Why to Spend Tax Money on Plant Microtubules?

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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 and gravity but also osmotic stress, cold, and pathogen attack. Many of these functions are specific for plants and involve unique 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.

1 Motivation: Plant Architecture Defines Yield

Plant architecture represents a target with high potential for plant biotechnology. When leaf angles can be manipulated, this will allow the sunlight to penetrate deeper into the canopy (Zheng et al. 2008). When internodes can be shortened, this will increase lodging resistance. When the formation of new tillers is suppressed,

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this will promote the filling of grains on the main ear (Sakamoto and Matsuoka 2004). These important traits for breeding of high-yielding cultivars have been modelled numerically to assess the impact of genetic traits on plant morphology and expected yield on a quantitative base (Xu et al. 2011). Plants with 'ideal architecture' would show reduced shoot length, reduced tiller number, and increased grain weight as traits (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, is still to be exploited motivating the current chapter.

Yield losses by lodging and windbreak are considerable: In rice, for example, they are estimated to range up to 40 % (Nishiyama 1986). Control of plant height has therefore 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 %. Thus, the agronomic importance of reduced shoot length cannot be overestimated, shifting the control of plant height into the centre of interest, especially for cereal crops. In fact, a central factor in the green revolution of cereals has been a mutation in the so-called DELLA regulators of gibberellin-dependent shoot elongation (Peng et al. 1999), and recently it was uncovered that during the domestication of japonica rice a semidwarf mutation linked to gibberellin synthesis had been selected (Asano et al. 2011).

However, the impact of plant architecture is not confined to lodging resistance. For instance, the resistance of crop plants to wind depends on the angle between main and branch roots (Stokes et al. 1995), and the marketable yield very often depends on the partitioning of biomass. As already pointed out in the 1920s as 'Law of Homologous Series' established by the Russian geneticist Vavilov (Vavilov 1922) for the domestication of crop plants, apical dominance represented a central factor. Instead of numerous, small axes bearing numerous, but smaller fruits, one main axis was established during domestication of many crops. This change of architecture is impressively illustrated by the transition from the ancestral teosinte to modern maize linked to mainly one locus controlling the formation of side branches (Doebley et al. 1995). 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.

Microtubules control plant architecture at two levels: (1) They act downstream as effectors. Microtubules control the axis of cell division and cell expansion and therefore link the output of signalling triggered by environment and development to a response of plant architecture. (2) They act upstream as sensors. Microtubules can integrate the mechanic load resulting from growth and architecture and feed this

information into the deposition of load-bearing elements. Both functions will be discussed in more details in the subsequent sections.

2 Plant Microtubules as Morphogenetic Tools

Plant microtubules pass through a series of different arrays during the cell cycle. These arrays are not only morphologically distinct but convey different cellular functions. Most of these functions are linked with cell axiality. Since cellular migrations, as central element of animal development, do not play a role in the walled plant cells, the spatial control of both cell expansion and cell division are the only mechanism at hand to control plant morphogenesis. The dynamically interchanging arrays of plant microtubule act as central morphogenetic effectors in the induction, maintenance, and perpetuation of cellular axiality.

During interphase, microtubules form arrays of parallel bundles oriented perpendicular to the axis of preferential cell expansion. These cortical microtubules define the biophysical properties of the yielding cell wall and thus the geometry of expansion. In fact, 'microtubules' were predicted to exist from merely biophysical considerations on expanding plant cells (Green 1962) and only later actually discovered exactly half a century ago by electron microscopy (Ledbetter and Porter 1963). The classical model assumes that cortical microtubules define the orientation in which newly synthesised cellulose microfibrils will be laid down (reviewed in Geitmann and Ortega 2009; Nick 2008a). In cylindrical cells, where isotropic action of turgor pressure is predicted to produce only half of the strain in the longitudinal direction relative to the transverse direction, a transverse orientation of cellulose microfibrils maintains the lateral reinforcement needed to drive elongation (Green 1980).

Cortical microtubules can change their orientation in response to a broad range of signals, both exogenous and endogenous, and thus allow to tune plant morphogenesis with the challenges of the environment. This signal-dependent reorientation is transformed into altered deposition of cellulose microfibrils, a mechanism that allows to adjust 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 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-synthesising 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 will determine cellulose orientation and thus the anisotropy of the cell wall. It is the direction of this movement where cortical microtubules interfere with the mechanical anisotropy of the expanding cell wall.

The direct contact between cortical microtubules and newly emerged cellulose microfibrils has been demonstrated by electron microscopy but is also 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, including 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: The original 'monorail' model proposed that motor proteins moving along cortical microtubules pull cellulose synthetases (Heath 1974). In contrast, the latter 'guardrail' model assumed that microtubules induce small protrusions in the plasma membrane constraining the movement of the enzyme complexes, whereas the actual movement is driven by the crystallising cellulose itself. This 'guardrail' model was stimulated by observations where the cellulose synthase complexes were found 'in gap' between adjacent microtubules (Giddings and Staehelin 1988). However, electron microscopy observation is prone to artefacts of chemical fixation, and great luck is required to locate the right section where the topological relation between microtubules and cellulose synthases can be assessed. Therefore, the results left space for controversial interpretations. The situation was further complicated by situations where the orientation of microtubules and cellulose microfibrils differs (for review, see Wasteneys 2004), casting doubt on microtubule-guided cellulose synthesis in general.

During the last decade, the classical 'monorail' model has recovered by evidence for a central role of kinesins and microtubule-binding proteins in cell-wall deposition (recently reviewed by Cai and Cresti 2012). A screen for mutants with reduced cell-wall integrity recovered the mutant *fragile fiber 2* with stunted and swollen cells. This phenotype was caused by a mutation in the microtubule-severing protein katanin, also affected in the mutant *botero* (Bichet et al. 2001). 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 a role of the FRA1/KIF4 motor in the movement of cellulose synthases (Zhong et al. 2002). In fact, fluorescently tagged cellulose synthases could later be shown to move in tracks adjacent to the subtending cortical microtubules (Paredez et al. 2006), and a cellulose synthase (CSI1) binds directly to microtubules (Li et al. 2012).

Based on situations where a transverse cellulose orientation persisted although microtubules had been eliminated by drug treatment or temperature-sensitive mutations, a self-organisation of cellulose has been proposed. During cell elongation, microtubules would sustain cellulosic self-organisation by constraining the secretion of noncellulosic polysaccharides (Fujita et al. 2011). Irrespective of the underlying mechanisms (that are not mutually exclusive), the cell axis seems to be linked to microtubules rather than to actin filaments.

However, the 'monorail' model suffers from a couple of 'chronic problems' that call for extensions and modifications. In polylamellate walls, layers with differing microfibril orientation coexist. This phenomenon could be plausibly explained by a

rotary movement of groups of microtubules (for review, see Lucas and Shaw 2008). A second 'chronic problem' arises from situations where a transverse cellulose orientation persisted although microtubules had been eliminated by drug treatment or temperature-sensitive mutations. These observations were explained by cellulosic self-organisation sustained by microtubules during cell elongation by constraining the secretion of noncellulosic polysaccharides (Fujita et al. 2011). A third problem is the observation that cellulose microfibrils are often observed to be intertwined (Preston 1988), again pointing to the self-organisation of cellulose synthesis. The microfibrils that are already laid down could act as templates for the synthesis of new microfibrils (for review, see Mulder et al. 2004). As a consequence, microtubules would not be required throughout all stages of cellulose deposition.

Last but not least, the relation between cell wall and cortical microtubules is bidirectional. Through transmembrane proteins, cortical microtubules are connected with the extracellular matrix. The molecular nature of these transmembrane proteins has remained elusive, but they share analogies with animal integrins (for review, see Pickard 2008). This link seems to stabilise cortical microtubules, because removal of the cell wall renders microtubules more cold sensitive in tobacco cells (Akashi et al. 1990). Moreover, cobtorin, a compound identified from a screen that specifically disturbs the parallelity of microtubules and microfibrils (Yoneda et al. 2007), has meanwhile been found to affect cell-wall pectins (Yoneda et al. 2010).

Thus, although often discussed in this manner, there is no reason why the 'monorail' model and cellulose self-organisation should be mutually exclusive. The 'chronic problems' of the original 'unified hypothesis' (Heath 1974) can be easily remedied by adding two aspects: (i) The deposition of cellulose microfibrils not only depends on microtubules but also on geometrical input from pre-existing microfibrils, and (ii) the link between microtubules and cellulose is not a one-way street, but bidirectional, i.e. the orientation and dynamics of microtubules depend on input from the cell wall.

Upon cell division, cortical microtubules are replaced by a rapidly changing sequence of diverse arrays: radial microtubules, preprophase band, spindle, and phragmoplast. In preparation for mitosis, the nucleus moves into the cell centre and somehow commits the site where the prospective cell plate will be formed (for review, see Nick 2008a). At the same time, radial microtubules are nucleated at the nuclear envelope and connect with the cortical cytoskeleton driving and stabilising nuclear migration. Once the nucleus has reached the cell centre, it will organise the preprophase band, a broad band of microtubules girdling the cell equator. The preprophase band predicts site and orientation of the prospective cell plate, although this will become manifest only much later, when mitosis has been completed. The preprophase band disappears when the division spindle forms, always orthogonal to the plane of the preprophase band. In late anaphase, 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, guides the growth of the expanding cell plate. The eclipse of the

preprophase band that nevertheless defines orientation and position of phragmoplast and cell plate has been a major enigma of plant cell biology. This mystery had been resolved by the discovery of an endosomal belt laid down adjacent with the preprophase band and persisting through mitosis (Dhonukshe et al. 2005). This endosomal belt is recognised during late anaphase by exploratory microtubules emanating from the cell poles throughout the dividing cell. Those microtubules that hit the endosomal belt defined by the preprophase band are stabilised, whereas those that fail to find their target will undergo catastrophic decay.

Microtubules are therefore used to establish and maintain the axis of cell expansion and division. This is important to adjust plant development with the variable challenges of the environment. In other words, microtubules are tools by which plants can exert control over their morphogenesis.

3 Plant Microtubules as Morphogenetic Sensors

The preceding section dealt with the classical role of microtubules as part of the response machinery that links signalling with cellular morphogenesis. However, microtubules play a second role that is more upstream and linked with morphogenetic signalling 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 allows to integrate mechanic load even across the borders of individual cells.

Mechanical tension is important to integrate the architecture of the expanding plant. In terrestrial plants, the considerable lever forces from branches and leaves are not compensated by buoyancy and require compensatory deposition of supportive structures. Mechanic force can reach instantaneously even the remotest parts of a tree and therefore provides an ideal signal to integrate compensation with mechanical load. Unlike the metazoan cell that is surrounded by a strictly regulated isotonic environment, the cells of multicellular plants are faced with a hypotonic environment leading to considerable turgor pressures of the expanding protoplasts upon the counteracting cell wall. The turgor of individual cells accumulates to considerable tissue tension on the organ level. It is this hydraulic principle that is used as signal to integrate the body plan of a plant (Niklas and Spatz 2004). When new organs are laid down, this will change the pattern of tissue tension, which in turn will guide the development of additional organs in a manner that a state of minimal energy is established. This has been intensively studied and modelled for phyllotaxis by Paul Green and co-workers (for review, see Green 1980).

Their work demonstrated that the buckling of the pre-existing older primordia altered the stress–strain pattern in the growing apical meristem and that the positions of incipient primordia could be predicted as the sites of the local energy minima. As to be expected from this model, local release of tissue tension by softening the cell wall using beads coated with expansin (Fleming et al. 1997) induced appendices that resembled primordia.

The first cellular event of primordial initiation is a reorientation of cortical microtubules that reorient perpendicular to the microtubules of the noncommitted neighbour cells. This difference is first sharp but later smoothened by a transitional zone of cells, where microtubules assume intermediate orientations. Eventually, a gradual, progressive change in microtubule reorientation is produced that extends over several tiers of cells (Hardham et al. 1980).

This phenomenon has been revisited using a combination of live-cell imaging with fluorescent microtubule markers modelling of stress–strain patterns in *Arabidopsis thaliana* (Hamant et al. 2008). Again, cortical microtubules were found to align in the direction of maximal mechanical stress in a transcellular pattern. In the next step, the outer meristem layer was removed locally by laser ablation, and the resulting changes of stress pattern were modelled. In fact, microtubules were then observed to reorient as predicted by these pattern, leading to a compensatory bulging of the apex. The impact of cortical microtubules is further corroborated by recent evidence for a role of the microtubule-severing protein katanin for meristem patterning (Uyttewaal et al. 2012). However, mechanic load can not only align cortical microtubules but also division-related microtubule arrays. Already three decades ago it could be demonstrated that new cell plates (oriented by phragmoplast microtubules) align with the force vector when a callus was subjected to compression forces (Lintilhac and Vesecky 1984). Furthermore, a mild centrifugation can align cell divisions parallel with the force vector, and this alignment requires microtubules to be present at the time, when this mechanic stimulus is administered (Wymer et al. 1996).

The ultimate reason, why plant microtubules are mechanosensitive, is gravity. Terrestrial plants must arrange force-bearing elements such that mechanic load by gravity is minimised. The pattern of mechanical strains (due to tension of the turgescent tissue) is used to guide the arrangement of supportive structures. As is valid for any physical stimulus, 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). When a plant is misoriented with respect to gravity, its flank will be subjected to the same gravitational field. This means that a gradient in the strength of the terrestrial gravitational field strength would not work. Gravitation as stimulus must therefore be first transformed into mechanical force by acting on heavy particles, the statoliths. In higher plants gravity susception is brought about by the amyloplasts as proposed simultaneously, but independently by Nemec (1900) and Haberlandt (1900) more than a century ago (see chapter by Opatrný, this volume), and finally proven in ingenious experiments using high-gradient magnetic fields by Kuznetsov and Hasenstein (1996). Of course, the statoliths (as well as their accessory structures) are not gravisensitive; they merely assist gravity 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 but also by taxol (for review, see Nick 2008b), indicating that microtubules not only have to be present but have to undergo dynamic turnover. A role of microtubules in the perception of gravity has also been identified for the gravimorphosis of germinating fern spores (Edwards and Roux 1994).

Since microtubules participate also in growth (see previous section), it is not trivial to discriminate their function in gravity sensing from their role in gravitropic curvature. Cortical microtubules reorient during the gravitropism of both shoots (Nick et al. 1991) and roots (Blancaflor and Hasenstein 1993) consistent with a model, where gravitropic stimulation causes a transverse flux of auxin from the upper to the lower flank of the organ (see chapter by Skůpa et al., this volume). Auxin depletion in the upper flank (shoots) or auxin accumulation in the lower flank (roots) will cause a microtubular orientation resulting in altered cellulose deposition and thus culminating in differential growth (Nick et al. 1990). When microtubules are eliminated, this will affect the mechanism of differential growth. The absence of gravitropic curvature therefore does not prove a role of microtubules in gravity sensing – when a prisoner without legs does not leave the prison, although the door has been opened for him, this does not mean that he cannot see that the door has been opened.

To pinpoint the sensory function of microtubules and their role in executing gravitropic bending, the lateral transport of auxin can be used because this response is upstream of differential growth (Godbolé et al. 2000; Gutjahr and Nick 2006). Using rice coleoptiles as experimental system, whereas the gravitropically induced lateral transport of radioactively labelled auxin can be easily measured, the elimination of microtubules suppressed auxin transport. Interestingly, taxol that acts as stabiliser of microtubules by suppressing their dynamic turnover blocked lateral auxin transport leaving longitudinal transport untouched. This not only unequivocally demonstrates a gravity-sensitive microtubule function that can be separated from the growth response but also suggests that microtubules have to be dynamic to sense gravity.

Not surprisingly, microtubules as mechanosensitive structures also participate in osmoadaptation. Osmotic stress induces massive bundles of microtubules termed macrotubules (Komis et al. 2002) that confer osmotic adaptation (Komis et al. 2006). It may appear less straightforward that the mechanosensitive nature of microtubules can also convey sensitivity to cold and pathogens. For instance, pharmacological manipulation of microtubules can be used to control cold hardiness (Kerr and Carter 1990; Abdrakhamanova et al. 2003). Since several events of plant defence can be triggered by localised mechanostimulation (Gus-Mayer et al. 1998), it is also possible to induce 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 plant microtubules are still to be explored. Generally, two paradigms are used to explain mechanosensing: 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 transduced to

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 be integrated into a so-called 'plasmalemmal reticulum' (for review, see Pickard 2008). This network might 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 signalling. Microtubules could act and focus mechanic stress upon mechanosensitive channels, similar to the set-up found in touch-sensitive cells of *Caenorhabditis elegans* (Savage et al. 1989). This would be a classical susceptor function. However, microtubules might be mechanosensors themselves: A growing microtubule is subjected to 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, mutation of EB1 genes renders *A. thaliana* 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 starlike 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.

4 Microtubules and Green-Revolution Architecture

Shorter plants are more resistant against windbreak and lodging. Reduction of stem length was therefore a central factor for the success of the green revolution. Lodging is inversely related to plant height by the relation

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

with W fresh weight, M bending momentum at breaking, l shoot length, and w dry weight of the shoot (Oda et al. 1966). Lodging will therefore decrease parabolically with decreasing shoot length. A very efficient strategy to increase lodging

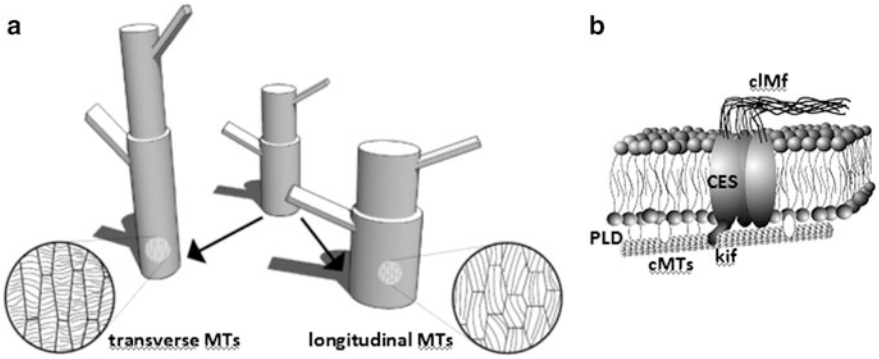

Fig. 1 Microtubules and lodging sensitivity. Elongation of internodes is promoted for transverse orientation of microtubules (MTs), whereas longitudinal microtubules repartition growth from elongation to produce thicker and shorter stems that are more resistant to lodging (**a**). The underlying mechanism is the movement of cellulose synthetases (CES) along cortical microtubules driven by the activity of specific kinesins (kif), such that cellulose microfibrils (clMf) are laid down parallel to cortical microtubules that are anchored to the plasma membrane by switchable linker proteins such as phospholipase D (PLD)

resistance is to repartition shoot growth from elongation to thickening, keeping fresh weight W constant (Fig. 1a). The conventional practice to use chemical growth regulators such as chlormequat chloride or ethephon (Luib and Schott 1990) follows exactly this strategy but is limited by undesired side effects on fertility. More relevant for the success of the Green Revolution was a genetic strategy based on semidwarf varieties (Wang and Li 2006). These varieties are either deficient in gibberellin synthesis (such as the green revolution rice *sd-1*) or constitutively repress gibberellin-responsive genes by dominant-negative mutation of DELLA genes (as in the case of the green revolution wheat *Rht-B1*/*Rht-D1*, Peng et al. 1999). Not only in cereals, reduced plant height is a desirable trait (Luib and Schott 1990). Shorter plants help light to penetrate into the canopy (rapeseed), improve the access of insecticides to the lower parts of the plant (cotton), or facilitate mechanical picking (fruit trees).

However, modern agriculture creates an environment that stimulates internode elongation through high nutrient influx and high canopy densities. Plants can sense competing neighbours through an increase of reflected far-red light using the phytochrome photoreceptor system (Smith 1981) and respond by activation of auxin synthesis through a non-canonical tryptophan-dependent pathway (Tao et al. 2008) promoting cell elongation in the internode. This shade-avoidance response protects plants against overgrowth by competitors but at the same time increases the risk of lodging. The increased levels of auxin produced by the phytochrome-triggered shade-avoidance response will sustain a transverse orientation of cortical microtubules in the outer epidermis (Nick et al. 1990). Since cortical microtubules guide the movement of cellulose synthases in the plasma membrane (Fig. 1b), the transverse microtubules will induce a transverse orientation of the

inner cellulosic layer of the epidermal cell wall. Since the epidermis mechanically constrains the expansion of the subtending tissues, the entire internode will become longer (Fig. 1a).

Can the desirable semidwarf trait be achieved through altering microtubular orientation? In fact, a screen for rice mutants that were resistant against ethyl-N-phenylcarbamate (EPC) (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). A similar situation has been reported for the hypocotyl of tubulin mutants in thale cress (Matsumoto et al. 2010). In this mutant, cortical microtubules were arranged in oblique or even longitudinal arrays and uncoupled from auxin. As to be expected, this resulted in reduced cell length and a semidwarf 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 mutant collection. The mutant was completely rescued by exogenous gibberellin (Li et al. 2011) pointing to a defect in gibberellin synthesis. Surprisingly, the mutation was located to a kinesin-like protein (BRITTLE CULM12) that controls the formation of secondary cell walls (Zhang et al. 2010). It turned out that this protein fulfils dual functions and also 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 semidwarf phenotype.

In addition to internode elongation, the inclination of leaves is crucial for yield, for it determines how far light can penetrate into a canopy and therefore limits the maximal density of planting. In cereal crops, leaf inclination is defined by the angle between leaf sheath and blade and by the inclination of the leaf sheath in the pulvinus (Fig. 2a). In fact, the pulvinus can respond to canopy density, with leaf sheaths becoming more erect, when canopy density increases (Gibson et al. 1992). This response is brought about by differential cell expansion at the two flanks of the pulvinus. 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 dichlorobenzonitrile, suggesting that the movement is driven by gravity-triggered microtubule orientation (Dayandanan and Kaufman 1984). The inclination of leaves is also adjusted by the collar region, delineating leaf sheath and blade (Fig. 2a). The angle between sheath and blade can be actively regulated by cell divisions in the adaxial epidermis of the collar (Zhao 2010), controlled by brassinosteroids. In fact, a classical bioassay for brassinosteroids makes use of this phenomenon and uses the inclination of the leaf lamina in rice for quantification (Takeno and Pharis 1982). Mutants of brassinosteroid synthesis in rice have steeper leaf blades and produce higher yields even in the absence of fertiliser (Sakamoto et al. 2006). Interestingly, 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). In the 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

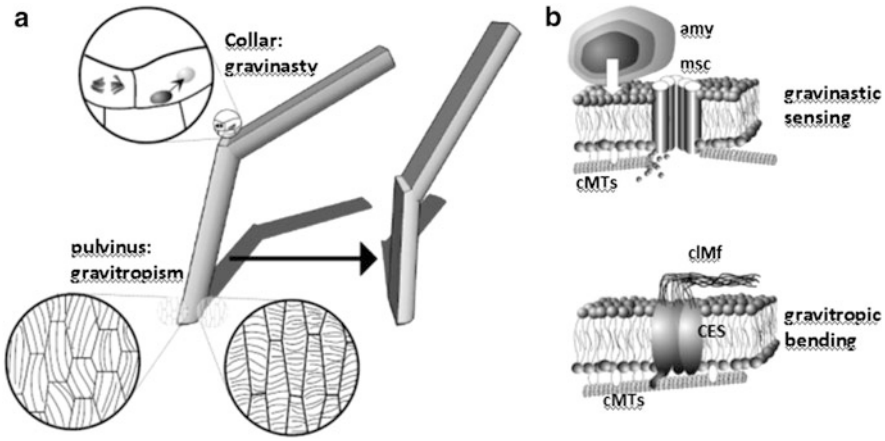

Fig. 2 Microtubules and leaf inclination. In cereals leaf inclination depends on a gravitropic bending (by inhibition of cell expansion at the adaxial flank of the pulvinus) of the pulvinus and a gravinastic orientation of the leaf blade (by inhibition of cell division at the adaxial flank of the leaf collar) (**a**). Both processes are controlled by microtubules (**b**). Gravitropic bending of the pulvinus depends on a gradient in the orientation of cellulose microfibrils (clMf) caused by the movement of cellulose synthetases (CES) along cortical microtubules (cMTs). The role of microtubules in gravinasty is sensory. Microtubules modulate the response of mechanosensitive channels (msc) to the pressure exerted by the sedimenting amyloplasts (amy)

(Abe et al. 1998), demonstrating that the inclination of the leaf blade represents an active gravinastic response. Again, microtubules seem to be involved because in rice mutants with reduced microtubule dynamics, the inclination of the leaf blade is significantly increased resulting in a fan-like appearance of the plant (Nick 2000). The underlying mechanism has to be sought in the modulation of gravity sensing by microtubules (Fig. 2b).

Thus, biotechnological control of microtubule dynamics could be used to control leaf inclination (Fig. 2), whereas control of microtubule orientation can constrain internode elongation and thus lodging resistance (Fig. 1). Microtubules represent therefore a crucial target to tune two central factors deciding yield especially in cereals as most important staple crops.

5 Microtubules and Cold Tolerance

Temperature limits crop yield in most temperate regions. Attempts to increase photosynthetic rates by conventional breeding programmes, although pursued over a long period, have not been successful, indicating that evolution has already reached the optimum (Evans 1975). Optimal photosynthesis requires that leaves are fully expanded, but the cold sensitivity of growth is much more pronounced than that of photosynthesis. This means that it is leaf growth which constrains

productivity (Watson 1952; Monteith and Elston 1971), a conclusion supported by the finding that in cool climates the production of biomass is not source but sink limited (Warren-Wilson 1966). The velocity of shoot development depends on the cold response of roots (Atkin et al. 1973), and cooling of the root can trigger adaptive responses in the shoot (Suzuki et al. 2008). However, the issue of cold sensitivity in agriculture is not confined to temperate regions. Many tropical and subtropical plants suffer severely when they are exposed to cool temperatures that are even still far above the freezing point. This poses extreme problems when fruits have to be harvested and cooled for transport and processing, because these fruits rot rapidly as soon as they return to warmer temperatures. This phenomenon has been known for a long time and was originally termed *Erkältung* (chilling damage) by Molisch (1897) in distinction from actual freezing damage. In extreme cases, even very moderate cooling can irreversibly damage a plant when it hits a very sensitive period of development. For instance, rice can lose fertility when temperature drops below 18°C during flower development. The economical consequences of this phenomenon can be drastic – for instance, according to estimates of the Japanese Ministry of Agriculture, Forestry and Fishery, during the cool summer of 1993, the rice yield was reduced by around 25 %. Insight into the mechanisms of cold sensitivity is therefore of high agronomical impact.

Since microtubules disassemble in the cold, they can limit the cold tolerance of a species. Despite the relatively high conservation of tubulin, cold sensitivity of microtubules is not constant but variable and thus subject to evolutionary change: Whereas mammalian microtubules disassemble already at temperatures below +20°C, the microtubules from poikilothermic animals maintain integrity at much lower temperatures (Modig et al. 1994). The cold stability of plant microtubules is generally more pronounced as compared to their animal counterpart, as to be expected from the higher temperature plasticity of plants. The critical temperature where microtubules disassemble varies between different plant species, and this is correlated with differences in chilling sensitivity (Jian et al. 1989).

The link between microtubule stability and cold hardiness is corroborated by the following observations:

- Treatment with abscisic acid that can stabilise microtubules against low temperature (Sakiyama and Shibaoka 1990; Wang and Nick 2001) also promotes cold hardiness (Irving 1969).
- Pharmacological manipulation of microtubules leads to corresponding changes of 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). 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, an inhibitor of 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 chilling, but not freezing temperature (Fig. 3a). 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 can 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 persist even during a freezing shock (Bartolo and Carter 1991a in spinach mesophyll; Pihakaski-Maunsbach and Puhakainen 1995 in roots of winter rye; Wang and Nick 2001; Abdrakhamanova et al. 2003 in roots of winter wheat). The development of acclimation was suppressed by taxol (Kerr and Carter 1990; Bartolo and Carter 1991b), indicating that microtubules have to disassemble to a certain degree in order to trigger cold hardening. Interestingly, abscisic acid, a well-known inducer of cold hardiness, has recently been found by live-cell imaging to trigger a transient disassembly of microtubules that is only at later stages followed by stabilisation (Seung et al. 2013). Thus, microtubules have to yield first to persist later.

How to explain this microtubule-based thermometer function? Cold perception is triggered by a loss of membrane fluidity (Los and Murata 2004). For instance, the input of low temperature can be mimicked by chemical compounds that decrease fluidity, such as dimethyl sulfoxide, whereas fluidity promotors such as benzyl alcohol 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). This calcium spike is not only a by-product of the cold response but necessary to trigger cold acclimation as demonstrated by pharmacological data (Monroy et al. 1993). Using a cold-responsive reporter system, it could be demonstrated that disassembly of microtubules by oryzalin or treatment with the calcium ionophore A23187 could mimic 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 are consistent with a model where microtubules constrain the permeability of mechanosensitive calcium channels that are triggered by membrane rigidification (Fig. 3b).

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 reduce the 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.

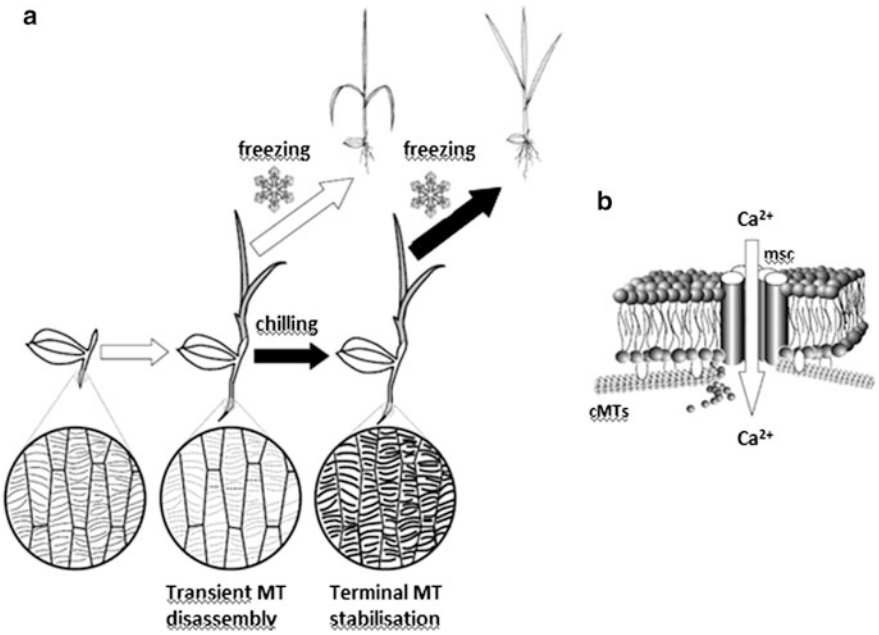

Fig. 3 Microtubules and cold hardening. Precultivation at chilling but nonfreezing temperature renders seedlings resistant against freezing. (**a**) Microtubules disassemble transiently during chilling but are replaced by stable microtubules that sustain vigorous growth even after freezing. The transient disassembly of microtubules is necessary and sufficient to trigger cold hardening and microtubule stabilisation and thus represents a 'thermometer function'. (**b**) Proposed mechanism for the microtubular 'thermometer': cold-sensitive calcium channels (msc) are gated by cortical microtubules (cMTs). The cold-induced reduction of membrane fluidity and the calcium influx cause a self-amplifying decay of microtubules that in turn will amplify calcium influx. This strong and sudden rise in cytosolic calcium will trigger the signalling culminating in cold hardening and stabilisation of microtubules (which in turn will also dampen the activity of the calcium channel)

Is it possible to use this microtubular thermometer function to improve cold tolerance (Fig. 3a, b)? This question was followed in a proof-of-principle experiment in three cultivars of winter wheat of different freezing tolerance (Abdrakhamanova et al. 2003). When these cultivars were exposed to 4°C, the growth rate of roots recovered at a rate that correlated with the degree of cold tolerance. 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 and partial, disassembly was observed in cultivars that were freezing tolerant but not in a cultivar that was freezing sensitive. However, a transient treatment with the antimicrotubular herbicide pronamide was able to confer freezing tolerance in the sensitive cultivar. This demonstrated that a transient, partial disassembly of microtubules is necessary and sufficient to trigger cold hardening. By engineering microtubule dynamics, it should be possible to induce more efficient cold hardening and thus to render crops more independent of climatic fluctuations.

6 Microtubules and Viral Resistance

Viruses exploit functions of the host for their own propagation cycle. Since viruses have to move from cell to cell, 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 they use for this purpose is probably the transport of mRNA, a central element of developmental signalling (reviewed in Martin and Ephrussi 2009). Signalling through RNA transport is also common in plants (reviewed in Lucas et al. 2001). Actually, it was in the green alga *Acetabularia* where for the first time mobile signals were discovered that later turned out to be RNA. During regeneration of the hat, a morphogenetic signal (untranslated mRNA) is transported from the nucleus into the stalk (Hämmerling 1934). Since actin and tubulin are highly conserved, also plant viruses might use the cytoskeleton to spread from the initial infection site through the rest of the plant (Fig. 4a).

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 (reviewed in 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 (Fig. 4b): Either microtubule might serve as tracks for translocation driven by molecular motors (Heinlein et al. 1995) or the viral particles bind to the treadmilling microtubule and are released at their destination (Sambade et al. 2008).

In order to discriminate motor-driven versus assembly-driven movement, viral spread was analysed in tobacco mutants with reduced microtubular turnover. These plants had been generated by T-DNA activation tagging and selected for their tolerance to 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 carboxy-terminus of α-tubulin (Wiesler et al. 2002; Morettini et al. 2013). However, since in activation tagging, any insertion of the tag into an exon would result

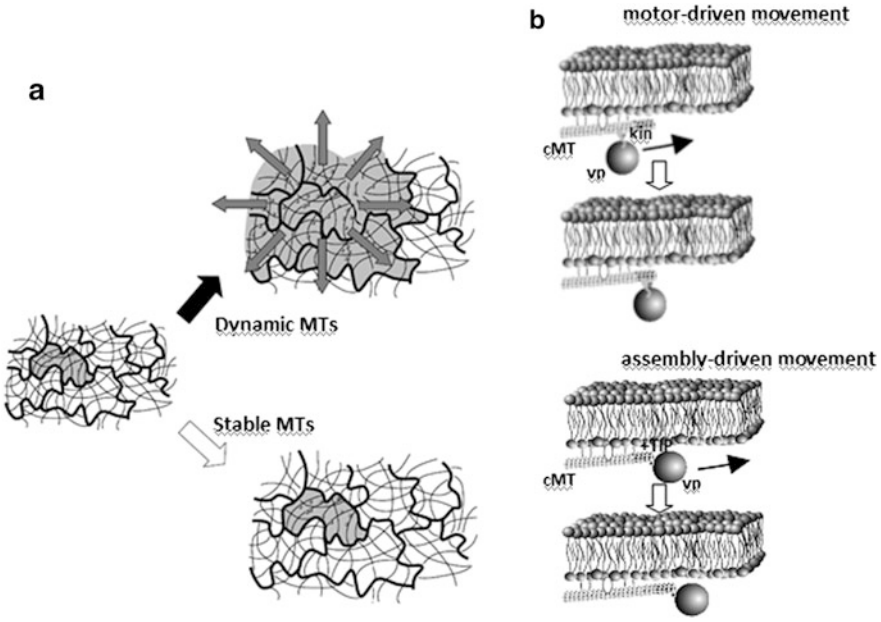

Fig. 4 Microtubules and viral movement. Many plant viruses usurp the microtubular cytoskeleton of their hosts to spread from the infected cell all over the tissue (**a**). This spread is correlated with microtubule dynamics. Stabilisation of microtubules can block viral movement. (**b**) Proposed mechanisms for microtubule-dependent viral movement. Cortical microtubules (cMTs) might serve as tracks along which viral particles (vp) move pulled by kinesin motors (kin). Alternatively, cortical microtubules that are anchored to the plasma membrane might push viral particles with their growing plus ent (+TIP). Reduction of microtubule dynamics should promote motor-driven movement, whereas assembly-driven movement should be impaired. In addition to movement itself, microtubules might participate in viral spread by controlling the release of viral particles to the ER or by constraining the aggregation of overexpressed proteins

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 turnover-dependent mechanism (Fig. 4b), it should be impaired in these mutants. 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 P_{450} found to be induced by EPC. The insertion by the tag resulted in a tenfold 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. In fact, 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. 4a). 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).

The link between host microtubules and viral spread is more complex though – since, during infection, they seem to play multiple roles: Microtubules tether and later release viral replication complexes adjacent to the endoplasmic reticulum in early infection, and later they anchor the maturating virus factories and eventually, in the centre of an infection site, bundle microtubules through their movement protein. Thus, there exist several specific targets for containment of plant viruses based on microtubular manipulation. Microtubular interference is not confined to TMV but is also found for other plant viruses such as cauliflower mosaic virus (Martinière et al. 2009) or the grapevine fanleaf virus (Laporte et al. 2003); the genetic or pharmacological manipulation of microtubule dynamics might be an efficient strategy to control viral spread in other crop plants as well.

7 Microtubules as Switches for Stress Resistance

Life is full of challenges, and there are basically two ways to cope with this: run away or adapt. Animals prefer to run away; plants are sessile and therefore have to go for the more heroic approach: adaptation. The developmental flexibility of plants allows them to overcome adverse environmental conditions. To achieve this, plants must integrate the signalling evoked by different stress factors into a balanced and appropriate response. For instance, osmotic adaptation requires transport of ions into the vacuole, whereas the most efficient way to encounter attacking biotrophic fungi is programmed cell death (Fig. 5a). The specificity of stress signalling might be brought about by specific molecular players and events that convey the signal to the downstream targets. Alternatively, signalling might utilise common, quite

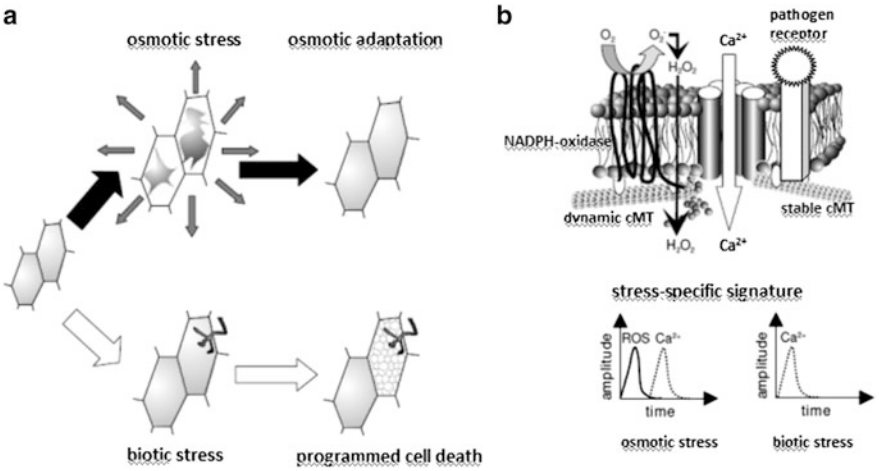

Fig. 5 Microtubular decoding of stress signatures. Many stress factors overlap in the signalling events they induce. Microtubules assist in the decoding of stress quality essential to select the appropriate cellular response. (**a**) Osmotic stress and biotic attack both induce an influx of calcium. The adaptive response must be different – osmotic adaptation has to restore the full turgescence of the cell achieved by transport of ions into the vacuole. In contrast, for biotic attack programmed cell death of the infected cell is the most efficient way to block the intruder. (**b**) Proposed mechanism for microtubule-dependent decoding of stress signatures. For osmotic stress, deformations of the membrane result in a transient decay of dynamic cortical microtubules (cMTs) that will be transduced by the microtubule-bound phospholipase function (*white ellipses*) upon the NADPH oxidase that then generates reactive oxygen species (ROS) that enter the cytoplasm. Cytoplasmic ROS species cause a decay of microtubules releasing the constraint of stable microtubules on calcium channels. As a result, an early peak of cytoplasmic ROS is followed by a transient peak of calcium. In case of biotic stress, a pathogen receptor binds a pathogen-derived elicitor and through accessory microtubules of the calcium channels triggers an early calcium peak (that *can* be followed by a later peak of cytoplasmic ROS in cases where a pathogen affects the integrity of the membrane)

general elements that are specifically recombined to produce appropriate outputs. A short survey on stress signalling yields a limited number of fairly general players including reactive oxygen species, calcium, or jasmonate. How can signalling be specific when the signals are so general? Specificity must derive from the context; the 'code' of stress signalling must be embodied in the spatiotemporal pattern (the so-called signature) rather than in the molecular nature of the signals. For instance, using transgenic plants expressing the aequorin reporter, it could be demonstrated that different stress factors induce different calcium signatures (Knight et al. 1991, for review, see McAinsh and Hetherington 1998). For reactive oxygen species, it is their subcellular distribution that confers specificity towards drought and salinity signalling (for review, see Miller et al. 2010) or towards programmed cell death (see chapter by Smertenko and Bozhkov, this volume). For the jasmonate pathway, it is the crosstalk of different transduction chains (i.e. the signalling history) converging at the proteasome that channels signalling into specific outputs (for

review, see Kazan and Manners 2008). Hence, specificity of stress signalling seems to rely on specific combinations of relatively general primary signals. A code requires a decoder, and there is evidence that microtubules can act as decoders of stress signals.

Hyperosmotic stress causes a strong and transient response of microtubules: Microtubules first disappear, but soon are replaced by massive bundles, the so-called macrotubules (Komis et al. 2002). Formation of macrotubules can be suppressed by oryzalin, which at the same time blocks osmoadaptation, demonstrating that this microtubule response is not a by-product of adaptation but represents an essential event. Inhibitors of phospholipase D, such as *n*-butanol or N-acetylethanolamine, suppress both macrotubule formation and osmotic adaptation (Komis et al. 2006). The product of phospholipase D, phosphatidic acid, can rescue the inhibition by *n*-butanol. As to be expected, T-DNA insertions into the PLD locus impair drought adaptation in thale cress (Hong et al. 2008).

Interestingly, the relationship between cortical microtubules and phospholipase D is bidirectional – on the one hand, microtubules can be detached from the membrane upon inhibition of phospholipase D; on the other hand, microtubules bind phospholipase D and thus might modulate enzymatic activity (Chae et al. 2005). Phospholipase D had originally been identified as membrane linker of plant microtubules (Gardiner et al. 2001), suggesting phospholipase D as signalling hub controlling the interaction between plasma membrane and cytoskeleton. Membrane deformations, for instance, imposed by osmotic challenge might render membrane lipids more accessible to phospholipase D, providing a mechanism to transduce mechanical load on the membrane into changes of cytoskeletal dynamics. The hub model is supported by the fact that phospholipase can trigger different signal pathways.

An attractive possibility, to be explored, would be a modulation of the phospholipase D signalling hub depending on interaction with microtubules. For instance, salt stress was shown to detach a plant-specific microtubule-associated protein, SPIRAL1, from microtubules followed by proteolytic degradation of this protein (Wang et al. 2011). This detachment renders microtubules unstable, which might then simultaneously activate phospholipase D-dependent signalling culminating in osmotic adaptation causing, among other responses, the formation of stable macrotubules. This mechanism would explain why microtubules have to yield in order to persist.

Microtubule decoding can be used not only to sense membrane load in the context of osmotic stress but also to sense the attack of pathogens. The role of microtubules in defence has been traditionally seen in their role for the formation of cell-wall papillae around sites of attempted pathogen penetration. The formation of these papillae is preceded by a reorganisation of the cytoskeleton causing redistribution of vesicle traffic 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). These responses could also be evoked by a localised mechanic stimulation in the parsley cell model and were accompanied by the formation of reactive oxygen

species and the induction of defence-related genes (Gus-Mayer et al. 1998). Mechanic stimulation could thus mimic several aspects of a pathogen attack and was equivalent to treatment with the corresponding chemical elicitor pep-13. These observations indicate a possible role of microtubules as decoders also in the context of defence.

If this link exists, it should be possible to manipulate defence responses through microtubules. This idea was tested using two cell lines from grapevine that differ in their microtubular dynamics and their susceptibility to Harpin elicitors (Qiao et al. 2010). In fact, pharmacological manipulation of microtubules could induce expression of defence genes in the absence of elicitor. This response was more pronounced in the cell line, where the elicitor triggered a transient elimination of microtubules – similar to the situation in cold adaptation (Abdrakhamanova et al. 2003) and osmotic adaptation (Wang et al. 2011), microtubules have to yield first in order to persist later. Similar to cold acclimation, it is a sensory role of microtubule that provides a promising target for biotechnological manipulation of plant defence.

A signature decoder must discriminate the history of a signal rather than its actual amplitude. To read history, some kind of feedback of downstream signalling upon perception is required. The microtubular stress decoder is endowed with these properties (Fig. 5b): Reactive oxygen species as those generated in response to osmotic stress would, through phospholipase-activated NADPH-oxidase activity (Guo et al. 2012), destabilise microtubules (Livanos et al. 2012) closing a self-referring signalling circuit, because microtubules modulate in turn the activity of phospholipase D. Disassembly of microtubules gating calcium channels (Ding and Pickard 1993; Mazars et al. 1997) would subsequently result in calcium influx. In contrast, biotic attack, through membrane-based receptors binding pathogen-derived elicitors, activates calcium influx more rapidly (Jeworutzki et al. 2010). Depending on the stimulus quality, microtubules therefore create different signal signatures that allow to activate the appropriate adaptive response.

8 New Tools for Cytoskeletal Manipulation

This chapter ventures to demonstrate that microtubules and their accessory proteins provide attractive targets to optimise plant architecture and stress tolerance. However, what tools and approaches do we have at hand to manipulate microtubules?

The most straightforward strategy is genetic engineering of tubulins. This has been actually employed already to engineer tolerance to dinitroanilines (Anthony et al. 1998) or aryl carbamates (Nick et al. 2003; Ahad et al. 2003) that bind to specific motives on α-tubulin. Since tubulins are very general players of the cellular lifecycle, unwanted side effects of mutated tubulins are an issue. This can be addressed by making use of 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. Alternatively, those proteins that have specifically evolved in higher plants and fulfil more confined tasks could be useful. 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).

A complementary route would be chemical engineering using microtubule-directed compounds. Antimicrotubular compounds have been traditionally used for growth control as potato sprouting suppressors or as herbicides (reviewed in 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 novel approach, described in the chapter by Sadot in this volume, is based on bioactivity screening cytoskeletal responses of mammalian cells as readout. A further promising development in chemical engineering are 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). We are presently adapting this approach to plant tubulin as a target by titration of specific domains on the tubulin heterodimer with ectopic peptides.

The plant microtubular cytoskeleton conveys a broad range of very specific functions that are not known in animal cells. Some of these functions are structural; others are of a sensory nature. This provides ample space for biotechnological manipulation that is subtle, specific, and safe (because these functions are not relevant for animals and man). The success of this approach will depend on the precision of our tools. This precision can only be reached when application is underlaid by solid pure science.

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