizing feedback loop. This idea has even been extended to reduce the multitude of microtubular reorientation responses to such a simple mechanosensory model (Fischer and Schopfer, 1998[30]). As interesting as this model may be, it is certainly oversimplified: growth responses and microtubule reorientation are often correlated, but they have been separated into a range of systems and under a range of conditions (Nick et al.,

1991[91]; Baluška et al., 1992[4]; Sauter et al., 1993[112]; Kaneta et al., 1993[58]; Sakiyama-Sogo and Shibaoka, 1993[109]; Nick and Schäfer, 1994[93]; Mayumi and Shibaoka, 1996[74]; Baskin, 1997[9]).

Response to hormones

Many of the signal responses mentioned above are accompanied by changes in the level of endogenous hormones and can be mimicked by addition of hormones. For instance, the reorientation of microtubules in response to phototropic or gravitropic stimulation can be mimicked by auxin or auxin gradients (Nick and Schäfer, 1994[93]); the induction of longitudinal microtubules during the barrier response of pea shoots by application of ethylene (Lang et al., 1982[62]); and the effect of red light on elongation and microtubule orientation in rice mesocotyls by an inhibition of gibberellin synthesis (Nick and Furuya, 1993[92]). These correlations do not prove that the hormones are actually the transducers for these different signals, but they do illustrate the strong response of the microtubular cytoskeleton to plant hormones. For almost all plant hormones a response of cortical microtubules has been described in one or the other system. These responses have been reviewed recently (Shibaoka, 1994[118]) and are therefore only briefly summarized.

Auxin produces transverse microtubules in shoots and coleoptiles (Bergfeld et al., 1988[10]; Nick et al., 1990[90]; Sakoda et al., 1992[110]) along with a loosening of actin arrays (Grabski and Schindler, 1996[38]; Wang and Nick, 1998[138]). In contrast, in roots, where cell elongation is inhibited, auxin induces the formation of longitudinal microtubules (Blancaflor and Hasenstein, 1995[13]). Gibberellins usually stimulate cell elongation in roots (Baluška et al., 1993[5]) and shoots, and this effect is accompanied by increased frequencies of transverse microtubules (reviewed in Shibaoka, 1993[117]). Brassinolides stimulate stem elongation and increase the frequency of transverse microtubules in azuki beans (Mayumi and Shibaoka, 1995[73]), whereas cytokinins typically suppress elongation and induce stem thickening along with longitudinal arrays of microtubules (Shibaoka, 1974[115]; Volfová et al., 1977[135]) and increased rigidity of actin microfilaments (Grabski and Schindler, 1996[38]). The same is true for ethylene (Lang et al., 1982[62]) and abscisic acid (Sakiyama and Shibaoka, 1990[108]; Sakiyama-Sogo and Shibaoka, 1993[109]). A further effect of abscisic acid that increases frost hardiness in many species is an increased cold resistance of cortical microtubules (Sakiyama and Shibaoka, 1990[108]).

Some of the hormonal effects, for instance that of gibberellin (Mizuno, 1994[83]) on microtubule reorientation, seem to involve protein kinase cascades and some, for instance that of auxin (Mayumi and Shibaoka, 1996[74]), do not. This suggests that different signal chains can interact at different sites with the microtubular cytoskeleton. Downstream of the kinase cascades post-translational modifications of tubulin such as a detyrosination of α-tubulin in response to gibberellin (Duckett and Lloyd, 1994[24]), and changes in the pattern of tubulin isotypes (Mizuno, 1994[83]) have been described. However, the functional significance of these responses is not understood.

Response to abiotic and biotic stresses

Animal microtubules depolymerize in the cold, whereas in plants many microtubules are found to be cold stable. This cold stability is variable and has been correlated to cold hardiness (Jian et al., 1989[52]). It is elevated upon cold acclimation (Pihakaski-Maunsbach and Puhakainen, 1995[98]). On the other hand, chilling injury is increased after treatment with antimicrotubular drugs (Rikin et al., 1980[105]), whereas abscisic acid that increases frost hardiness in many species can induce increased cold resistance of cortical microtubules (Sakiyama and Shibaoka, 1990[108]).

The exact mechanism of cold adaptation of microtubules is far from being understood but seems to involve active signalling. Pharmacological evidence suggests a role for the phosphoinositide pathway (Bartolo and Carter, 1992[7]), and calcium/calmodulin (Fisher et al., 1996[31]) in the cold-induced depolymerisation of microtubules. Conversely, a role of microtubules for the control of the temperature sensing itself has been proposed from experiments with aequorin-expressing tobacco cells (Mazars et al., 1997[75]). The expression patterns of the different tubulin genes is changed during cold acclimation (Chu et al., 1993[17]), and a certain degree of tubulin depolymerisation is required to trigger the acclimation response. If microtubule disassembly is suppressed by taxol, chilling resistance becomes markedly reduced (Bartolo and Carter, 1991[6]) indicating that, in fact, existing microtubules have to be replaced by new microtubules with changed isotype composition. Whether these isotypes confer higher cold stability *per se* or whether they interact with a different set of associated proteins that mediate the increased cold stability (Akashi et al., 1990[2]) remains to be elucidated.

In addition to abiotic stresses, plant life is endangered by biotic stresses such as wounding by herbivores, fungal and viral attack. Again, the plant response to these stresses involves the cytoskeleton. The response of microtubules during wound healing (for instance as consequence of herbivore attack) has already been discussed above (Hush et al., 1990[47]). When plants are attacked by pathogens such as fungi, the nucleus and the cytoplasm of the host cell move rapidly towards the penetration site (Gross et al., 1993[40]), and a plant homologue of the cytoskeletal protein centrin is among the earliest genes that are induced in response to bacterial inoculation (Cordeiro et al., 1998[22]). This nuclear movement is driven by actin filaments and accompanied by a local depolymerization of cortical microtubules around the penetration site. When the nuclear movement is blocked by cytoskeletal drugs, fungi that normally are not able to infect the host cells become pathogenic (Kobayashi et al., 1997[60]), and the formation of callose around the penetration site is impaired (Kobayashi et al., 1997[60]), suggesting a role for the cytoskeleton in redistribution of vesicle transport towards the penetration site. A rapid depolymerisation of the actin cytoskeleton is also a central step in successful penetration of *Rhizobium* bacteria into root hairs during nodule formation of Leguminosae triggered by the lipochitooligosaccharides, the so called Nod factors (Ruijter et al., 1998[106]; Cárdenas et al., 1998[14]).

Microtubules play a positive role in the response to fungal attack. Plant viruses, in contrast, usurp microtubules for their own purpose: Fusions of the viral movement proteins with

the green fluorescent protein were found to be aligned along microtubules (Heinlein et al., 1995[45]; Blanc et al., 1996[11]), and the movement protein behaved as a MAP *in vitro*. These observations suggest that viruses use the microtubular cytoskeleton to be targeted and transported across the cell towards the plasmodesmata (Heinlein et al., 1995[45]).

Response to developmental signals

The developmental responses of the cytoskeleton will be treated here in a separate section, although some of the triggering signals are possibly identical to those that have already been discussed above.

The formation of new leaf primordia involves a shift in the axis of growth and division heralded by a reorientation of microtubules in a ring of cells around the margins of the prospective primordium that are otherwise undistinguishable from their neighbours with respect to dimension or growth axis (Hardham et al., 1980[42]). A similar reorientation of cortical microtubules, followed by a reorientation of the preprophase band, is observed during stomata formation that involves a switch in the axis and symmetry of cell division (reviewed in Wick, 1991[142]). The cell cycle-dependent protein kinase $p34^{cdc2}$ has been found to be co-localized with the preprophase band in maize root tips and cells of the stomatal guard cells and might couple the formation of the preprophase band to signal transduction (Mineyuki et al., 1991[81]; Colasanti et al., 1993[20]). The orientation and density of cortical microtubules often changes during differentiation or ageing of cells. This phenomenon can be demonstrated impressively in tissues where differentiation can be followed over files of cells, such as root tissue (Baluška et al., 1992[4]), conifer wood (Abe et al., 1995[1]) or Graminean leaves (Jung et al., 1993[55]).

The formation of tubers or bulbs is accompanied by a shift in the growth axis from elongation towards lateral growth that, in the case of potatoes, can be triggered by jasmonic acid and suppressed by gibberellin. Whereas jasmonic acid causes the disruption of cortical microtubules and thus disturbs the reinforcement of elongation growth leading to lateral swelling as a first step of tuber formation (Shibaoka, 1991[116]), gibberellins, in contrast, maintain microtubules in a transverse orientation and suppress tuber formation (Sanz et al., 1996[111]).

From Biochemistry to Shape: a Key Role for Cytoskeletal Motors?

Reorientation of cortical microtubules is a common theme in most of the signal responses listed above. This raises the question how these microtubules actually reorient. The first model that emerged from pioneering immunofluorescence studies (Lloyd and Seagull, 1985[65]) assumed that the cortical microtubules are organized into mechanically coupled helicoidal arrays forming a dynamic spring of variable pitch. When the microtubules slide in such a way that the helix is tightened, this will result in a steep pitch and in longitudinal microtubules. If they slide in the opposite direction, the spring will relax resulting in an almost transverse pitch. According to this elegant model the molecular mechanism of reorientation is expected to be based upon microtubule motors. The observation that microtubules of different orientation can coexist within one cell (Bergfeld et al., 1988[10]; Nick et al., 1990[90]; Wymer and Lloyd, 1996[144]), and more recently, studies based on the microinjection of fluorescent-labeled neurotubulin into living epidermal cells (Yuan et al., 1994[145]; Wymer and Lloyd, 1996[144]) lead to alternative ideas that involve direction-dependent assembly and disassembly. Depending on its orientation, a given microtubule might be in a growing state (assembly dominating over disassembly) or in a shrinking state (so called catastrophe with disassembly dominating over assembly), depending on its orientation. If this model is correct, the target for signals interfering with microtubule orientation has to be sought in the first place among those factors that control assembly and disassembly of microtubules.

Assembly and disassembly are biochemical processes that do not convey directional information *per se*. This review attempted to show that the reorientation of cortical microtubules, the nuclear migration, and the assembly of preprophase band and spindle, are events that are strictly regulated in space. In other words: the transition from biochemistry to shape requires some kind of spatial information. Direction-dependent stability is a factor that cannot be intrinsic to microtubules themselves. There must be some kind of either lattice or field that is responsible for the directional component of microtubule dynamics. This lattice might be either cytoskeletal lattices (e.g., actin microfilaments), physical fields (e.g., mechanical strains, bound dipoles) or apoplastic structures (e.g., cell wall components). This lattice or field somehow participates in the spatial organization of microtubule nucleation and, possibly, elongation. A flexible pattern of microtubule nucleation could be produced when MTOCs were redistributed along such a lattice (Fig. **2A**). It is more difficult to conceive a model explaining the control of microtubule elongation. One possibility could be the arrangement of cross-linking MAPs that support microtubule stability along a cytoskeletal lattice. When the lattice is bundled in response to a signal (Fig. **2B**), the minimal distance between the MAPs would change in direction (and thus the direction of preferential microtubule stability). Such a lattice could consist of a stable subpopulation of microtubules themselves that would not be seen upon microinjection of fluorescent-labeled tubulin into living cells (Wasteneys et al., 1993[139]). Alternatively, it might be actin microfilaments that are rapidly bundled in response to growth-inhibiting signals (Waller and Nick, 1997[137]), and that have already been discussed as an organizing lattice for the microtubule arrays related to cell division (Lloyd, 1991[66]).

This model calls for a special role of cytoskeletal motors in cellular morphogenesis. In fact such motors are prime candidates to organize biochemical events into defined spatial patterns. Both microtubules and actin microfilaments are endowed with a distinct polarity. The difference between the poles becomes manifest as a shift in the equilibrium between assembly and disassembly, with the positive pole being defined as the growing pole. The movement of cytoskeletal motors is guided by this cytoskeletal polarity.

In fact cytoskeletal motors have been identified as essential, early elements of axis formation in animals: the microtubule motor kinesin has been found to transport mRNA coding for *oskar*, a morphogenetic determinant of the posterior pole in the *Drosophila* oocyte (Clark et al., 1994[18]). The *inversus viscerum* mutation of mice causing an inversion of left–right asymmetry has been cloned and identified as the microtubule mo-

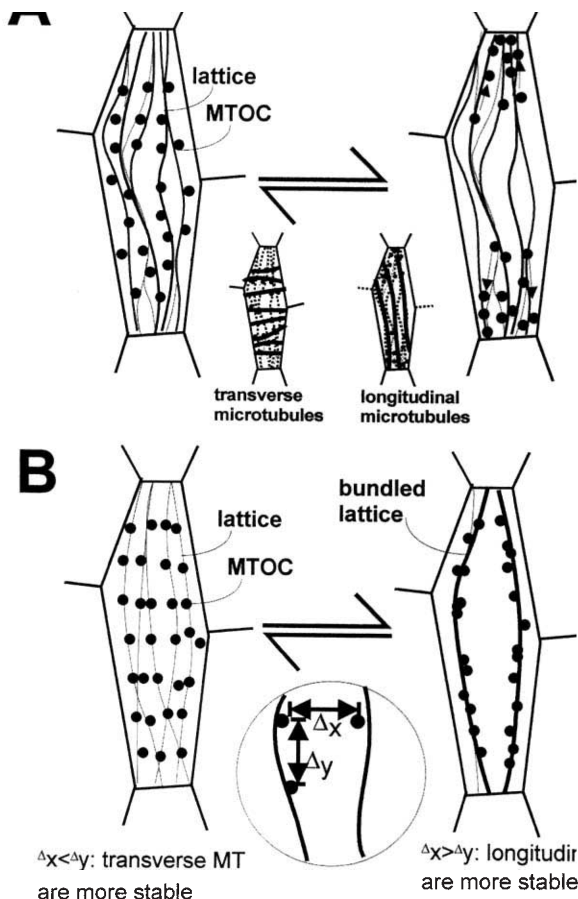

Fig. 2 Potential mechanisms responsible for directional stability of microtubules. (**A**) Microtubule-organizing centres could move along a directional matrix/lattice resulting in a redistribution of microtubule nucleating sites. (**B**) Microtubule-stabilizing factors are arranged along a directional matrix/lattice and this lattice becomes bundled. As a consequence, longitudinal microtubules will be favoured over transverse microtubules due to a lower minimal distance between the microtubule-stabilizing factors.

tor left-right dynein (Supp et al., 1997[126]). The analysis of morphogenetic genes in *Arabidopsis* has uncovered genes that seem to be involved in vesicle transport and secretion (Shevell et al., 1994[114]; Lukowitz et al., 1996[69]). These findings have to be seen in the context of intracellular traffic – whereas vesicles are transported from the cell centre to the periphery during cell growth, the direction of this transport has to be inverted during the formation of the cell plate. This must involve a corresponding response of cytoskeletal motors that are expected to play a key role as transducers between biochemistry and shape. In plants the transition between cell division and growth can be shifted by signals such as light (Gendreau et al., 1998[34]) or hormones (Jones et al., 1998[54]). Recently, an analysis of the three-dimensional structures of cytoskeletal motors has uncovered striking similarities with well known signalling proteins of the G-protein family (reviewed in Vale, 1996[132]). This finding points to the exciting possibility that cytoskeletal motors directly couple signal transduction to cell polarity.

Outlook: Molecular Cell Biology, Single Cell, Real Time

In the years to come the search for microtubule-associated proteins, actin-binding proteins and tubulin isotypes is expected to provide new molecular tools for a molecular analysis of microtubular signalling. However, *in vitro* assays such as microtubule-binding assays (Nick et al., 1995[94]) or microtubule-bundling approaches (Cyr and Palevitz, 1989[23]) are not sufficient to develop functional approaches for this problem. On the other hand, transgenic approaches based on over-expression MAPs or tubulin isotypes and/or transformation with the respective antisense constructs are expected either to produce phenotypes that are characterized by extreme pleiotropy or, even worse, the lack of a phenotype (in case of mutual replacement of signal chains or gene silencing). To circumvent this drawback of a transgenic approach, an *in vivo* assay for microtubule function must be developed that ideally should meet the following requirements:

(i) It should work in the natural tissue context to allow for intercellular signalling.
(ii) It should be confined to alteration of individual cells to minimize pleiotropic effects on development.
(iii) It can be manipulated by exogenous signals.
(iv) It can be observed and analyzed over the time that is typical for the signal response, i.e., up to several hours.
(v) It should be possible without extensive wounding in order to avoid artifacts caused by stress responses.
(vi) It should allow for simultaneous observation of at least two cytoskeletal components.

This type of *in vivo* assay is not yet available. Nevertheless, important steps towards such assays have been accomplished during the last years. There are two principal approaches:

(1) Microinjection into intact plant tissue to circumvent the production of protoplasts (the removal of the cell wall alters the behaviour of the cytoskeleton completely and protoplasts are therefore inappropriate models for the intact plant). Microinjection of fluorescent-labelled animal tubulin has been successfully used for the study of microtubular dynamics *in vivo* in dividing (Zhang et al., 1990[148]; Vantard et al., 1990[133]) as well as in elongating cells (Wasteneys et al., 1993[139]; Yuan et al., 1994[145]). Upon microinjection, the labelled neurotubulin is rapidly inserted into the microtubular system of the host cell and seems to participate in the dynamic behaviour of the host cytoskeleton. In epidermal cells, for instance, the reorientation of cortical microtubules could be visualized by this system (Yuan et al., 1994[145]; Wymer and Lloyd, 1996[144]). Similarily, microinjection has been used successfully to visualize actin *in vivo* (Wasteneys et al., 1996[140]; Ren et al., 1997[103]; Cardenas et al., 1998[14]).

(2) Transient transformation of intact cells in the tissue context with fusion constructs between the green fluorescent protein (GFP), and microtubule-binding or actin-binding proteins. Fusions between GFP and MAPs have already been successful in demonstrating the dynamics of microtubules in living animal cells (Kaech et al., 1996[57]), and, by a similar approach, the dynamics of cortical actin filaments can be visualized in living tobacco cells (Freudenreich et al., manuscript submitted).

Plant morphogenesis is characterized by a developmental plasticity that involves the ability to align the direction of cell division and cell expansion with signals that are perceived from the environment. Signalling to the cytoskeleton and intracellular traffic along the cytoskeleton are key events in this process. There remains a lot to be learnt about the plant cytoskeleton, but already it has now become evident that it is organized and governed by different principles from microtubules in animal cells.

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