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Microtubules as Sensors for Abiotic Stimuli

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Abstract Microtubules are generally perceived as structural, static elements that basically function either as supporting scaffolds or barriers. This view has been increasingly challenged during the last decade of the last century, when in-vivo imaging of microtubules revealed that they are endowed with complex and highly nonlinear dynamics. This indicates that, in addition to their traditional structural functions, microtubules must play a role in more volatile events that have to be organized in space and time. It has become clear that microtubules are subject to numerous signalling chains and that this is especially important in plants, where morphogenesis is under tight control of a broad panel of environmental cues. However, it has remained a bit more implicit that microtubules are not only targets for signalling, but participate very actively in signal transduction itself. This work ventures to review and to emphasize this aspect. It begins with a survey of the physiological and molecular evidence of microtubules as targets for signalling, but then changes perspective focussing on the mechanosensory properties of microtubules. It is proposed that the nonlinear dynamics of microtubule assembly provide the strong and sensitive signal amplifier necessary for the sensing of minute mechanic stimuli. Using gravi- and cold-sensing as examples, it is shown, how this mechanism can be used very efficiently to detect abiotic stimuli and to adapt to even harsh environments.

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Microtubules as Sensors: Physiological Mechanisms

The perception of abiotic stimuli other than light poses demanding challenges to signalling: a physical stimulus has to be transformed into a biochemical output. It is generally believed that the original inputs are minute changes in geometry of the membrane, where the perception mechanism is located. In other words: the energy of the primary input is extremely small and has to be efficiently amplified.

This problem is even accentuated in plants, because plant cells are subject to continuous pressure from inside (produced by the expanding protoplast) and outside (produced by the expanding neighbouring cells). These pressures are in the range of several bars and are responsible for large background forces against which the minute changes of mechanical energy have to be discriminated. A second challenge is the lack of specialized sensory organs—sensing is diffusely spread over a large number of cells. Thus, each individual cell has to produce a sufficient sensory output by itself. The maximally possible input energies are therefore limited and hardly exceed thermal noise.

Mechanochemical sensors in higher plants are involved in numerous responses to physical stimuli including sensing of gravity, touch, wind, (for review see Telewski 2006), but also cold and salt. Experiments with aequorin-transformed plants have shown that these stimuli trigger the release of calcium in specific time signatures (Knight et al. 1991). Therefore, mechanosensitive calcium channels have been proposed to transduce these physical stimuli into calcium influx as chemical signals. In fact, when touch-insensitive mutants were isolated in *Arabidopsis*, three of the four genes identified in this approach turned out to be calmodulins (Braam and Davis 1990), and touch-responses can be suppressed by inhibitors of calmodulin (Jones and Mitchell 1989). However, the touch-sensitive calcium channels that are postulated to trigger this signal chain have remained elusive, at least in plants. This might be related to the highly artificial conditions required to identify stretch-activated ion fluxes by patch-clamp techniques. Removal of the cell wall, isotonic conditions, and suction by the holding electrode create conditions, where most ion channels would be defined as mechanosensitive (Gustin et al. 1991).

The transition to terrestrial life forms required very efficient systems to efficiently sense and respond to gravity and mechanical tension. It is even possible to understand plant evolution in terms of adaptation to this task (Niklas 1997). Despite this impact, mechanosensing has remained obscure so far. A simple stretch-activated ion-channel system is certainly not sufficient to cope with the challenge to detect a minute input (deformation of a membrane) against a background of fairly large turgor pressures. Thus, efficient systems of input amplification are required. It is likely that similar systems operate in other organisms as well, however, in plants they have to be particularly effective.

What are the requirements for such input amplifiers? (1) They should be able to collect small and diffuse mechanic energies (for instance from changes in membrane fluidity, Los and Murata 2004) and to concentrate them into a local, stronger stimulus (stress-focussation). (2) They should be anisotropic to efficiently transfer mechanical translocations. (3) They should be endowed with a certain rigidity. (4) They should be endowed with positive autoregulation to efficiently amplify small inputs.

These four preconditions are met by microtubules that therefore represent good candidates for such input-amplifiers. Their bending modulus corresponds to that of glass (Gittes et al. 1993)—unlike actin, for instance. They are long, hollow cylinders and their growth and shrinkage is not a continuous process, but subject to catastrophic phase transitions. A recent publication (Grishuk et al. 2005) could show that disassembly of microtubules can generate substantial forces that are about tenfold higher than even those caused by microtubule motors.

A range of observations from different organisms suggests that microtubules are involved in the perception of abiotic stimuli:

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1. *Perception of touch in Caenorhabditis*: A screen for mutants that are insensitive to touch, Chalfie and coworkers identified transmembrane proteins that possibly represent elements of a mechanosensitive channel (Chalfie and Au 1989). However, they also recovered a couple of mutants in tubulins that participated in specialized microtubule bundles characteristic for the touch-sensitive cells (e.g. Fukushige et al. 1999). These observations led to a working model, where the primary input—a deformation of the membrane—was amplified by a microtubule-based lever system ("toilet-flush system") into a focussed mechanical force that is large enough to open the putative ion channel (Chalfie 1993).
2. *Gravitropism*: The trigger (susception in sensu Björkman 1988) is the sedimentation of statoliths. The force exerted by these statoliths is believed to be sensed by mechanosensitive ion channels. This gravitropic sensing can be blocked by antimicrotubular drugs in the rhizoid of *Chara* (Friedrich and Hertel 1973) as well as in moss protonemata (Schwuchow et al. 1990; Walker and Sack 1990) or in coleoptiles of maize (Nick et al. 1991) and rice (Godbolé et al. 2000; Gutjahr and Nick 2006) at concentrations that leave the machinery for growth and bending essentially untouched. Conversely, when the dynamics of microtubules is reduced either as a consequence of a mutation (Nick et al. 1994) or treatment with taxol, this results in a strong inhibition of gravitropic responses (Nick et al. 1997; Godbolé et al. 2000; Gutjahr and Nick 2006).
3. *Mechanic stimuli affect microtubule orientation*: The application of mechanical fields (Hush and Overall 1991), high pressure (Cleary and Hardham 1993) or artificial bending of coleoptiles (Zandomeni and Schopfer 1994) can induce a reorientation of cortical microtubules. Centrifugation experiments in regenerating tobacco protoplasts (Wymer et al. 1996) suggest that microtubules are aligned in parallel to the administered centrifugal force. Although the stimuli used in these studies were several orders of magnitude above those that typically occur in a physiological context, there are indications that microtubules are aligned by mechanical strain during development as well. For instance, when new leaf primordia are laid down, sharp transitions in microtubule orientation arise at the boundary of the incipient primordium. These sharp transitions are subsequently smoothened by realignments of microtubules such that the pitch of cortical microtubules changes gradually over several tiers of cells in parallel to the stress-strain pattern predicted for the environment of a protruding primordium (Hardham et al. 1980).
4. *Volume regulation*: The ability to regulate cell volume depending on the osmolarity of the environment represents an evolutionary ancient achievement and can be observed already in bacteria, where osmoregulation involves mechanosensitive channels of large conductance (MscL) that can open and allow mass transport of osmotically active ions parallel to the gradient of chemical potential for those particles (Chang et al. 1998). Again,

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the primary stimulus is a deformation of the membrane. In the case of MscL, this has to be quite drastic nearly in the range of membrane breakage to trigger opening of the channel. In eukaryotes, more subtle inputs are sufficient to trigger volume regulation. Recent studies on the role of the cytoskeleton in the volume regulation of mammalian spermatozoa (Petrunkina et al. 2004) or plant protoplasts (Komis et al. 2002) indicate that microtubules participate in the earliest events of volume regulation.

5. *Temperature sensing*: Since microtubules disassemble in the cold, there exist a couple of studies, where their behaviour was followed in the context of low-temperature responses. When microtubules were manipulated pharmacologically, this was accompanied by changes in cold hardiness. For instance, a treatment with taxol was reported to reduce freezing tolerance in rye roots (Kerr and Carter 1990) or spinach mesophyll (Bartolo and Carter 1991b). Conversely, freezing tolerance could be induced by a mild treatment with pronamide (a herbicide that affects microtubule assembly) in a way similar to cold acclimation (Abdrakhamanova et al. 2003) indicating that a sensory microtubule population acts as a "thermometer" that triggers or modulates adaptive responses to low temperature.

These examples may suffice to illustrate the importance of microtubules for the sensing of abiotic stimuli. The primary sensors of these responses have remained obscure so far, but it seems that microtubules act as amplifiers in concert with these primary sensors. For several of the responses described above, the removal of microtubules cannot completely interrupt sensing, but results in a decreased sensitivity and thus in a delay of the response. For instance, treatment with microtubule assembly-blockers delays the onset of gravitropic bending in coleoptiles (Nick et al., unpublished results), but eventually gravitropism initiates suggesting that microtubules are not the conditio sine qua non, but rather act as positive modulators of the primary sensing response.

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2 Microtubules as Sensors: Molecular Mechanisms

Although a sensory function of microtubules in the sensing of abiotic stimuli is supported by a large number of observations from different organisms, the molecular base of this sensory function has remained enigmatic so far. Principally, there are two possible routes and at the present (Fig. 1), limited, state of knowledge it is not possible to rule out any of those. And this may not be necessary, because these routes are not mutually exclusive:

1. Microtubules as Susceptors for Mechanosensitive Ion Channels.
   In the first model, the actual perception of the mechanic stimulus occurs through mechanosensitive ion channels (Fig. 1A). The primary input are minute deformations of the membrane with energies that are, in

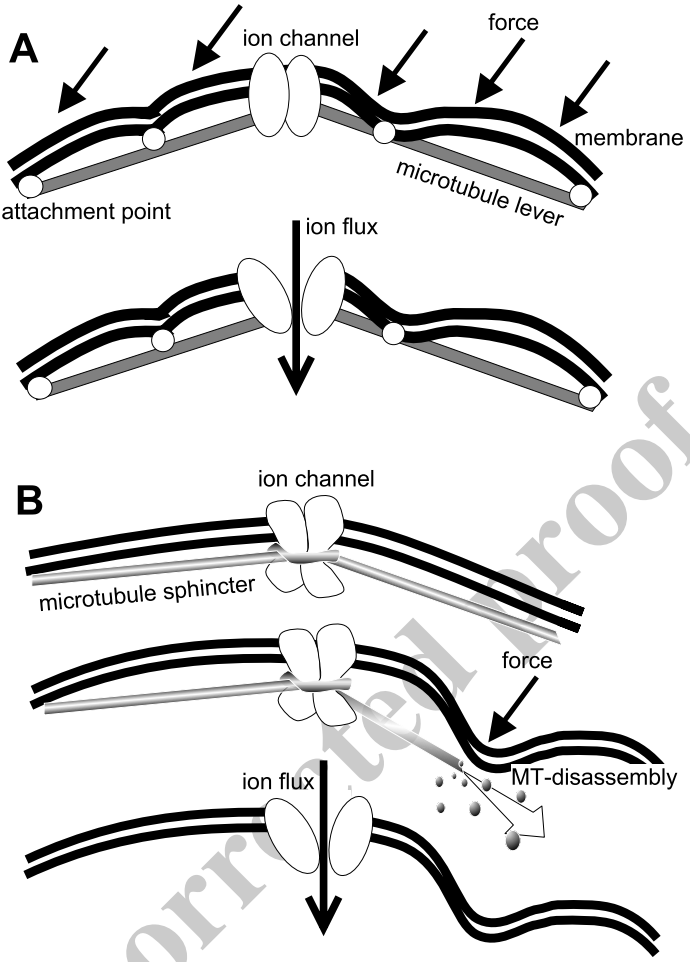

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Fig. 1 Models for the role of microtubules in mechanosensing. **A** Microtubules acting as mechanosusceptors in sensu (Björkman 1988). Membrane deformations are collected and focussed by a microtubule lever system towards a mechanosensitive ion channel such that the input energy exceeds thermal noise. **B** Microtubules acting as mechanoreceptors in sensu strictu. Microtubules constrict the opening of ion channels and disassemble upon mechanic load. Note that, in this model, the ion channel acts as a transducer, not as a receptor [in contrast to the model depicted in (**A**)]

most cases, below the fluctuations due to thermal noise. In order to obtain a sensible signal, these primary deformations have to be focussed by a lever system. The role of microtubules in this model would be that of a (mechanic) susceptor in sensu (Björkman 1988).

Elimination of microtubules has been repeatedly found to activate calcium channels. Antimicrotubular compounds such as oryzalin, ethyl-*N*-

phenylcarbamate, or colchicine induce a six- to tenfold increase in the activity of calcium channels (Ding and Pickard 1993; Thion et al. 1996, 1998). Moreover, cold-induced calcium fluxes are amplified conspicuously by these drugs in tobacco cells (Mazars et al. 1997). However, the characterization of a channel activity as mechanosensitive is usually based on patch-clamp experiments and does not prove any physiological function in mechanosensing.

These pharmacological findings from plant cells are supported by the results from genetic screens for touch-sensitive ion channels in *Caenorhabditis elegans*. Using a system, where the phobic response to a specific touch stimulus was screened, so-called mechanosensation defective (*mec*) mutants could be recovered (Chalfie and Au 1989; for review see Chalfie 1993). Some of the mutated genes encoded a novel class of transmembrane proteins, the so-called degerins, that might represent components of a touch-sensitive ion channel. However, two of these mutants, *mec7* and *mec12* were affected in a unique set of microtubules consisting of 15 protofilaments that were confined to the axons of the touch-sensitive neurons responsible for the phobic response (Chalfie and Thomson 1982). MEC12 and MEC7 were later shown to encode specific isotypes of α-tubulin (Savage et al. 1989) and β-tubulin (Fukushige et al. 1999), respectively. In both mutants, the loss of mechanosensation was correlated by specific changes in the organization of these 15-protofilament microtubules. This led to a model, where the microtubules act through specific linker proteins as a kind of lever system that amplifies minute deformations of the perceptive membrane into a strong aperture of the putative channels (Chalfie 1993). A similar set-up, where specialized microtubules are able, via an intermediate protein to induce a functional spatial arrangement of receptors or ion channels has been proposed for the clustering of glycine receptors in rat spinal cord synapses, where the microtubule-associated protein gephyrin plays the role of the intermediate linker (Kirsch et al. 1993).

2. Microtubules as Primary Deformation Sensors.

In the model sketched above, microtubules act as signal amplifiers for mechanosensitive ion channels. However, microtubules might be the sensors themselves (Fig. 1B). Even in vitro the assembly of microtubules from soluble tubulin heterodimers could be shown to depend on vectorial forces. The preferential direction of the assembled microtubules could be modulated by centrifugal force (Tabony and Job 1992) although one should note that the forces acting here are orders of magnitude above the minute inputs that trigger the perception of abiotic stimuli in a physiological context. The same argument is true for experiments, where the microtubules could be reoriented by bending of maize coleoptiles by application of defined weights (Zandomeni and Schopfer 1994). The forces acting in those experiments approached the limits of membrane integrity

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and thus are far beyond the physiologically relevant range. This emphasizes the necessity of efficient signal amplification in deformation sensing. As will be explained in more detail later, due to their nonlinear dynamics microtubules themselves should be able to amplify small mechanic stimuli into clear net outputs that can be processed by downstream signalling cascades. It should also be kept in mind that microtubules can generate force not only through microtubule-motors such as kinesins or dyneins. In addition, microtubule disassembly could be recently shown to generate a force that is quite considerable and even exceeds the forces produced by motor proteins (Grishchuk et al. 2005).

Summarizing, both models for the sensory role of microtubules are compatible with our (admittedly still limited) knowledge on the molecular base of abiotic sensing. Both models rely on positive feedback circuits that are able to amplify the minute inputs (small deformations of the perceptive membranes in the first model or changes in the dynamic equilibrium between assembly and disassembly of microtubules themselves in the second model) into clear and nearly qualitative outputs that can then be processed by downstream signalling cascades. The distinction between the two models described above was introduced for the sake of conceptual clarity, it might be not as pronounced in the biological context of a cell, where both mechanisms could act in a complementary fashion. It will be a challenge for the next years not only to identify the molecular elements acting in the perception and modulation of abiotic stimuli, but to understand their interaction and systemic properties. This will require integration of molecular data with cell biological and physiological analysis and even mathematical modelling.

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3 Plant Microtubules as Mechanosensors

Plant growth and development responds very sensitively to mechanical stimulation, a phenomenon that has been termed thigmomorphogenesis (Jaffe 1973). For instance, cell expansion is redistributed from elongation towards lateral thickening, when a shoot is repeatedly touched or bent. Later, it was possible to demonstrate thigmomorphogenesis on the cellular level as well. For instance, when a protonema of the fern *Adiantum* was squeezed by a needle, chloroplasts "fled" from the contact site (Sato et al. 1999), whereas in parsley cells, the nucleus, probably as an element of a defence response to pathogen invasion (see Chapter "Microtubules and pathogen defence", in this volume), approached the needle (Gus-Mayer et al. 1998). Alternatively, cells (protoplasts) were embedded in agar and mildly centrifuged or squeezed, resulting in a respective alignment of cell division or cell expansion with the force vector (Wymer et al. 1996; Zhou et al. 2007).

It has long been speculated that calcium fluxes are involved in the signal transduction that culminates in thigmomorphogenesis. By generating transgenic tobacco plants that expressed the luminescent calcium reporter aequorin (Knight et al. 1991), it became possible to observe these fluxes directly and to demonstrate that the signature triggered by a touch stimulus was specifically different from those induced by other stimulation qualities such as cold. Since these changes of intracellular calcium levels occur rapidly after stimulation (Legue et al. 1997), mechanosensitive calcium channels have been postulated as the primary element of mechanosignalling.

Recently, homologues of the bacterial MscS (for mechanosensitive channel of small conductance) have been identified in *Arabidopsis thaliana*. One of these homologues, MSL3, could functionally complement a bacterial mutant affected in the function of mechanosensitive channels suggesting that MSL3 is indeed a mechanosensitive ion channel. GFP fusions of MSL3 and a second homologue, MSL2, were demonstrated, by fluorescence microscopy, and by subcellular fractionation, to be localized in discrete patches in the plastid envelope. Moreover, they colocalized with the plastid division factor MinE (see Chapter "Microtubules and the Evolution of Mitosis", in this volume) indicating an interaction of MSL2 and MSL3 with plastid division. In fact, mutants in these bona-fide channels harboured chloroplasts that were irregular in size, shape and partially number. Thus, these channels regulate morphogenesis and development of plastids. In other words: during endosymbiosis of the prokaryotic plastid ancestors, these channels underwent a shift in function from osmoregulation (that has been taken over by the eukaryotic "host" cell) towards regulation of plastid morphogenesis. An attractive model assumes that MSL2 and MSL3 sense membrane tension in the plastid envelope and feed this information through interactions with MinE (and, indirectly, MinD) into the machinery that defines the location of the plastid division ring (see Chapter "Microtubules and the Evolution of Mitosis", in this volume).

Thus, homologues of prokaryotic mechanosensitive channels seem to exist in plants. The putative channels that are responsible for thigmomorphogenesis, have remained elusive, though. By patch-clamp analysis, it was possible to detect mechanosensitive calcium fluxes in membrane preparations (Ding and Pickard 1993). These fluxes could be inhibited by lanthanoid ions and were capacitated by antimicrotubular agents indicating that microtubules control the permeability of these channels for calcium.

In the context of an expanding tissue (that is characterized by considerable tension), microtubules seem to be directly involved in osmoadaptation. By application of osmotic stress to root tips of *Triticum turgidum* microtubules could be induced to disassemble and to reorganize in massive bundles, the so-called macrotubules (Komis et al. 2002). When this response was suppressed by treatment with oryzalin, the protoplasts were not any longer able to adapt by controlled swelling to the osmotic stress and perished. A pharmacolog-

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ical study (Komis et al. 2006) revealed that inhibitors of phospholipase D, such as butanol-1 or *N*-acetylethanolamine, suppressed osmotic adaptation as well as the formation of the macrotubules. In contrast, phosphatidic acid, a product of phospholipase D, enhanced osmoadaptation and macrotubule formation and was able to overcome the inhibitory effect of butanol 1. These observations demonstrate that the microtubule response (formation of macrotubules) is essential for osmoadaptation, and that signalling through phospholipase D acts upstream of microtubules in this response.

A recent study (Zhou et al. 2007) highlights the interaction of microtubules with the cell-wall-cytoplasmic continuum during mechanosensing. Agarose-embedded suspension cells of chrysanthemum were subjected to compression force. Under these conditions, the axis of cell expansion could be aligned in a direction perpendicular to the vector of force. When microtubules were removed by oryzalin prior to the treatment or when the cell-wall cytoplasmic continuum was impaired by treatment with RGD-peptides (that, in animal cells, interfere with adhesion sites), this alignment response was interrupted. Elimination of actin filaments by cytochalasin B did not produce this effect. Thus, microtubules, probably in conjunction with the cell wall, are essential for the cellular response to mechanic stimulation. However, as in the macrotubule system, it is not clear, whether microtubules act as transducers or even effectors of the mechanic stimulus or whether they convey a true sensory function.

Using tension-free protoplasts, Wymer et al. (1996) were able to align microtubules by a short centrifugation and thus to orient the axis of cell expansion in a direction perpendicular to the force vector (Fig. 2). They used this system to dissect a possible sensory role of microtubules. Since microtubules are necessary for the directional synthesis of cellulose (see Chapter "Control of cell axis", in this volume), a transient elimination of microtubules using the herbicide amiprophosmethyl was used. After washing out the herbicide, microtubules recovered such that the directionality of cellulose synthesis and thus the cell axis could become manifest. Using this approach, microtubules were eliminated for the short interval corresponding to the time of centrifugation and then allowed to recover without any significant effect on viability or regeneration of the protoplasts. This transient microtubule elimination was then administered either immediately before or immediately after the centrifugation stimulus (Fig. 2). When microtubules were eliminated subsequent to the centrifugation, the alignment of cell axis by the stimulus was not impaired. However, when microtubules were eliminated just prior to the centrifugation and allowed to recover immediately after the end of stimulation, the alignment disappeared completely. This demonstrated clearly that microtubules are essential for the sensing of this mechanic stimulus.

Thus, there is clear evidence for a microtubule function in the mechanosensing of plants, and experimental systems have been developed, where this question can be addressed. It should now become possible to discriminate

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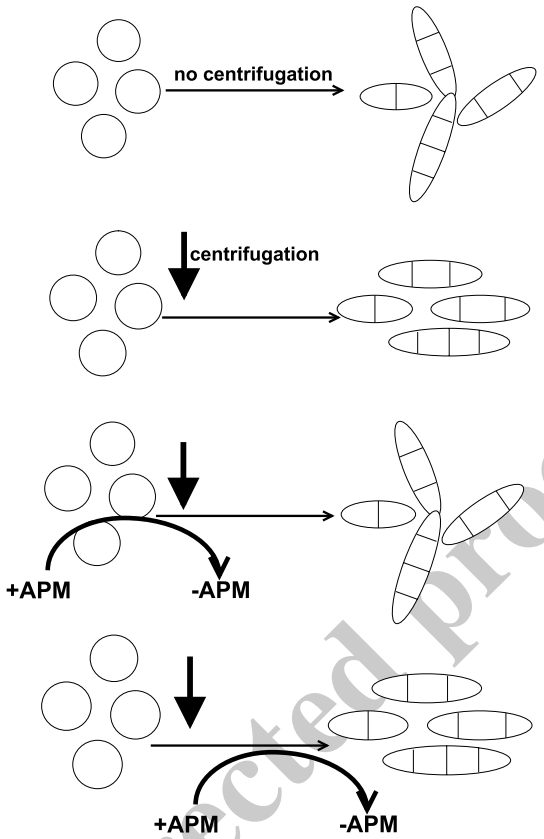

Fig. 2 Demonstration of a true sensory role for microtubules in plant mechanoperception. Tobacco protoplasts were embedded in agarose and then subjected to a short mechanic stimulation (mild centrifugation). Cell expansion and cell division were subsequently aligned in a direction perpendicular to the vector of force. When microtubules were eliminated by amiprophosmethyl (APM) prior to centrifugation and allowed to recover afterwards, cells elongated and divided normally, but without alignment. APM treatment of identical duration but administered following the centrifugation was not effective

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between mechanosusception by microtubules (Fig. 1A) and a microtubular mechanoreceptor function in *sensu strictu* (Fig. 1B).

4 Plant Microtubules as Gravisensors

Mechanosensation is not confined to the perception of touch, but includes a range of abiotic stimuli with gravity certainly being of central importance. Upon the transition to terrestrial life, plants had to cope with the loss of

buoyancy as an important supportive element. This required the development of mechanical support, the vascular bundle as the central element of the telomes that represent the modular elements of all land plants (Zimmermann 1965). However, it is not sufficient to generate a force-bearing element, what matters, is the spatial arrangement of these elements in a manner such that they provide optimal mechanical support, but simultaneously consume minimal biomass and are as light as possible. This optimization task can only be achieved, when the arrangement of supportive structures is guided by the pattern of mechanical strain. The ultimate source of these strains is gravity. Thus, gravity has to be perceived very efficiently and it has, in addition, to be linked to morphogenesis.

This link becomes manifest in two basic phenomena:

1. When the orientation of a plant is changed with respect to gravity, it will respond by bending that will restore the original orientation and thus will minimize mechanical stress (gravitropism).
2. When new organs are laid down and oriented, these processes are often adjusted with respect to gravity (gravimorphosis).

Microtubules and gravitropism: For the rhizoid of *Chara* the classical experiments of Johannes Buder (1961) have shown that barium-sulfate containing vesicles, the *Glanzkörperchen*, are necessary and sufficient for gravisusception. For higher plants the classical starch-statolith theory (Nemec 1900; Haberland 1900) postulated that amyloplasts in the perceptive tissues (e.g. root cap or bundle sheath cells) are responsible for the susception of the gravitropic stimulus. A long tradition of experimentation demonstrated that amyloplasts are necessary for efficient gravitropism. For instance, gravitropic sensitivity was reduced in starch-deficient mutants. However, it took almost a century until it could be shown that amyloplast translocation is sufficient to trigger a tropistic growth response. By using high-gradient magnetic fields, Kuznetsov and Hasenstein (1996) succeeded to induce bending in vertically oriented roots and thus were able to proof the starch-statolith concept for gravisusception. It represents an irony of science history that this breakthrough was not achieved by the elaborate and expensive microgravity experiments in the context of space research, but instead through a very cheap, but well-designed ground experiment. Thus, in higher plants as well, the primary stimulus is produced by statolithic particles (the amyloplasts), but the actual perception event remains to be revealed. It had been postulated for the rhizoid of *Chara* that a sedimentation of the *Glanzkörperchen* to the lower flank of the rhizoid would divert vesicle flow towards the upper side such that more material is intussuscepted into the upper flank resulting in a growth differential driving downward bending (Sievers and Schröter 1971). This hypothesis was later extended to negative gravitropism by combining sedimentation with a different mode of growth (Hodick 1994). According to this model, the actual perception of gravity would rely upon a proximity

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mechanism. It is to be doubted that proximity is used for graviperception in higher plants, since already classical studies (Rawitscher 1932) using intermittent stimulation could show that perception can occur in the absence of amyloplast sedimentation. Moreover, dose-response studies employing centrifugation show that the output (gravitropic curvature) is dose-dependent even for stimuli that completely saturate amyloplast sedimentation. Even for the rhizoid of *Chara*, for which the proximity mechanism had been postulated originally, it could be demonstrated that stimuli that produce a complete sedimentation of the *Glanzkörperchen* can nevertheless be discriminated (Hertel and Friedrich 1973). This suggests that the actual perception of gravity is not based on proximity, but on pressure exerted by the statoliths to a mechanosensitive receptor.

If gravity is not perceived by proximity, but by pressure, this poses a big challenge to the sensing mechanism. Since gravity is sensed by individual cells (in contrast to the direction of light in phototropism—Buder 1920; Nick and Furuya 1995), the maximal energy available for stimulation is the potential energy of the sensing cell. This energy barely exceeds thermal noise, if it is not focussed upon small areas. These considerations stimulated research on a potential role of microtubules as amplifiers of gravitropic perception. In fact, gravitropism can be blocked by antimicrotubular drugs in the rhizoid of *Chara* (Hertel and Friedrich 1973) as well as in moss protonemata (Schwuchow et al. 1990; Walker and Sack 1990) or in coleoptiles of maize (Nick et al. 1991) and rice (Godbolé et al. 2000; Gutjahr and Nick 2006) at concentrations that leave the machinery for growth and bending essentially untouched. Conversely, when the dynamics of microtubules is reduced either as a consequence of a mutation (Nick et al. 1994) or treatment with taxol, this results in a strong inhibition of gravitropic responses (Nick et al. 1997; Godbolé et al. 2000; Gutjahr and Nick 2006).

The gravitropically induced reorientation of cortical microtubules has been observed for both shoot (Nick et al. 1991) and root gravitropism (Blancaflor and Hasenstein 1993). In maize coleoptiles, the microtubules in the epidermal cells of the upper flank of the stimulated organ assumed a longitudinal orientation, whereas the microtubules in the lower flank remained transverse. By microinjection of fluorescent tubulin into epidermal cells of intact maize coleoptiles it was later even possible to demonstrate the gravitropic microtubule reorientation in vivo (Himmelspach et al. 1999). The time course of this response was consistent with a model where gravitropic stimulation induced a lateral shift of auxin transport towards the lower organ flank and, as a consequence, a depletion of auxin in the upper flank. The microtubular response was thought to be primarily by this decrease in auxin concentration rather than by gravity itself. In maize roots, however, where a similar reorientation could be observed in the cortex (Blancaflor and Hasenstein 1993), the time course of reorientation was found to be slower than the changes in growth rate induced by gravity.

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This leads to the question, whether the gravitropic response of microtubules is direct or whether microtubules merely respond to changes in growth rate. In fact, it is possible to induce microtubule reorientation by bending coleoptiles with manual force (Zandomeni and Schopfer 1994)—microtubules will then become longitudinal in the concave flank, but remain transverse in the convex flank. To dissect the gravitropic response and a potential response to changed growth rate, microtubule behaviour was followed in coleoptiles that were prevented by a surgical adhesive from elongation and either kept in horizontal orientation (such that a gravitropic stimulation occurred) or in vertical orientation (such that growth was inhibited in the absence of a gravitropic stimulus). In this setup, a microtubule reorientation from transverse to longitudinal could be observed only in the horizontal orientation (Himmelspach and Nick 2001) demonstrating unequivocally that microtubules, at least in this system, responded to gravity rather than to the inhibition of growth.

Microtubules and gravimorphosis: The impact of gravimorphosis is already illustrated by the simple observation that roots form at the lower pole of a plant. Although a considerable amount of phenomenological work has been dedicated to this problem at the turn of the century (Vöchting 1878; Sachs 1880; Goebel 1908), the underlying mechanisms have remained obscure so far. One reason for this problem has been certainly the use of adult organs, where polarity has already been fixed and is hard to invert. In the meantime, new systems have been introduced that may be more suited to study gravimorphosis. Germinating fern spores, for instance, initiate their development with a first asymmetric division that separates a larger, vacuolated rhizoid precursor from a smaller and denser thallus precursor. This first cell division is clearly of formative character—when it is rendered symmetric by treatment with antimicrotubular herbicides (Vogelmann et al. 1981), the two daughter cells both give rise to thalloid tissue. The axis of the first division is strictly aligned with gravity. When the spore is tilted after the axis of the first division has been determined, the rhizoid will grow in the wrong direction and cannot adjust this error (Edwards and Roux 1994). Prior to division, at the time when the spore is competent to the aligning influence of gravity, a vivid migration of the nucleus towards the lower half of the spore is observed. This movement is not a simple sedimentation process because it is oscillatory and interrupted by short periods of active sign reversal, indicating that the nucleus is tethered to a motive force (Edwards and Roux 1997). The action of antimicrotubular compounds strongly suggests that this guiding mechanism is based on microtubules that probably align with the gravity vector. It should be mentioned that a similar mechanism of gravimorphosis has been described for the determination of the grey crescent in frog eggs (Gerhart et al. 1981), where the dorsiventral axis is determined by an interplay of gravity-dependent sedimentation of yolk particles, sperm-induced nucleation of microtubules, and self-amplifying alignment of newly formed microtubules that drive cortical rotation (Elinson and Rowning 1988).

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Microtubules versus mechanosensitive channels in gravisensing: Since microtubules guide the anisotropic deposition of cellulose in the cell wall (see Chapter "Control of cell axis", in this volume), it is not trivial to discriminate their function in gravity-sensing from their participation in the control of axial cell expansion. When gravitropic bending is inhibited by antimicrotubular agents, this might be caused by a block of the sensory or of the effector function of microtubules. To discern these microtubular functions, lateral transport of auxin induced by gravitropic stimulation was analyzed as an event situated upstream of differential growth using radioactively labelled auxin in rice coleoptiles (Godbolé et al. 2000). Lateral auxin transport could be blocked by ethyl-*N*-phenylcarbamate (EPC), a herbicide that binds to the carboxyterminus of α-tubulin and inhibits assembly of tubulin heterodimers to the growing ends of microtubules (Wiesler et al. 2002). Interestingly, taxol inhibited lateral transport partially without any inhibition of longitudinal transport of auxin. This indicates that the presence of sensory microtubules is not sufficient for gravity sensing—they have to be endowed with turnover to fulfil their function. The high dynamics of this sensory microtubule population might also explain the extreme sensitivity of gravisensing to low temperature that would be otherwise difficult to explain (Taylor and Leopold 1992). These observations favour a model, where microtubules are actively sensing gravity (Fig. 1B) rather than merely acting as gravisusceptors (Fig. 1A).

The gravisensory function of microtubules can be specifically blocked by acrylamide (Gutjahr and Nick 2006), a widely used inhibitor of intermediate-filament function in mammalian cells (Eckert and Yeagle 1988). Similar to EPC, acrylamide interrupts a very early step in the gravitropic response chain, clearly upstream of auxin redistribution and differential growth. There are no clear homologues of intermediate-filament proteins known in the plant kingdom, but acrylamide treatment specifically disrupts microtubules, leaving, for instance, actin filaments, untouched (Gutjahr and Nick 2006). The immediate target of acrylamide in mammalian cells seems to be a kinase that phosphorylates keratin (Eckert and Yeagle 1988). Since kinases and phosphatases have been shown to regulate the organization of plant microtubules (Baskin and Wilson 1997), the inhibition of gravitropism by acrylamide might be caused by interference with the regulatory circuits active in the highly dynamic microtubule population responsible for gravisensing.

By application of artificial bending stress in antagonism to a gravitropic stimulus, it is possible to separate the response of gravity from the secondary mechanic stimulus that is induced by the differential growth during gravitropic bending (Ikushima and Shimmen 2005). When, under these conditions, the activity of mechanosensitive channels was suppressed by gadolinium in hypocotyls of adzuki beans, this suppressed the (mechanically induced) reorientation of microtubules in the effector tissue, whereas gravitropic curvature proceeded unaltered (indicating that the microtubule

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population resident in the inner tissues of the apical hook that is responsible for gravisensing remained functional). Thus, at least in this system, mechanosensing is sensitive to gadolinium, gravisensing is not.

Although our knowledge on the primary events of mechano- and gravisensing in plants is extremely limited, it is clear already at this stage that the role of microtubules might differ qualitatively. In mechanosensing, microtubules seem to act as susceptor structures that focus deformation stress towards ion channels (Fig. 1A). In contrast, in gravisensing, the necessity for high dynamics and dimer turnover favours a direct sensory role of microtubules (Fig. 1B). Thus, nature might utilize both mechanisms simultaneously to sense (and possibly to discriminate) different stimuli. The challenge for future research in this field will be to design experimental approaches with clear outputs based on clear concepts on the sensing mechanism. Only in a second step it will become possible to define and test molecular and cellular candidates.

5 Microtubules as Thermometers

In temperate regions, temperature poses major constraints to crop yield. Attempts to increase photosynthetic rates by conventional breeding programs, although pursued over a long period, were not very successful, which indicates that evolution has already reached the optimum (Evans 1975). However, optimal photosynthetic rates can be reached only, when the leaves are fully expanded. The cold sensitivity of growth is much more pronounced than that of photosynthesis. This means that, in temperate regions, productivity is limited by the cold sensitivity of leaf growth (Watson 1952; Monteith and Elston 1971). This conclusion is supported by the finding that in cool climates the production of biomass is not source-, but sink-limited (Warren-Wilson 1966). The major target seems to be the root—it is thus the cold response of roots that defines the velocity of shoot development (Atkin et al. 1973).

However, the issue of cold sensitivity in agriculture is not confined to the 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) to distinguish it from the damage that is caused by actually freezing the tissue. In extreme cases, even very moderate cooling can produce irreversible damage, when it hits a very sensitive period of development. For instance, the fertility of rice is extremely and irreversibly reduced when temperature drops below 18 °C during flower development. The economical consequences of this phenomenon can be drastic—for instance, during the

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cool summer of 1993, the rice yield was reduced by around 25% according to estimates of the Japanese Ministry of Agriculture, Forestry and Fishery.

The extent of cold sensitivity varies between different species and even between different cultivars of the same crop. Those plants that can cope with cool, but non-freezing temperatures below 10 °C, are termed chilling-resistant, whereas the term freezing-resistant is used for plants that can survive temperatures below zero, such as winter wheat or rye (Lyons 1973). It should be kept in mind that the degree of cold sensitivity can change depending on development and environment. For instance, the freezing resistance of many species can be increased by pretreatment with cool, but non-freezing temperature. This so-called cold acclimation or cold hardening during the autumn determines the survival during the winter (Stair et al. 1998). The problem of cold tolerance is not only important for agronomy, but represents an interesting scientific issue as well, because similar to mechanic and gravity-stimulation, a physical signal has to be transformed into a cascade of biochemical signalling events.

In contrast to chilling damage, the cellular consequences of freezing injury are well understood. Especially during rapid freezing, ice crystals form and disrupt internal and external membranes which will kill the cell instantaneously (Burke et al. 1976). As long as freezing occurs at a slow pace and does not exceed a certain limit, the ice will form outside the cell and will remain on the surface of the cell walls, in vessel elements and on the external surface. This does not kill the cell as long as the ice crystals do not penetrate the plasma membrane. However, the plant will dry out in the long term because the access of water to the roots is impaired (Mazur 1963). Therefore, reduced dehydration by reduced transpiration is a strategy to cope with freezing stress. This is a major reason for the dominance of xeromorphic species (such as the conifers) in subarctic or subalpine forests. A second strategy against freezing injury seems to be the expression of specific hydrophilic proteins (Hughes and Dunn 1996). Some of these proteins seem to correspond to the antifreezing proteins found in Antarctic fishes (Kurkela and Frank 1990). These proteins are thought to reduce the threshold temperature for the phase transition into the solid state in membranes and cytoplasm. For instance, one of these proteins, COR15a stabilizes the lamellar phase of chloroplasts in low temperatures (Steponkus et al. 1998).

The actual reason for chilling injury is less evident and has not received the same degree of attention as freezing injury. An apparently trivial consequence of low temperature are reduced rates of biochemical processes. This should result in a reduced metabolic activity, but cannot explain irreversible damages. However, the temperature dependence of different enzymes varies considerably (Guy 1990): whereas maize starch synthetase is inhibited already at 12 °C, and the rice tonoplast proton-ATPase at 10 °C, the maize PEP carboxylase is still active at 4 °C. Thus, when the temperature drops below 10 °C, the enzymes of the respiratory chain will be more affected (possibly because

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they are bound to membranes), whereas some of the glycolytic enzymes are still active. This will result in metabolic imbalance and the accumulation of ethanol and acetaldehyde (Lyons 1973).

Membrane-bound enzymes are more susceptible to chilling injury as compared to soluble enzymes (Lyons 1973). The reason has to be sought in the fluidity of the membranes which is tightly coupled to the abundance in unsaturated fatty acids. A membrane that is composed exclusively from fully saturated lipids should exhibit phase separation at around 30 °C, whereas the introduction of one *cis*-double bond in the centre of the molecule would decrease this phase transition down to 0 °C (Ishizaki-Nishizawa et al. 1996). The degree of lipid saturation can be manipulated by overexpression of desaturases in plants and this has been shown repeatedly to modify chilling sensitivity in plants (Murata et al. 1992; Wolter et al. 1992; Kodama et al. 1994; Ishizaki-Nishizawa et al. 1996).

One of the most pronounced and rapid cellular responses to chilling is the cessation of cytoplasmic streaming (Kamiya 1959; Woods et al. 1984; Tucker and Allen 1986) which occurs within a few minutes after a drop to 10 °C in chilling-sensitive species such as cucumber or tomato (Sachs 1865), whereas it can proceed in chilling-resistant plants down to 0 °C (Lyons 1973). When the period of chilling exceeds a few hours, cytoplasmic streaming fails to recover. Kinetic studies in subtropical species such as maize, lima bean and cotton (Lyons 1973) showed developmental differences with high sensitivity during periods of elevated cell growth. Additionally, high chilling sensitivity is characteristic for morphogenetic responses to light such as axis formation in lower plants (Haupt 1958) or phototropism in maize (Nick and Schäfer 1991).

Microtubules of both plants and animals disassemble in response to low temperature, but the degree of cold sensitivity depends on the type of organism. Whereas mammalian microtubules disassemble already at temperatures below +20 °C, the microtubules from poikilothermic animals remain intact below that temperature (Modig et al. 1994). In plants, the cold stability of microtubules is more pronounced as compared to animals (Juniper and Lawton 1979) reflecting the higher developmental plasticity. However, the critical temperature where microtubule disassembly occurs varies between different plant species (Jian et al. 1989; Chu et al. 1992; Pihakaski-Maunsbach and Puhakainen 1995): In chilling-sensitive plants such as maize, tomato or cucumber, microtubules disassemble already at temperatures above 4 °C, whereas they can withstand 0 °C in moderately resistant plants such as spinach and beet. In cold-resistant species such as winter wheat or winter rye even temperatures as low as –5 °C will not eliminate microtubules. Even within a given species, the cold sensitivity of microtubules can vary considerably (Abdrakhamanova et al. 2003).

The close correlation between microtubular cold sensitivity and the chilling sensitivity of cell growth is supported by the observation that abscisic

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acid, a hormonal inducer of cold hardiness (Holubowicz and Boe 1969; Irving 1969; Rikin et al. 1975; Rikin and Richmond 1976) can stabilize cortical microtubules against low temperature (Sakiyama and Shibaoka 1990; Wang and Nick 2001). Tobacco mutants, where microtubules are more cold stable due to expression of an activation tag, are endowed with cold resistant leaf expansion (Ahad et al. 2003). Conversely, destabilization 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 1986), renders cortical microtubules more cold-susceptible (Akashi and Shibaoka 1987).

It is possible to increase the cold resistance of an otherwise chilling-sensitive species by precultivation at moderately cool temperature. The genes that are activated during this so-called cold hardening are partially identical to those that respond to abscisic acid (for a review see Hughes and Dunn 1996), and the tissue content of abscisic acid increases during cold hardening (Lalk and Dörffling 1985; Lång et al. 1994). On the other hand, mutants that are not able to sense abscisic acid are nevertheless capable of cold hardening (Gilmour and Thomashow 1991) indicating the coexistence of at least two parallel pathways that differ with respect to their dependency on abscisic acid.

Cold hardening can be detected on the level of microtubules as well. Microtubules of cold-acclimated spinach mesophyll cells coped better with the consequences of a freeze-thaw cycle (Bartola and Carter 1991a). Although abscisic acid can increase the cold resistance of microtubules (Sakiyama and Shibaoka 1990), it seems not to be the only trigger. When the microtubular response to abscisic acid was compared to the response to cold hardening, it differed in both orientation and degree of bundling (Wand and Nick 2001), again supporting the existence of a pathway that is independent of abscisic acid.

Microtubules are not only the target of cold stress, they seem, in addition, to participate in cold sensing itself, triggering a chain of events that culminates in increased cold hardiness. When microtubule disassembly is suppressed by taxol, this is reported to suppress cold hardening (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. To test this hypothesis, 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 which would impair growth irreversibly in nonacclimated 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, when a transient disassembly was artificially

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generated by a pulse treatment with the antimicrotubular herbicide pronamide in the sensitive cultivar, this could induce freezing tolerance. This demonstrates that a transient, partial disassembly of microtubules is necessary and sufficient to trigger cold hardening suggesting that microtubules act as "thermometers".

Similar to mechano- and gravity-sensing this leads to the question, whether microtubules act as susceptors (Fig. 1A) or as true receptors (Fig. 1B) for low temperature. The primary signal for cold perception is thought to consist of increased membrane rigidity (Los and Murata 2004; Sangwan et al. 2001). 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). Using aequorin as a reporter in transgenic plants, rapid and transient increases of intracellular calcium levels in response to a cold shock could be demonstrated monitoring changes of bioluminescence (Knight et al. 1991). Pharmacological data (Monroy et al. 1993) confirmed that this calcium peak is not only a byproduct of the cold response, but necessary to trigger cold acclimation. This peak is generated through calcium channels in conjunction with calmodulin. Calcium/calmodulin in turn are intimately linked to microtubule dynamics. Immunocytochemical data show that microtubules are decorated with calmodulin depending on the concentration of calcium (Fisher and Cyr 1993). It was further suggested that the dynamics of microtubules is regulated via a calmodulin-sensitive interaction between microtubules and microtubule-associated proteins such as the bundling protein EF-1α (Durso and Cyr 1994). However, the interaction could be even more direct, because cleavage of the C-terminus of maize tubulin was shown to render microtubules resistant to both low temperature and calcium (Bokros et al. 1996). If the release of calcium from intracellular pools was blocked by treatment with lithium, an inhibitor of polyphosphoinositide turnover (Berridge and Irvine 1984), this resulted in increased cold stability of microtubules in spinach mesophyll (Bartolo and Carter 1992). Using a cold-responsive reporter system it could be demonstrated that disassembly of microtubules by oryzalin could mimick the effect of low temperature, whereas suppression of microtubule disassembly by taxol suppressed the activation of this promotor by low temperature (Sangwan et al. 2001). In the same system, treatment with the calcium ionophore A23187 was observed to be inductive, whereas the Ca-channel blocker gadolinium suppressed cold induction of the reporter. These data favour a model, where microtubules act as receptors that limit the permeability of calcium channels that are triggered by membrane rigidification (Fig. 3).

When microtubules function as modulators of calcium-channel activity and when microtubule integrity is regulated through calcium/calmodulin this would set up a regulatory circuit capable of self-amplification: Stable microtubules that limit the activity of cold-induced voltage-dependent calcium

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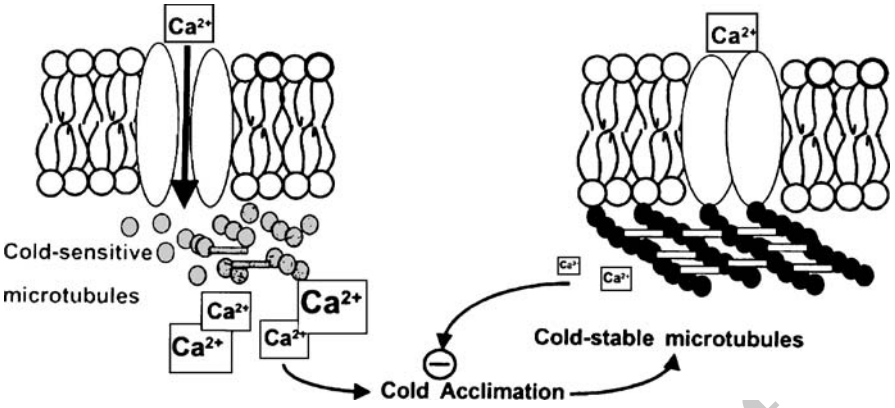

Fig. 3 Perceptive role of microtubules in cold sensing. Cold induced disassembly of specific microtubules that control the permeability of mechanosensitive calcium channels amplifies the influx of calcium, triggering further disassembly of microtubules in a positive feedback loop. The calcium-triggered transduction cascade culminates in changes of gene expression that will produce cold-hardening, including the formation of cold-stable microtubules such that the microtubule-dependent perception of cold will be alleviated (sensory adaptation)

channels, would, upon disassembly, release this constraint and this would elevate the activity of the channels resulting in an increased influx of calcium. This calcium influx, in turn would result in further disintegration of the microtubular cytoskeleton and thus trigger by this positive feedback the influx of additional calcium. A very small initial calcium influx might thus be amplified into a strong signal that can be easily processed by the activation of calcium-dependent signalling cascades. 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 endow cold sensing with high sensitivity, but, in addition, with the ability to downregulate sensitivity upon prolonged stimulation, a key requirement for any biological sensory process.

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6 Outlook

Comparison of the Three Sensory Mechanisms

This work summarizes evidence for a microtubule function in the sensing of touch, gravity and low temperature. Although we are still far from under-

standing the actual setup of the sensory machinery, already at this stage first differences between the different stimulus qualities emerge (Fig. 4):

For mechanoperception, microtubules seem to interact with stretch-activated ion channels. They might act as mechanosusceptors (Fig. 4A) focussing diffuse membrane deformations upon specific membrane areas. Since the definition of a stretch-activated channel is experimentally very problematic and prone to artifacts (Gustin et al. 1991), the possibility should be tested seriously that there are no a priori stretch-activated channels in plants. It might be the combination of a channel with a microtubule lever system that generates the mechanogating of the channel. The same channel would not be defined as mechanosensitive, if the membrane would be stripped of microtubules.

The situation might be different for the sensing of gravity (Fig. 4B). Here microtubules themselves could act as primary sensors. The few experiments, where the involvement of ion channels has been addressed experimentally (Ikushima and Shimmen 2005) suggest that these channels might be dispensable for gravity sensing. The necessity of microtubule turnover in the sensing of gravity indicates a true perceptive function rather than stimulus susception.

The sensing of cold (Fig. 4C) seems to represent a third mechanism, where the gating of cold-sensitive channels (that probably respond to membrane rigidity as an input) is limited by microtubules. When these microtubules disassemble in response to cold, this will release the constraints upon the activity of the ion channels such that calcium can enter which will facilitate, through interaction with calmodulin, further disassembly of microtubules and thus trigger a positive feedback loop. In this system microtubules would play a dual function—in the first phase perception in sensu strictu and susception in the subsequent phase.

It is their innate molecular properties that render microtubules ideal for the sensing of minute and noisy inputs. Microtubule dynamics are nonlinear and endowed with autocatalytic properties: (A) Under cellular conditions, microtubules of different orientation compete for the incorporation of free heterodimers. In all organisms investigated so far tubulin synthesis is tightly regulated by an elaborate system of transcriptional and post-transcriptional controls, probably to avoid the accumulation of (highly toxic) supernumerous free heterodimers (for details refer to Chapter "Plant tubulin genes: regulatory and evolutionary aspects", in this volume). (B) Although the term "cytoskeleton" evokes the idea of a rigid framework that stabilizes the structure of a cell, such associations are far from reality. The half-time of a plant microtubule, for instance, has been calculated to be in the range of 30–60 s (Hush et al. 1994). Therefore, it is more appropriate to conceive microtubules as states of dynamic equilibrium between assembly and disassembly of tubulin heterodimers. It is this dynamic equilibrium that provides the major source for the characteristic nonlinearity of microtubule dynamics.

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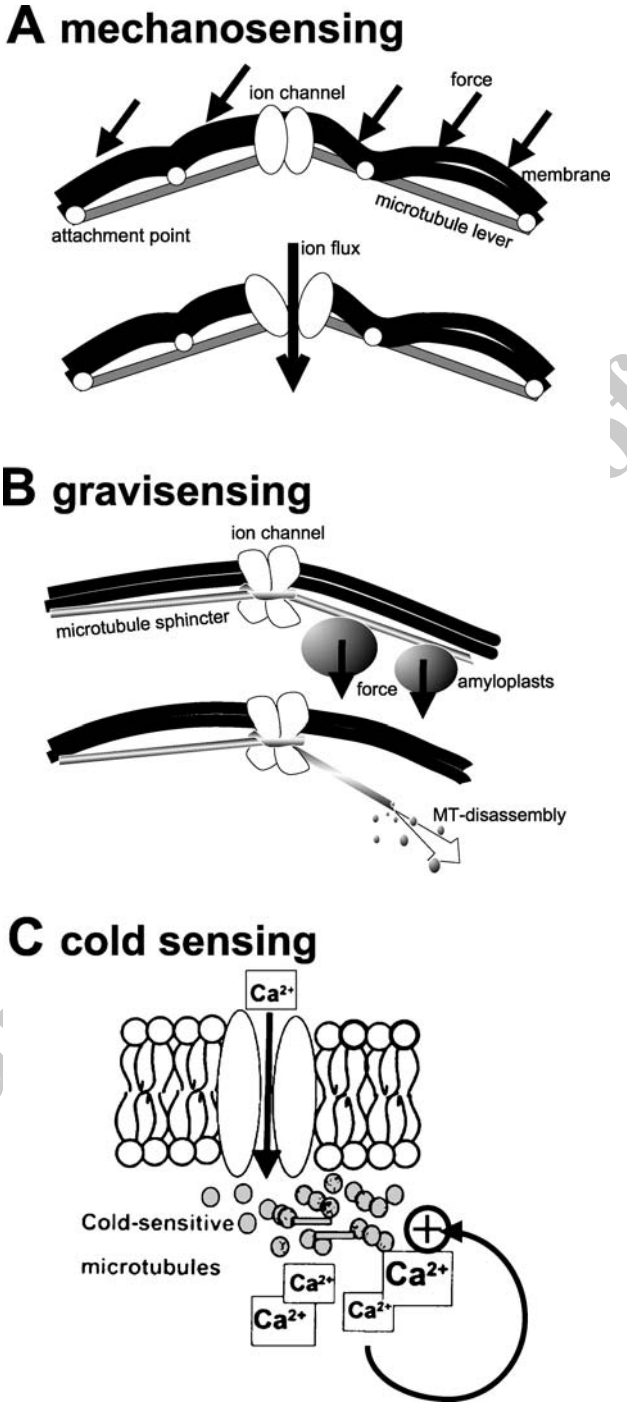
A mechanosensing
ion channel
force
membrane
microtubule lever
attachment point
ion flux
B gravisensing
ion channel
microtubule sphincter
force
amyloplasts
MT-disassembly
C cold sensing
Ca2+
Cold-sensitive
microtubules
Ca2+
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◄ **Fig. 4** Potential differences in microtubule function during the sensing of touch, gravity and cold. **A** Mechanosusceptor function of microtubules during stretch-activation of mechanosensitive ion channels. **B** Microtubules as primary gravity sensors, possibly in interaction with microtubule-gated ion channels. **C** Dual function of microtubules as primary sensors and amplifiers (susceptors) during cold sensing

Interestingly, the relation between assembly and disassembly is practically never balanced—there is always one dominating over its antagonist. This statement is valid in both space and time. In space, because dimer addition and dispersal define a distinct polarity of each individual microtubule with dimer addition dominating at the plus end, dimer dissociation at the minus end. In time, because each microtubule can switch between a growing state, when dimer addition at the plus end predominates over dimer dissociation at the minus end, and a shrinking state, when dimer dissociation at the minus end exceeds dimer addition at the plus end. The switch between both states is swift and dramatic, so dramatic that it has been termed microtubule catastrophe. These conversions depend on associated proteins that can increase or decrease the frequency of transition between growth and shrinkage.

Because of their nonlinear growth microtubules are often involved in developmental patterning. For instance, the induction of the grey crescent in the developing frog egg is produced in an epigenetic process, where a gravity-dependent gradient of developmental determinants in the central yolk interacts with a second, displaced, gradient in the egg cortex (Gerhart et al. 1981). The displacement of the egg cortex is driven by microtubules and triggered by the penetration of the sperm. The sperm induces the nucleation of microtubules that act as tracks for a kinesin-driven movement (Elinson and Rowning 1988). The movement, in turn, triggers shear forces that align the nucleation of additional microtubules in a direction parallel to the movement, whereas deviant microtubules more frequently undergo catastrophic transitions. The resulting net alignment of tracks increases the efficiency of movement and thus the aligning force. This culminates in a rapid rotation of the cortical plasma in a direction from the sperm towards the more remote equator of the egg. This movement will then cause an overlap of upper cortex with a small region of the lower core and eventually trigger inductive events that define the Spemann organisator.

The combination of nonlinear, autocatalytic dynamic states of individual microtubules with the tight control of free heterodimers accentuating mutual competition between individual microtubules generates system properties that are highly relevant for sensory processes. Microtubules fulfil all formal criteria of a reaction-diffusion system *in sensu* Turing (1952). This means that they can be understood as ideal pattern-generators that are able to produce qualitatively clear, neat outputs from minute, and highly noise-contaminated inputs. It can be modelled how due to their innate dynamic

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properties microtubules will spontaneously self-organize in response to even weak external factors such as gravity or mechanic fields (Tabony et al. 2004). It thus seems that nature has made ample use of these unique molecular properties to build sensory systems that are both sensitive and robust against stochastic noise.

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