Mechanics of the Cytoskeleton

Peter Nick

Abstract This chapter summarizes evidence for a cytoskeletal function in tensegral integration on both the organismal and the cellular levels. The plant cytoskeleton consists of two major elements, microtubules and actin filaments. The spatial organization of these elements is highly dynamic and changes fundamentally during the cell cycle, with conspicuous effects on the predicted stress–strain patterns. In interphase cells, microtubule bundles are thought to control the direction of cellulose deposition and thus to reinforce the axiality of cell growth. By microtubule–actin linkers such as the novel class of plant-specific kinesins with a calponin-homology domain, the rigid microtubules and the flexible actin bundles can be integrated into a system endowed with mechanical tensegrity. Because the plant cytoskeleton is relieved of the load-bearing task it fulfils in the non-walled animal cells, it has adopted sensory functions. Stretch-induced changes of protein conformation and stretch-activated ion channels seem to act in concert with the cytoskeleton, which acts either as a stress-focussing susceptor of mechanical force upon mechanosensitive ion channels or as a primary sensor that transduces mechanical force into differential growth of microtubule plus ends. This cytoskeletal tensegrity sensor is used both to integrate the growth of individual cells with mechanical load of tissues and organs and as an intracellular sensor used to control holistic properties of a cell such as organelle positioning. The distinct nonlinearity of microtubules in particular renders them an ideal tool for self-organization in response to mechanical input from the exterior.

P. Nick
Botanical Institute and Center of Functional Nanostructures, Karlsruhe Institute of Technology, Kaiserstr. 2, 76128 Karlsruhe, Germany
e-mail: peter.nick@kit.edu

P. Wojtaszek (ed.), *Mechanical Integration of Plant Cells and Plants*,
Signaling and Communication in Plants 9, DOI 10.1007/978-3-642-19091-9_3,

1 Prologue: The Sensitive Cytoskeleton or the Hidden Face of Plant Tensegrity

Even before Ledbetter and Porter (1963) described "microtubules" in plant cells, which they had observed by transmission electron microscopy, the plant cytoskeleton was predicted to exist on the basis of biomechanical considerations. It was Paul Green who concluded from their geometry that growing plant cells repartition growth by an unknown "reinforcement mechanism" from the spontaneously preferred lateral expansion in favour of elongation, and he predicted that the cell can establish and maintain the mechanical anisotropy of cell walls through an as-yet-unknown lattice of tubular elements that are oriented in an ordered fashion (Green 1962). Thus, ever since its discovery, the plant cytoskeleton has been intimately linked with mechanical aspects of morphogenesis, and this chapter will therefore not follow the usual approach to describe the plant cytoskeleton from its molecular basis and then derive structures and functions. It will rather assume a view of the cytoskeleton that derives from its function in mechanical integration of cell and organ morphogenesis.

As a consequence of their photosynthetic lifestyle, plants increase their surface by folding outwards, producing a considerable degree of mechanical load. As long as they remained aquatic, this load was partially relieved by buoyancy, allowing considerable body sizes even for fairly simple architectures. However, when plants began to move into terrestrial habitats, they had to develop flexible yet robust mechanical supports. The invention of vasculature-based modules, the so-called *telomes* (Zimmermann 1965), was the decisive factor for the evolutionary success of the cormophytic land plants.

Mechanical load shaped plant architecture down to the cellular level. Plant cells are endowed with a rigid cell wall, and this affects cell division and cell expansion both specifically and fundamentally (see also chapter "Micromechanics of Cell Walls"). The deposition of a new cross wall will define the patterns of mechanical strain that, during subsequent cell expansion, will guide the complex interplay between the expanding protoplast and the yielding cell wall. It is even possible to describe the shape of individual cells in a plant tissue as a manifestation of minimal mechanical tension (Thompson 1959), emphasizing the strong influence of mechanical load on plant development.

When plants are challenged by mechanical stimuli, they respond by changing the architecture, which will allocate load-bearing elements (vessels and fibres on the organ level, cellulose microfibrils and lignin incrustations on the cellular level) guided by the imposed field of forces. An impressive example is the formation of tension and compression wood (for a recent review, see Funada 2008). This architectural response ensures that mechanical strains are balanced in an optimal fashion for minimal investment of energy and biomatter. Moreover, this mechanical balance is continuously adjusted to the current environment – a must in a system endowed with open morphogenesis, where the *Bauplan* is continually extended by addition of newly formed organs (see also chapter "Mechanical Force Responses of Plant Cells and Plants").

or the Hidden

tubules" in plant cells,
the plant cytoskeleton
derations. It was Paul
cells repartition growth
preferred lateral
cell can establish and
as-yet-unknown lattice
Green 1962). Thus, ever
linked with mechani-
not follow the usual
basis and then derive
cytoskeleton that derives
morphogenesis.
plants increase their surface
mechanical load. As long
buoyancy, allowing
However, when plants
develop flexible yet robust
modules, the so-called
the evolutionary success

the cellular level. Plant cells
sion and cell expansion
Micromechanics of Cell
patterns of mechanical
the complex interplay
It is even possible
as a manifestation of
the strong influence of

respond by changing the
tissues and fibres on the
the cellular level)
is the formation
Hanada 2008). This
enhanced in an optimal
However, this mechanical
a must in a system
continually extended by
Force Responses of

It was only in the 1920s when such self-supporting, flexible structures were deliberately constructed by mankind (Robby 1996). Starting from the Equilibrist Studies (consisting of three solid sticks interconnected by ropes) of the Soviet artist Karl Joganson, it was mainly the American architect and engineer Richard Buckminster Fuller (1895–1983) who systematically adopted biological principles and developed self-supportive structures that all consisted of a continuous network of tensile elements (which can transmit forces by pulling) linked to a discontinuous system of stiff elements (which can transmit forces by pushing). It was also Buckminster Fuller who later coined the term "tensegrity" as a combination of "tension" and "integrity".

A few years later it became clear that animal cells are shaped by tensegrity (for reviews see Ingber 2003a, b; chapter "Introduction: Tensegral World of Plants"). The part of the tensile elements is played by actin microfilaments, which are not only contractile, but are also mechanically comparable to silk fibres (Gittes et al. 1993). The part of the stiff elements is played by the microtubules, which are not only hollow cylinders, but are also mechanically much more rigid than actin filaments and can be approximated as very delicate glass fibres (Gittes et al. 1993). The actin filaments are connected through the membrane with a supporting scaffold – the extracellular matrix.

What is the secret that renders biological tensegrity so successful? Biological tensegrity is not constructed a priori, but emerges a posteriori from reorganization in response to ever-changing stress–strain patterns. To use a term of Jacob (1977), biological shape is not produced by intelligent design but rather from *bricolage*, and therefore it oscillates around the state minimal energy without every reaching it.

Plant cells are endowed with a cell wall that is built as a composite structure with elongate load-absorbing elements (cellulose microfibers) that are embedded in an amorphous matrix (hemicelluloses, pectins, proteins). It has been shown for technical applications that such composite materials optimally combine bending flexibility with mechanical stability (Niklas 1992). This means that the tensegrity function fulfilled by the interphase cytoskeleton in animal cells is replaced by tensegrity of the cell wall. The plant cytoskeleton is therefore not directly required to support cellular architecture and is therefore free to adopt other functions (Fig. 1).

Cellular architecture uses the tensegral principle to achieve maximal mechanical stability and, simultaneously, flexibility on the basis of parsimonious use of resources and load-bearing elements. In addition, it adapts continuously to the ever-changing conditions of growing and developing cells. This requires efficient sensing of forces and strains followed by appropriate reorganization of the tensegral elements. Thus, the tensegral cytoskeleton is not only a device to provide mechanical stability, it must also participate in the sensing of stress and strain patterns. This mechanical stimulation feeds back to the organization of the cytoskeleton in such a way that a stable minimum of mechanical energy is reached and continuously adjusted. It is this hidden face of tensegrity that becomes especially important in the walled plant cells that are under continuous turgor pressure and use this pressure for regulated expansion. The evolution of the interphasic plant cytoskeleton was therefore shaped by selective pressures towards optimized sensing and integration

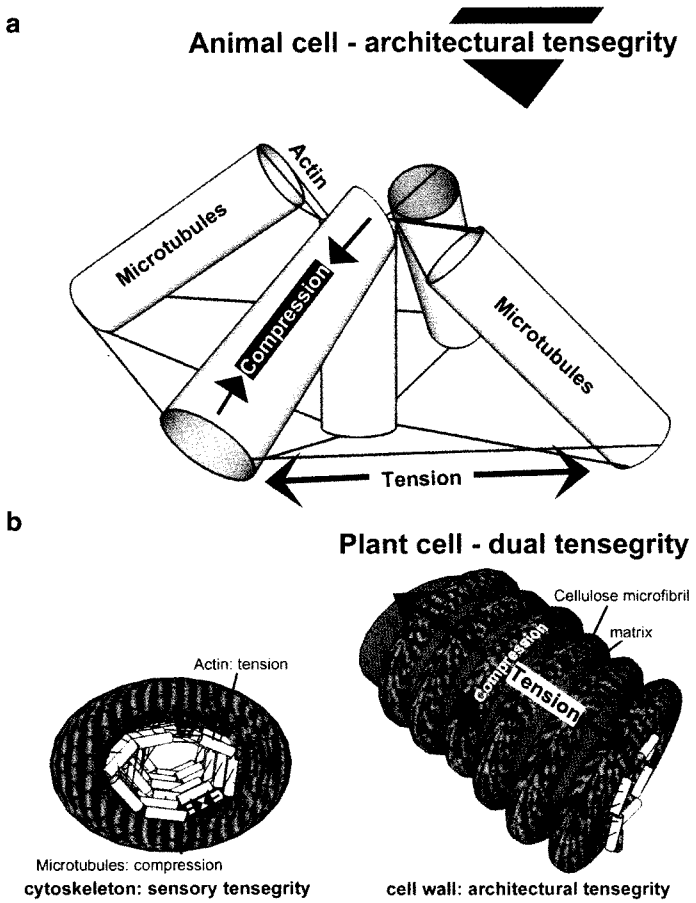


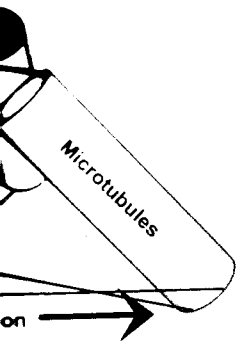
Fig. 1 Functional shift of the cytoskeleton from architectural tensegrity in animal cells towards sensory tensegrity in plant cells. In animal cells (**a**), architecture results from the interplay between stiff microtubules absorbing compressive forces and flexible actin filaments elastically tethering the cells through focal adhesions to the substrate. This set-up is used both to maintain cell shape and to sense and to adjust to mechanical force. In plant cells (**b**), cellulosic microfibers in combination with a flexible cell wall matrix maintain cell shape, such that the tensegral cytoskeleton is released from its architectural function and can be optimized for sensory integration of mechanical stimuli

of mechanical stimuli. The aim of this review is to give a survey of the role of the cytoskeleton in mechanical integration of plant cells, tissues and organs.

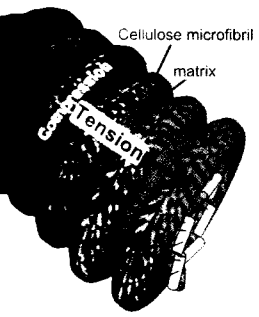
2 Cellular Players: The Microtubule–Actin Tensegrity System

The plant cytoskeleton consists of two major elements, microtubules and actin filaments – intermediate filaments have remained elusive in plants. The spatial organization of these elements is highly dynamic and changes fundamentally

tectural tensegrity



ell - dual tensegrity



architectural tensegrity

grity in animal cells towards
from the interplay between
aments elastically tethering
to maintain cell shape and
microfibers in combination
egral cytoskeleton is released
egration of mechanical stimuli

survey of the role of the
ssues and organs.

-Actin Tensegrity System

microtubules and actin
in plants. The spatial
changes fundamentally

during the cell cycle, with conspicuous effects on the predicted stress–strain patterns. Since microtubules and actin filaments are functionally, but probably also structurally, interconnected (Wasteneys and Galway 2003; Collings 2008), it seems more appropriate to describe their dynamics as a functional entity. The molecular players have been extensively reviewed elsewhere (for recent reviews see Hamada 2007; Sedbrook and Kaloriti 2008), and thus the focus of this chapter is on cellular function, rather than on molecular composition.

2.1 *Cell Expansion*

2.1.1 Microtubules

In interphase cells, microtubules are organized in arrays of parallel bundles perpendicular to the axis of preferential cell expansion (Fig. 2a). These bundles are thought to control the direction of cellulose deposition and thus to reinforce the axiality of cell growth. Cortical microtubules can change their orientation in response to various external and internal stimuli, and this reorientation will shift the preferential direction of cellulose deposition and thus the mechanical anisotropy of the yielding cell wall such that the proportionality of cell expansion can be altered in response to the stimulus (for a recent review see Nick 2008a). The cell wall in cells that are not endowed with tip growth is formed by apposition of cellulose on the inner surface of the cell wall. Cellulose is synthesized by specialized enzyme complexes that, in freeze-fracture preparations, appear as rosettes of six subunits of about 25–30-nm diameter around a central pore (Kimura et al. 1999) and are therefore designated as terminal rosettes. The terminal rosettes are integrated into the membrane by fusion of exocytotic vesicles. UDP-glucose is transported towards the central pore and polymerized in a β-1,4 configuration. Each subunit of the cellulose synthase will produce six cellulose chains that will be integrated by hydrogen bonds into a long and fairly stiff cellulose microfibril. These enzyme complexes are thought to move within the fluid membrane and leave a "trace" of crystallizing cellulose behind them. This movement will therefore determine the orientation of cellulose microfibrils and thus the anisotropy of the cell wall. It is at this point that the microtubules come into play. In fact, it was cell-wall anisotropy that led Green (1962) to predict that microtubules must exist even before they were actually discovered microscopically by Ledbetter and Porter (1963).

The intimate link between cortical microtubules and the preferential axis of growth is supported by the following main arguments (for a review see Nick 2008a) (1) upon plasmolysis, direct contact between cortical microtubules and newly formed cellulose microfibrils can be demonstrated by electron microscopy; (2) when the axis of cell expansion changes in response to a stimulus or during development, this is accompanied by a corresponding switch in the preferential axis of cellulose deposition, preceded by a corresponding reorientation of cortical microtubules; (3) elimination of cortical microtubules by inhibitors produces a

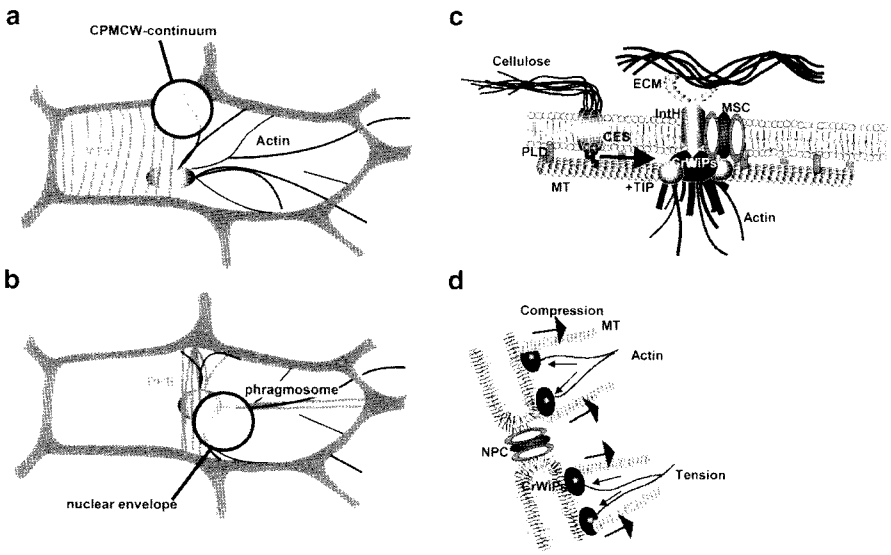
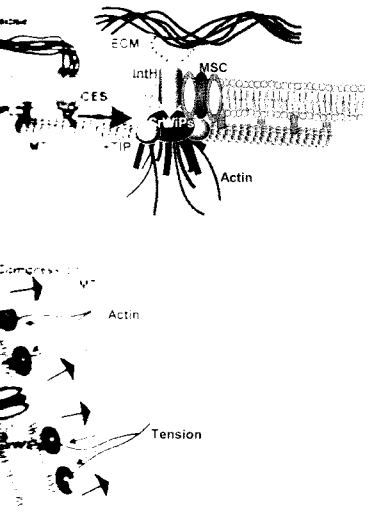


Fig. 2 Molecular players of mechanointegration in expanding (**a**, **c**) and dividing (**b**, **d**) plant cells. Microtubules and actin filaments cooperate to form tensegral structures together with other proteins. (**a**) Organization of microtubules (*MTs*) and actin filaments in an expanding cell; molecular details of the cell wall–plasma membrane–cytoskeleton (*WMC*) continuum are shown in (**c**). (**b**) Organization of the microtubular preprophase band (*PPB*), and the actin-based phragmosome in a cell preparing for mitosis; molecular details of the nuclear envelope are shown in (**d**). (**c**) Molecular details of the cell wall–plasma membrane–cytoskeleton continuum. *PLD* phospholipase D anchor for microtubules, *CES* cellulose synthase complexes that are pulled by kinesins of the KIF4 family along microtubules towards the plus-end complexes (*+TIP*) that are linked via cross-wiring proteins (*CrWiPs*) such as kinesins containing a calponin-homology domain with actin, putative plant homologues of integrins (*IntH*) and mechanosensitive ion channels (*MSC*). The functional integrin homologues are linked with the extracellular matrix (*ECM*), e.g. arabinogalactan proteins, and cellulose microfibrils. (**d**) Molecular details of the link between the nuclear envelope and the cytoskeleton. Through cross-wiring proteins, a tensegral link between actin filaments and microtubules is generated, whereby microtubules might confer compression forces between the periphery and the nucleus, whereas the flexible actin filaments transfer tension forces. *NPC* nuclear pore complex

progressive loss of ordered cellulose texture and the axiality of cell expansion, leading, in extreme cases, to lateral swelling and bulbous growth. The mode of action of several herbicide classes, such as the phenyl carbamates or the dinitroanilines, is based on the elimination of cortical microtubules and the subsequent inhibition of elongation growth.

The striking parallelism between cortical microtubules and newly deposited cellulose microfibrils stimulated two alternative models: The original "monorail" model postulated that cortical microtubules adjacent to the plasma membrane guide the movement of the cellulose-synthesizing enzyme complexes and thus generate a pattern of microfibrils that parallels the orientation of microtubules (Heath 1974). The driving force for the movement of cellulose synthases in the monorail model



...a, c) and dividing (**b**, **d**) plant ...structures together with other ...aments in an expanding cell; ...*MC*) continuum are shown ...*B*, and the actin-based phrag- ...ar envelope are shown in (**d**). ...n continuum. *PLD* phospho- ...s that are pulled by kinesins of ...es (+*TIP*) that are linked via ...min-homology domain with ...sensitive ion channels (*MSC*). ...matrix (*ECM*), e.g. arabino- ...the link between the nuclear ...tegral link between actin ...confer compression forces ...ents transfer tension forces.

...ality of cell expansion, ...s growth. The mode of ...rbamates or the dinitro- ...es and the subsequent

...s and newly deposited ...the original "monorail" ...asma membrane guide ...es and thus generate a ...tubules (Heath 1974). ...the monorail model

would be an active transport through microtubule motors. Alternatively, the interaction between microtubules and cellulose synthases could be more indirect, whereby the microtubules act as "guardrails" inducing small folds of the plasma membrane that confine the movement of the enzyme complexes (Giddings and Staehelin 1991). The driving force for the movement would be the crystallization of cellulose. The solidifying microfibril would thus push the enzyme complex through the fluid plasma membrane, and the role of microtubules would be limited to delineating the direction of this movement.

The practical discrimination between these two models is far from straightforward because experimental evidence was mostly based on electron microscopy observation and thus prone to fixation artefacts. Moreover, great luck was required to locate the right section. For instance, the newly synthesized cellulose microfibrils formed after treatment with taxol were sometimes found to be directly adjacent to individual microtubules, for instance in tobacco BY-2 cells (Hasezawa and Nozaki 1999), supporting the monorail model. On the other hand, the cellulose synthase complexes were observed "in gap" between adjacent microtubules in the alga *Closterium* (Giddings and Staehelin 1988), favouring the guardrail model. The situation was further complicated by situations where the orientations of microtubules and cellulose microfibrils differ (for a review see Baskin 2001; Wasteneys 2004), leading to a debate on the role of microtubules in guiding cellulose synthesis. This debate stimulated a key experiment exploiting the potential of live-cell imaging in *Arabidopsis thaliana* (Paredez et al. 2006). A component of the terminal rosette, cellulose synthase subunit A6 (CESA6), was expressed as fusion with yellow fluorescent protein under the native promoter in the background of a *cesa6*-null mutant, such that overexpression artefacts could be excluded. The resulting punctuate signal was observed to be localized adjacent to the plasma membrane and to move along parallel pathways that resembled cortical microtubules. By crossing this line into a background, where one of the α-tubulins was expressed as fusion with a blue fluorescent protein, it became possible to follow this movement under simultaneous visualization of CESA6 and microtubules. This dual visualization demonstrated very clearly that CESA6 was moving along individual microtubule bundles. Moreover, in a recent publication, a central problem of the monorail model, i.e. the existence of polylamellate walls with layers of differing microfibril orientation, could be plausibly explained by a rotary movement of groups of microtubules (Chan et al. 2007; for a recent review see Lucas and Shaw 2008).

The original monorail model postulated a microtubule motor that pulls the cellulose synthase complex along the microtubules. If this motor were defective, cellulose microfibrils would deviate from the orientation of microtubules. A screen for reduced mechanical resistance in *A. thaliana* yielded a series of so-called *fragile fiber* mutants (Burk et al. 2001; Burk and Ye 2002) that were shown to be completely normal in terms of cell wall thickness or cell wall composition, but were affected in terms of wall texture. One of these mutants, *fragile fiber 2*, allelic to the mutant *botero* (Bichet et al. 2001), was affected in the microtubule-severing protein katanin, leading to swollen cells and increased lateral expansion. A second

mutant, *fragile fiber 1*, was mutated into a kinesin-related protein belonging to the KIF4 family of microtubule motors (Fig. 2c). As expected, the array of cortical microtubules was completely normal; however, the helicoidal arrangement of cellulose microfibrils was messed up in these mutants. This suggests that this KIF4 motor is involved in guiding cellulose synthesis and might be a component of the monorail complex. Thus, the original monorail model for microtubule guidance of the terminal rosettes (Heath 1974) was rehabilitated after more than three decades of dispute.

However, the microtubule–microfibril model is still far from complete. In addition to occasionally discordant orientations of microtubules, there are cell wall textures that are difficult to reconcile with a simple monorail model. For instance, cellulose microfibrils are often observed to be intertwined (Preston 1988). This has stimulated views that claim that microtubules are dispensable for the correct texture of microfibrils. The self-organization of cellulose synthesis would be sufficient to perpetuate the pattern because the geometrical constraints from microfibrils that are already laid down would act as templates for the synthesis of new microfibrils (for a review see Mulder et al. 2004). There are two problems with this model. First, it ignores that microtubules and microfibrils are actually parallel in most cases, at least if cells in a tissue context are analysed. Second, it ignores that disruption of microtubules either by inhibitors (for a review see Nick 2008a, b) or by mutations that impair the formation of ordered microtubule arrays (Burk et al. 2001; Bichet et al. 2001 for katanin; Whittington et al. 2001 for *mor1*) is accompanied by a progressive loss of ordered cell wall texture and a loss of growth axiality. Nevertheless, the issue of cellulose self-organization highlights that the original microtubule–microfibril model has to be extended by a feedback control of microfibrils upon cortical microtubules.

2.1.2 Actin Filaments

Similar to microtubules, actin is organized into several distinct arrays that presumably exert different functions. For cells with pronounced tip growth, such as pollen tubes or root hairs, actin functions as a track for the transport of vesicles with cell-wall material that are inserted into the tip by intussusception (reviewed in Hepler et al. 2001; chapter "Generating a Cellular Protuberance. Mechanics of Tip Growth") and are probably conveyed by actin polymerization (Gossot and Geitmann 2007; Cárdenas et al. 2008). However, cells growing in a tissue context grow by apposition to the stretched cell wall rather than by intussusception. The role of actin therefore must be different and is not as obvious as for tip growth. During the diffuse elongation of tissue cells, longitudinal actin bundles prevail, especially in vacuolated cells (Parthasarathy et al. 1985; Sonobe and Shibaoka 1989). The rigidity of these transvacuolar strands and the degree of their bundling is regulated by signals such as plant hormones (Grabski and Schindler 1996), kinase cascades (Grabski et al. 1998) or light (Waller and Nick 1997). In addition to the transvacuolar bundles, a fine network of highly dynamic microfilaments can be detected in

...n-related protein belonging to the
...As expected, the array of cortical
...r the helicoidal arrangement of
... mutants. This suggests that this
...thesis and might be a component
...mor rail model for microtubule
...was rehabilitated after more than

...e, is still far from complete. In
...s of microtubules, there are cell
...th a simple monorail model. For
...rved to be intertwined (Preston
... microtubules are dispensable for
...ganization of cellulose synthesis
...cause the geometrical constraints
...d act as templates for the synthesis
...al. 2004). There are two problems
...ules and microfibrils are actually
...e context are analysed. Second, it
... inhibitors (for a review see Nick
...ion of ordered microtubule arrays
...Whittington et al. 2001 for *mor1*) is
... wall texture and a loss of growth
...reorganization highlights that the
...extended by a feedback control of

...several distinct arrays that presum-
...ished tip growth, such as pollen
...the transport of vesicles with cell-
...dissociation (reviewed in Hepler
...ence "Mechanics of Tip Growth")
...tion (Gossot and Geitmann 2007;
...a tissue context grow by apposi-
...dissociation. The role of actin
...as in tip growth. During the
...actin bundles prevail, especially in
...Schibaoka 1989). The
...their bundling is regulated
...Schindler 1996), kinase cascades
...In addition to the transva-
...filaments can be detected in

the cortical cytoplasm of elongating cells often accompanying cortical microtubules (for a review see Collings 2008). This cortical network can be rendered visible after pretreatment with protein cross-linkers (Sonobe and Shibaoka 1989), upon very mild fixation (Waller and Nick 1997) or by fusing binding domains of actin-binding proteins to fluorescent proteins (Voigt et al. 2005; Nick et al. 2009).

The transvacuolar actin cables were suggested to limit cell expansion by their rigidity and auxin was thought to stimulate growth by releasing this rigidity (Grabski and Schindler 1996). This mechanical model for the growth control through actin was supported by experiments where growth was modulated by light (Waller and Nick 1997). In the dark, when cells underwent rapid elongation, actin was organized into fine strands that became bundled in response to light-induced inhibition of growth. This actin reorganization was rapid and preceded the changes in growth rate (Waller and Nick 1997). Moreover, this response was confined to the epidermis, the target tissue for the signal control of growth. However, the mechanical model of actin function was shattered by experiments with actin inhibitors. The mechanical model predicted that elimination of actin cables should release the rigidity that limits growth. However, experiments with cytochalasin D (Thimann et al. 1992; Wang and Nick 1998) and latrunculin B (Baluška et al. 2001) revealed that even mild elimination of actin inhibited, rather than promoted, cell elongation. Thus, actin, possibly in combination with directional vesicle transport (Baskin and Bivens 1995), is a positive regulator of growth.

Subsequently, two actin populations could be separated owing to differences in sedimentability (Waller et al. 2002). Whereas the fine actin filaments correlated with a cytosolic fraction of actin, actin became progressively trapped on the endomembrane system and partitioned into the microsomal fraction when bundling was induced by light (perceived by phytochrome), by fluctuations of auxin content or by brefeldin A. This bundling of actin was accompanied by a reduced auxin sensitivity of cell elongation. This led to a model where auxin-signalling triggered the reorganization of actin bundles into finer filaments that more efficiently transported auxin-signalling/transport components towards the cell pole. The debundling in response to auxin predicted by this model was later demonstrated in intact rice coleoptiles in vivo using the actin-binding domain of mouse talin in fusion with yellow fluorescent protein first upon transient expression (Holweg et al. 2004) and later after stable expression (Nick et al. 2009).

To understand the link between actin and the auxin response of growth, excessive actin bundling was induced by overexpression of the actin-binding domain of talin in tobacco BY-2 cells (Maisch and Nick 2007) and in rice plants (Nick et al. 2009). In both systems, the reversion of a normal actin configuration can be restored by addition of exogenous auxin and this fully restores the respective auxin-dependent functions. These findings led to a model of a self-referring regulatory circuit between polar auxin transport and actin organization. Thus, although actin can stimulate growth by virtue of its mechanical properties in tip-growing cells (Gossot and Geitmann 2007), within a tissue context it does not act through mechanics, but acts by controlling the proper localization and thus activity of the signalling machinery that regulates cell expansion.

2.1.3 Actin–Microtubule Interaction

For cell growth, coordination and cross talk between microtubules and actin filaments have been inferred from their close coalignment and structural interaction (for reviews see Wasteneys and Galway 2003; Collings 2008) that seem to be ubiquitous and have been observed in different species and cell types. This conclusion has been supported by experiments involving pharmacological manipulation, where both microfilament- and microtubule-eliminating agents reduced cell elongation, often accompanied by a loss of growth anisotropy (*Arabidopsis*: Baskin and Bivens 1995; Collings et al. 2006; Graminean seedlings: Gianí et al. 1998; Wang et al. 2004; Blancaflor 2000; cotton fibres: Seagull 1990). Recently, it has even been proposed that microtubule reorientation is caused by detachment of microtubules from membrane-associated contact points (controlled by specific isoforms of phospholipase D; Fig. 2c) followed by their realignment with the direction of actin-based streaming (Sainsbury et al. 2008). Despite extensive studies on microtubular association of actin filaments, the proteins that mediate these interactions have remained elusive. Interactions between microtubules and actin filaments could be mediated either by bifunctional proteins that can bind to both cytoskeletal elements or, alternatively, by a connecting complex of two or more monofunctional proteins harbouring a microtubule-binding or an actin-binding domain, respectively. In animal and fungal cells, a number of proteins have been identified that mediate such interactions, either in a bifunctional way or as complexes consisting of monofunctional proteins (Goode et al. 2000; Rodriguez et al. 2003). In plants, however, only a few candidates such as MAP190 have been proposed so far (Igarashi et al. 2000).

In animal cells, the microtubular minus-end motor dynein is connected with and activated through the dynactin complex (for a review see Karki and Holzbaur 1999). The dynactin complex is further linked to the microtubule tip component EB1 and thus regulates the stability of microtubules (for a review see Tirnauer and Bierer 2000). Dyneins as central elements of the dynactin complex are not found in plants. This is probably the consequence of the loss of flagellate cells and centrioles (for a review see Schmit and Nick 2008). Plants must have evolved a functional compensation for the loss of dyneins in the form of other minus-end-directed motors that are able to interact, either directly or indirectly, with actin filaments. Recently, kinesins with a calponin-homology domain (KCH kinesins) were identified as plant-specific subset of the kinesin-14 family (Tamura et al. 1999; Preuss et al. 2004; Frey et al. 2009; Xu et al. 2009). In addition to the characteristic microtubule-binding kinesin motor domain, these proteins possess a conserved calponin-homology domain, well known as an actin-binding domain from a variety of actin-associated proteins such as α-actinin, spectrin and fimbrin. Thus, KCHs are strong candidates for a bifunctional mediation between both cytoskeletal elements, and several studies confirmed that they can bind, in fact, both elements of the cytoskeleton (cotton: Preuss et al. 2004; Xu et al. 2009; rice: Frey et al. 2009). These cross-linking microtubule motors are present in higher plants, but also in *Physcomitrella patens* (Richardson et al. 2006; Frey et al. 2009). Their cellular function is not really understood, but for one of the cotton KCHs, a role in cell

elongation through cross-linking of microtubules and microfilaments has been proposed, however without experimental evidence (Xu et al. 2009). For the rice homologue OsKCH1, the phenotype of insertion mutants and a KCH overexpression line generated in tobacco BY-2 cells (Frey et al. 2010) suggest that this kinesin stimulates cell elongation, although this stimulation might be a secondary phenomenon caused by changes in the timing of cell division.

2.1.4 The Cell-Wall Cytoskeletal Continuum

A continuum between the cytoskeleton and the extracellular matrix is central for mechanosensing in animal cells (for a review see Geiger and Bershadsky 2001) and involves interaction between integrins and extracellular matrix proteins that contain Arg–Gly–Asp (RGD) motifs (for a review see Giancotti and Ruoslahti 1999). Plants seem to lack integrin homologues, but there is evidence for cytoskeletal reorganization in response to treatment with RGD peptides (Canut et al. 1998; Wang et al. 2007). As a molecular basis for the cytoskeleton–plasma membrane–cell wall continuum in plants (Fig. 2c), cell-wall-associated kinases, arabinogalactan proteins, pectins and cellulose synthases themselves have been discussed (for a review see Baluška et al. 2003; chapter "Introduction: Tensegral World of Plants"). Recently, the rich but circumstantial evidence for such transmembrane interactions was assembled into a model for a so-called plasmalemmal reticulum as a third element of the plant cytoskeleton (Pickard 2008). This plasmalemmal reticulum is considered to be a tensile structure and to participate in the control of cellulose deposition. It can be visualized as a reticulate structure by antibodies raised against components of the mammalian extracellular matrix and also contains arabinogalactan proteins. The reticulum is reorganized in the context of cell elongation in a manner similar to cellulose deposition and is suggested to represent a morphological manifestation of lipid rafts.

2.2 *Cell Division*

2.2.1 Microtubules

In dividing cells, the ensuing mitosis is heralded by a displacement of the nucleus to the cell centre, where the prospective cell-plate will be formed (for a review see Nick 2008a). Simultaneously, radial microtubules emanate from the nuclear surface and merge with the cortical cytoskeleton, tethering the nucleus to its new position (Fig. 2b). In the next step, the preprophase band is organized by the nucleus as a broad band of microtubules around the cell equator, marking the site where after completed mitosis the new cell plate will be formed. In fern protonemata, where the formation of the preprophase band can be manipulated by centrifugation of the nucleus to a new location (Murata and Wada 1991), a causal relationship between

the preprophase band and cell-plate orientation was elegantly demonstrated. Moreover, in cells where the axis or symmetry of cell division changes, this change is always predicted by a corresponding localization of the preprophase band. The division spindle is always laid down perpendicular to the preprophase band, with the spindle equator located in the plane of the preprophase band. As soon as the chromosomes have separated, a new array of microtubules, the phragmoplast, appears at the site that had already been marked by the preprophase band. This microtubular structure consists of a double ring of interdigitating microtubules that increases in diameter with increasing size of the cell plate. New microtubules are organized along the edge of the growing phragmoplast (Vantard et al. 1990). The phragmoplast targets vesicle transport to the periphery of the expanding cell plate. Microtubules seem to pull at tubular–vesicular protrusions emanating from the endoplasmic reticulum (Samuels et al. 1995). The guiding function of the preprophase band is supported by evidence from *Arabidopsis* mutants (*tonneau/fass*), where the preprophase band is absent owing to a mutation in a phosphatase PP2A regulatory subunit (Camilleri et al. 2002). In these mutants, the ordered pattern of cell divisions that characterizes the development of the wild type is replaced by a completely randomized pattern of cross walls (Traas et al. 1995; McClinton and Sung 1997). It should be mentioned, however, that during meiosis the division plane can be controlled in the absence of a preprophase band (for a recent review see Brown and Lemmon 2007), suggesting that there exist additional mechanisms of spatial control.

2.2.2 Actin Filaments

In contrast to the obvious and dramatic reorganization of the microtubule during mitosis, actin filaments seem to be more persistent. Two decades ago, actin filaments were shown to accompany mitotic microtubule arrays such as preprophase band, spindle and phragmoplast (Kakimoto and Shibaoka 1987; Lloyd and Traas 1988). However, there is still some controversy as to the exact behaviour, persistence and orientation of actin filaments during M phase (for a recent review see Panteris 2008). The microtubular preprophase band that disappears with the breakdown of the nuclear envelope leaves a so-called actin-depleted zone as a negative imprint that later, upon reestablishment of the daughter nuclei, will be the site where the new cell plate is formed. Despite some debate regarding to what extent this zone is completely void of actin or whether it just contains fewer actin filaments, there is a clear correlation between the actin-depleted zone and the site of the prospective cell plate. When the actin filaments lining this depletion zone are eliminated by inhibitor treatment, this will affect the subsequent cell division when the treatment occurs during the presence of the microtubular preprophase band. However, actin inhibitors will have only a marginal effect once the preprophase band has disappeared (Sano et al. 2005). What is the actin-dependent function that defines the cell plate? It might be linked to a belt composed of endosomes that is laid down adjacent to the preprophase band by joint action of microtubule-driven and actin-driven transport (Dhonukshe et al. 2005). This belt persists during

as elegantly demonstrated. More-
l division changes, this change is
of the preprophase band. The
ar to the preprophase band, with
preprophase band. As soon as the
microtubules, the phragmoplast,
ed by the preprophase band. This
of interdigitating microtubules that
cell plate. New microtubules are
noplast Vantard et al. 1990). The
iphery of the expanding cell plate.
protrusions emanating from the
guiding function of the preprophase
mutants (*tonneau/fass*), where the
in a phosphatase PP2A regulatory
the ordered pattern of cell divisions
type is replaced by a completely
995, McClinton and Sung 1997). It
is the division plane can be con-
or a recent review see Brown and
ular mechanisms of spatial control.

ization of the microtubule during
tent two decades ago, actin fila-
tubule arrays such as preprophase
Shibaoka 1987; Lloyd and Traas
as to the exact behaviour, persis-
M phase (for a recent review see
and that disappears with the break-
actin-depleted zone as a negative
daughter nuclei, will be the site
debate regarding to what extent
ther it just contains fewer actin
e actin-depleted zone and the site
ments during this depletion zone are
the subsequent cell division when
e microtubular preprophase band.
guiding effect once the preprophase
the actin-dependent function that
is composed of endosomes that is
joint action of microtubule-driven
2005). This belt persists during

mitosis, and is, upon completed separation of the chromosome, "read out" by a new set of microtubules emerging from the spindle poles that "explore" the cell periphery in different directions. The lifetime of these exploratory microtubules is increased when they hit the endosomal belt, whereas microtubules that fail to interact with the endosomes are prone to undergo catastrophic decay (Dhonukshe et al. 2005). Thus, the actin-depleted zone is rather a manifestation of and not the cause for the correct positioning of the cell plate.

2.2.3 Nuclear Migration

In cells that prepare for mitosis, the nucleus is tethered by the so-called phragmosome forming the characteristic "Maltese cross" to its new position in the cell centre, and persists during the subsequent mitosis (Fig. 2b). The cytoskeletal reorganizations accompanying mitosis assign a central role to nuclear migration in the control of division symmetry, and to the preprophase band for the control of division axis. Nuclear migration can be blocked by actin inhibitors such as cytochalasin B (Katsuta and Shibaoka 1988). However, microtubules also seem to be involved in nuclear positioning, since antimicrotubular compounds such as colchicine (Thomas et al. 1977) and pronamide (Katsuta and Shibaoka 1988) have been found to loosen the nucleus such that it can be displaced by mild centrifugation. This indicates again that proteins mediating actin–microtubular interaction are relevant for nuclear positioning. In fact, the plant-specific KCHs (Frey et al. 2009) were found to be dynamically repartitioned during the cell cycle: in premitotic cells, KCH1 was clearly aligned in a punctuate pattern along filamentous, mesh-like structures on both sides of the nucleus and on perinuclear filaments spanning over and surrounding the nucleus. At the onset of mitosis, KCH1 retracted to both sides of the nucleus, but not in preprophase bands nor in the spindle apparatus nor at the division plate. During late telophase and the beginning of cytokinesis, KCH1 was shifted towards the newly forming nuclei and lined the filaments that tethered these nuclei to the periphery and the new cell wall (Frey et al. 2010). Tos17 *kch1* knockout in rice showed increased cell numbers in the seedling shoot, whereas overexpression of KCH1 in tobacco BY-2 cells reduced the cell number. This effect was assigned to a delay in the premitotic nuclear migration towards the cell centre, suggesting that KCH regulates nuclear positioning and thus the progression of mitosis (Fig. 2d).

3 Molecular Players: Protein Conformation Versus Stretch-Activated Channels

Mechanical integration requires the perception of stress–strain patterns. In turgescent plant cells, the expanding protoplast exerts considerable turgor pressure upon the yielding cell wall. This cell-autonomous component of mechanical load is

complemented by mechanical tension across the tissue that is caused by the limiting extensibility of the epidermis (for a recent review see Kutschera 2008). Thus, any mechanism for plant mechanosensing has to cope with this strong, but tonic background stimulus.

Essentially, there are two basic models for the molecular basis of mechanoperception:

1. Stretching of proteins will change their conformation and create new binding sites for the recruitment of associated proteins (for reviews see Janmey and Weitz 2004; Orr et al. 2006).
2. Mechanosensitive channels are able to directly detect and respond to forces from the lipid bilayer. Such channels will open when the plasma membrane is deformed or when the channel is pulled by a tether (for a review see Kung 2005).

Mechanosensing by stretch-induced conformational changes is well supported for the adhesion of mammalian cells (for a review see Geiger and Bershadsky 2001). Here, the growth of focal contacts, where the actin cytoskeleton is tethered to the extracellular matrix through a complex of associated proteins and integrins, is promoted by local mechanical force (Riveline et al. 2001). A similar mechanosensing network was proposed for plant cells, where analogues of integrins link the cytoskeleton at the inner face with the cell wall at the outer face of the plasma membrane (Jaffe et al. 2002). However, the transfer of this model from mammalian cells to plants is not straightforward because the molecular components differ considerably (for a review see Baluška et al. 2003). It is the cell wall with a completely different set of molecules that replaces the extracellular matrix of mammalian cells. Furthermore, canonical integrins are obviously absent from plants, suggesting that the link between actin filaments and the extracellular binding sites must use different groups of molecules. Further, although plants and animals share several actin-binding proteins, important components of focal contacts, such as talin, do not exist in plants. On the other hand, there seem to exist integrin analogues that can bind to RGD tripeptides in a way similar to the way integrins do in animal cells. It seems that class VIII myosins and formins might act as linkers between the actin cytoskeleton and the plant analogues of the extracellular matrix, such as cell-wall-associated kinases and arabinogalactan proteins (Baluška and Hlavačka 2005). Additionally, cellulose synthases that move along microtubules (Paredez et al. 2006) tether the cytoskeleton to the cell wall, and several microtubule-associated proteins, for instance phospholipase D (Gardiner et al. 2001) and MAP18 (Wang et al. 2007), act as linkers between cortical microtubules and the plasma membrane Thus, although the molecular components differ considerably from their animal counterpart, a contiguous link between the cytoskeleton and the cell wall does exist and is commonly referred to as the cell wall–plasma membrane-cytoskeleton interface (Telewski 2006).

The existence of mechanosensitive channels in plants was originally discovered in specialized cells, where a touch stimulus induced an action potential (Shibaoka 1966) such as in the seismonastic leaves of *Mimosa pudica* (Toriyama and Jaffe 1972), or internodal cells of *Chara* (Kishimoto 1968). From electrophysiological

...sue that is caused by the limiting
... see Kutschera 2008). Thus, any
... with this strong, but tonic

...ecular basis of mechano-

... and create new binding
... reviews see Janmey and

... and respond to forces from
... the plasma membrane is
... a review see Kung 2005).

...hanges is well supported
... see Geiger and Bershadsky
... the actin cytoskeleton is tethered
... proteins and integrins, is
... A similar mechanosen-
...gues of integrins link the
... outer face of the plasma
... this model from mammalian
... molecular components differ
... the cell wall with a com-
... cellular matrix of mamma-
... absent from plants,
... extracellular binding sites
... plants and animals share
... focal contacts, such as
... seem to exist integrin
... similar to the way integrins
... mins might act as linkers
... the extracellular matrix,
... proteins (Baluška and
... move along microtubules
... and several microtu-
... Gardiner et al. 2001) and
... microtubules and the
... ents differ considerably
... the cytoskeleton and the
... wall plasma membrane-

... originally discovered
... on potential (Shibaoka
... Toriyama and Jaffe
... electrophysiological

and pharmacological evidence, a model emerged where these touch-sensitive channels mediate an influx of calcium (for a review see Jaffe et al. 2002). In fact, with use of aequorin-transformed plants, mechanical stimulation was demonstrated to trigger calcium influx with stimulus-specific signatures (Knight et al. 1991). A causative role of calcium fluxes was supported by the isolation of touch-insensitive *Arabidopsis* mutants affected in calmodulin genes (Braam and Davis 1990), and inhibition of touch responses by inhibitors of calmodulin (Jones and Mitchell 1989). A mechanosensitive calcium channel was demonstrated for epidermal cells of onion (Ding and Pickard 1993), but the molecular identity of mechanosensitive calcium channels has remained elusive. Molecular identification is also hampered by 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).

Although both mechanisms, stretch-induced conformational changes and stretch-activated ion channels, are often discussed separately, they might, in fact, act in concert, as components of a so-called plasmalemmal reticulum (for a review see Pickard 2008). This integrative structure comprises adhesive components (among others arabinogalactan proteins and wall-associated kinases) that link the plasma membrane with the cell wall, and are also connected with mechanosensory calcium channels. This structure has been demonstrated and described for tobacco BY-2 as a cell biological model system (Gens et al. 2000; Pickard and Fujiki 2005). The plant integrin analogues have been reported to connect microtubules, plasma membrane, actin filaments and stretch-activated membrane channels (Telewski 2006). It is highly conceivable that such a network could act, on the one hand, as a tensegral entity that can convey and focus mechanical force upon stretch-activating membrane channels and, simultaneously, transduce forces into conformational changes that can result in differential decoration with associated proteins that can act as a trigger for signalling. The necessity for stress-focussing is supported by estimations of the activation energies for mechanosensitive channels (around 1 mN m^{-1}, Sachs and Morris 1998) in a range not far below the lytic tension of plant membranes (around 4 mN m^{-1}, Kell and Glaser 1993).

If the tensegral cytoskeleton is linked to the cell wall through such integrative linkers, this should become manifest as organizing influences of the cell wall upon the cytoskeleton. This has been observed. For instance, removal of the cell wall renders microtubules cold-sensitive in tobacco cells (Akashi et al. 1990). When in the same cells the incorporation of UDP-glucose into the cell wall was blocked by the herbicide isoxaben (Fisher and Cyr 1993), this impaired the axiality of cell expansion, resulting in isodiametric cells and disordered cortical arrays of microtubules. Thus, the mechanical strains produced by cellulose microfibrils align cortical microtubules, closing a regulatory circuit between the cell wall and the cytoskeleton. Since expansion is reinforced in a direction perpendicular to the orientation of microtubules and microfibrils, forces will be generated parallel to the major strain axis. These forces are then relayed back through the plasma

membrane upon cortical microtubules that are aligned in relation to these strains. Since individual microtubules mutually compete for tubulin heterodimers, and since the number of microfibrils is limited by the quantity of cellulose synthase rosettes, this regulatory circuit should follow the rules of a reaction–diffusion system (Turing 1952) and should therefore be capable of self-organization and patterning.

How could mechanical strains from the cell wall cause a corresponding alignment of cortical microtubules? The so-called microtubule plus-end tracking proteins (+TIP proteins) seem to play an important role in this context. These proteins associate with growing plus ends of microtubules and form complexes that control microtubule dynamics, organization and the interaction of microtubules with membranes, organelles and proteins (for a review see Akhmanova and Steinmetz 2008). EB1, a central component of this complex, is important in searching for so-called exploratory microtubules for intracellular capture sites that are often marked by specific actin structures. For instance, such capture sites are laid down by the preprophase band and the phragmosome prior to mitosis and are recognized by exploratory microtubules emanating from the cell poles during telophase (Dhonukshe et al. 2005). Because of this function, several members of +TIP proteins interact with the actin cytoskeleton. Some +TIP proteins, such as adenomatous polyposis coli (Moseley et al. 2007) and CLIP-associated protein (Tsvetkov et al. 2007), interact with actin filaments directly, others interact through the actin-binding formins. A third group, including EB1, interact with the dynactin complex linking the minus-end microtubule motor dynein with actin filaments (for a review see Tirnauer and Bierer 2000). 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 to sustain microtubule growth. The +TIP complex, in general, and EB1, in particular, are therefore subject to mechanical tension and must be considered as primary targets for mechanical strains on microtubules. In fact, *Arabidopsis* mutants in members of the EB1 family have been found to be touch-insensitive (Bisgrove et al. 2008).

Summarizing, in plant cells both stretch-induced changes of protein conformation and stretch-activated ion channels seem to act in concert during the perception of mechanical stimuli. The cytoskeleton can participate in both pathways, either as a stress-focussing susceptor of mechanical force upon mechanosensitive ion channels or as a primary sensor that transduces mechanical force into differential growth of microtubule plus ends. Microtubules are endowed with nonlinear dynamics, leading to phase transitions between growth and catastrophic shrinkage. In addition, they have to compete for a limited pool of free heterodimers. Microtubules are therefore ideal devices to amplify the minute inputs from mechanical stimulation (small deformations of the perceptive membranes, changes in the dynamic equilibrium between assembly and disassembly of microtubules at the microtubule plus end) into clear and nearly qualitative outputs that can then be processed by downstream signalling cascades (for a review see Nick 2008b).

aligned in relation to these strains.
ete for tubulin heterodimers, and
the quantity of cellulose synthase
the rules of a reaction–diffusion
capable of self-organization and

will cause a corresponding align-
ner tubule plus-end tracking pro-
role in this context. These proteins
s and form complexes that control
interaction of microtubules with
e.g. see Akhmanova and Steinmetz
is important in searching for so-
capture sites that are often marked
capture sites are laid down by
for to mitosis and are recognized
the cell poles during telophase
tion. several members of +TIP
me +TIP proteins, such as adeno-
CLIP-associated protein (Tsvetkov
others interact through the actin-
interact with the dynactin complex
with actin filaments (for a review
otubule plus ends at the seam that
. 2006) and is therefore a good
During microtubule catastrophe,
that they have to be actively tied
P complex in general, and EB1, in
ens and must be considered as
ar tubules. In fact, *Arabidopsis*
een found to be touch-insensitive

ed changes of protein conforma-
t is relevant during the perception
icipate in both pathways, either as
or mechanosensitive ion chan-
nical force into differential growth
ked with nonlinear dynamics,
talastic shrinkage. In addition,
e heterodimers. Microtubules are
puts in mechanical stimulation
changes in the dynamic equilib-
rotubules at the microtubule plus
that can then be processed by
e Nick 2009).

4 The Cytoskeleton as a Sensor: Intercellular Sensing

It was the loss of buoyancy as a supporting force that drove the evolution of mechanical integration in terrestrial plants. Signalling through a mechanical signal is much faster than any diffusion-based process and its velocity equals or even exceeds that of electric signalling. Moreover, mechanical integration is holistic in nature, since it allows the sensing almost simultaneously of the presence or absence of building elements even if they are remote from the sensing cell (as long as they are mechanically coupled). The stiffer this mechanical coupling, the less energy is dissipated during signalling. Microtubules are endowed with a high degree of rigidity (Gittes et al. 1993) and therefore represent ideal transducers for mechanical integration even across the borders of individual cells.

Such microtubule orientations that transcend the borders of individual cells have been reported during phyllotaxis (Hardham et al. 1980; Hamant et al. 2008). In wounded pea roots, a supracellular alignment of microtubules heralds corresponding changes of cell axis and cell divisions such that the wound is efficiently closed (Hush et al. 1990). A curious case of microtubule patterning was discovered in the *Arabidopsis* mutants *spiral*, *lefty* and *tortifolia* (Furutani et al. 2000; Thitamadee et al. 2002; Buschmann et al. 2004). In these mutants, microtubules are aligned over many cells in the distal elongation zone of the root (*spiral* and *lefty*) or the petiole (*tortifolia*), accompanied by twisted growth.

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

The impact of microtubules for mechanointegration can be exemplarily studied in the context of gravity responses (see chapter "Mechanical Aspects of

Gravity-Controlled Growth, Development and Morphogenesis"). To compensate for mechanical load by gravity, plants have to optimize the arrangement of force-bearing elements in space 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. Thus, gravity has to be perceived very efficiently and it has, in addition, to be linked to morphogenesis. When the orientation of a plant is changed with respect to gravity, the plant will respond by bending in such a way as to restore the original orientation and thus to minimize mechanical stress (gravitropism). When new organs develop, they are often adjusted with respect to gravity (gravimorphosis). When the mechanointegrative role of the cytoskeleton is discussed with respect to gravity, it is important to separate so-called susception from perception in the strict sense. "Susception" means transformation of physical energy into a different type of energy that can be perceived by the perceptive system (Björkman 1988). For instance, the difference in gravitational field strength between the two flanks of a misoriented plant would be certainly far too small to be sensed by any biochemical process. It is generally accepted that gravity is first transformed into mechanical force by acting on heavy particles, the so-called statoliths. These statoliths (as well as their accessory structures) themselves are not gravisensitive, but they assist sensing by acting as susceptors.

4.1 Microtubules and Gravitropism

For the rhizoid of *Chara*, experiments by Johannes Buder (1961) demonstrated that vesicles filled with barium sulfate, 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 was shown that susception of energy by amyloplasts is sufficient to trigger a curvature response. By using high-gradient magnetic fields, Kuznetsov and Hasenstein (1996) succeeded in inducing bending in vertically oriented roots and thus were able to prove very elegantly that the generation of mechanical force by statoliths is sufficient for gravisusception. It is 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), although the actual perception event has remained elusive so far.

For the rhizoid of *Chara* the sedimentation of the *Glanzkörperchen* to the lower flank of the rhizoid was found to 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 mechanism. It is doubtful that proximity is used for graviperception in higher plants because classical studies (Rawitscher 1932) using intermittent stimulation showed that perception can occur in the absence of amyloplast sedimentation. Moreover, dose–response studies employing centrifugation have shown 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 was originally postulated, it was demonstrated that strong stimuli that saturate the sedimentation of the *Glanzkörperchen* can still be discriminated (Hertel and Friedrich 1973). This suggests that the actual perception of gravity is not based on proximity, but is based on the force exerted by the statoliths on a mechanosensor such as stretch-activated ion channels and/or the cell wall–plasma membrane–cytoskeleton interface.

If gravity is perceived not by proximity but by pressure, this poses a big challenge for the sensing mechanism. Since gravity is sensed by individual cells (in contrast to the direction of light in phototropism; Buder 1920; Nick and Furuya 1996), 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 as a consequence of either 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 gravitropism (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 was 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.

This leads to the question of 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) – the 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 elongating and were kept either in a horizontal orientation (such that a gravitropic stimulation occurred) or in a vertical orientation (such that growth was inhibited in the absence of a gravitropic stimulus). In this set-up, microtubule reorientation from transverse to longitudinal was 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.

4.2 *Microtubules and Gravimorphosis*

It seems trivial that roots form at the lower pole of a plant organ, but this is a manifestation of gravimorphosis. Although a considerable amount of phenomenological work was dedicated to this problem at the turn of the nineteenth century (Vöchting 1878; Sachs 1880; Goebel 1908), the underlying mechanisms are still far from being understood. This is partially due to the use of adult organs, where polarity has already been fixed and is hard to invert. However, in germinating fern spores, the first asymmetric division that separates a larger, vacuolated rhizoid precursor from a smaller and denser thallus precursor can be oriented by gravity (Edwards and Roux 1994). 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. When this spore is tilted after the axis of the first division has been determined, the rhizoid will grow in the wrong direction and cannot adjust for 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, resembling the determination of the grey crescent in amphibian eggs (Gerhart et al. 1981), where the dorsoventral axis is determined by an interplay among 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).

...ir ... response of microtubules is
... changes in growth rate. In fact, it
... bending coleoptiles with manual
... tubules will then become longi-
... in the convex flank. To dissect
... changed growth rate, microtu-
... that were prevented by a surgical
... in a horizontal orientation (such
... a vertical orientation (such that
... tropic stimulus). In this set-up,
... longitudinal was observed only in
... demonstrating unequivo-
... responded to gravity rather than to

...s

... of a plant organ, but this is a
... considerable amount of phenomeno-
... the turn of the nineteenth century
... underlying mechanisms are still far
... the use of adult organs, where
... ver. However, in germinating fern
... ates a larger, vacuolated rhizoid
... ...s can be oriented by gravity
... is clearly of formative character;
... antimicrotubular herbicides
... to give rise to thalloid tissue.
... ...sis has been determined, the
... adjust for this error (Edwards
... the spore is competent to the
... of the nucleus towards the lower
... a simple sedimentation process
... periods of active sign reversal,
... Edwards and Roux 1997).
... suggests that this guiding
... with the gravity vector,
... in amphibian eggs (Gerhart et al.
... by an interplay among gravity-
... induced nucleation of micro-
... microtubules that drive

4.3 *Microtubules and the Sensing of Gravity*

Since microtubules guide the anisotropic deposition of cellulose in the cell wall, it is not trivial to discriminate their function in gravity sensing from their role in the response to gravity during the development of tropistic curvature. When gravitropic bending is inhibited by antimicrotubular agents, this might be caused by a block of either the sensory function or the effector function of microtubules. To discern these microtubular functions, the lateral transport of auxin can be used as a response upstream of differential growth. With use of radioactively labelled auxin in rice coleoptiles (Godbolé et al. 2000), lateral auxin transport was found to be blocked by ethyl-*N*-phenylcarbamate, a herbicide that binds to the carboxy terminus 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 sensory microtubules have to be not only present, but, in addition, also dynamic 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 difficult to explain otherwise (Taylor and Leopold 1992). The necessity of high microtubular turnover favours a model where microtubules sense gravity-induced forces actively rather than merely acting as gravisusceptors. 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 ethyl-*N*-phenylcarbamate, acrylamide interrupts a very early step in the gravitropic response chain, clearly upstream of auxin redistribution and differential growth. No clear homologues of intermediate-filament proteins are known in the plant kingdom, but acrylamide treatment specifically disrupts microtubules, leaving actin filaments, for instance, 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.

4.4 *Microtubules and Mechanosensing*

Gravity is not the only source of mechanical stimulation used to integrate plant architecture. In contrast to terrestrial animals, the cells of land plants are not surrounded by an isotonic medium, but are surrounded by a hypotonic medium, with the consequence that their plasma membrane is under continuous tension from the expanding cytoplasm that is counterbalanced by the cell wall. On the level of organs, considerable tissue tensions develop that can be used for

mechanointegration when, for instance, new organs emerge and will thus generate local tension. This phenomenon has been intensively studied and modelled for phyllotaxis by Paul Green and co-workers, who showed that models of stress-strain patterns could perfectly predict the positions of incipient primordia (for a review see Green 1980). In the growing meristem, the formation of new primordia is suppressed by the older primordia. The tissue tension present in an expanding meristem produces mechanical stresses resulting from buckling of the preceding primordia. One of the earliest events of primordial initiation is a reorientation of cortical microtubules that are perpendicular with respect to the microtubules of their non-committed neighbours. This difference is sharp, but later it is smoothed by a transitional zone of cells with oblique microtubules, such that eventually a gradual, progressive change in microtubular reorientation emerges over several rows of cells (Hardham et al. 1980). This phenomenon has been revisited recently making use of microtubule marker lines labelled with green fluorescent protein in the developing shoot apex of *A. thaliana* (Hamant et al. 2008), where a feedback between microtubule orientation and organ growth was demonstrated. Mechanical modelling of the expanding shoot meristem predicted the transcellular pattern of microtubule orientation that was predicted and observed to be aligned with the directions of maximal stress. By ablation of specific cells in the outer meristem layer of the meristem, a redistribution of stress was induced and modelled. As expected, this redistribution caused a corresponding redistribution of microtubular orientation.

4.5 Candidates for the Underlying Mechanism

The sensing of gravity relies on the mechanical forces suscepted by the amyloplasts. This would, at first sight, suggest that the sensing of gravity and the sensing of mechanical stimuli should run in parallel. This can be tested by antagonistic application of artificial bending stress in antagonism to a gravitropic stimulus; it is possible to separate the response of gravity from the secondary mechanical 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 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, whereas gravisensing is not. Similarly, when the gravitropic bending of maize coleoptiles was inhibited by a surgical adhesive, the gravity-induced reorientation of microtubules was nevertheless developed (Himmelspach and Nick 2001), suggesting different signal chains for gravisensing and mechanosensing. Although the responses of plant cells to gravity and mechanical stimuli are generally discussed in the context of stretch-activated ion channels (Ding and Pickard 1993), the protein conformation paradigm of sensing

...erge and will thus generate
...studied and modelled for
...that models of stress-strain
...primordia (for a review see
...of new primordia is sup-
...in an expanding meristem
...the preceding primordia.
...reorientation of cortical
...microtubules of their non-
...later it is smoothed by a
...such that eventually a gradual,
...over several rows of cells
...ted recently making use of
...protein in the developing
...feedback between microtu-
...Mechanical modelling of the
...pattern of microtubule orien-
...with the directions of maximal
...stem layer of the meristem, a
...expected, this redistribution
...orientation.

...echanism

...susceptied by the amylo-
...sensing of gravity and the
...can be tested by antago-
...to a gravitropic stimulus;
...secondary mechanical
...gravitropic bending
...conditions, the activity of
...in hypocotyls of adzuki
...orientation of microtubules in
...ted unaltered (indicating
...of the apical hook that
...at least in this system,
...sensing is not. Similarly,
...inhibited by a surgical
...was nevertheless devel-
...signal chains for grav-
...cells to gravity and
...stretch-activated ion
...paradigm of sensing

should not be neglected. This is emphasized by the phenotype of *Arabidopsis* mutants, where the microtubule plus-end protein EB1 is affected (Bisgrove et al. 2008). In these mutants, both the initiation of gravitropic curvature and a specific touch-induced waving of roots on inclined agar plates were affected. Although our knowledge of the primary events of mechanosensing and gravisensing in plants is extremely limited, it is clear even at this stage that the roles of microtubules might differ qualitatively. In mechanosensing, microtubules seem to act as susceptor structures that focus deformation stress towards ion channels. In contrast, in gravisensing, the necessity for high dynamics and dimer turnover favours a direct sensory role of microtubules. 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 of the sensing mechanism. Only in a second step will it become possible to define and test molecular and cellular candidates.

5 The Cytoskeleton as a Sensor: Intracellular Sensing

The previous section dealt with the mechanical integration of individual cells into a coordinated response of the entire organ. However, mechanosensing is also used to integrate the different components of a cell into an individual. This becomes evident as redistribution of organelles in response to mechanical stimulation (thigmomorphogenesis; chapter "Mechanical Force Responses of Plant Cells and Plants"), but also involves responses that are less evident, such as the adaptation to cold, the induction of plant defence, osmoregulation (chapter "Osmosensing") and the regulation of division symmetry. The fundamental role of intracellular mechanosensing has emerged in recent years, but its full impact is still far from being recognized.

5.1 Thigmomorphogenesis

Morphological responses to mechanical stimulation have been demonstrated not only on the supracellular level, but also on the sub-cellular level. When fern protonemata are squeezed by a needle, chloroplasts avoid the site of contact (Sato et al. 1999). In epidermal cells (Kennard and Cleary 1997) or in suspension cells of parsley (Gus-Mayer et al. 1998) such a local pressure can induce nuclear movement towards the contact point. When regenerating protoplasts or cells are challenged by either mild centrifugation or touch, the axis of cell division is aligned with the force vector (Lintilhac and Vesecky 1984; Wymer et al. 1996; Zhou et al. 2007). Using tension-free protoplasts, Wymer et al. (1996) aligned microtubules by a short centrifugation and thus oriented the axis of cell expansion in a direction perpendicular to the force vector. They used this system to demonstrate a role of microtubules

in mechanosensing. To separate this sensory role from the microtubular function in cell-wall synthesis, microtubules were eliminated transiently during the application of force by amiprophos-methyl and allowed to recover by washing out the herbicide, such that cellulose synthesis could occur and thus the cell axis could develop. When this transient microtubule elimination was performed either immediately before or immediately after the centrifugation, the alignment of cell division was not observed. In a control, microtubules were eliminated subsequent to the centrifugation, which did not impair the alignment of the cell axis by the mechanical stimulus. This demonstrated clearly that microtubules are necessary for the sensing of this mechanical stimulus. A recent study in agarose-embedded walled cells of *Chrysanthemum* (Zhou et al. 2007) extends these findings by the interaction with the cell-wall cytoplasmic continuum. Here, cell divisions were aligned by compression force (Lintilhac and Vesecky 1984). When microtubules were removed by oryzalin prior to the treatment or when the cell-wall cytoplasmic continuum was impaired by treatment with RGD peptides, this alignment response was interrupted. In contrast, elimination of actin filaments by cytochalasin B was not effective.

5.2 *Cold Sensing*

The sensing of temperature must occur cell-autonomously. This 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 has been shown repeatedly to modify chilling sensitivity in plants (Murata et al. 1992). Since microtubules disassemble in the cold, they have long been discussed as alternative sensors for low temperatures. In fact, when microtubules were manipulated pharmacologically, this was accompanied by changes in cold hardiness (Kerr and Carter 1990). Microtubule disassembly of plants and of animals in the cold differ depending on the type of organism. Whereas mammalian microtubules disassemble at temperatures below 20°C, the microtubules from poikilothermic animals remain intact far below that temperature (Modig et al. 1994). In plants, the cold stability of microtubules is generally more pronounced than in animals, reflecting the higher developmental plasticity. However, the critical temperature where microtubule disassembly occurs varies between different plant species, which is correlated with differences in chilling sensitivity (Jian et al. 1989). The close correlation between microtubular cold sensitivity and general chilling sensitivity is supported by the observation that abscisic acid, a hormonal inducer of cold hardiness (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 owing 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 and podophyllotoxin increased the chilling sensitivity of cotton seedlings, and

e to the microtubular function in
ed transiently during the application
re-over by washing out the herbi-
nd thus the cell axis could develop.
as performed either immediately
the alignment of cell division was
minated subsequent to the centri-
of the cell axis by the mechanical
ubules are necessary for the sensing
agarose-embedded walled cells of
se findings by the interaction with
divisions were aligned by compres-
en microtubules were removed by
l-wall cytoplasmic continuum was
alignment response was interrupted.
tochalasin B was not effective.

l-autonomously. This is generally
that will alter the activity of ion
[97]. For instance, overexpression
rmidity chilling sensitivity in plants
sensitive in the cold, they have long
temperatures. In fact, when micro-
his was accompanied by changes in
ubule disassembly of plants and of
organism. Whereas mammalian
ow 20°C, the microtubules from
at temperature (Modig et al.
ules is generally more pronounced
cortical plasticity. However, the
ity occurs varies between different
es of chilling sensitivity (Jian et al.
ular cold sensitivity and general
that abscisic acid, a hormonal
e [see Irving 1969; Rikin et al.
e of the microtubules against low
Wang and Nick 2001). Tobacco
stant owing to expression of an
leat expansion (Ahad et al. 2003).
assembly blockers such as colchi-
sensitivity of cotton seedlings, and

this effect could be rescued by addition of abscisic acid (Rikin et al. 1980). Conversely, gibberellin, a hormone that has been shown in several species to reduce cold hardiness (Rikin et al. 1975; Irving and Lanphear 1968), renders cortical microtubules more cold-susceptible (Akashi and Shibaoka 1987).

It is possible to increase the cold resistance of an otherwise chilling-sensitive species by pre-cultivation at moderately cool temperature. 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 (Bartolo and Carter 1991a). 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 can suppress cold hardening (Kerr and Carter 1990; Bartolo and Carter 1991b). This indicates that microtubules have to disassemble to a certain degree 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 (-7°C) which would impair growth irreversibly in non-acclimated roots. When microtubules were monitored during cold hardening, a rapid, but transient partial disassembly was observed in cultivars that were freezing-tolerant, but not in a cultivar that was freezing-sensitive. However, when a transient disassembly was artificially generated by a pulse treatment with the antimicrotubular herbicide pronamide in the sensitive cultivar, this induced 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 mechanosensing and gravisensing, this leads to the question of whether microtubules act as susceptors or as true receptors for low temperature. The primary signal for cold perception is thought be 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 dimethyl sulfoxide, whereas benzyl alcohol, a compound that increases membrane fluidity, can block cold signalling (Sangwan et al. 2001). With use of aequorin as a reporter in transgenic plants, rapid and transient increases of intracellular calcium levels in response to a cold shock were demonstrated by monitoring changes of bioluminescence (Knight et al. 1991). Pharmacological data (Monroy et al. 1993) confirmed that this calcium peak is not only a by-product of the cold response, but is also necessary to trigger cold acclimation. This peak is generated through calcium channels in conjunction with calmodulin. Calcium and 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 carboxy terminus of maize tubulin was shown to render microtubules resistant to both low temperature and calcium (Bokros et al. 1996). When the release of calcium from intracellular pools was blocked by treatment with lithium, an inhibitor of polyphosphoinositide turnover, this resulted in increased cold stability of microtubules in spinach mesophyll (Bartolo and Carter 1992). With use of a cold-responsive reporter system it was 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 promoter by low temperature (Sangwan et al. 2001). These data favour a model where microtubules act as receptors that limit the permeability of calcium channels that are triggered by membrane rigidification. 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 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 signalling 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 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, would also endow it with the ability to downregulate sensitivity upon prolonged stimulation, a key requirement for any biological sensory process.

5.3 Plant Defence

The interaction of pathogens and their hosts is subject to an evolutionary arms race, where the pathogens develop various strategies to circumvent or suppress defence responses of the host, whereas the host develops various strategies to sense and attack the invading pathogen or its effector molecules. Cellular responses to elicitors include formation of cell-wall papillae around sites of pathogen penetration. The formation of these papillae is preceded by a reorganization of the cytoskeleton causing a redistribution of vesicle traffic and a 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 a review see Schmelzer 2002). By localized mechanical stimulation of parsley

cells, it was possible to partially mimic an attack by *Phytophthora sojae* and to induce several aspects of a non-host resistance, including nuclear migration, cytoplasmic reorganization, formation of reactive oxygen species and the induction of several defence-related genes (Gus-Mayer et al. 1998). In contrast, localized application of the corresponding elicitor (pep-13) failed to induce the morphological changes, although it induced the full set of defence-related genes and the formation of reactive oxygen species. Interestingly, the elicitor completely inhibited cytoplasmic aggregation and nuclear migration in response to the mechanical stimulus. Since pep-13 induces in this system the activity of a mechanosensitive calcium channel (Zimmermann et al. 1997), it seems that chemical and mechanical signalling converge during the cytoskeletal response to pathogen attack. Neither the mechanical stimulus nor the elicitor nor their combination was able to induce hypersensitive cell death in these experiments, leading the investigators to conclude that additional chemical signals are required to obtain the complete pathogen response. This suggests an interaction between microtubules and mechanosensitive ion channels that are important for the induction of defence.

5.4 *Osmoregulation*

The ability to maintain ionic balance represents a basic capacity of all living beings. Prokaryotes have already developed osmoregulation. In plants, the mechanical tensions produced in the context of an expanding tissue have to be balanced by osmotic pressure (chapter "Osmosensing"). Microtubules seem to be directly involved in osmoadaptation. By application of osmotic stress to root tips of *Triticum turgidum*, microtubules were induced to disassemble and to reorganize into massive bundles (Komis et al. 2002). When the formation of these so-called macrotubules was suppressed by treatment with oryzalin, the protoplasts were no longer able to adapt to osmotic stress by controlled swelling and perished. A pharmacological study (Komis et al. 2006) revealed that inhibitors of phospholipase D, such as butan-1-ol and *N*-acetylethanolamine, suppressed osmotic adaptation as well as the formation of the macrotubules. In contrast, phosphatidic acid, a product of the action of phospholipase D, enhanced osmoadaptation and macrotubule formation and was able to overcome the inhibitory effect of butan-1-ol. 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.

5.5 *Division Symmetry*

A homologue of the bacterial mechanosensitive channel MscS, MSL3, localized in discrete patches in the plastid envelope and co-localized with the plastid division

factor MinE, indicating an interaction with plastid division (Vitha et al. 2003). When this bona fide channel was mutated, this resulted in chloroplasts that were irregular in size, shape and partially number. Thus, these channels regulate morphogenesis and development of plastids, suggesting a functional shift from osmoregulation towards regulation of plastid morphogenesis. However, it is not only in the symmetry of organelle division where mechanosensing seems to play a role. Mechanosensing seems to be the integrator that allows the nucleus to determine the correct position prior to mitosis – this premitotic nuclear movement is an active process and is driven by the cytoskeleton. It will determine the symmetry of the ensuing cell division and thus the basic morphology of the prospective daughter cells. The discovery that overexpression or knockout of the plant-specific kinesin motor KCH1 (Frey et al. 2010), which binds to actin filaments, retards or accelerates premitotic nuclear positioning, indicates that cytoskeletal tensegrity is used to determine the correct position of the nucleus. Two principal modes are conceivable that are not necessarily mutually exclusive. Microtubules and actin filaments might transmit forces that are generated by the KCH1 motor at the perinuclear contact sites to the cortex such that the nucleus is either pulled or pushed, or both (Fig. 2c, d). Alternatively, KCH1 might simply anchor the perinuclear network at the cell cortex and move the nucleus by mutual sliding of actin filaments and microtubules in the cortical cytoplasm. From studies in yeast, filamentous fungi and a variety of animal cells, the molecular mechanisms that orient and move nuclei were found to be moderately conserved and involve as key players dynein, dynactin and other proteins at the plus ends of astral microtubules, mediating interaction with the cell cortex and actin filaments (Morris 2003; Yamamoto and Hiraoka 2003). Both repulsive and attractive forces are generated by a combination of microtubule polymerization and de-polymerization events, complemented by dynein-mediated sliding of microtubules along the cell cortex (Adames and Cooper 2000). In plants, which lack dynein and its associated proteins (Lawrence et al. 2001), the mechanisms for nuclear movement must involve fundamentally different players that are able to interact with both premitotic microtubules and actin filaments. Could KCH proteins be these missing links as functional homologues of dyneins by anchoring minus-end-directed motor activity to the cortex?

6 Evolutionary Perspective: The Cytoskeleton as a Central Integrator

This chapter summarized evidence for a cytoskeletal function in tensegral integration on both the organismal and the cellular level. We are still far from understanding the molecular set-up of tensegral sensing. But even at this stage, the first differences between the different stimulus qualities have emerged.

For mechanoperception, microtubules seem to interact with stretch-activated ion channels, probably focussing minute deformations of the membrane or changes in

...astid division (Vitha et al. 2003).
...s resulted in chloroplasts that were
Thus, these channels regulate mor-
...esting a functional shift from osmo-
...ogenesis. However, it is not only in
...chanosensing seems to play a role.
...as the nucleus to determine the
...otic nuclear movement is an active
...determine the symmetry of the
...logy of the prospective daughter
...ut of the plant-specific kinesin
...actin filaments, retards or accel-
...that cytoskeletal tensegrity is used
...Two principal modes are conceiv-
...Microtubules and actin filaments
...he KCH1 motor at the perinuclear
...is either pulled or pushed, or both
...anchor the perinuclear network at
...sliding of actin filaments and
...studies in yeast, filamentous fungi
...hanisms that orient and move nuclei
...olve as key players dynein, dynactin
...otubules, mediating interaction with
...3, Yamamoto and Hiraoka 2003).
...ed by a combination of microtubule
...complemented by dynein-mediated
...dames and Cooper 2000). In plants,
...Lawrence et al. 2001), the mechan-
...amentally different players that are
...es and actin filaments. Could KCH
...gues of dyneins by anchoring

...toskeleton

...etal function in tensegral integra-
...We are still far from understand-
...but even at this stage, the first
...ities have emerged.
...interact with stretch-activated ion
...s of the membrane or changes in

membrane fluidity towards specific membrane areas. Because the demonstration of mechanosensitivity by patch-clamp experiments is experimentally very problematic and prone to artefacts (Gustin et al. 1991), the molecular identity of stretch-activated channels has remained elusive in plants. When mechanosensitivity is not an intrinsic property of such channels, but is conferred by accessory structures (such as cytoskeletal tensegrity), the identification of these channels will go beyond simple expression in heterologous systems (such as frog oocytes), and will require the reconstitution of the entire structure, i.e. it will rely on synthetic biology.

The situation might be different for the sensing of gravity. Here microtubules themselves could act as primary sensors. The findings of 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 seems to represent a third mechanism, where the gating of cold-sensitive channels (which probably respond to membrane rigidity as an input) is limited by microtubules. When these microtubules disassemble in response to cold, the constraints upon the activity of the ion channels will be released 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 – first as a perceptive device and later as accessory structures for the perceptive channels.

Why is the cytoskeleton central for mechanical integration? The reason is probably linked to the innate properties of cytoskeletal dynamics that render microtubules and actin filaments ideal for the sensing of minute and noisy inputs. These dynamics are nonlinear and endowed with autocatalytic properties. In the cell, the abundance of monomers is limited, which means that different polymers compete for the incorporation of free monomers. For instance, 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 a review see Breviario 2008). Although the term "cytoskeleton" was coined in the model 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 example, has been estimated to be in the range of 30–60 s (Hush et al. 1990). Therefore, it is more appropriate to conceive of microtubules and actin filaments as states of dynamic equilibrium between assembly and disassembly of monomers. It is this dynamic equilibrium that provides the major source for the characteristic nonlinearity of cytoskeletal dynamics.

Interestingly, the relation between assembly and disassembly is practically never balanced –one always dominates 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 cytoskeletal polymer, with dimer addition dominating at the plus end and dimer dissociation dominating at the minus end; in time, because each polymer 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 so swift and 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 its nonlinear growth, the cytoskeleton is an ideal tool for developmental patterning. This has been exemplarily shown for the induction of the grey crescent in developing frog eggs. This manifestation of dorsoventrality 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 is 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 lay down the Spemann organizer.

The combination of nonlinear, autocatalytic dynamic states of individual cytoskeletal polymers with the tight control of free monomers accentuating mutual competition generates system properties that are highly relevant for sensory processes. Microtubules and actin filaments fulfil all formal criteria of a reaction–diffusion system (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. One can model how owing to their innate dynamic properties microtubules will spontaneously self-organize in response to even a weak external factor such as gravity or mechanical 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 and can be used to integrate even minute mechanical strains on the level of individual cells as well as on the level of entire organs.

References

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

Adames NR, Cooper JA (2000) Microtubule interactions with the cell cortex causing nuclear movements in Saccharomyces cerevisiae. J Cell Biol 149:863–874

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

end, and a shrinking state,
addition at the plus end.
that it has been termed
associated proteins that
een growth and shrinkage.
an ideal tool for develop-
the induction of the grey
dorsoventrality is produced
gradient of developmental
d, displaced, gradient in the
the egg cortex is driven by
sperm. The sperm induces
a kinesin-driven movement
triggers shear forces that
direction parallel to the move-
undergo catastrophic transi-
the efficiency of movement
rotation of the cortical plasma
equator of the egg. This
with a small region of the
that lay down the Spemann

states of individual cyto-
omers accentuating mutual
relevant for sensory pro-
criteria of a reaction–
can be understood as ideal
clear, neat outputs from
model how owing to their
spontaneously self-organize in
or mechanical fields
ample use of these unique
both sensitive and robust
minute mechanical strains
entire organs.

assembly a trigger for
cortex causing nuclear
are tolerant to antimicro-
12:615–629

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

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

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

Baluška F, Hlavačka A (2005) Plant formins come of age: something special about cross-walls. New Phytol 168:499–503

Baluška F, Jasik J, Edelmann HG, Salajová T, Volkmann D (2001) Latrunculin B-induced plant dwarfism: plant cell elongation is F-actin-dependent. Dev Biol 231:113–124

Baluška F, Šamaj J, Wojtaszek P, Volkmann D, Menzel D (2003) Cytoskeleton-plasma membrane-cell wall continuum in plants. Emerging links revisited. Plant Physiol 133:482–491

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

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

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

Bartolo ME, Carter JV (1992) Lithium decreases cold-induced microtubule depolymerization in mesophyll cells of spinach. Plant Physiol 99:1716–1718

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

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

Baskin TI, Wilson JE (1997) Inhibitors of protein kinases and phosphatases alter root morphology and disorganize cortical microtubules. Plant Physiol 113:493–502

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

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

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

Blancaflor EB (2000) Cortical actin filaments potentially interact with cortical microtubules in regulating polarity of cell expansion in primary roots of maize (*Zea mays* L.). J Plant Growth Regul 19:406–414

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

Bokros CL, Hugdahl JD, Blumenthal SSD, Morejohn LC (1996) Proteolytic analysis of polymerized maize tubulin: regulation of microtubule stability to low temperature and Ca^{2+} by the carboxyl terminus of β-tubulin. Plant Cell Environ 19:539–548

Braam J, Davis RW (1990) Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. Cell 60:357–364

Breviario D (2008) Plant tubulin genes: regulatory and evolutionary aspects. Plant Cell Monogr 11:207–232

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

Buder J (1920) Neue phototropische Fundamentalversuche. Ber Dtsch Bot Ges 38:10–19

Buder J (1961) Der Geotropismus der Characeenrhizoide. Ber Dtsch Bot Ges 74:14–23

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

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

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

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

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

Canut H, Carrasco A, Galaud J-P, Cassan C, Bouyssou H, Vita N, Ferrara P, Pont-Lezica R (1998) High affinity RGD-binding sites at the plasma membrane of *Arabidopsis thaliana* links the cell wall. Plant J 16:63–71

Cárdenas L, Lovy-Wheeler A, Kunkel JG, Hepler PK (2008) Pollen tube growth oscillations and intracellular calcium levels are reversibly modulated by actin polymerization. Plant Physiol 146:1611–1621

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

Collings DA (2008) Crossed-wires: interactions and cross-talk between the microtubule and micro. lament networks in plants. Plant Cell Monogr 11:47–79

Collings DA, Lill AW, Himmelspach R, Wasteneys GO (2006) Hypersensitivity to cytoskeletal antagonists demonstrates microtubule-microfilament cross-talk in the control of root elongation in *Arabidopsis thaliana*. New Phytol 170:275–290

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

Ding JP, Pickard BG (1993) Mechanosensory calcium-selective cation channels in epidermal cells. Plant J 3:83–110

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

Eckert BS, Yeagle PL (1988) Acrylamide treatment of PtK1 cells causes dephosphorylation of keratin polypeptides. Cell Motil Cytoskelet 11:24–30

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

Edwards ES, Roux SJ (1997) The influence of gravity and light on developmental polarity of single cells of *Ceratopteris richardii* gametophytes. Biol Bull 192:139–140

Elinson RP, Rowning B (1988) Transient array of parallel microtubules in frog eggs: potential tracks for a cytoplasmic rotation that specifies the dorso-ventral axis. Dev Biol 128:185–197

Fisher DD, Cyr RJ (1993) Calcium levels affect the ability to immunolocalize calmodulin to cortical microtubules. Plant Physiol 10:543–551

Frey N, Klotz J, Nick P (2009) Dynamic bridges – a calponin-domain kinesin from rice links actin filaments and microtubules in both cycling and non-cycling cells. Plant Cell Physiol 50: 1493–1506

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

Funada R (2008) Microtubules and the control of wood formation. Plant Cell Monogr 11:83–119

Furutani I, Watanabe Y, Prieto R, Masukawa M, Suzuki K, Naoi K, Thitamadee S, Shikanai T, Hashimoto T (2000) The *SPIRAL* genes are required for directional control of cell plates elongation in *Arabidopsis thaliana*. Development 127:4443–4453

Gardiner JC, Harper JDI, Weerakoon ND, Collings DA, Ritchie S, Gilroy S, Cyr RJ, Marc J (2001) A 90-kD phospholipase D from tobacco binds to microtubules and the plasma membrane. Plant Cell 13:2143–2158

...-like protein regulates normal
...
... CW, Schäffner AR (2004)
... a plant-specific microtubule-
...
... Bouchez D (2002) The Arabi-
... regulatory subunit essential for the
...
... the pattern of cell division in
...
... N, Ferrara P, Pont-Lezica R (1998)
... *Arabidopsis thaliana* links the cell
...
... Pollen tube growth oscillations and
... actin polymerization. Plant Physiol
...
... arrays undergo rotary movements in
...
... between the microtubule and
...
... Hypersensitivity to cytoskeletal
... in the control of root elonga-
...
... Microtubule plus-ends reveal essential
... modulation of endocytosis during
...
... cation channels in epidermal
...
... between microtubules and a higher
...
... causes dephosphorylation of
...
... in germinating spores of
...
... on developmental polarity of
... 192:139–140
... microtubules in frog eggs: potential
... axis. Dev Biol 128:185–197
... immunolocalize calmodulin to
...
... kinesin from rice links actin
... cells. Plant Cell Physiol 50:
...
... domain is involved in
...
... Plant Cell Monogr 11:83–119
... S, Thitamadee S, Shikanai T,
... directional control of cell plates
...
... Cyr RJ, Marc J (2001)
... the plasma membrane. Plant

Geiger B, Bershadsky A (2001) Assembly and mechanosensory function of focal contacts. Curr Opin Cell Biol 13:584–592
Gens JS, Fujiki M, Pickard BG (2000) Arabinogalactan protein and wall-associated kinase in a plasmalemmal reticulum with specialized vertices. Protoplasma 212:115–134
Gerhart J, Ubbeles G, Black S, Hara K, Kirschner M (1981) A reinvestigation of the role of the grey crescent in axis formation in *Xenopus laevis*. Nature 292:511–516
Giancotti FG, Ruoslahti E (1999) Integrin signaling. Science 285:1028–1032
Gianí S, Qin X, Faoro F, Breviario D (1998) In rice, oryzalin and abscisic acid differentially affect tubulin mRNA and protein levels. Planta 205:334–341
Giddings TH, Staehelin A (1988) Spatial relationship between microtubules and plasmamembrane rosettes during the deposition of primary wall microfibrils in *Closterium spec*. Planta 173: 22–30
Giddings TH, Staehelin A (1991) Microtubule-mediated control of microfibril deposition. A re-examination of the hypothesis. In: Lloyd CW (ed) The cytoskeletal basis of plant growth and form. Academic, London, pp 85–99
Gierer A (1981) Generation of biological patterns and form: some physical, mathematical, and logical aspects. Progr Biophys Mol Biol 37:1–47
Gittes F, Mickey B, Nettleton J, Howard J (1993) Flexual rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. J Cell Biol 120:923–934
Godbolé R, Michalke W, Nick P, Hertel R (2000) Cytoskeletal drugs and gravity-induced lateral auxin transport in rice coleoptiles. Plant Biol 2:176–181
Goebel K (1908) Einleitung in die experimentelle Morphologie der Pflanzen. Teubner, Leipzig, pp 218–251
Goode BL, Drubin DG, Barnes G (2000) Functional cooperation between the microtubule and actin cytoskeletons. Curr Opin Cell Biol 12:63–71
Gossot O, Geitmann A (2007) Pollen tube growth: coping with mechanical obstacles involves the cytoskeleton. Planta 226:405–416
Grabski S, Schindler M (1996) Auxins and cytokinins as antipodal modulators of elasticity within the actin network of plant cells. Plant Physiol 110:965–970
Grabski S, Arnoys E, Busch B, Schindler M (1998) Regulation of actin tension in plant cells by kinases and phosphatases. Plant Physiol 116:279–290
Green PB (1962) Mechanism for plant cellular morphogenesis. Science 138:1404–1405
Green PB (1980) Organogenesis – a biophysical view. Annu Rev Plant Physiol 31:51–82
Gus-Mayer S, Naton B, Hahlbrock K, Schmelzer E (1998) Local mechanical stimulation induces components of the pathogen defense response in parsley. Proc Natl Acad Sci USA 95:8398–8403
Gustin MC, Sachs F, Sigurdson WJ, Ruknudin A, Bowman C (1991) Technical comments. Single channel mechanosensitive currents. Science 253:1195–1197
Gutjahr C, Nick P (2006) Acrylamide inhibits gravitropism and destroys microtubules in rice coleoptiles. Protoplasma 227:211–222
Haberland G (1900) Über die Perzeption des geotropischen Reizes. Ber Dtsch Bot Ges 18:261–272
Hamada T (2007) Microtubule-associated proteins in higher plants. J Plant Res 120:79–98
Hamant O, Heisler MG, Jönsson H, Krupinski P, Uyttewaal M, Bokov P, Corson F, Sahlin P, Boudaoud A, Meyerowitz EM, Couder Y, Traas J (2008) Developmental patterning by mechanical signals in Arabidopsis. Science 322:1650–1655
Hardham AR, Green PB, Lang JM (1980) Reorganization of cortical microtubules and cellulose deposition during leaf formation of *Graptopetalum paraguayense*. Planta 149:181–195
Hasezawa S, Nozaki H (1999) Role of cortical microtubules in the orientation of cellulose microfibril deposition in higher-plant cells. Protoplasma 209:98–104
Hashimoto T, Kato T (2006) Cortical control of plant microtubules. Curr Opin Plant Biol 9:5–11
Heath IB (1974) A unified hypothesis for the role of membrane bound enzyme complexes and microtubules in plant cell wall synthesis. J Theor Biol 48:445–449

Hepler PK, Vidali L, Cheung AY (2001) Polarized cell growth in higher plants. Annu Rev Cell Dev Biol 17:159–187

Hertel R, Friedrich U (1973) Abhängigkeit der geotropischen Krümmung der *Chara*-Rhizoide von der Zentrifugalbeschleunigung. Z Pflanzenphysiol 70:173–184

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

Himmelspach R, Nick P (2001) Gravitropic microtubule reorientation can be uncoupled from growth. Planta 212:184–189

Hodick D (1994) Negative gravitropism in *Chara* protonemata: a model integrating the opposite gravitropic responses of protonemata and rhizoids. Planta 195:43–49

Holubowicz T, Boe AA (1969) Development of cold hardiness in apple seedlings treated with gibberellic acid and abscisic acid. J Am Soc Hortic Sci 94:661–664

Holweg C, Süßlin C, Nick P (2004) Capturing in-vivo dynamics of the actin cytoskeleton. Plant Cell Physiol 45:855–863

Hush JM, Hawes CR, Overall RL (1990) Interphase microtubule re-orientation predicts a new cell polarity in wounded pea roots. J Cell Sci 96:47–61

Igarashi H, Orii H, Mori H, Shimmen T, Sonobe S (2000) Isolation of a novel 190 kDa protein from tobacco BY-2 cells: possible involvement in the interaction between actin filaments and microtubules. Plant Cell Physiol 41:920–931

Ikushima T, Shimmen T (2005) Mechano-sensitive orientation of cortical microtubules during gravitropism in azuki bean epicotyls. J Plant Res 118:19–26

Ingber DE (2003a) Tensegrity I: cell structure and hierarchical systems biology. J Cell Sci 116:1157–1173

Ingber DE (2003b) Tensegrity II: how structural networks influence cellular information processing networks. J Cell Sci 116:1397–1408

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

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

Jacob F (1977) Evolution and tinkering. Science 196:1161–1166

Jaffe MJ, Leopold AC, Staples RA (2002) Thigmo responses in plants and fungi. Am J Bot 89:375–382

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

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

Jones RS, Mitchell CA (1989) Calcium ion involvement in growth inhibition of mechanically stressed soybean *Glycine max* seedlings. Physiol Plant 76:598–602

Kakimoto T, Shibaoka H (1987) Actin filaments in the preprophase band and phragmoplast of tobacco cells. Protoplasma 140:151–156

Karki S, Holzbaur EL (1999) Cytoplasmic dynein and dynactin in cell division and intracellular transport. Curr Opin Cell Biol 1:45–53

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

Kell A, Glaser RW (1993) On the mechanical and dynamic properties of plant-cell membranes: their role in growth, direct gene transfer and protoplast fusion. J Theor Biol 160:41–62

Kennard JL, Cleary AL (1997) Pre-mitotic nuclear migration in subsidiary mother cells of *Tradescantia* occurs in G1 of the cell cycle and requires F-actin. Cell Motil Cytoskelet 36:55–67

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

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

...igher plants. Annu Rev Cell
...ung der *Chara*-Rhizoide von
...duced reorientation of cortical
...tation can be uncoupled from
...model integrating the opposite
...apple seedlings treated with
...the actin cytoskeleton. Plant
...reorientation predicts a new cell
...tion of a novel 190 kDa protein
...tion between actin filaments and
...cortical microtubules during
...systems biology. J Cell Sci
...ed cellular information proces-
...nhibitor in the induction of cold
...*gundo*. Plant Physiol 43:9–13
...plants and fungi. Am J Bot
...s of force transduction in cells.
...ibility in relation to plant cold
...inhibition of mechanically
...se band and phragmoplast of
...division and intracellular
...wall in nuclear positioning
...ces of plant-cell membranes:
...Exper Biol 160:41–62
...subsidiary mother cells of
...Cell Motil Cytoskelet
...root-tip cells and cold
...Physiol 93:77–82
...Immunogold labeling of
...*Vigna angularis*. Plant

Kishimoto U (1968) Response of *Chara* internodes to mechanical stimulation. Ann Rep Biol Works Fac Sci Osaka Univ 16:61–66

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

Kobayashi I, Kobayashi Y (2008) Microtubules and pathogen defence. Plant Cell Monogr 11:121–140

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

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

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

Kutschera U (2008) The outer epidermal wall: design and physiological role of a composite structure. Ann Bot 101:615–621

Kuznetsov OA, Hasenstein KH (1996) Magnetophoretic induction of root curvature. Planta 198:87–94

Lawrence CJ, Morris NR, Meagher RB, Dawe RK (2001) Dyneins have run their course in plant lineage. Traffic 2:362–363

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

Lintilhac PM, Vesecky TB (1984) Stress-induced alignment of division plane in plant tissues grown in vitro. Nature 307:363–364

Lloyd CW, Traas JA (1988) The role of F-actin in determining the division plane of carrot suspension cells. Drug Stud Dev 102:211–221

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

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

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

Maisch J, Nick P (2007) Actin is involved in auxin-dependent patterning. Plant Physiol., Plant Physiol 143:1695–1704.

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

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

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

Morris NR (2003) Nuclear positioning: the means is at the ends. Curr Opin Cell Biol 15:54–59

Moseley JB, Bartolini F, Okada K, Wen Y, Gundersen GG, Goode BL (2007) Regulated binding of adenomatous polyposis coli protein to actin. J Biol Chem 282:12661–12668

Mulder B, Schell J, Emons AM (2004) How the geometrical model for plant cell wall formation enables the production of a random texture. Cellulose 11:395–401

Murata T, Wada M (1991) Effects of centrifugation on preprophase-band formation in *Adiantum* protonemata. Planta 183:391–398

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

Nemec B (1900) Über die Art der Wahrnehmung des Schwerkraftreizes bei den Pflanzen. Ber Dtsch Bot Ges 18:241–245

Nick P (2008a) Control of cell axis. Plant Cell Monogr 11:3–46

Nick P (2008b) Microtubules as sensors for abiotic stimuli. Plant Cell Monogr 11:175–203

Nick P, Furuya M (1996) Buder revisited – cell and organ polarity during phototropism. Plant Cell Environ 19:1179–1187

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

Nick P, Yatou O, Furuya M, Lambert AM (1994) Auxin-dependent microtubule responses and seedling development are affected in a rice mutant resistant to EPC. Plant J 6:651–663

Nick P, Godbolé R, Wang QY (1997) Probing rice gravitropism with cytoskeletal drugs and cytoskeletal mutants. Biol Bull 192:141–143

Nick P, Han M, An G (2009) Auxin stimulates its own transport by actin reorganization. Plant Physiol 151:155–167

Niklas KJ (1992) Plant biomechanics. An engineering approach to plant form and function. University of Chicago Press, Chicago

Orr AW, Helmke BP, Blackman BR, Schwartz MA (2006) Mechanisms of mechanotransduction. Dev Cell 10:11–20

Panteris E (2008) Cortical actin filaments at the division site of mitotic plant cells: a reconsideration of the 'actin-depleted zone'. New Phytol 179:334–341

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

Parthasarathy MV, Perdue TD, Witztum A, Alvernaz J (1985) Actin network as a normal component of the cytoskeleton in many vascular plant cells. Am J Bot 72:1318–1323

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

Pickard BG, Fujiki M (2005) Ca^{2+} pulsation in BY-2 cells and evidence for control of mechanosensory Ca^{2+}-selective channels by the plasmalemmal reticulum. Funct Plant Biol 32:863–879

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

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

Preuss ML, Kovar DR, Lee YR, Staiger CJ, Delmer DP, Liu B (2004) A plant-specific kinesin binds to actin microfilaments and interacts with cortical microtubules in cotton fibers. Plant Physiol 136:3945–3955

Rawitscher F (1932) Der Geotropismus der Pflanzen. Fischer, Jena

Richardson D, Simmons M, Reddy A (2006) Comprehensive comparative analysis of kinesins in photosynthetic eukaryotes. BMC Genomics 7:18

Rikin A, Richmond AE (1976) Amelioration of chilling injuries in cucumber seedlings by abscisic acid. Physiol Plant 38:95–97

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

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

Riveline D, Zamir E, Balaban NQ, Schwarz US, Ishizaki T, Narumiya S, Kam Z, Geiger B, Bershadsky AD (2001) Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J Cell Biol 153:1175–1185

Robby T (1996) A new architecture. Yale Academic Press, New Haven

Rodriguez OC, Schaefer AW, Mandato CA, Forscher P, Bement WM, Waterman-Storer CM (2003) Conserved microtubule-actin interactions in cell movement and morphogenesis. Nat Cell Biol 5:599–609

Sachs J (1880) Stoff und Form der Pflanzenorgane. Arb Bot Inst Würzburg 2:469–479

Sachs F, Morris CE (1998) Mechanosensitive ion channels in nonspecialized cells. Rev Physiol Biochem Pharmacol 132:1–77

Sainsbury F, Collings DA, Mackun K, Gardiner J, Harper JDI, Marc J (2008) Developmental reorientation of transverse cortical microtubules to longitudinal directions: a role for actomyosin-based streaming and partial microtubule-membrane detachment. Plant J 56:116–131

of microtubules in gravitropism

dent microtubule responses and
EPC. Plant J 6:651–663
with cytoskeletal drugs and

by actin reorganization. Plant

ch to plant form and function.

Mechanisms of mechanotransduction.

mitotic plant cells: a reconsidera-

of cellulose synthase demon-
1491–1495
Actin network as a normal
Am J Bot 72:1318–1323
for orienting cellulose: model-
–29
and evidence for control of mechan-
m. Funct Plant Biol 32:863–879
exposure on cortical microtubules of
Physiol Plant 93:563–571
in plant cell walls. Planta

B (2004) A plant-specific kinesin
microtubules in cotton fibers. Plant

mparative analysis of kinesins in

cucumber seedlings by abscisic

al regulation of morphogenesis
gibberellic acid in alfalfa (*Medicago*

pium hirsutum L.): effects of

Naramiya S, Kam Z, Geiger B,
externally applied local mechanical
dependent and ROCK-independent

New Haven
WM, Waterman-Storer CM
ment and morphogenesis. Nat

Würzburg 2:469–479
specialized cells. Rev Physiol

Mutz J (2008) Developmental
functions: a role for actomyosin-
Plant J 56:116–131

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

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

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

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

Sano T, Higaki T, Oda Y, Hayashi T, Hasezawa S (2005) Appearance of actin microfilament 'twin peaks' in mitosis and their function in cell plate formation, as imaged in tobacco BY-2 cells expressing GFP-fimbrin. Plant J 44:595–605

Sato Y, Kadota A, Wada M (1999) Mechanically Induced Avoidance Response of Chloroplasts in Fern Protonemal Cells. Plant Physiol 121:37–44

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

Schmit AC, Nick P (2008) Microtubules and the evolution of mitosis. Plant Cell Monogr 11: 1500 233–266

Schwuchow J, Sack FD, Hartmann E (1990) Microtubule disruption in gravitropic protonemata of the moss *Ceratodon*. Protoplasma 159:60–69

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

Sedbrook JC, Kaloriti D (2008) Microtubules, MAPs and plant directional cell expansion. Trends Plant Sci 13:303–310

Shibaoka T (1966) Action potentials in plant organs. Symp Soc Exp Biol 20:165–184

Sievers A, Schröter K (1971) Versuch einer Kausalanalyse der geotropischen Reaktionskette im *Chara*-Rhizoid. Planta 96:339–353

Sonobe S, Shibaoka H (1989) Cortical fine actin filaments in higher plant cells visualized by rhodamine-phalloidin after pretreatment with m-maleimidobenzoyl-N-hydrosuccinimide ester. Protoplasma 148:80–86

Tabony J, Glade N, Papaseit C, Demongeot J (2004) Microtubule self-organization as an example of the development of order in living systems. J Biol Phys Chem 4:50–63

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

Tamura K, Nakatani K, Mitsui H, Ohashi Y, Takahashi H (1999) Characterization of katD, a kinesin-like protein gene specifically expressed in floral tissues of *Arabidopsis thaliana*. Gene 230:23–32

Taylor DP, Leopold AC (1992) Offset of gravitropism in maize roots by low temperature. ASGSB Bull 6:75

Telewski FW (2006) A unified hypothesis of mechanoperception in plants. Am J Bot 93:1466–1476

Thimann KV, Reese K, Nachmikas VT (1992) Actin and the elongation of plant cells. Protoplasma 171:151–166

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

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

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

Tirnauer JS, Bierer BE (2000) EB1 proteins regulate microtubule dynamics, cell polarity, and chromosome stability. J Cell Biol 149:761–766

Toriyama H, Jaffe MJ (1972) Migration of calcium and its role in the regulation of seismonasty in the motor cell of *Mimosa pudica* D. Plant Physiol 49:72–81

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

Tsvetkov AS, Samsonov A, Akhmanova A, Galjart N, Popov SV (2007) Microtubule-binding proteins CLASP1 and CLASP2 interact with actin filaments. Cell Motil Cytoskelet 64:519–530
Turing AM (1952) The chemical basis of morphogenesis. Philos Trans R Soc Lond Ser B 237:37–72
Vantard M, Levilliers N, Hill AM, Adoutte A, Lambert AM (1990) Incorporation of *Paramecium* axonemal tubulin into higher plant cells reveals functional sites of microtubule assembly. Proc Natl Acad Sci USA 87:8825–8829
Vitha S, Froehlich JE, Koksharova O, Pyke KA, van Erp H, Osteryoung KW (2003) ARC6 is a J-domain plastid division protein and an evolutionary descendant of the cyanobacterial cell division protein Ftn2. Plant Cell 15:1918–1933
Vöchting H (1878) Über Organbildung im Pflanzenreich. Cohen, Bonn
Vogelmann TC, Bassel AR, Miller JH (1981) Effects of microtubule-inhibitors on nuclear migration and rhizoid formation in germinating fern spores (*Onoclea sensibilis*). Protoplasma 109:295–316
Voigt B, Timmers ACJ, Šamaj J, Müller J, Baluška F, Menzel D (2005) GFP-FABD2 fusion construct allows *in vivo* visualization of the dynamic actin cytoskeleton in all cells of *Arabidopsis* seedlings. Eur J Cell Biol 84:595–608
Walker LM, Sack FD (1990) Amyloplasts as possible statoliths in gravitropic protonemata of the moss *Ceratodon purpureus*. Planta 181:71–77
Waller F, Nick P (1997) Response of actin microfilaments during phytochrome-controlled growth of maize seedlings. Protoplasma 200:154–162
Waller F, Riemann M, Nick P (2002) A role for actin-driven secretion in auxin-induced growth. Protoplasma 219:72–81
Wang QY, Nick P (1998) The auxin response of actin is altered in the rice mutant *Yin-Yang*. Protoplasma 204:22–33
Wang QY, Nick P (2001) Cold acclimation can induce microtubular cold stability in a manner distinct from abscisic acid. Plant Cell Physiol 42:999–1005
Wang YS, Motes CM, Mohamalawari DR, Blancaflor EB (2004) Green fluorescent protein fusions to *Arabidopsis* fimbrin 1 for spatio-temporal imaging of F-actin dynamics in roots. Cell Motil Cytoskelet 59:79–93
Wang X, Zhua L, Liu B, Wang C, Jin L, Zhao Q, Yuan M (2007) Arabidopsis microtubule-associated protein18 functions in directional cell growth by destabilizing cortical microtubules. Plant Cell 19:877–889
Wasteneys GO (2004) Progress in understanding the role of microtubules in plant cells. Curr Opin Plant Biol 7:651–660
Wasteneys GO, Galway ME (2003) Remodeling the cytoskeleton for growth and form: an overview with some new views. Annu Rev Plant Biol 54:691–722
Whittington AT, Vugrek O, Wei KJ, Hasenbein NG, Sugimoto K, Rashbrooke MC, Wasteneys GO (2001) MOR1 is essential for organizing cortical microtubules in plants. Nature 411:610–613
Wiesler B, Wang QY, Nick P (2002) The stability of cortical microtubules depends on their orientation. Plant J 32:1023–1032
Wymer C, Wymer SA, Cosgrove DJ, Cyr RJ (1996) Plant cell growth responds to external forces and the response requires intact microtubules. Plant Physiol 110:425–430
Xu T, Qu Z, Yang X, Qin X, Xiong J, Wang Y, Ren D, Liu G (2009) A cotton kinesin GhKCH2 interacts with both microtubules and microfilaments. Biochem J 421:171–180
Yamamoto A, Hiraoka Y (2003) Cytoplasmic dynein in fungi: insights from nuclear migration. J Cell Sci 116:4501–4512
Zandomeni K, Schopfer P (1994) Mechanosensory microtubule reorientation in the epidermis of maize coleoptiles subjected to bending stress. Protoplasma 182:96–101
Zhou J, Wang B, Li Y, Wang Y, Zhu L (2007) Responses of *Chrysanthemum* cells to mechanical stimulation require intact microtubules and plasma membrane-cell wall adhesion. J Plant Growth Regul 26:55–68
Zimmermann W (1965) Die Telomtheorie. Fischer, Stuttgart
Zimmermann S, Nürnberger T, Frachisse JM, Wirtz W, Guern J, Hedrich R, Scheel D (1997) Receptor-mediated activation of a plant Ca^{2+}-permeable ion channel involved in pathogen defense. Proc Natl Acad Sci USA 94:2751–2755