Protoplasma (2012) 249 (Suppl 2):S81–S94
DOI 10.1007/s00709-011-0339-5

REVIEW ARTICLE

Microtubules and the tax payer

Peter Nick

Received: 30 August 2011 / Accepted: 10 October 2011 / Published online: 18 October 2011

Abstract Plant microtubules have evolved into a versatile tool to link environmental signals into flexible morphogenesis. Cortical microtubules define the axiality of cell expansion by control of cellulose orientation. Plant-specific microtubule structures such as preprophase band and phragmoplast determine symmetry and axiality of cell divisions. In addition, microtubules act as sensors and integrators for stimuli such as mechanic load, gravity, but also osmotic stress, cold and pathogen attack. Many of these functions are specific for plants and involve specific proteins or the recruitment of proteins to new functions. The review aims to ventilate the potential of microtubule-based strategies for biotechnological application by highlighting representative case studies. These include reorientation of cortical microtubules to increase lodging resistance, control of microtubule dynamics to alter the gravity-dependent orientation of leaves, the use of microtubules as sensitive thermometers to improve adaptive cold tolerance of chilling and freezing sensitive plants, the reduction of microtubule treadmilling to inhibit cell-to-cell transport of plant viruses, or the modulation of plant defence genes by pharmacological manipulation of microtubules. The specificity of these responses is controlled by a great variety of specific associated proteins opening a wide field for biotechnological manipulation of plant architecture and stress tolerance.

Keywords Biotechnology · Gravisensing · Microtubules · Plant defence · Rice (*Oryza sativa*)

Handling Editor: Kang Chong

P. Nick (✉)
Molecular Cell Biology, Karlsruhe Institute of Technology,
Kaiserstr. 2,
76128 Karlsruhe, Germany
e-mail: peter.nick@bio.uni-karlsruhe.de

Plant architecture defines yield

Plant architecture has emerged as a new and promising issue in plant biotechnology. In rice, for instance, steeper leaf angles that allow the sunlight to penetrate deeper into the canopy (Zheng et al. 2008), shorter internodes that increase lodging resistance, or suppression of tillers with unfilled grains (Sakamoto and Matsuoka 2004) have been identified as important traits for breeding high yielding cultivars. The rapid progress in functional genomics and morphological modelling has even resulted in numerical models that allow simulation of the impact of genetic traits on plant morphology and expected yield on a quantitative base (Xu et al. 2011). The ultimate goal is plants with "ideal architecture" including reduced shoot length, reduced tiller number, and increased grain weight (Jiao et al. 2010). Microtubules, as central regulators of plant growth and development, provide an important target for biotechnological applications aiming to change plant architecture. However, the potential of microtubules for plant biotechnology, so far, has been only marginally exploited.

Control of plant height has been a major topic in cereal crops, because the resistance of a plant to lodging and windbreak is inversely related to the square of plant height (Oda et al. 1966). This means that a reduction of internode elongation by 50% will reduce lodging to 25%. The yield losses by lodging are considerable: in rice, for example, they are estimated to range up to 40% (Nishiyama 1986). Thus, the agronomic importance of reduced shoot length cannot be overestimated.

The impact of plant architecture includes additional traits beyond the intensively studied dependence of lodging on plant height. The resistance of crop plants to wind depends on the angle between main and branch

roots (Stokes et al. 1995). Moreover, the marketable yield very often depends on the partitioning of biomass. Production of fewer, but larger fruits, tubers or grains facilitates processing, whereas in other cases, such as breeding of potatoes or tulips, the advantage might be on the side of more, but smaller structures. The morphogenetic events involved in the formation of tubers, fruits, or side branches might thus be manipulated to recruit biomass optimally between product quantity, size, and quality, without the need to interfere with source–sink relations or photosynthetic efficiency in general.

Plant microtubules—different arrays, different functions

During the cell cycle, plant microtubules are dynamically reorganised into different arrays that convey different cellular functions. In interphase, microtubules form arrays of parallel bundles oriented perpendicular to the axis of preferential cell expansion (Fig. 1a). These so-called cortical microtubules control the direction of cellulose deposition and reinforce axial cell expansion. Cortical microtubules can change their orientation in response to a

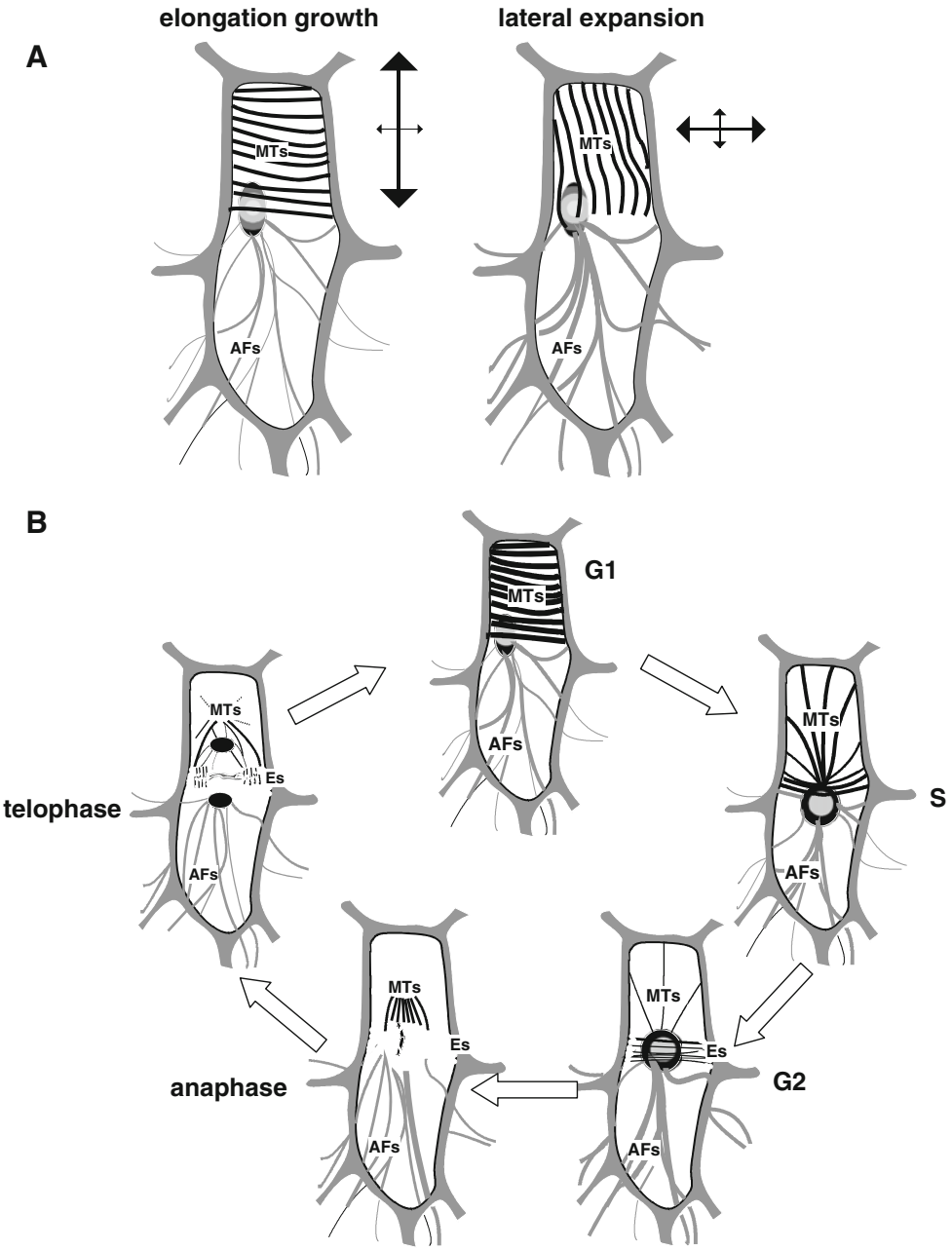

Fig. 1 Cellular functions of plant microtubules. **a** Cortical interphase microtubules (MTs) can reorient and guide the axis of preferential cell expansion through controling the movement of cellulose-synthetising enzyme complexes. Actin filaments (AFs) do not change orientation, but bundle in cells that cease elongation growth. **b** Mitotic microtubule arrays controling axis and symmetry of cell division. Es endosomal belt laid down by the preprophase band

broad range of signals, both exogenous and endogenous. The signal-dependent reorientation is transformed into changed cellulose deposition. This mechanism allows adjustment to the direction in which the cell wall yields to the turgor pressure exerted by the expanding protoplast and eventually alters the proportionality of cell expansion in response to the stimulus (for a recent review see Nick (2008a)). Except for cells that undergo tip growth, the cell wall is formed by apposition of textured cellulose layers to the inner surface of the cell wall. The cellulose-synthetising enzyme complexes are integrated into the membrane by fusion of exocytotic vesicles, and are thought to move within the fluid membrane leaving a "trace" of crystallising cellulose. The movement of the enzyme complex determines cellulose orientation and thus the anisotropy of the cell wall. The direction of this movement, and thus, the mechanical anisotropy of the expanding cell wall are defined by cortical microtubules. The direct contact between cortical microtubules and newly emerged cellulose microfibrils has not only been shown by electron microscopy, but is supported by a wealth of data where signal responses of cell expansion were preceded by a corresponding reorientation of cortical microtubules. As to be expected from a microtubule-based mechanism for cellulose orientation, elimination of cortical microtubules by inhibitors produces a progressive loss of ordered cellulose texture. The resulting loss of axiality causes lateral swelling and bulbous growth. The mode of action of several herbicide classes, such as the phenyl carbamates or the dinitroanilines, is based on the microtubule dependency of cell wall texture.

The striking parallelism between cortical microtubules and newly deposited cellulose microfibrils stimulated two alternative models: according to the original "monorail" model, motor proteins moving along cortical microtubules pull cellulose synthetases (Heath 1974) whereas the "guardrail" model assumes that microtubules induce small protrusions in the plasma membrane that confine the movement of the enzyme complexes that is driven by the crystallising cellulose itself. The guardrail model was stimulated by observations where the cellulose synthase complexes were found "in gap" between adjacent microtubules (Giddings and Staehelin 1988). However, since electron microscopical observation is prone to fixation artifacts, and great luck is required to locate the right section where the topological relation between microtubules and cellulose synthases can be assessed, the question remained unresolved. The situation was further complicated by situations where the orientation of microtubules and cellulose microfibrils differ (for review see Wasteneys (2004)), casting doubt on microtubule-guided cellulose synthesis. However, by in vivo imaging of the cellulose synthase subunit A6 (CESA6) simultaneously with microtubules (Paredez et al. 2006), the movement of CESA6 along cortical microtubules could be unequivocally demonstrated. A second chronical problem of the "monorail" model, how layers of differing microfibril orientation can be generated in polylamellate walls, could be plausibly explained by a rotary movement of groups of microtubules (for review see Lucas and Shaw (2008)). In the meantime, molecular players for the microtubule–"monorail" complex have been identified: a screen for reduced mechanical resistance in *Arabidopsis thaliana* yielded a series of so-called *fragile fiber* mutants that were specifically 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. The phenotype of this mutant suggested that the FRA1/KIF4 motor is a component of the "monorail" complex (Zhong et al. 2002).

In dividing cells, cortical microtubules are replaced by a rapid sequence of diverse arrays: radial microtubules, preprophase band, spindle, and phragmoplast (Fig. 1b). The preparation for mitosis is heralded by a displacement of the nucleus to the cell centre where the prospective cell plate will be formed (for review see Nick (2008a)). Simultaneously, radial microtubules are nucleated at the nuclear rim and merge with the cortical cytoskeleton driving and stabilising nuclear migration (Fig. 1b). Once the nucleus has reached the cell centre, it organises a broad band of microtubules girdling the cell equator. This preprophase band predicts location and orientation of the prospective cell plate that will form after mitosis has been completed. However, the preprophase band disappears when the division spindle forms in a direction perpendicular to the preprophase band. Upon separation of the chromosomes, the microtubular phragmoplast array is organised at the site that had been predicted by the preprophase band. The phragmoplast, a double ring of interdigitating microtubules, targets vesicles containing carbon-hydrate monomers to the periphery of the expanding cell plate. The enigmatic finding that the preprophase band defines orientation and position of phragmoplast and cell plate, although it disappears prior to mitosis proper, has been resolved by the discovery of an endosomic belt laid down adjacent with the preprophase band and persisting through mitosis (Dhonukshe et al. 2005). This endosomic belt is recognised during late anaphase by exploratory microtubules radiating from the cell poles through the dividing cell. Those microtubules that hit the endosomal belt defined by the preprophase band are stabilised over those that fail. The persistent impact of the rather ephemeric preprophase band is emphasised by mutants

in *A. thaliana* where the preprophase band is absent due to a mutation in a phosphatase PP2A regulatory subunit (Camilleri et al. 2002) leading to a completely randomised pattern of cross walls causing the loss of organ axiality underlying the name of these mutants: *tonneau/fass*. It should be mentioned, however, that, during meiosis, the division plane can be controlled in the absence of a PPB (for review see Brown and Lemmon (2007)), suggesting that there exist additional mechanisms of spatial control.

A new function—microtubules as sensors

In addition to their classical role as part of the response machinery that links signaling with cellular morphogenesis, microtubules have emerged as an important element of signaling itself. This sensory function is linked to the high stiffness of microtubules (Gittes et al. 1993). The combination of mechanic rigidity with high dynamics of assembly and disassembly renders microtubules ideal transducers for mechanic integration even across the borders of individual cells (Hardham et al. 1980; Hamant et al. 2008).

Gravity responses provide a case study for this sensory role of microtubules. Terrestrial plants optimise the arrangement of force-bearing elements in space to compensate for mechanic load by gravity. The arrangement of supportive structures is guided by the pattern of mechanical strain. Gravity-sensing requires a transformation of the physical stimulus into a different type of energy that can be perceived by a biological receptor, a process termed susception (Björkman 1988). For instance, the difference in gravitational field strength between the two flanks of a misoriented plant would be certainly far too small to be sensed by any biochemical process. It is generally accepted that gravity is first transformed into mechanical force by acting on heavy particles, so-called statoliths. These statoliths (as well as their accessory structures) themselves are not gravisensitive, but they assist sensing by acting as susceptors.

Since gravity is sensed by individual cells, the maximal energy available for stimulation is the potential energy of the sensing cell. This energy must be focussed upon small areas to exceed thermal noise. Microtubules as rigid, elongate structures would be ideal levers for gravitropic perception. In fact, gravitropism can be blocked by antimicrotubular drugs (for review see Nick (2008b)). Conversely, the dynamics of microtubules is reduced either as a consequence of a mutation or by treatment with taxol results in a strong inhibition of gravitropic responses. A microtubular function in graviperception has also been identified for gravimorphosis of germinating fern spores (Edwards and Roux 1994).

Since microtubules also guide the anisotropic deposition of cellulose in the cell wall, it is not trivial to discriminate their function in gravity-sensing from their role in downstream events of the gravity response. In fact, a gravitropically induced reorientation of cortical microtubules has been observed for both shoot (Nick et al. 1991), and root gravitropism (Blancaflor and Hasenstein 1993) and could be also demonstrated in vivo by microinjection of fluorescent tubulin (Himmelspach et al. 1999). This led to a model where gravitropic stimulation depleted auxin from the upper flank, causing microtubular orientation culminating in differential growth. To discriminate between the sensory function of microtubules and their role in executing gravitropic bending, the lateral transport of auxin was used as response upstream of differential growth (Godbolé et al. 2000; Gutjahr and Nick 2006) to demonstrate a sensory microtubule function that required microtubule dynamics. Mechanosensing by microtubules has been also demonstrated using the alignment of cell divisions parallel with the force vector after mild centrifugation (Wymer et al. 1996).

As to be expected from their role in mechanosensitivity, microtubules also participate in osmoadaptation. Osmotic stress causes massive microtubule bundling (Komis et al. 2002). These so called macrotubules have been shown to be necessary for osmotic adaptation (Komis et al. 2006). Mechanosensing not only allows detection of physical force, but can be used to sense numerous other stimuli. For instance, reductions in membrane fluidity can be perceived by microtubules as signal for low temperature. In fact, pharmacological manipulation of microtubules can be used to control cold hardiness (Kerr and Carter 1990; Abdrakhamanova et al. 2003). Several events of plant defence including nuclear migration, cytoplasmic reorganization, formation of reactive oxygen species, and the induction of several defence-related genes can be triggered by localised mechanostimulation (Gus-Mayer et al. 1998). As to be expected from a mechanosensory function of microtubules, the cellular competence to induce defence genes in response to an elicitor correlates with microtubule stability. Conversely, it is even possible to induce certain defence genes in the absence of elicitors by mere pharmacological manipulation of microtubules (Qiao et al. 2010).

The molecular details of this sensory role of microtubules are not fully understood. Mechanosensing is generally explained by two paradigms: stretching of proteins will change their conformation and create new binding sites for the recruitment of associated proteins (for review see Janmey and Weitz (2004)). Alternatively, forces from the lipid bilayer can be directly detected by mechanosensitive ion channels. Such channels will open when the plasma membrane is deformed, or when the channel is pulled by a tether (for review see Kung (2005)). In plants, both mechanisms seem to act in concert, as components of

a so-called 'plasmalemmal reticulum' (for review see Pickard (2008)). This network is thought to focus mechanic force upon stretch-activating membrane channels, and simultaneously might transduce forces into conformational changes that can result in differential decoration with associated proteins triggering signaling. Microtubules could act as accessory machinery with mechanosensitive channels that focus mechanic stress, similar to the set-up found in touch-sensitive cells of *Caenorhabditis* (for review see Nick (2011)). This would be a classical susceptor function. However, microtubules themselves might be mechanosensors, since they are charged by considerable mechanic tension. This tension is caused by transition of the tubulin dimers into a kinked conformation when the GTP residue of newly inserted dimers is progressively dephosphorylated into GDP with increasing distance of the dimer from the growing tip (Akhmanova and Steinmetz 2008). Microtubule plus-end tracking proteins (+TIP proteins) form complexes that counteract this innate tension and thus stabilise the growing microtubules. One of these proteins, EB1, binds to microtubule plus ends at the seam that joins the tubulin protofilaments (Sandblad et al. 2006) and is therefore a good candidate for a conformational mechanosensor. During microtubule catastrophe, the protofilaments bend outwards, which means that they have to be actively tied together in order to sustain microtubule growth. The + TIP complex in general, and EB1 in particular are therefore subject to mechanic tension and must be considered as primary targets for mechanic strains on microtubules. In fact, *Arabidopsis* mutants in members of the EB1 family have been found to be touch-insensitive (Bisgrove et al. 2008). Imaging of tobacco protoplasts expressing fluorescently tagged cytoskeletal markers by Total Internal Reflection Microscopy (TIRF) shows that the microtubules adjacent to the membrane emanate in a star-like manner from specific focal points that are also subtended by actin filaments (Hohenberger et al. 2011). It remains to be elucidated whether these foci contain ion channels that might be rendered mechanosensitive by a microtubule-based accessory system.

A second new function—microtubules as cell cycle regulators

Microtubules build up the division spindle, and this function has been traditionally used as target for pharmacological manipulation of the cell cycle. For instance, the widely used tobacco cell line BY-2 has emerged as the classical model for the plant cell cycle because here cells can be synchronised by reversible elimination of spindle microtubules using the herbicide propyzamide (for review see Nagata et al. (1992)). In modern breeding, somatic embryogenesis from haploid male gametophytes is induced by spindle blockers such as colchicin (Twell et al. 1998).

Important players of mitosis microtubules participate in the execution of the cell cycle. However, more recently, they have been found to mediate also the regulation of the cell cycle. Very recently, plant homologues of the retinoblastoma protein (an important cell cycle regulator in mammalian cells) have been found to interact with the microtubular cytoskeleton and to control stem cell fate, lateral branching and cell differentiation (Borghi et al. 2010). In addition, the timing of cell division depends on cytoskeleton-dependent changes of cellular architecture. Mechanic strains are integrated by the cytoskeletal arrays that tether the nucleus. During the G2 phase, the nucleus migrates in non-continuous manner until it has reached the cell centre, corresponding to the mechanic equilibrium. If this movement is delayed by either overexpression or knockout of the plant-specific kinesin motor KCH1 (Frey et al. 2010), a central component of cytoskeletal tensegrity, the transition from G2 into M phase is delayed for up to 1 day. This shows that the correct position of the nucleus is used as input for central checkpoints during the cell cycle, the G2/M transition. In this context, microtubules and their associated proteins act as cell cycle regulators, and this function is clearly distinct from their classical role for the execution of mitosis.

Microtubules and the tax payer 1: green revolution architecture

As pointed out above, the impact of Green Revolution was intimately linked with changes in plant architecture. Here, internode elongation and leaf angles were the central factors defining yield. Both can be manipulated through the microtubular cytoskeleton (Fig. 2a, b). Losses by windbreak and lodging are inversely related to plant height by the relation:

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

with W=fresh weight, M=bending momentum at breaking, L=shoot length and w=dry weight of the shoot (Oda et al. 1966). 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. Traditionally, lodging resistance in graminean crops has been reduced either chemically by growth regulators such as chlormequat chloride or ethephone (Luib and

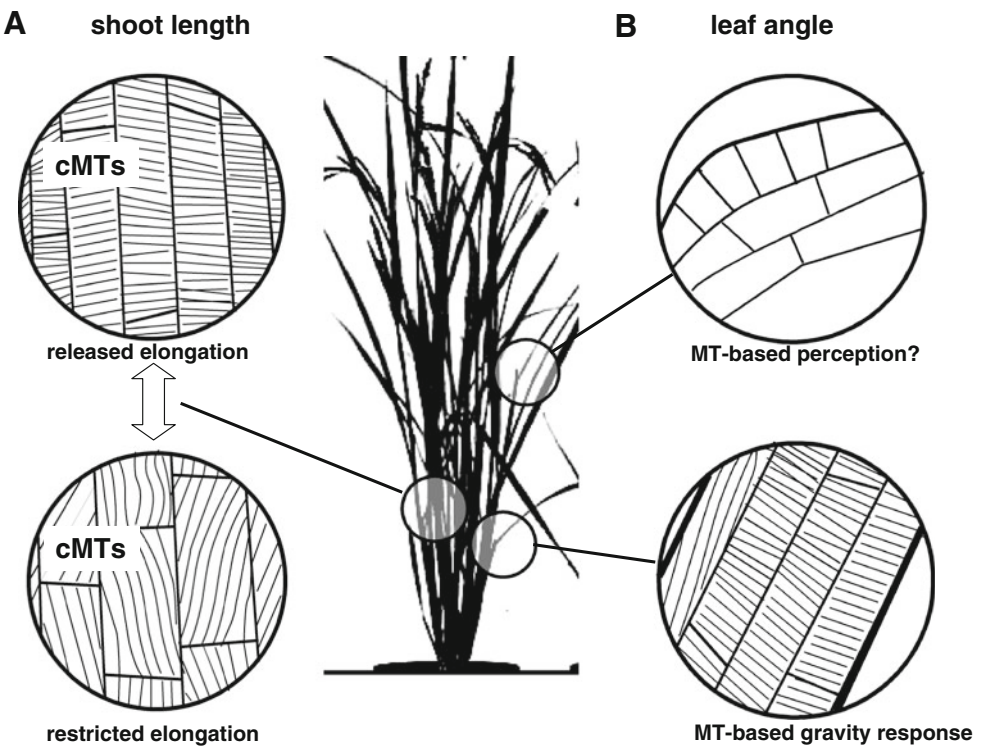

Fig. 2 Control of internode elongation (**a**) and leaf angle (**b**) as central factors defining yield by the microtubular cytoskeleton. a Longitudinal microtubules restrict cell elongation, and hormone-induced formation of transverse microtubule arrays releases this constraint resulting in stimulated cell elongation and increased lodging. b Gravity-dependent reorientation of microtubules in the pulvinus and gravity-dependent control of cell division in the leaf collar represent two pivotal mechanisms to control leaf orientation

Schott 1990) or genetically by using semi-dwarf varieties (Wang and Li 2006). These semi-dwarf varieties were central for the success of the Green Revolution and can be due to deficiencies in gibberellin synthesis (such as the green revolution rice *sd-1*) or to constitutive repression of gibberellin-responsive genes (as in case of the green revolution wheat *Rht-B1/Rht-D1*). Reduced plant height is a desirable trait in other crops as well (Luib and Schott 1990). In rapeseed, shorter internodes improve yield, probably due to an increased penetration of light into the canopy. In cotton, compact plant posture improves penetration of insecticides to the lower parts of the plants, and in fruit trees, reduced plant height is a prerequisite for mechanical picking.

The specific environment generated by modern agriculture such as high nutrient influx and high canopy densities even stimulate internode elongation. For instance, plants can sense their neighbours through subtle changes in the ratio between red and far red light through the phytochrome photoreceptor system (Smith 1981), and they respond to this spectral change in light quality by stimulated cell elongation. This so-called shade avoidance response has the function to protect these plants against overgrowth by neighbouring plants, but at the same time, it will render them prone to lodging. The increased proportion of far red light (reflected from neighbouring plants in a canopy) has been found to trigger auxin synthesis through a novel tryptophan-dependent pathway (Tao et al. 2008). Increased levels of auxin will sustain a transverse orientation of cortical microtubules in the outer epidermis (Nick et al. 1990) where shape of shoot morphogenesis is controlled. The transverse microtubules cause a transverse orientation of the inner cellulosic layer of secondary walls and thus reinforce cell elongation (Fig. 2a).

Can the desirable semi-dwarf trait be achieved through altering microtubular orientation? In fact, a screen for rice mutants that were resistant against ethyl-N-phenylcarbamate (a traditional potato anti-sprouting agent acting on plant microtubules) yielded a mutant where the microtubular reorientation in response to auxin was interrupted by mutation (Nick et al. 1994). In this mutant, cortical microtubules were arranged in oblique or even longitudinal arrays and were uncoupled from auxin. As to be expected, this resulted in reduced cell length and a semi-dwarf phenotype of leaves and culms. Recently, a similar observation was made with respect to gibberellins, a second central regulator of elongation growth in rice. Here, a mutant termed *gibberellin-deficient dwarf 1* (*gdd1*) was isolated from a T-DNA mutagenesis screen. The mutant could be completely rescued by exogenous gibberellin (Li et al. 2011), and turned out to be affected in a kinesin-like protein (BRITTLE CULM12) that controls the formation of secondary cell walls (Zhang et al. 2010). However, simultaneously, this protein acts as transcriptional regulator of ent-kaurene oxidase, a key enzyme of gibberellin synthesis. Again, the orientation of cortical microtubules was altered into oblique and longitudinal arrays accounting for the observed semi-dwarf phenotype.

Leaf inclination determines how far light can penetrate into a canopy and therefore limits the maximal density of

planting. In the Poaceae, leaf inclination is defined by two pivotal points, one at the ligule defining the angle between sheath and blade, the other at the base of the leaf sheath in the pulvinus (Fig. 2b). The leaf-sheath pulvinus can respond to environmental signals such as canopy density. With increasing density, the leaf sheaths are more erect (Gibson et al. 1992) due to differential cell expansion. The apical region of the upper flank of the leaf-sheath pulvinus does not elongate in contrast to the remaining regions, and this asymmetry is enhanced by antimicrotubular herbicides such as isopropylphenylcarbamate or dichlorobenzonitril suggesting that the movement is driven by gravity-triggered microtubule orientation (Dayandanan and Kaufman 1984). A second site of gravity adjustment resides in the collar region where the leaf sheath borders to the leaf blade. The angle between sheath and blade can be actively regulated by cell divisions in the adaxial epidermis of the collar (Zhao 2010). This division is under control of brassinosteroids—a classical bioassay for brassinosteroids measuring the inclination of the leaf lamina in rice makes use of this phenomenon (Takeno and Pharis 1982). Brassinosteroid synthesis mutants in rice with steeper leaf blades have been shown to generate higher yields even in the absence of fertiliser (Sakamoto et al. 2006). Unlike the leaf sheath pulvinus, the leaf blade collar is not exhibiting a gravitropic response. However, it is able to sense gravity and to respond by a preformed gravinastic movement (Maeda 1965). Again, microtubules seem to be involved because in rice mutants obtained from a screen for reduced microtubule dynamics, the inclination of the leaf blade is significantly increased resulting in a fan-like appearance of the plant (Nick 2000). Interestingly, in so-called *lazy* mutants where gravitropic responses are impaired, inversion of plants causes a curious stimulation of leaf blade growth and elevated levels of gibberellins (Abe et al. 1998) indicating a link between microtubule-dependent gravity sensing and gibberellin synthesis.

Microtubules and the tax payer 2: cold tolerance

Microtubules disassemble in the cold and this has been found to limit the cold tolerance of a species. The cold sensitivity of microtubules is subject to evolutionary change. Whereas mammalian microtubules disassemble already at temperatures below +20°C, the microtubules from poikilothermic animals remain intact far below that temperature (Modig et al. 1994). In plants, the cold stability of microtubules is generally more pronounced as compared to animals reflecting the higher developmental plasticity. However, the critical temperature where microtubule disassembly occurs varies between different plant species, which is correlated with differences in chilling sensitivity (Jian et al. 1989). The close correlation between microtubular cold sensitivity and general chilling sensitivity is supported by the observation that abscisic acid, a hormonal inducer of cold hardiness (Irving 1969), can stabilise cortical microtubules against low temperature (Sakiyama and Shibaoka 1990; Wang and Nick 2001). In fact, when microtubules were manipulated pharmacologically, this was accompanied by changes in cold hardiness (Kerr and Carter 1990). Tobacco mutants where microtubules are more cold stable due to expression of an activation tag show cold-resistant leaf expansion (Ahad et al. 2003). Conversely, destabilisation of microtubules by assembly blockers such as colchicine or podophyllotoxin increased the chilling sensitivity of cotton seedlings, and this effect could be rescued by addition of abscisic acid (Rikin et al. 1980). Gibberellin, a hormone that has been shown in several species to reduce cold hardiness (Rikin et al. 1975; Irving and Lanphear 1968), renders cortical microtubules more cold susceptible (Akashi and Shibaoka 1987).

Cold-resistant species are able to sense low temperature and to respond by adaptation. It is possible to increase the cold resistance of an otherwise chilling-sensitive species by precultivation at moderately cool temperature. Cold sensing is generally ascribed to a reduced fluidity of membranes that will alter the activity of ion channels or the balance of metabolites (Lyons 1973). For instance, overexpression of desaturases reducing membrane fluidity has been shown repeatedly to modify chilling sensitivity in plants (Murata et al. 1992). Cold hardening can be detected on the level of microtubules as well. Microtubules of cold-acclimated cells cope better with a freezing shock (spinach mesophyll: Bartolo and Carter 1991a; rye roots: Pihakaski-Maunsbach and Puhakainen 1995; wheat roots: Wang and Nick 2001; Abdrakhamanova et al. 2003). The development of acclimation was suppressed by taxol (Kerr and Carter 1990; Bartolo and Carter 1991b). This indicates that microtubules have to disassemble to a certain degree in order to trigger cold hardening.

What might be the mechanism for this microtubule-based thermometer function? The primary signal for cold perception consists of increased membrane rigidity (Los and Murata 2004). For instance, the input of low temperature can be mimicked by chemical compounds that increase rigidity, such as demethylsulfoxide, whereas benzyl alcohol, a compound that increases membrane fluidity, can block cold signalling (Sangwan et al. 2001). The fluidity change triggers a spike of intracellular calcium as shown in classical experiments with tobacco plants expressing the bioluminescent aequorin reporter (Knight et al. 1991). Pharmacological data (Monroy et al. 1993) confirmed that this calcium spike is not only a byproduct of the cold response but necessary to trigger cold acclimation. Using a cold-responsive reporter system it could be demonstrated that disassembly of micro-

tubules by oryzalin or treatment with the calcium ionophore A23187 could mimick the effect of low temperature, whereas the calcium channel inhibitor gadolinium or suppression of microtubule disassembly by taxol prevented the activation of this promotor by low temperature (Sangwan et al. 2001). These data favour a model where microtubules limit the permeability of mechanosensitive calcium channels that are triggered by membrane rigidification (Fig. 3a).

As to be expected from this model, the activity of cold-triggered calcium channels is negatively modulated by pharmacological stabilisation of microtubules but amplified by microtubule elimination (Mazars et al. 1997). The resulting signal cascade will activate cold-hardening as an adaptive response to cold stress. Interestingly, microtubules will be rendered cold stable as a consequence of this cold hardening (Pihakaski-Maunsbach and Puhakainen 1995; Abdrakhamanova et al. 2003), which in turn, should result in a reduced activity of the calcium channels that respond to membrane rigidification. Thus, microtubules would not only mediate cold sensing with high sensitivity but, in addition, trigger adaptation by downregulating sensitivity upon prolonged stimulation, a key requirement for any biological sensory process.

This microtubule-dependent thermometer function is of agronomical relevance. To demonstrate this, cold hardening was followed in three cultivars of winter wheat that differed in freezing tolerance (Abdrakhamanova et al. 2003). During cultivation at 4°C, the growth rate of roots recovered progressively as a manifestation of cold hardening. In parallel, the roots acquired progressive resistance to a challenging freezing shock (−7°C) that would impair growth irreversibly in non-acclimated roots. When microtubules were monitored during cold hardening, a rapid but transient partial disassembly was observed in cultivars that were freezing-tolerant but not in a cultivar that was freezing-sensitive. However, transient treatment of seedlings with the antimicrotubular herbicide pronamide was able to induce freezing tolerance in the sensitive cultivar. This demonstrates that a transient, partial disassembly of microtubules is necessary and suffcient to trigger cold hardening.

Microtubules and the tax payer 3: viral resistance

Viruses exploit functions of the host for their own propagation cycle. The cytoskeleton as a central element of motility represents an ideal target for this viral usurpation. In fact, many animal viruses spread through interaction with host microtubules (Greber and Way 2006; Leopold and Pfister 2006; Radtke et al. 2006). The cellular function usurped here might be motor-driven transport of mRNA (for a recent review, see Martin and Ephrussi (2009)). Signalling through RNA transport is common in plants as well (for review see Lucas et al. (2001)). Actually, the first investigated example for transmissible signals that later turned out to be RNA was from plants: the transport of morphogenetic signals from the nucleus into the hood-forming stalk in the green alga *Acetabularia* (Hämmerling 1934). Based on the evolutionary conservation of actin and microtubules, plant viruses are predicted to use the cytoskeleton for propagation in a way similar to that observed for animal hosts. Viral infection usually initiates from only few cells from where the virus has to move to adjacent cells and, eventually, through the rest of the plant. Viral transport has been most intensively studied in the case of tobacco mosaic virus (TMV) moving by virtue of a virus-encoded movement protein (TMV-MP). The complex of viral RNA and TMV-MP assembles near the endoplasmic reticulum, probably anchored to microtubules, and is then translocated to the plasmodesmata by a mechanism dependent on the ER and microtubules (for a recent review see Heinlein (2008)).

The interaction of the TMV-MP with microtubules is based on molecular mimicry of the TMV-MP with a motif in α-tubulin involved in lateral interactions of microtubule protofilaments. Transmission of TMV-MP viral RNA has been shown to be closely linked to the ability of MP to interact with microtubules (Boyko et al. 2000). The microtubule-dependent transport might be caused by two possible mechanisms: either microtubule might serve as tracks for translocation driven by molecular motors (Heinlein et al. 1995) or the viral particles are hooked up to the treadmilling microtubule and released at their destination point (Sambade et al. 2008).

In order to discriminate between motor-driven and assembly-driven movement, viral movement was assessed in tobacco mutants where microtubular turnover was reduced. These plants had been generated by T-DNA activation tagging and selected for their tolerance to ethyl-N-phenylcarbamate (EPC), a traditional inhibitor of potato sprouting that sequesters tubulin dimers and therefore eliminates microtubules depending on their innate turnover (Ahad et al. 2003). Principally, resistance of a mutant to antimicrotubular compounds could be caused by altered binding sites as it has been found for mutants of goosegrass (*Eleusine indica*) resistant to microtubule-eliminating dinitroaniline herbicides (Anthony et al. 1998). The binding site of EPC has been located to the carboxyterminus of α-tubulin (Wiesler et al. 2002). However, since in activation tagging, any insertion of the tag into an exon would result in a knockdown of the gene function, the tolerance of these plants to EPC is rather expected to be caused by reduced microtubular dynamics (Ahad et al. 2003). If viral movement is brought about by a polymerisation-dependent mechanism, it should be impaired in these mutants.

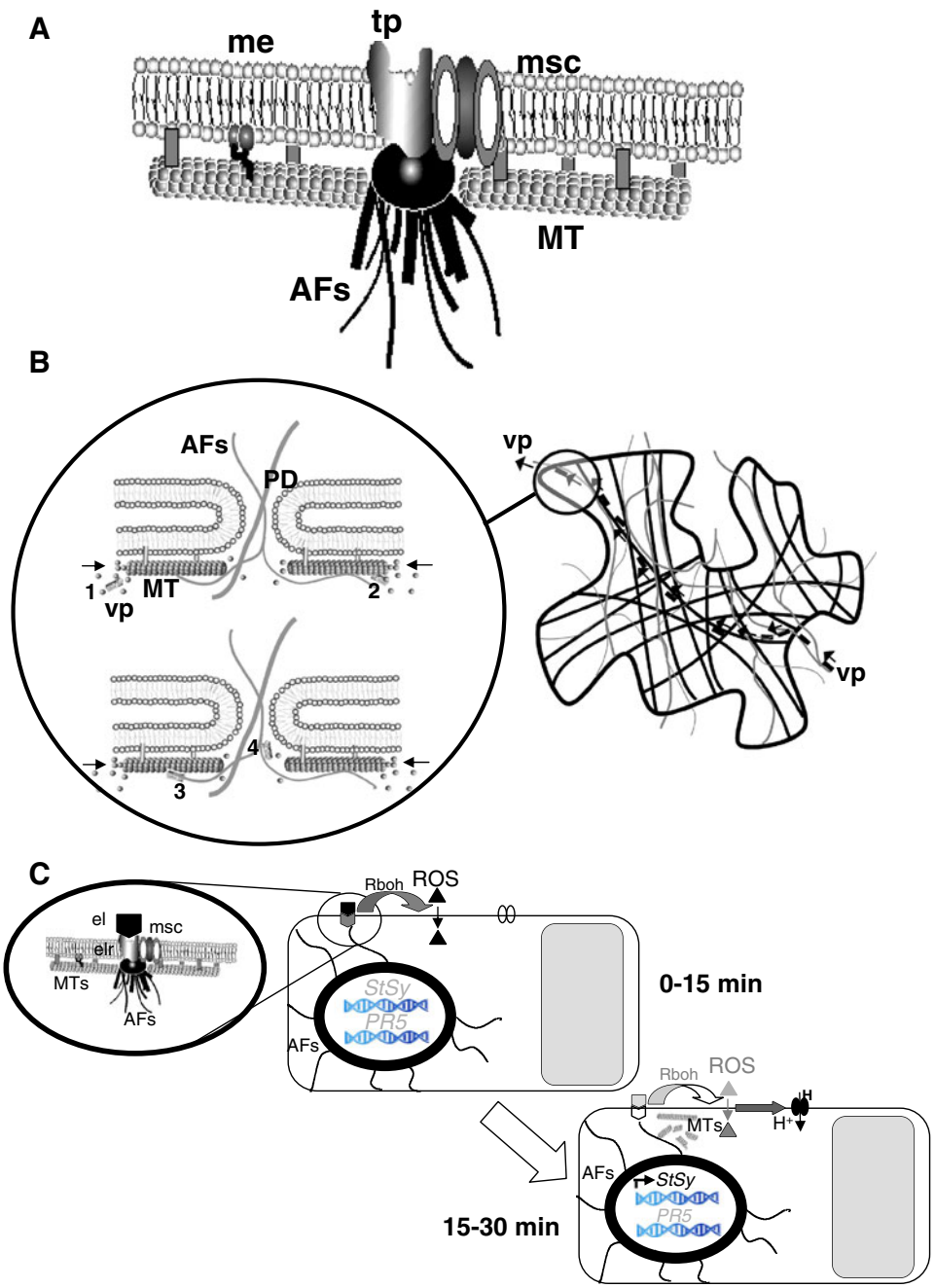

Fig. 3 Microtubules as targets for biotechnological manipulation. **a** Microtubule-based gating of mechanosensitive ion channels (msc) as target to engineer cold resistance. *MTs* microtubules, *AFs* actin filaments, *me* plasma membrane, *tp* (integrin-analogous) transmembrane protein. **b** Microtubule-treadmilling as a target to engineer viral resistance. Viral particles (vp) travel long distance along microtubules (MTs, *black*), but are reloaded onto actin filaments (AFs, *grey*) to cross the cell borders; the *arrows* indicate the track of a particle through a pavement cell, detail shows the transport of a viral particle across the plasmodesmon (PD), numbers show different stages of viral transport across the plasmodesmon (not drawn to scale), and the *arrows* indicate microtubular dynamics. **c** Microtubule-dependent sensing of biotic attack as a target to engineer plant defence. Perception is triggered by binding of an elicitor (el) to a membrane-bound receptor (elr) that interacts with a mechanosensitive ion channel (msc) and submembraneous microtubules (MTs) and actin filaments (AFs). The binding activates the NADPH oxidase Rboh leading to apoplastic reactive oxygen species (ROS), which can permeate into the cytoplasm. A primary signal is generated through disruption of the microtubules culminating in the activation of defence-related genes, such as the phytoalexin forming stilbene synthases (StSy). In parallel, the ROS activate a proton influx causing the alkalinisation of the medium used as rapid assay for the induction of defence

In one of these mutants, *ATER2* (for activation-tagged EPC resistance), the activation tag was inserted into an intron of *CYP87A3*, a gene encoding a cytochrome P450 found to be induced by EPC. The insertion by the tag

resulted in a ten-fold upregulation of this transcript in the *ATER2* mutant. The biological function of the tagged gene is not fully understood, but the rice homologue of CYP87A3 had been isolated originally by fluorescent differential display based on a rice mutant that had been recovered from a screen for EPC resistance (Wang and Nick 1998). This gene might act as a regulator for synthesis or activity of microtubule-associated proteins that control the dynamic equilibrium between assembly and disassembly of microtubules. Microtubule lifetimes are increased in the *ATER2* mutant as evident from increased resistance of growth to EPC and oryzalin, increased ratios of detyrosinated tubulin monitoring elevated activity of tubulinyl-tyrosine decarboxylase (an enzyme that binds preferentially to assembled microtubules), and reduced movement of the microtubule plus end marker EB1 (Ouko et al. 2010). Based on the evidence for reduced microtubule turnover, it was possible to use *ATER2* as a tool to assess the role of reduced microtubule treadmilling in the movement of TMV using MP-GFP-tagged viruses.The cell-to-cell movement was reduced in the *ATER2* mutant by about 25%. This reduced cell-to-cell movement was accompanied by a strongly reduced expression of systemic disease symptoms. Thus, although the reduced microtubule turnover did not prevent viral infection per se, it did impair cell-to-cell movement (Fig. 3b).

What are the consequences of this slower viral spread on the level of the whole plant? The SR1 line used as background for the mutants is susceptible to TMV because it lacks a functional N resistance gene (Dinesh-Kumar et al. 2000). Following TMV infection, the virus is capable of replication and systemic spread culminating in terminal necrosis as final stage (not caused by a systemic hypersensitive response). This necrotic response was strongly reduced in the *ATER2* mutant as compared to the wild type (Ouko et al. 2010). Thus, a partial viral resistance could be engineered into tobacco by genetic engineering of a regulatory factor for microtubule dynamics. Since other plant viruses such as the grapevine fanleaf virus (Laporte et al. 2003) employ similar mechanisms for movement, the genetic or pharmacological manipulation of microtubule dynamics might be an efficient strategy to control viral spread in other crop plants as well.

Microtubules and the tax payer 4: switches for plant defence

The interaction of pathogens and their hosts is subject to an evolutionary race of arms where the pathogens developed various strategies to circumvent or suppress defence responses of the host, whereas the host developed various strategies to sense and attack the invading pathogen or its effector molecules. For example, cell wall papillae form around sites of attempted pathogen penetration. The formation of these papillae is preceded by a reorganisation of the cytoskeleton causing redistribution of vesicle traffick and cytoplasmic aggregation towards the penetration site (for reviews see Takemoto and Hardham (2004), Kobayashi and Kobayashi (2008)), and a somewhat slower migration of the nucleus (for review see Schmelzer (2002)). By localised mechanic stimulation of parsley cells, it was possible to partially mimic an attack by *Phytophthora sojae*, and to induce several aspects of a non-host resistance including nuclear migration, cytoplasmic reorganization, formation of reactive oxygen species, and the induction of several defence-related genes (Gus-Mayer et al. 1998). In contrast, localised application of the corresponding elicitor (pep-13) failed to induce the morphological changes, although it induced the full set of defence-related genes and the formation of reactive oxygen species. Interestingly, the elicitor completely inhibited cytoplasmic aggregation and nuclear migration in response to the mechanic stimulus. Since pep-13 induces in this system the activity of a mechanosensitive calcium channel (Zimmermann et al. 1997), it seems that chemical and mechanical signaling converge during the cytoskeletal response to pathogen attack. Neither the mechanical stimulus nor the elicitor nor their combination was able to induce hypersensitive cell death in these experiments leading the authors to conclude that additional chemical signals are required to obtain the complete pathogen response. This suggests an interaction between microtubules and mechanosensitive ion channels that are important for the induction of defence (Fig. 3c).

If this link exists, it should be possible to manipulate defence responses through microtubules. This idea was tested using two cell lines from *Vitis* that differ in their microtubular dynamics. The line *Vitis vinifera* cv. 'Pinot Noir' is susceptible to pathogens such as *Plasmopara viticola* and *Erysiphe necator*, whereas *Vitis rupestris* efficiently copes with infection by these pathogens (Jürges et al. 2009). Using this system, defence responses were triggered using the elicitor Harpin, a powerful so-called type III effector from *Erwinia amylovora*, the bacterium that causes fire blight of Rosaceae (Wei et al. 1992a) and can trigger a hypersensitive response in nonhost plants (Wei et al. 1992b). Both cell lines responded to the elicitor by a rapid alkalinisation of the medium (generally used as faithful reporter to monitor plant defence, Felix et al. 1993), and the activation of defence-related genes. However, these defence responses were weaker and less sensitive in the pathogen-susceptible cv. 'Pinot Noir' as compared to the disease resistant *V. rupestris* (Qiao et al. 2010). The differences in defence activity were correlated with a difference of microtubule dynamics. Microtubules were more dynamic in 'Pinot Noir' as evident from a higher

fraction of tyrosinylated α-tubulin, and an increased sensitivity to oryzalin. However, in response to the elicitor, the cortical microtubules were disrupted, most pronounced in *V. rupestris*. This correlation indicates that stable microtubules act as negative regulators of defence signaling and are disrupted in response to the elicitor. If this is more than a correlation, pharmacological manipulation of microtubules should be able to activate defence genes. In fact, using resveratrol synthase and stilbene synthase as key genes of phytoalexin induction, it could be shown that pharmacological manipulation of microtubules could induce gene expression in the absence of elicitor. Similar to cold acclimation, it is a sensory role of microtubule that provides a promising target for biotechnological manipulation of plant defence.

Outlook: new tools for cytoskeletal manipulation

As illustrated by these examples, microtubules and their accessory proteins provide attractive targets to optimise plant architecture and stress tolerance. What tools and approaches can be used to manipulate microtubules? Genetic engineering of tubulins has been used to generate tolerance to dinitroanilines (Anthony et al. 1998) or to aryl carbamates (Nick et al. 2003; Ahad et al. 2003) that bind to specific motives on α-tubulin. To reduce unwanted side effects caused by interference of the mutated tubulin with essential cellular functions, the mutated tubulins might be placed under the specific and versatile regulatory features of innate tubulin promotors (Breviario and Nick 2000). Nevertheless, the relative evolutionary conservation of tubulins has led to a highly efficient design of protein structure that leaves only limited flexibility for engineering without impairing the core functions of the protein. So, genetic engineering would either require inducible promotors or should be directed to those proteins that have specifically evolved in higher plants and fulfill more confined tasks. Among those, the highly diverse and apparently functionally flexible kinesins are certainly key targets as exemplified by the newly discovered gdd1-kinesin (Li et al. 2011).

Chemical engineering using microtubule-directed compounds might provide a second route and has been traditionally used for growth control as potato sprouting suppressors or as herbicides (for review see Vaughn (2000)). Screening of chemical libraries has identified new promising compounds such as cobtorin that specifically interferes with the microtubule guidance of cellulose synthesis (Yoneda et al. 2007). A new promising field of genetic engineering is designed peptides that can be tailored to interfere with specific targets in the host cells. A drawback of chemical engineering through peptides is the difficulty of membrane passage. So-called cell-penetrating peptides (CPPs) provide an attractive tool to overcome this bottleneck. They share common structural features such as short size and a positive charge usually stemming from multiple lysine or arginine residues (Su et al. 2009). Several CPPs such as transportan, pVEC, arginine-rich peptides or BP100 have already been introduced into plant cells (for instance, Mizuno et al. 2009), but without the attempt to introduce a functional cargo. Recently, we were able to fuse the novel actin-binding peptide Lifeact with BP100. This fusion was imported rapidly, efficiently, and specifically into tobacco BY-2 cells that successfully labelled the phragmosomal actin cables that tether the nucleus in the cell centre (Eggenberger et al. 2011). This approach could now be adapted to plant tubulin, for instance, to outcompete the interaction of specific accessory proteins with their recognition sites on the microtubule.

Acknowledgements The content of this article were presented at the Sino-German Symposium "Applied Plant Cell Biology" funded by the SinoGerman Center for the Promotion of Science (GZ628).

Conflict of interest The authors declare that they have no conflict of interest.

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 K, Takahashi H, Suge H (1998) Gravimorphism in rice and barley: promotion of leaf elongation by vertical inversion in agravitropically growing plants. J Plant Res 111:523–530

Ahad A, Wolf J, Nick P (2003) Activation-tagged tobacco mutants that are tolerant to antimicrotubular herbicides are cross-resistant to chilling stress. Transgenic Res 12:615–629

Akashi T, Shibaoka H (1987) Effects of gibberellin on the arrangement and the cold stability of cortical microtubules in epidermal cells of pea internodes. Plant Cell Physiol 28:339–348

Akhmanova A, Steinmetz MO (2008) Tracking the ends: a dynamic protein network controls the fate of microtubule tips. Nat Rev Mol Cell Biol 9:309–322

Anthony RG, Waldin TR, Ray JA, Bright SWJ, Hussey PJ (1998) Herbicide resistance caused by spontaneous mutation of the cytoskeletal protein tubulin. Nature 393:260–263

Bartolo ME, Carter JV (1991a) Microtubules in the mesophyll cells of nonacclimated and cold-acclimated spinach. Plant Physiol 97:175–181

Bartolo ME, Carter JV (1991b) Effect of microtubule stabilization on the freezing tolerance of mesophyll cells of spinach. Plant Physiol 97:182–187

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

Bisgrove SR, Lee YRJ, Liu B, Peters NT, Kropf DL (2008) The microtubule plus-end binding protein EB1 functions in root responses to touch and gravity signals in *Arabidopsis*. Plant Cell 20:396–410

Björkman T (1988) Perception of gravity by plants. Adv Bot Res 15:1–4

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

Borghi L, Gutzat R, Futterer J, Laizet Y, Hennig L, Gruissem W (2010) *Arabidopsis* RETINOBLASTOMA-RELATED is required for stem cell maintenance, cell differentiation, and lateral organ production. Plant Cell 22:1792–1811

Boyko V, Ferralli J, Heinlein M (2000) Cell-to-cell movement of TMV RNA is temperature-dependent and corresponds to the association of movement protein with microtubules. Plant J 22:315–325

Breviario D, Nick P (2000) Plant tubulins: a melting pot for basic questions and promising applications. Transgenic Res 9:383–393

Brown RC, Lemmon BE (2007) The pleiomorphic plant MTOC: an evolutionary perspective. J Int Plant Biol 49:1142–1153

Camilleri C, Azimzadeh J, Pastuglia M, Bellini C, Grandjean O, Bouchez D (2002) The *Arabidopsis TONNEAU2* gene encodes a putative novel PP2A regulatory subunit essential for the control of cortical cytoskeleton. Plant Cell 14:833–845

Dayandanan P, Kaufman PB (1984) Analysis and significance of gravity-induced asymmetric growth in the grass leaf-sheath pulvinus. Ann Bot 53:29–44

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

Dinesh-Kumar SP, Tham WH, Baker BJ (2000) Structure-function analysis of the tobacco mosaic virus resistance gene N. Proc Natl Acad Sci U S A 97:14789–14794

Edwards ES, Roux SJ (1994) Limited period of graviresponsiveness in germinating spores of *Ceratopteris richardii*. Planta 195:150–152

Eggenberger K, Mink C, Wadhwani P, Ulrich AS, Nick P (2011) Using the peptide BP100 as a cell penetrating tool for chemical engineering of actin filaments within living plant cells. ChemBioChem 12:132–137

Felix G, Regenass M, Boller T (1993) Specific perception of subnanomolar concentrations of chitin fragments by tomato cells: induction of extracellular alkalinization, changes in protein phosphorylation, and establishment of a refractory state. Plant J 4:307–316

Frey N, Klotz J, Nick P (2010) A kinesin with calponin-homology domain is involved in premitotic nuclear migration. J Exp Bot 61:3423–3437

Gibson D, Casal JJ, Deregibus A (1992) The effects of plant density on shoot and leaf lamina angles in *Lolium multiflorum* and *Paspalum dilatatum*. Ann Bot 70:69–73

Giddings TH, Staehelin LA (1988) Spatial relationship between microtubules and plasmamembrane rosettes during the deposition of primary wall microfibrils in *Closterium spec*. Planta 173:22–30

Gittes F, Mickey B, Nettleton J, Howard J (1993) Flexual rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. J Cell Biol 120:923–934

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

Greber UF, Way M (2006) A superhighway to virus infection. Cell 124:741–754

Gus-Mayer S, Naton B, Hahlbrock K, Schmelzer E (1998) Local mechanical stimulation induces components of the pathogen defense response in parsley. Proc Natl Acad Sci U S A 95:8398–8403

Gutjahr C, Nick P (2006) Acrylamide inhibits gravitropism and destroys microtubules in rice coleoptiles. Protoplasma 227:211–222

Hamant O, Heisler MG, Jönsson H, Krupinski P, Uyttewaal M, Bokov P, Corson F, Sahlin P, Boudaoud A, Meyerowitz EM, Couder Y, Traas J (2008) Developmental patterning by mechanical signals in *Arabidopsis*. Science 322:1650–1655

Hämmerling J (1934) Entwicklungsphysiologische und genetische Grundlagen der Formbildung bei der Schirmalge *Acetabularia*. Naturwissenschaften 22:55–63

Hardham AR, Green PB, Lang JM (1980) Reorganization of cortical microtubules and cellulose deposition during leaf formation of *Graptopetalum paraguayense*. Planta 149:181–195

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

Heinlein M (2008) Microtubules and viral movement. Plant Cell Monogr 143:141–173

Heinlein M, Epel BL, Padgett HS, Beachy RN (1995) Interaction of tobamovirus movement proteins with the plant cytoskeleton. Science 270:1983–1985

Himmelspach R, Wymer CL, Lloyd CW, Nick P (1999) Gravity-induced reorientation of cortical microtubules observed *in vivo*. Plant J 18:449–453

Hohenberger P, Eing C, Straessner R, Durst S, Frey W, Nick P (2011) Plant actin controls membrane permeability. Biochim Biophys Act 1808:2304–2312

Irving RM (1969) Characterization and role of an endogenous inhibitor in the induction of cold hardiness in *Acer negundo*. Plant Physiol 44:801–805

Irving RM, Lanphear FO (1968) Regulation of cold hardiness in *Acer negundo*. Plant Physiol 43:9–13

Janmey PA, Weitz DA (2004) Dealing with mechanics: Mechanisms of force transduction in cells. Trends Biochem Sci 29:364–370

Jian LC, Sun LH, Lin ZP (1989) Studies on microtubule cold stability in relation to plant cold hardiness. Acta Bot Sin 31:737–741

Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, Qian Q, Li J (2010) Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. Nat Genet 42:541–545

Jürges G, Kassemeyer HH, Dürrenberger M, Düggelin M, Nick P (2009) The mode of interaction between *Vitis* and *Plasmopara viticola* Berk. & Curt. Ex de Bary depends on the host species. Plant Biol 11:886–898

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

Knight MR, Campbell AK, Smith SM, Trewavas AJ (1991) Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. Nature 352:524–526

Kobayashi I, Kobayashi Y (2008) Microtubules and pathogen defence. Plant Cell Monogr 143:121–140

Komis G, Apostolakos P, Galatis B (2002) Hyperosmotic stress induces formation of tubulin macrotubules in root-tip cells of *Triticum turgidum*: their probable involvement in protoplast volume control. Plant Cell Physiol 43:911–922

Komis G, Quader H, Galatis B, Apostolakos P (2006) Macrotubule-dependent protoplast volume regulation in plasmolysed root-tip cells of *Triticum turgidum*: involvement of phospholipase D. New Phytol 171:737–750

Kung Ch (2005) A possible unifying principle for mechanosensation. Nature 436:647–654

Laporte C, VetterG LoudesAM, RobinsonDG HS, Stussi-Garaud C, Ritzenthaler C (2003) Involvement of the secretory pathway and the cytoskeleton in intracellular targeting and tubule assembly of grapevine fanleaf virus movement protein in tobacco BY-2 cells. Plant Cell 15:2058–2075

Leopold PL, Pfister KK (2006) Viral strategies for intracellular trafficking: motors and microtubules. Traffic 7:516–523

Li J, Jiang J, Qian Q, Xu Y, Zhang C, Xiao J, Du C, Luo W, Zou G, Chen M, Huang S, Feng Y, Cheng Z, Yuan M, Chong K (2011) Mutation of rice *BC12/GDD1*, which encodes a kinesin-like protein that binds to a GA biosynthesis gene promoter, leads to dwarfism with impaired cell elongation. Plant Cell 23:628–640

Los DA, Murata N (2004) Membrane fluidity and its roles in the perception of environmental signals. Biochim Biophys Acta 1666:142–157

Lucas J, Shaw SL (2008) Cortical microtubule arrays in the *Arabidopsis* seedling. Curr Opin Plant Biol 11:94–98

Lucas WJ, Yoo BC, Kragler F (2001) RNA as a long-distance information macromolecule in plants. Nat Rev Mol Cell Biol 2:849–857

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

Lyons JM (1973) Chilling injury in plants. Annu Rev Plant Physiol 24:445–466

Maeda E (1965) Rate of lamina inclination in excised rice leaves. Physiol Plant 18:813–827

Martin KC, Ephrussi A (2009) mRNA localization: gene expression in the spatial dimension. Cell 136:719–730

Mazars C, Thion L, Thuleau P, Graziana A, Knight MR, Moreau M, Ranjeva R (1997) Organization of cytoskeleton controls the changs in cytosolic calcium of cold-shocked *Nicotiana plumbaginifolia* protoplasts. Cell Calcium 22:413–420

Mizuno T, Miyashita M, Miyagawa H (2009) Cellular internalization of arginine- rich peptides into tobacco suspension cells: a structure–activity relationship study. J Pept Sci 15:259–263

Modig C, Strömberg E, Wallin M (1994) Different stability of posttranslationally modified brain microtubules isolated from cold-temperate fish. Mol Cell Biochem 130:137–147

Monroy AF, Sarhan F, Dhindsa RS (1993) Cold-induced changes in freezing tolerance, protein phosphorylation, and gene expression. Plant Physiol 102:1227–1235

Murata N, Ishizaki-Nishizawa O, Higashi H, Tasaka Y, Nishida I (1992) Genetically engineered alteration in chilling sensitivity of plants. Nature 356:710–713

Nagata T, Nemoto Y, Hasezawa S (1992) Tobacco BY-2 cell line as the 'Hela' cell in the cell biology of higher plants. Int Rev Cytol 132:1–30

Nick P (2000) Control of Plant Shape. In: Nick P (ed) Plant microtubules—potential for biotechnology. Springer, Berlin, pp 24–46

Nick P (2008a) Control of cell axis. Plant Cell Monogr 143:3–46

Nick P (2008b) Microtubules as sensors for abiotic stimuli. Plant Cell Monogr 143:175–203

Nick P (2011) Mechanics of the cytoskeleton. In: Wojtaszek P (ed) Mechanical integration of plant cells and plants. Springer, Berlin-Heidelberg, pp 53–90

Nick P, Bergfeld R, Schäfer E, Schopfer P (1990) Unilateral reorientation of microtubules at the outer epidermal wall during photo- and gravitropic curvature of maize coleoptiles and sunflower hypocotyls. Planta 181:162–168

Nick P, Schäfer E, Hertel R, Furuya M (1991) On the putative role of microtubules in gravitropism of maize coleoptiles. Plant Cell Physiol 32:873–880

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, Christou P, Breviario D (2003) Generating transgenic plants by minimal addition of exogenous DNA—a novel selection marker based on plant tubulins. AgBiotechNet 5:105

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

Ouko MO, Sambade A, Brandner K, Ahad A, Heinlein M, Nick P (2010) Tobacco mutants with reduced microtubule dynamics are less susceptible to TMV. Plant J 62:829–839

Paredez AR, Somerville CR, Ehrhardt DW (2006) Visualization of cellulose synthase demonstrates functional association with microtubules. Science 312:1491–1495

Pickard BG (2008) "Second extrinsic organizational mechanism" for orienting cellulose: modeling a role for the plasmalemmal reticulum. Protoplasma 233:7–29

Pihakaski-Maunsbach K, Puhakainen T (1995) Effect of cold exposure on cortical microtubules of rye (*Secale cereale*) as observed by immunocytochemistry. Physiol Plant 93:563–571

Qiao F, Chang X, Nick P (2010) The cytoskeleton enhances gene expression in the response to the Harpin elicitor in grapevine. J Exp Bot 61:4021–4031

Radtke K, Dohner K, Sodeik B (2006) Viral interactions with the cytoskeleton: a hitchhiker's guide to the cell. Cell Microbiol 8:387–400

Rikin A, Waldman M, Richmond AE, Dovrat A (1975) Hormonal regulation of morphogenesis and cold resistance. I. Modifications by abscisic acid and gibberellic acid in alfalfa (*Medicago sativa* L.) seedlings. J Exp Bot 26:175–183

Rikin A, Atsmon D, Gitler C (1980) Chilling injury in cotton (*Gossypium hirsutum* L.): effects of antimicrotubular drugs. Plant Cell Physiol 21:829–837

Sakamoto T, Matsuoka M (2004) Generating high-yielding varieties by genetic manipulation of plant architecture. Curr Op Biotech 15:144–147

Sakamoto T, Morinaka Y, Ohnishi T, Sunohara H, Fujioka S, Ueguchi-Tanaka M, Mizutani M, Sakata K, Takatsuto S, Yoshida Sh, Tanaka H, Kitano H, Matsuoka M (2006) Brassinosteroid-induced bending of the leaf lamina of dwarf rice seedlings: an auxin-mediated phenomenon. Nature Biotechnol 24:105–109

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

Sambade A, Brandner K, Hofmann C, Seemanpillai M, Mutterer J, Heinlein M (2008) Transport of TMVmovementprotein particles associated with the targeting of RNA to plasmodesmata. Traffic 9:2073–2088

Sandblad L, Busch KE, Tittmann P, Gross H, Brunner D, Hoenger A (2006) The *Schizosaccharomyces pombe* EB1 homolog Mal3p binds and stabilizes the microtubule lattice seam. Cell 127:1415–1424

Sangwan V, Foulds I, Singh J, Dhindsa RS (2001) Cold-activation of *Brassica napus BN115* promoter is mediated by structural changes in membranes and cytoskeleton, and requires Ca^{2+} influx. Plant J 27:1–12

Schmelzer E (2002) Cell polarization, a crucial process in fungal defence. Trends Plant Sci 7:411–415

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

Stokes A, Fitter AH, Coutts MP (1995) Responses of young trees to wind: effects on root architecture and anchorage strength. J Exp Bot 46:1139–1146

Su Y, Doherty T, Waring AJ, Ruchala P, Hong M (2009) Roles of arginine and lysine residues in the translocation of a cell-penetrating peptide from 13C, 31P, and 19F solid-state NMR. Biochem 48:4587–4595

Takemoto D, Hardham AR (2004) The cytoskeleton as a regulator and target of biotic interactions in plants. Plant Physiol 136:3864–3876

Takeno K, Pharis RP (1982) Brassinosteroid-induced bending of the leaf lamina of dwarf rice seedlings: an auxin-mediated phenomenon. Plant Cell Physiol 23:1275–1281

Tao Y, Ferrer JL, Ljung K, Pojer F, Hong F, Long JA, Li L, Moreno JE, Bowman ME, Ivans LJ, Cheng Y, Lim J, Zhao Y, Ballaré CL, Sandberg G, Noel JP, Chory J (2008) Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. Cell 133:164–176

Twell D, Park SK, Lalanne E (1998) Asymmetric division and cell-fate determination in developing pollen. Trends Plant Sci 3:305–310

Vaughn KC (2000) Anticytoskeletal herbicides. In: Nick P (ed) Plant microtubules—potential for biotechnology. Springer, Berlin, pp 193–206

Wang Y, Li J (2006) Genes controlling plant architecture. Curr Op Biotech 17:123–129

Wang QY, Nick P (1998) The auxin response of actin is altered in the rice mutant *Yin-Yang*. Protoplasma 204:22–33

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

Wei ZM, Laby RJ, Zumoff CH, Bauer DW, He SY, Collmer A, Beer SV (1992a) Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. Science 257:85–88

Wei ZM, Sneath BJ, Beer SV (1992b) Expression of *Erwinia amylovora hrp* genes in response to environmental stimuli. J Bacteriol 174:1875–1882

Wiesler B, Wang QY, Nick P (2002) The stability of cortical microtubules depends on their orientation. Plant J 32:1023–1032

Wymer C, Wymer SA, Cosgrove DJ, Cyr RJ (1996) Plant cell growth responds to external forces and the response requires intact microtubules. Plant Physiol 110:425–430

Xu L, Henke M, Zhu J, Kurth W, Buck-Sorlin G (2011) A functional–structural model of rice linking quantitative genetic information with morphological development and physiological processes. Annals Bot 107:817–828

Yoneda A, Higaki T, Kutsuna N, Kondo Y, Osada H, Hasezawa S, Matsui M (2007) Chemical genetic screening identifies a novel inhibitor of parallel alignment of cortical microtubules and cellulose microfibrils. Plant Cell Physiol 48:1393–1403

Zhang M, Zhang B, Qian Q, Yu Y, Li R, Zhang J, Liu X, Zeng D, Li J, Zhou Y (2010) Brittle Culm 12, a dual-targeting kinesin-4 protein, controls cell-cycle progression and wall properties in rice. Plant J 63:312–328

Zhao SQ (2010) Rice leaf inclination2, a VIN3-like protein, regulates leaf angle through modulating cell division of the collar. Cell Res 20:935–947

Zheng B, Shi L, Ma Y, Deng Q, Li B, Guo Y (2008) Comparison of architecture among different cultivars of hybrid rice using a spatial light model based on 3-D digitising. Funct Plant Biol 35:900–910

Zhong R, Burk DH, Morrison WH, Ye ZH (2002) A kinesin-like protein is essential for oriented deposition of cellulose microfibrils and cell wall strength. Plant Cell 14:3101–3117

Zimmermann S, Nürnberger T, Frachisse JM, Wirtz W, Guern J, Hedrich R, Scheel D (1997) Receptor-mediated activation of a plant Ca^{2+}-permeable ion channel involved in pathogen defense. Proc Natl Acad Sci U S A 94:2751–2755