Signaling and Communication in Plants

Przemysław Wojtaszek *Editor*

Mechanical Integration of Plant Cells and Plants

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Mechanical Integration of Plant Cells and Plants

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Preface

> (. . .) the quest for an answer to the riddle, "What is Life?" is one of the grand themes that resonate through the scientific conversation of this century (. . .). That riddle embraces and transcends the subject matter of all the biological sciences, and much of physical science as well. A physics that has no place for life is as impoverished as would be a biology not informed by chemistry. The study of life as a natural phenomenon, a fundamental feature of the universe, must not be allowed to slip into the black hole of departmental tribalism.
>
> Franklin M. Harold (2001)

The great successes of science in the last one and a half century built a strong conviction that chemical reactions and interactions between molecules lie at the basis of life. Starting with physiological chemistry, through biochemistry and physiology, up to molecular biology, -omics, systems biology, and now also synthetic biology, they all provided a very detailed picture of the chemical nature of cells and organisms. Only in some areas of natural sciences, the emerging data were suggesting that biology means more than chemistry itself. Electrophysiology, bioenergetics, the phenomenon of photosynthesis on one side, and the properties of wood, cotton fibers, silk, or spiderweb as construction and engineering materials on the other, are only a handful of such cases. Research of recent years, however, is more and more evidently indicating that physical forces are profoundly affecting the functioning of life at all levels of its organization. To detect and to respond to such forces, cells and organisms, among them plants, need to be organized physically, and mechanically in particular (Wang et al. 2009). Although the structure–function relationship is studied for decades at all levels of hierarchical organization, the knowledge about its physical aspects is still in the making. Macromolecular crowding, the importance of electrical forces, regulation of molecular machineries via structural organization of cellular compartments, direct mechanical connections between the interior and peripheries of the cell, cellular adhesion, and mechanical integration of cells within multicellular organism are examples of the recently investigated processes with strong physical side. Due to historical reasons, and also significant medical potential, the importance of mechanical environment on the functioning/fate of the cells is becoming very well recognized in animal cell

biology, and also for bacterial cells. The least documented are the mechanobiological phenomena in plant cells and plants itself. The interplay between cell walls and the turgor pressure, the basis for creation of the biggest organisms on Earth, is on the other hand the major barrier in revealing the mysteries of the structural and functional integrity of the cells.

The mechanical aspects of plant life could be analyzed at many different levels of hierarchical organization. Here, we are trying to demonstrate how the awareness of the physical side of life is affecting the interpretation of biological phenomena. This book gathers contributions from many authors describing the importance of mechanical forces/stimuli for or mechanical organization of (1) supramolecular structures, like the cytoskeleton or cell walls; (2) cellular integrity, like cytoplasmic streaming and movement of organelles; (3) supracellular coordination in the processes of plant organ growth and development; (4) integration of plant functioning, e.g., in long-range water transport or plant responses to physical forces or environmental stimuli. The chapters are organized in a way to give the reader the possibility to travel along the ladder of hierarchical levels in a bottom-up approach, i.e., from molecules through cells and organs, and up to plants interacting with their immediate neighborhood or responding to stresses. Thus, the book covers all the major aspects of mechanobiological phenomena, providing also direct or indirect evidence for the organismal nature of plants – a feature which could only very rarely be seen in multicellular animals.

Immanuel Kant, in his *Metaphysische Anfangsgründe der Naturwissenschaft* (1786), noted "(. . .) in any special doctrine of nature there can be only as much *proper* science as there is *mathematics* therein". Using this saying, one can observe that in any biological process there might be only as much freedom as the physical laws would allow. Mechanical integration of living cells and organisms constitutes a visible expression of the unity and intertwining connections between the living and inanimate parts of nature.

Poznań, January 2011 Przemysław Wojtaszek

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Contents

Introduction: Tensegral World of Plants

Anna Kasprowicz, Michalina Smolarkiewicz, Magdalena Wierzchowiecka, Michał Michalak, and Przemysław Wojtaszek

Abstract In this chapter, we are providing a brief overview of the tensegral concept as applied to plants. Starting with a short introduction to the history of the idea of mechanical integration of the cell and the organism, we then discuss the mechanical design of the plant body. The importance of the mechanical properties of cells, tissues, organs, and their domains is indicated, and the systems of detection of mechanical stimuli are briefly discussed. Finally, the mechanical integration of plant cells is presented based on the various aspects of the functioning of the cell wall–plasma membrane–cytoskeleton continuum spanning the whole cell. The initial stage of knowledge within this area is indicated with special attention paid to different modes of inter- and intracellular communication as well as the utilization of the continuum to functional organization and integration of the whole cell.

1 Introduction

The significant role of mechanical forces in growth and development has been studied for over a century in a wide range of plant and animal species (Darwin and Darwin 1880; Thomson 1992). In the last decade, noticeable progress has been made in understanding the molecular background of these mechanical responses (Kasprowicz et al. 2009; Monshausen and Gilroy 2009). Mechanical forces influence living organisms at all levels of organization, from organismal through tissue and organ down to the cellular level. The specific reactions can be evoked either by macroenvironmental stimuli, such as wind, or microenvironmental stimuli resulting from, for example, differential pressure exerted by neighboring cells. These responses

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are thought to regulate not only cell growth and development, but also affect their shape or fate. Although significant knowledge has been gathered about such mechanisms in animals, the full picture of plant reactions is only starting to be built (Vogel and Sheetz 2006; Ingber 2008; Monshausen and Gilroy 2009).

The idea that changes in local mechanical environment can regulate growth, fate, shape, and pattern formation is not a new one. Even before the famous D'Arcy Thompson's book *On Growth and Form* (1992), first published in 1917, such suggestions could be found in literature. Many of the biophysical considerations, for example, linking the properties of the cell walls to plant morphogenesis, were later elaborated in the papers by Paul Green (1962, 1996, 1999), and elegantly summarized by Karl J. Niklas' book *Plant Biomechanics. An Engineering Approach to Plant Form and Function* (1992). However, it was Donald Ingber who first paid particular attention to the role of the cytoskeleton in the responses to mechanical stimuli and the maintenance of structural integrity of animal cells (reviewed in Ingber 2003a, b). Using several diverse approaches to study the spreading of animal cells on patterned substrata, equivalent to extracellular matrix (ECM) islands, his group demonstrated that such differentiated microcontacts affected not only the shape of individual cells, but also cell fate. The mechanical factor was sufficient to direct the cells to proliferation, differentiation, or death (Singhvi et al. 1994; Chen et al. 1997; Parker et al. 2002; Brock et al. 2003). It means that mechanical stress must have been somehow perceived by cell surface and then translated into biochemical message, which altered cell metabolism. To explain this phenomenon, a new conceptual framework was needed. Instead of elastic balloon (plasma membrane) filled with viscous cytoplasm, Ingber visualized cell as a tent (membrane) outstretched on the cytoskeletal backbone (Ingber 1993, 1998). The idea was generally based on a tensegral architecture model. The term "tensegrity" was introduced by American architect Buckminster Fuller, and referred to structures relying on the balance between tensile and compressive components, which results in a dynamic state of constant prestress that mechanically stabilizes all constituents (Fuller 1961). In the animal cell, contractile actomyosin filaments are responsible for generating tension forces which are resisted by ECM, neighboring cells, and other cytoskeletal components, such as microtubules which are considered to be resistant to compression. All elements are in a state of isometric tension and disturbance of one building block immediately induces alterations in other elements. The whole system is well balanced and becomes highly responsive to external mechanical stimuli (reviewed in Ingber et al. 1994; Ingber 2003b). In the tensegral model, a special emphasis is put on the internal, but not a cortical, actin network. This is especially important when the transduction of mechanical signal is considered, and distinguishes the tensegral model among others. In a more classical view, the assumption is that stress is equally distributed to the whole surface of plasma membrane and the primary load-bearing structure is a dense network of cortical actin filaments (Schmid-Schönbein et al. 1995; Ingber 2003a). To the opposite, the tensegral model assumes that mechanical stress is perceived by specialized receptor proteins spanning the

plasma membrane (integrins in animals), and then transmitted through cortical and internal cytoskeleton networks to the interior of the cell, most often directly to nucleus (Ingber 2008, 2009; Wang et al. 2009). Thus, a whole living organism can be perceived as not only a hierarchical but also tensegral structure (Ingber 2006). If so, then the mechanical disturbance at the organismal level can be simply transduced to tissue, cellular and finally molecular levels. These general theses of the tensegrity model were formulated based on the experiments done on animal cells. However, after some reconsideration, they can also be applied to plants and plant cells.

2 Mechanical Design of Plant Body

Living organisms must follow physical laws limiting their size, form, and structure. The shape of a plant is defined by growth rate and growth direction (Hamant and Traas 2010), although orientation of cellular divisions is also an important variable. Plant mechanical design relies on cell wall properties, existence of supporting tissues as well as spatial organization of plant body (Niklas 1992; Nachtigal 1994). Biochemical/mechanical relationships influence the morphological/anatomical features that scale up with respect to body size, geometry and shape rather than with achievement of an organized body plan. In Plant Kingdom, one noticeable observation is that different clades have adopted body architectures allowing for survival in a particular ecological niche, due to diversification within and convergence among different lineages (Niklas 2000). In contrast to animals, depending on various nutrient sources (e.g., carnivores and herbivores), all plants require the same resources for growth and development (light, water, atmospheric gases, minerals, space) and are thus more influenced by the abiotic factors than by the biotic ones. This is also the reason for a much higher level of developmental plasticity, as elegantly summarized by E.J.H. Corner (2002): "A plant is a living thing that absorbs in microscopic amounts over its surface what it needs for growth. It spreads therefore an exterior whereas the animal develops, through its mouth, an interior."

Plant form arises from the multitude of molecular/biochemical and physical interactions that occur within a growing assembly of cells. Although molecular techniques are now providing large amounts of information about the behavior of cells and their functioning, little is known about how those processes integrate within whole plant tissues and organs. Plant organogenesis as well as patterning is dependent on mechanical forces acting inside and outside of the plant body. In complex plant organisms, the position and shapes of cells, tissues, meristems, and organs bear repeated and regular relationships with one another. The spatial organization and regularity of patterns are apparent not only at the macroscopic level, but also in meristems and tissues, which contain repeated and predictable arrangements of various types of cells. Patterning in plants is highly ordered and the mechanisms of interaction and communication between cells are crucial to

understanding how cellular activities are coordinated during development (Dupuy et al. 2008). Specific patterns of plant organ emergence and formation are dependent on a complex network of hormonal (mainly auxin) and mechanical signaling (Kuhlemeier 2007). Manual bending of roots can induce lateral root formation (Ditengou et al. 2008). Stress-driven buckling is a model proposed for primordium initiation in sunflower capitulum and phyllotaxial events on shoot apical meristem (Dumais 2007; Newell et al. 2008; Yin et al. 2008; see also Chapter 6).

Because plants germinate only with simple embryonic root and cotyledons, they have the potential to respond to changing environmental conditions during their development and formation of plant body. Roots, stem, branches, leaves, and flowers emerge during plant life and, although genetically encoded, are characterized by developmental plasticity optimized to fit into particular ecological niche (Deak and Malamy 2005). For example, even genetically identical plants grown in slightly different conditions of nutrient availability will develop different root architectures (López-Bucio et al. 2003). Organ plasticity is also important for effective relations between neighboring plants. As plants develop larger branches on less shaded side, the branches of neighboring trees can influence the growth direction of other tree branches (Novoplansky et al. 1989; Henriksson 2001). For sessile organisms, such adaptive strategies are crucial to survive in ever-changing climatic and environmental conditions (Deak and Malamy 2005; Malamy 2005).

Plants consist of a number of tissues characterized by different stiffness and mechanical properties. Sclerified tissues, e.g., sclerenchyma and wood fibers, are composed mainly of dead cell fibers with much stiffer cell walls (Niklas 1992). Hydrostatic and sclerified tissues play the supporting role. In living hydrostatic tissues the flexural and torsional rigidities of cells and tissues are a consequence of active interplay between turgor pressure and mechanical properties of cell walls (Niklas 1992). The hydrostatic pressure across the plasma membrane exceeding 2 MPa could be used for mechanical stabilization of plant bodies (Peters et al. 2000). Small herbs are mostly hydrostatic, whereas trees rely mostly on sclerified tissues. Intermediate situations could be found in grass stems or midribs (Niklas 1992; Moulia and Fournier 1997), where stiff sclerenchymous hypodermic exoskeleton and internal spongy parenchymous tissues are necessary for bending (Moulia et al. 2006). In aqueous environment, hydrostatic, buoyant plant cells do not exert significant pressure on themselves and neighboring cells, and different design of cell walls is required. On the other hand, the terrestrial environment requires adaptive solutions allowing the cell, that is approximately 1,000 times more dense than surrounding air, to withstand internal compressive forces (Niklas 2000). Compression-resistant turgid protoplast is surrounded by and pressed against tension-resistant and mechanically stable cell walls (Wojtaszek 2000; Zonia and Munnik 2007). Lignified and thick cell walls provide tissues with higher resistance to changing water conditions or elastic deformation, and this is indispensible when coping with herbivoral or microbial attack.

To resist exposure to external bending and twisting forces resulting from water flow in aqueous environment or wind in terrestrial one, a cylindrical body shape

with thick boundary layer is best suited (Niklas 2000). Compared with the cell walls of the internal cell types, the outer epidermal walls are up to ten times thicker and more resistant to mechanical stress. Other cells are tightly packed inside this rigid cylinder of epidermis. Growth of elongated organs such as cylindrical stem or coleoptiles composed of different tissues with different mechanical properties causes longitudinal tissue tensions, leading to transfer of wall stress from inner to external cell layers that take control over organ growth (Schopfer 2006). This creates an additional supracellular pressure allowing the cells of the organ's interior to sense and adjust their mechanical balance to direct physical environment as well as to transduce mechanical stimuli throughout the organ (Kutschera 1995, 2008). A very good example illustrating this phenomenon is the reorientation of the cell fate. Laser ablation of selected cells from the root meristem of *Arabidopsis thaliana* leads to reorganization of the cell division planes in remaining cells, enabling effective and rapid filling of the empty space. Interestingly, daughter cells change their original fate and adopt a new one according to their new position in the root (van den Berg et al. 1995, 1997).

Plants possess very sensitive detectors of mechanical stimuli. Even subtle, short touch can induce not only mechanisms of signal transduction, but also immediate wall remodeling as evidenced by an induced expression of *TCH4* gene coding for xyloglucan endotransglycosidase (XTH; Braam and Davis 1990; Braam 2005). Genome-wide analysis of expression patterns in touch-stimulated *Arabidopsis* plants revealed that expression of 589 genes was upregulated within 30 min of touch stimulation, while 171 genes were downregulated. Importantly, relatively high proportion of upregulated genes coded for proteins involved in cell wall synthesis and modification (Lee et al. 2005). It was also demonstrated that even slight change in the growth pattern with respect to gravity vector (plants growing on normal or on a slope conditions) is immediately reflected by changes in the root proteome (Di Michele et al. 2006). Upon mechanical stresses a tree modifies its growth to minimize the risk of failure by an increase in secondary growth at the side of the prevailing wind direction (Mattheck and Breloer 1995). As for mechanical stability of plant body, a balance between belowground and aboveground parts is essential, counterbalancing mechanisms exist to ensure simultaneous adaptation of root system to wind (Fourcaud et al. 2008). Tree growth response to wind can be controlled by a reorientation of secondary axes, which is possible through the formation of reaction wood (Fournier et al. 2006; Moulia et al. 2006; Sellier and Fourcaud 2009). When the angle between the primary branches and the stem is small, the resulting wind speed near the distal parts of these branches is higher. Therefore trees with plagiotropic branches appear better suited to withstand high, dynamic winds than trees with orthotropic ones (Sellier and Fourcaud 2009). Trees exposed to changing wind conditions can even reversibly rearrange their crowns. Brittle branches break easily in high winds, decreasing the crown exposure, and preventing damage in more critical zones, such as in the stem or the root system (Niklas 2000; Sellier and Fourcaud 2009).

3 Mechanical Properties of Plant Cells

3.1 Mechanical Properties of Plant Cell Walls

The turgor pressure is an isodiametric force, and thus protoplasts, devoid of cell walls, as well as the cells equipped with ideally homogenous walls develop an energetically optimal spherical shape (Baluška et al. 2003b; Mathur 2006). However, plant cells are usually surrounded by the cell walls composed of diverse wall domains exhibiting different mechanical properties (Wojtaszek et al. 2007), and an interplay with the turgor pressure leads to an establishment of anisotropic cell growth (Wojtaszek 2000). The micromechanical design of cell walls relies mostly on their biochemical composition (see also chapter "Micromechanics of Cell Walls"). Although cellulose is the strongest biopolymer in terms of tension resistance, it is rather weak when compressed along its backbone. Therefore, in algal cells surrounded by cellulosic walls, when the turgor pressure is lost, protoplasts deflate, the cell wall stiffness is lost, and, in consequence, the collapse of the cell can be observed (Niklas 2000). Primary cell walls exhibit physical properties enabling for plastic expansion (Niklas 1992). Upon extension, primary cell walls resemble viscoelastic composite that undergoes stress relaxation in a time-dependent manner after stretching (Schopfer 2006). Enrichment of the walls with lignin helps to slide cellulose microfibrils against lateral bending, and, due to its hydrophobic properties, protects cellulose from moisture and thus increases wall stiffness (Niklas 2000).

It is commonly agreed that in the elongating cells, the cellulose microfibrils are located perpendicularly to the growth axis, determining the direction of growth. However, the mechanism of their orientation remains elusive. The classical point of view is that the deposition of cellulose microfibrils is affected by the alignment of cortical microtubules (Wymer and Lloyd 1996). The geometrical model assumes that new microfibrils are oriented by the cell geometry together with existing wall components, while orientation of microtubules is a simple reflection of the directed delivery of cellulose synthase complexes to the plasma membrane (reviewed by Emons and Mulder 2000; Emons et al. 2007). The orientation of the cellulose microfibrils during deposition might be therefore controlled by the number of cellulose synthase complexes and their distance (Emons and Mulder 1998). However, according to recent biochemical and genetic data, bidirectional flow of information between cortical microtubules and cellulose microfibrils exists. In tobacco suspension-cultured cells, biophysical forces are responsible for the spatial organization of microtubules and microtubules themselves can respond to vectorial changes of such forces (Wymer et al. 1996). Moreover, cellulose microfibrils through localization of the cellulose synthesis machineries provide spatial cues for the internal organization of microtubules (Fisher and Cyr 1998; Paredez et al. 2006, 2008). In effect, microtubules change dynamically their orientation in response to internal and/or external stimuli, such as directional mechanical stress (Fischer and Schopfer 1998). It should be mentioned, however, that filamentous

actin is also essential for cell elongation (Baluška et al. 2001) and for the directed delivery of cellulose synthase complexes to the sites of wall synthesis (Wightman and Turner 2008).

In maturing plant cells, the processes of specific orientation of cellulose microfibrils during wall formation enable plant to control the mechanical properties of the apoplast at the tissue/organ/plant levels (Reiterer et al. 1999; see also chapter "Micromechanics of Cell Walls"). The deposition of cellulose in the cell wall can be also adjusted to prestressed tissues or to actuate movement of the organ upon swelling or shrinking of the cell wall (reviewed in Burgert and Fratzl 2009). For the establishment and maintenance of mechanical properties of plants, the angle of microfibrils in the wood is crucial. Tissues with lower microfibril angle reveal higher modulus of elasticity (Cave 1969; Reiterer et al. 1999; Burgert et al. 2002; Groom et al. 2002). The microfibril angle is also specific to the age of the tree. In young trees high microfibril angles can be found, allowing for plastic deformations after yielding and leading to higher flexibility and streamlining of the stem, whereas in mature trees the wood containing the walls built of small angle microfibrills is stiffer and able to withstand the wind (Lindström et al. 1998; Bonham and Barnett 2001). For the reaction wood in leaning stems and branches on their upper side (tension wood) and lower side (compression wood), respectively, extremely low or high cellulose microfibril angles are specific (Côté and Day 1965; Wardrop 1965). The variations between microfibril angles are crucial for generating stresses causing bending movements of plant organs (Burgert et al. 2007; Goswami et al. 2008; Burgert and Fratzl 2009). The orientation of cellulose microfibrils is crucial during and even after senescence, allowing for passive actuation of organs under changes of humidity. This mechanics is based upon properties of cellulose microfibrils that do not swell axially, thus allowing for swelling/shrinking only in the directions perpendicular to the fibrils. The composition of tissues and cells with cell walls of different orientations of cellulose fibrils allows for complex movements at the organ level. This mechanism is used to control seed dispersal including the spore capsules of mosses that show a moisture-dependent seed dispersal mechanism with hygrosensitive openings and closures of the capsule (Ingold 1959), the release of ripe seeds from conifer cones (Dawson et al. 1997), and enables the motility of seed dispersal units of wheat (Elbaum et al. 2007).

3.2 Linking Cell Walls and Cell Interior

Due to the tensegral organization of the plant cell, the main mechanical force influencing cellular properties is the turgor pressure acting on the plasma membrane and the nonextensible cell walls. Looking from the opposite direction, many external perturbation acts on cell walls, and because of internal pressure caused by turgor, local deformations of the wall domains are conveyed to the plasma membrane. This could trigger specific biochemical responses. During cell growth, localized loosening of the wall structure is a common phenomenon, and it could

also be sensed by potential receptors (Monshausen and Gilroy 2009). Opposite to the animal model where tensed elements are pulling against compressed ones (Ingber 2009), in plant cells compression elements push outward the cell wall introducing tension. The interplay between turgor pressure and cell wall mechanics is crucial in determining growth and development patterns in plant. The idea that in plants mechanical stimuli are converted to biochemical information in a similar way as in the animal systems seems very promising. In animal cells, the molecular bridge between ECM and cytoskeleton is known as focal adhesion. The central point in this bridge is formed by transmembrane proteins – integrins – mechanoreceptors converting mechanical forces to biochemical signals such as protein phosphorylation or activation of calcium influx. At the cytoplasmic side, integrins are physically linked with the cytoskeleton through a macromolecular complex, which includes, among others, actin-associated proteins, such as talin, zynxin, and vinculin. The generated biochemical signal is then transduced into changes in gene expression. Moreover, because the cell elements are prestressed, mechanical forces loaded on integrins can easily and quickly move through the cytoskeleton network all over the cell (Geiger et al. 2001; Ingber 2006). This kind of structural and functional continuum between ECM, plasma membrane, and cytoskeleton in animal cells is well documented. The existence of analogous functional network connecting cell *w*all, plasma *m*embrane and *c*ytoskeleton (WMC) in plant cells has also been postulated (Wyatt and Carpita 1993), but knowledge about individual elements of this continuum is rather poor (Wojtaszek et al. 2004). For a long time cell wall was recognized as a dead structure surrounding living protoplast. This view of the walls is systematically evolving to a highly responsive, dynamic structure responsible for many fundamental events including perception of environmental stimuli. Accordingly, identification of specific or general molecular linkers connecting cell walls with plasma membrane and cytoskeleton is crucial in our understanding of an interplay between external and internal environments of the cell. Available experimental data point to several proteins which could function as a potential linker candidates (Baluška et al. 2003a; Gouget et al. 2006; Humphrey et al. 2007).

Despite intensive research efforts, none of the characterized higher plant genomes seems to contain true integrin homologues. One of the possible reasons might lie in the different chemical composition of exocellular matrices: plant cell wall is composed mainly of carbohydrates, while animal ECM is of proteinaceous nature. This chemical diversity might lead to an adaptation of completely different molecules to perform the same receptor functions (Baluška et al. 2003a; Monshausen and Gilroy 2009). Interestingly, however, some experimental data show the presence of integrin-like proteins in plants. In animal cells, the Arg–Gly–Asp (RGD) motif can be found in the ECM proteins responsible for adhesion, and this motif is normally recognized by integrins. Senchou et al. (2004) identified the protein in *A. thaliana* that specifically bound peptides containing RGD sequence. Moreover, addition of such peptides to plasmolysed *Arabidopsis* cells disrupted adhesion sides between cell wall and plasma membrane. Phage display approach showed that among the 12 specific RGD-binding proteins identified in this experiment, eight

belong to the receptor-like kinases (RLK) superfamily. Plant RLKs are transmembrane proteins with extracellular domains located at amino-terminal part of protein, and intracellular kinase domains forming the C terminus. This protein architecture shows some similarity to domain organization in the animal receptor tyrosine kinases, such as the receptor of epidermal growth factor. In *Arabidopsis* RLKs belong to a large gene family with over 600 members, but the function of individual proteins is still poorly understood (Shiu and Bleecker 2001). Four of the previously mentioned RLKs identified by phage display technique (Senchou et al. 2004) contained specific lectin-like extracellular domains which were responsible for RGD binding as well as interactions with carbohydrates (Gouget et al. 2006; Humphrey et al. 2007; Bouwmeester and Govers 2009). Although lectin receptor kinases (LecRKs) are relatively well characterized at the molecular level, very little is known of their exact function in plants. They are thought to be involved in processes such as hormone responses, disease resistance, and stress adaptation (Wan et al. 2008). It seems, however, that the ability to bind RGD-containing proteins, combined with kinase activity makes them good candidates for mechanical signal receptors as well.

Another example of RLK which may potentially be involved in mechanical signal transduction between cell wall and the cellular interior are wall-associated kinases (WAK). WAKs are till date the best characterized potential receptor proteins. Five direct WAK isoforms and 22 WAK-like genes have been identified in *A. thaliana* (Anderson et al. 2001; Verica and He 2002). WAKs are defined by the presence of a highly conserved C-terminal kinase domain, transmembrane region, and relatively variable N-terminal sequence responsible for cell wall interactions (Wagner and Kohorn 2001). This amino-terminal region resembles the vertebrate epidermal growth factor motifs, which, in all cases studied, are involved in protein–protein interactions. The presence of other motifs, specific for metazoan proteins, e.g., collagen-like or neurexine-like sequences has also been documented, but their function remains unclear (He et al. 1999; Kohorn 2000; Anderson et al. 2001). Initial data suggested covalent interactions of WAKs with cell walls (Kohorn 2001). However, it was later shown that they interact through the N-terminal domain via noncovalent binding to pectins, and this interaction was shown to be calcium dependent (Decreux and Messiaen 2005; Humphrey et al. 2007). Expression profiling also indicates the role of WAK family proteins in different environmentally induced processes, such as responses to aluminum or pathogens. Interestingly, the expression of WAKs is most noticeably induced in organs undergoing expansion, such as meristems (Humphrey et al. 2007). It was also shown that in protoplasts WAK1 fusion with GFP tended to accumulate in specific cytoplasmic compartments that also contained pectin. Moreover, migration of WAK1 complex to the cell surface was shown to be related to cellulose synthesis (Kohorn et al. 2006). Combined with its pectin-binding properties, the data strongly suggest the possible role of WAKs in the emerging cell wall–plasma membrane functional integrity.

In recent years, very interesting data are emerging from studies on the family of CrRLK1 proteins. The name of the family originates from RLK1 from

Catharanthus roseus, a novel protein kinase of unknown function (Schulze-Muth et al. 1996). In Arabidopsis there are 17 proteins which share similarity with CrRLK1, and few of them have been studied intensively (Hématy and Höfte 2008). THESEUS 1 (THE1) is thought to act as a sensor of the cell wall integrity and mediator of signaling induced by the cell wall damage. The protein was shown to inhibit cell elongation in case of impaired cellulose synthesis (Hématy et al. 2007). FERONIA (FER), another member of this family, was shown to be involved in elongation, and its possible function as a growth arrest factor in the elongating pollen tube was indicated. This growth cessation is crucial at the last step of fertilization process (Hématy and Höfte 2008). Similarly, the HERCULES1 (HERK1) was shown to be involved in cell elongation during vegetative growth (Guo et al. 2009a). More importantly, all three kinases are transcriptionally induced by brassinosteroids (Guo et al. 2009b).

Plant proteins belonging to the formin family seem to have a potential to act as putative linker elements within the WMC continuum. Formins are actin-binding proteins responsible for nucleation and elongation of actin microfilaments. They are defined by the presence of a conserved FH2 domain (formin homology 2), as well as other domains characteristic for distinct types of proteins (Paul and Pollard 2009). There are over 20 formin-coding genes in the *A. thaliana* genome. According to predicted domain architecture they are divided into two classes. Class I formins in plants share the presence of N-terminal transmembrane domain, and a short proline-rich region located at the extracellular side of plasma membrane. The FH2 domain, responsible for interactions with the cytoskeleton is located at the C-terminus of protein, and protrudes to cytoplasm. Class II formins are located in the cytoplasm and, apart from FH2 domain, they usually contain PTEN-like domain. However, due to mutation, this latter domain probably lacks conventional phosphatase activity (Deeks et al. 2002; Cvrcková et al. 2004; Blanchoin and Staiger 2008; Grunt et al. 2008). Class I formins are involved in the tip growth, particularly of pollen tubes and root hairs (Cheung and Wu 2004; Deeks et al. 2005), as well as in cytokinesis (Ingouff et al. 2005). An intriguing observation is that some formins tend to localize at the cross-walls which, in axial organs such as roots, are thought to be involved in signal transduction between neighboring cells (Deeks et al. 2005; Wojtaszek et al. 2007). Because of experimentally confirmed localization at the plasma membrane, and specific domain architecture with one end potentially interacting with the walls and the other with the cytoskeleton, class I formins are often indicated as potential linkers within the WMC continuum. Unfortunately, probably because of the high redundancy, clear phenotypes of individual mutants are unavailable at this moment, and the estimation of the impact of a particular protein for cellular functioning is still a challenge. Recent data on AtFH1 protein indicate that this is, however, possible (Martinière et al. 2011).

Among the variety of cell wall proteins that could also mediate wall–membrane interactions, *a*rabino*g*alactan *p*roteins (AGP) deserve a special attention. A category of classical AGPs groups hydroxyproline-rich glycoproteins (HRGPs), mostly highly glycosylated, that could be localized predominantly at the cell surfaces. Carbohydrates constitute up to 90% of single arabinogalactan protein mass. Polysaccharide units vary in size between 30 and 150 monomers and are attached to

multiple sides on core of the protein (Showalter 2001; Seifert and Roberts 2007). Patterns of sugar moieties on AGPs are thought to be information-bearing structures, and the information could be conveyed via direct interactions with putative membranous receptors. Small carbohydrate fragments, released by an enzymatic cleavage from large oligosaccharide side-chains, could bind to putative receptors and trigger generation of biochemical signaling (Showalter 2001). In fact, such mechanism is suggested as a possible explanation for the regulatory role of chitinases in somatic embryogenesis (van Hengel et al. 2001). At least some AGPs contain an additional *g*lycosyl*p*hosphatidyl*i*nositol (GPI) anchor at the C-terminus that allows them to stay attached to the extracellular side of the plasma membrane (Kohorn 2000; Majewska-Sawka and Nothnagel 2000). This GPI anchor can be removed in a controlled manner by phospholipase C. Such action releases AGPs from the plasma membrane to the cell wall enabling signaling of another type (Borner et al. 2002). In addition to the signaling properties, AGPs are also considered as adhesive molecules connecting plasma membrane with the cell wall. AGPs can bind to pectins (Nothnagel 1997), and potentially interact with WAKs (Gens et al. 2000). A family of fasciclin-like arabinogalactan proteins (FLA) contain fasciclin-like domain which, in animal cells, is responsible for promoting cell adhesion (Johnson et al. 2003). Very importantly, it was recently shown that application of Yariv reagent, which specifically binds to AGPs, leads to disorganization of cortical microtubules in *A. thaliana* roots (Nguema-Ona et al. 2007). The connection between AGPs and cortical microtubules and F-actin was also proved in BY-2 suspension cells (Sardar et al. 2006).

3.3 Actin Cytoskeleton as Mechanical Integrator of Plant Cell

Cytoskeleton plays a very important role in the formation of cellular shape, cell polarity, and functional organization by, among others, the perception of physical forces and different mechanical stimuli. Numerous examples confirm that cytoskeleton is implicated in the maintenance of mechanical integrity of the cell, driving its growth, differentiation, and cell-to-cell communication. In the bacterial model, the actin homolog, MreB, contributes nearly as much to the stiffness of a cell as the peptidoglycan cell wall. MreB is rigidly linked to the cell wall, increasing the mechanical stiffness of the whole cell. These data provide the first evidence that in walled cells the cytoskeleton contributes to the mechanical integrity in similar way as it does in naked animal cells (Wang et al. 2010). It still remains to be demonstrated if the same is true for plant cells.

From studies on animals and some bacteria it is known that cytoskeleton, mainly actin, is involved in the generation of forces necessary for cell movement or shape reorganization (van der Honing et al. 2007). Interestingly, plant cells are equipped with most of the homologues of the actin-binding proteins typical for animals, which are responsible for force generation. Although such force generation by the actin polymerization in plant cells has not been studied yet, it is possible that it

plays a role in the organization of their cytoplasm. Evidence for that comes from several experiments on plant mutants. One of the most interesting observations derives from analysis of *Arabidopsis* lines devoid of the subunits of Arp2/3 complex. Such mutation caused disturbances in trichomes development and in root hairs. Trichomes were twisted or with short branches, and the cytoplasmic streaming was largely limited, while root hairs were wavy and had a variable diameter. Epidermal cells of leaves and dark-grown hypocotyls were also affected and displayed abnormal organization (Le et al. 2003; Li et al. 2003; Mathur et al. 2003a, b; El-Din El-Assal et al. 2004). These data suggest that Arp2/3 complex might be involved in the organization of the subapical fine F-actin and, in consequence, in the determination of the architecture of expanding cells (van der Honing et al. 2007). On the other hand, *a*ctin *d*epolymerization *f*actors (ADF) are known to be responsible for the turnover of actin filaments by increasing depolymerization at the pointed end. This provides actin monomers for the elongating barbed end, which, in animal cells, is usually pointing towards the direction of the cellular movement. When ADFs are differentially expressed in plant cells, the amounts of the available G-actin are also changed, and, in effect, the rates of cells and organs development are also affected. Overexpression of ADFs resulted in disappearance of actin bundles, causing reduction of cell expansion and organ growth. Interestingly, inhibition of ADFs expression stimulated cell expansion and organ growth (Dong et al. 2001). These and other analyses of plants lacking or overexpressing actin-binding proteins turn attention to their possible role in force generation. In animal cells, actin filaments are necessary for cell shape changes. The question is, however, how plants may utilize these proteins in cells with the determined cell shape and surrounded by the rigid cell walls? It is possible that the role of actin polymerization-based system is limited just to the directional delivery of exocytic vesicles to cellular peripheries. Differentiation of the rates of exocytosis in various directions within plant cell will provide diverse amounts of cell wall material to different wall domains thus providing the way for creation of the cellular shape. Search for other functions of actin-based force generation in plant cells is now under way (Emons and Mulder 2000; Hussey et al. 2006; van der Honing et al. 2007). One of the possibilities has been just indicated. In plant cells, microfilaments span the whole cell and enable effective intracellular communication through the formation of actin-based transvacuolar strands (van der Honing et al. 2010).

Actomyosin system is involved in the regulation of protoplast volume during plasmolysis (Komis et al. 2003; Wojtaszek et al. 2005). Cells subjected to hyperosmotic conditions reorganize their actin filaments network, and thin cortical F-actin is replaced by cortical, subcortical, and endoplasmic well-organized and thick actin bundles (Komis et al. 2002). The amount of F-actin is generally higher than in control cells, and some of the filaments traverse the Hechtian strands connecting the retracted protoplast with cellular peripheries. Application of anti-actin drug leads to dramatic changes in the pattern of plasmolysis, with resulting greater decrease of the protoplast volume. In some experiments protoplasts adopted amoeboid form or were subdivided into subprotoplasts (Komis et al. 2002). What is even more important is the reorganization of actin is to some extent cell wall

dependent as the organization and composition of wall domains surrounding individual cells affect the anchorage of actin bundles (Wojtaszek et al. 2005, 2007). The dynamic reorganization of actin filaments is also essential for the transduction of gravitropic stimuli in root cells (Kordyum 2003; Volkmann and Baluška 2006). Actin mediates positioning, transport, and sedimentation of statoliths, which are the specialized form of amyloplasts, involved in gravity perception by plants (Kordyum 2003). Statocytes, which contain statoliths, have to polarize their protoplast to function as graviperceptive cells. In that way, statoliths sediment in the distal part of the cell in the direction of a gravitational vector, and the nucleus is positioned in the proximal part. It is believed that structures responsible for this positioning and polar arrangement of organelles are actin filaments (Kordyum 2003). Changes in the cytoskeleton architecture were also observed upon localized mechanical stimuli such as pressing the cells with microcapillary. Such stress conditions activate the avoidance response of the chloroplasts, i.e. movement of organelles away from the site of stimulation. As treatment with cytochalasin B (the actin inhibitor) or 2,3-butanedione monoxime (the myosin inhibitor) stopped movements upon stimulation, it is thought that actomyosin motile system plays a role in plant response to touch (Sato et al. 1999). On the other hand, localized mechanical stimulation, which could also be treated as a mimic of fungal pathogen attack, induces very rapid focusing of actin microfilaments beneath the contact site (Hardham et al. 2008).

3.4 Mechanics of Intercellular Communication

The cellular distribution of organelles seems to be essential for proper functioning of living cells and has a great role in maintaining many activities of plants (see chapter "Intracellular Movement: Integration at the Cellular Level as Reflected in the Organization of Organelle Movements"). In animal cells, microtubules rather than actin filaments are considered to be responsible for organelle movements; in contrast, actin filaments are believed to play mostly such a function in plant cells (Muthugapatti et al. 1999; Wada et al. 2003; Kadota et al. 2009). Thus, they are believed to be not only major players in vesicle trafficking between endomembranes compartments, but also responsible for the spatial distribution and movements of most organelles (Chuong et al. 2006; Boutté et al. 2007). Microfilaments congregate in densely packed actin cables, and form the tracks for intracellular organelle trafficking (Schmidt and Panstruga 2007; van der Honing et al. 2007).

In animal and yeast cells, two types of actin-based organelle movements have been identified. The first one is based on myosins, which bind tail domain of organelle cargos and transport them by sliding on actin cables. The second mechanism refers to ARP2/3 complex, which can nucleate actin filaments at the organelle edge, forming "comet tails" generating the motive force to push the organelle (Kadota et al. 2009). It is still unclear how these processes occur in plant cells, but it is likely that they involve the role of myosins, and with usage of the energy of ATP hydrolysis, maintain movement along microfilaments (Vidali et al. 2001; Holweg

and Nick 2004; Schmidt and Panstruga 2007). Various organelle movements in plants have been shown to be dependent on the cytoskeleton. Some of the actin-dependent movements of, e.g., peroxisomes (Collings et al. 2002; Jedd and Chua 2002) and mitochondria (Van Gestel et al. 2002) are the part of an active and continuous mass movement called the cytoplasmic streaming (Shimmen and Yokota 2004; Schmidt and Panstruga 2007). Actin filaments are responsible for the movements of both ER tubules and individual Golgi stacks (Knebel et al. 1990; Boevink et al. 1998; Boutté et al. 2007). It was observed that actin inhibitor cytochalasin D causes accumulation of the ER into patches, a fusion of tubules into cisternae and changes in the ER overall shape as well as disruption of Golgi stacks (Knebel et al. 1990; Satiat-Jeunemaitre et al. 1996). In the absence of actin, Golgi bodies clump together and stop moving. Similarly, the movement of the ER tubules were stopped. However, depolymerization of the microtubules had no effect on the ER and Golgi movements (Brandizzi et al. 2003). Interestingly, the organization of ER–Golgi complexes seems to be differentiated: the transport of cargo between ER and Golgi stacks is not dependent on cytoskeleton, while the transport of secretory vesicles from the Golgi stacks to different compartments is the sole responsibility of actin (Boutté et al. 2007). Internalization of small particles from the cell surface is in most cases performed by membrane carrier proteins. Specific uptake of these molecules together with polymers (i.e. pectins) is crucial for cell growth, metabolism, and signaling (Müller et al. 2007). In contrast to animal or fungal endocytosis, where vesicles movement is organized by dyneins and kinesins along microtubules, in plant cells, the pivotal role is played by F-actin and its inherent interactions with plant cell-specific class VIII myosins (Baluška et al. 2002). Latrunculin B treatment revealed a crucial role of F-actin in vesicle docking, fusion as well as endocytic vesicle formation. Inhibition of actin cytoskeleton in pollen tubes affects organelle motility, the vesicle, and small endosome movement pathways in the clear zone and their ability to fuse with the plasma membrane. Moreover, endosomes could play a role as actin nucleation hot spots. Therefore, actin polymerization could underlie directed vesicle movement. In addition, F-actin depolymerization changed tubular and dynamic vacuole into stationary round structure in the subapical region. Hence, motility of vacuole is also actin dependent (Ovecka et al. 2005).

Intracellular distribution of chloroplasts, controlled by actin cytoskeleton, depends mainly on the intensity and spectral quality of light. Chloroplasts move away from strong light irradiation to avoid photodamage, and move towards weak light to maximize photosynthesis (Muthugapatti et al. 1999; Kasahara et al. 2002; Wada et al. 2003; Suetsugu and Wada 2007; Kadota et al. 2009). Application of actin inhibitors caused aberrant distribution of these organelles. On the other hand, microtubules were very rarely observed in connection with chloroplasts, and, accordingly, oryzalin treatment did not affect chloroplasts distribution (Muthugapatti et al. 1999; Chuong et al. 2006). It was also observed that short actin filaments, called cp-actin filaments, form connections between chloroplasts' peripheries and the plasma membrane. The cp-actin filaments appear immediately after inducing signal in the form of light irradiation, and their formation depends on *ch*loroplast *u*nusual *p*ositioning *1* (CHUP1) proteins, which are localized at the chloroplast

envelope. Mutation of CHUP1 resulted in disappearance of cp-actin filaments and normal cytoplasmic actin filaments were not influenced (Kadota et al. 2009). Other experiments showed that CHUP1 have not only F-actin binding motif, but it can also bind G-actin or profilin, suggesting that CHUP1 may regulate cp-actin filament dynamics at the chloroplasts envelope (Oikawa et al. 2003, 2008; Schmidt von Braun and Schleiff 2008). Moreover, basket-like structures of actin filaments were noticed around the chloroplasts, anchoring organelles during streaming and allowing for control over proper three-dimensional orientation of chloroplasts with respect to light (for details see Chapter "Intracellular Movement: Integration at the Cellular Level as Reflected in the Organization of Organelle Movements").

The movements of mitochondria are mediated by two components of the cytoskeleton – actin filaments and microtubules, and the involvement of each element varies, depending on a specific cell type and organism (Zheng et al. 2009). For example, it was reported that plant mitochondria move on actin filaments, but their positioning in cortical part of the cell is the responsibility of both actin and myosin (Van Gestel et al. 2002). Myosin inhibitor reduced mitochondrial velocity in a manner similar to that observed in *Arabidopsis* myosin knock-out mutant, confirming that the actomyosin system is the main driving force of mitochondrial movement (Peremyslov et al. 2008; Prokhnevsky et al. 2008; Zheng et al. 2009). Recent analysis also showed that microtubules affect mitochondrial velocity, trajectory, and positioning because they direct the positioning of actin polymerization events (Zheng et al. 2009).

Plant cytoskeleton, especially actin filaments, is responsible for both positioning and movements of nuclei in normal conditions and in response to external stimuli as well as during cell division. It was demonstrated in animal cells that this actin-dependent repositioning of nuclei had an effect on chromatin organization, but affected nuclear movement to a lesser extent (Maniotis et al. 1997a, b; Yang et al. 2008). Interestingly, in plant cells, in some processes, e.g., during cytokinesis, actin cytoskeleton is absolutely required for proper partitioning of organelles (Sheahan et al. 2004). However, microtubules were also observed to function in organelle positioning, and this suggested that both actin and microtubules might function cooperatively during organelle movement. It was thus proposed that the actin-based system provides the mechanism for moving organelles during the early stage of cellular partitioning, whereas microtubules are implicated in proper spatial relationship of organelles at subcellular location (Chuong et al. 2006).

3.5 Maintenance of Hydromechanical Integrity of Plant Cells Due to Balanced Endo- and Exocytosis

Because of the high turgor pressure endocytic processes have been disputed in plant cells on an assumption that the amount of energy needed to aggregate clathrin triskelions is very low. However, it was shown that the energy is inadequate to form

a vesicle even in the absence of turgor pressure (Meckel et al. 2005). Adequate energy for pit and consequently vesicle formation is delivered by generating a molecular imbalance in plasma membrane bilayer. Adding phospholipids to the inner leaflet generates enough force for vesicle formation even in unilamellar giant vesicles containing no proteins. Therefore phospholipids asymmetry within bilayer generates enough energy for vesiculation during endocytosis (Meckel et al. 2005). It was proposed that plant cells possess standard, constantly maintained plasma membrane tension. Any changes of tension are detected and followed by adequate response. Therefore, increasing membrane tension triggers exocytic secretion of membrane material, subsequently decreasing tension until standard membrane tension is re-established. Osmotic or pressure stress prompts swelling or shrinking of guard cells protoplasts. Patch clamp experiments revealed that surface changes of these cells are accompanied by removal of membrane material from or its incorporation to plasma membrane. Moreover, in most cases fusion and fission events are not associated with the visible vesicle movement. Therefore, it was proposed that the surface change in guard cell protoplasts is reached mostly via small vesicles of size below diffraction limit (ca. 300 nm) (Meckel et al. 2005). Intact guard cells in hyperosmotic conditions showed uptake of membrane styryl dye FM4-64 in objects whose size varied between 1 μm and diffraction-limited size under 270 nm. Also invaginated tubular structures in the plasma membrane were found (Meckel et al. 2005). Indeed, precise measurements of the size of endocytic vesicles in guard cells with the fluorescent dye Alexa 488 hydrazide provided estimation of the minimal size to be at least 87 nm (Gall et al. 2010).

Endocytic processes are operative in turgid plant cells. They were shown to function during wall remodeling, enabling utilization of cell surface material for the construction of a new cell plate (Dhonukshe et al. 2006). Clathrin-mediated endocytosis constitutes the basis for recycling of PIN auxin efflux carriers (Dhonukshe et al. 2007), and this process itself is regulated by auxin (Paciorek et al. 2005). Finally, endocytic vesicles in intact guard cells are able to carry GFP-tagged plasma membrane K^+ channel KAT1, which shows that endocytosis is also operating in cells with the highest turgor pressure (Meckel et al. 2004). Investigation of the rates of membrane material addition in guard cells showed that the new material is present in the plasma membrane instantly after hydrostatic pressure stressing. Hence, it was proposed that guard cells possess the reservoir of membrane material disposable for fusion with swelling plasma membrane. Moreover, membrane material internalized during cell shrinking could be secreted while cell swells. This shall enable the guard cell protoplasts to swell and shrink several times. Unfortunately, there is still no data showing the origin and quality of membrane material transported to and from the plasma membrane in tension-induced surface changes (Meckel et al. 2005).

The elongation of the pollen tube seems to be orchestrated by transcellular hydrodynamic flow coordinating exocytosis and endocytosis (Zonia and Munnik 2008). Hyperosmotic treatment of pollen tube cells leads to cell shrinking, which stimulates endocytosis at the apex and also arrests exocytic processes. Hyperosmotic conditions induce the reduction of cellular volume and, in consequence, halt cell

elongation. On the contrary, hypoosmosis increases the exocytic membrane flow, and decreases endocytosis (Zonia and Munnik 2007). It was proposed that because of the exposure to mechanical agents and therefore possibility of structural defects in expansion, the pollen tube apex is not the place where the secretion of new cell wall compounds occurs and that cell wall synthesis is localized distal from the apex. It was shown that exocytosis takes place adjacent to the apical dome (Zonia and Munnik 2008). Endocytosis was observed in the pollen tube apex in the form of small vesicles that underwent retrograde transport. Respectively, exocytic activity was shown next to the cell apex. During cell elongation excess plasma membrane was moved to the apex and taken up through endocytosis. Therefore predominantly unorganized pollen tube growth seems to be directed by vectorial hydrodynamic flow coupled with exo- and endocytic processes occurring adjacent and at the apex, respectively (Zonia and Munnik 2007). Newly mounted cell wall is more viscoplastic than mature cell wall; therefore it is likely to undergo directed expansion driven by hydrodynamic flux and cooperative exo- and endocytosis. It was assumed that exocytic vesicles are very appropriate for cell wall material transport and showed that they belong to a large class of vesicles with intermediate size (Zonia and Munnik 2008). It should also be added that the cooperative exo- and endocytosis needs to be tightly regulated. Recent estimates of the rates of both processes in root hairs and pollen tubes of *Arabidopsis* showed that 9,204 and 2,686 exocytic vesicles are consumed per minute at 20°C during growth of root hairs and pollen tubes, respectively. More importantly, however, the recycling process involves 86.7% of newly inserted membranes in root hairs, and 79.0% in pollen tubes (Ketelaar et al. 2008).

During plant cell development there are constant changes in cell wall composition realized by directed transport of polysaccharides and proteins in transport vesicles. It is known that internalized membrane arabinogalactan proteins are trafficked to MVB and consequently to vacuoles. Cellulose syntase complex activity is regulated throughout orchestrated recycling of its subunits between plasma membrane and internal membrane compartments. Moreover, ingredients of cell plate during plant cell cytokinesis are partially delivered in endosomes containing components of existing cell walls (Dhonukshe et al. 2006). Endocytic uptake of cell wall pectins and their redirection to the cell plate is important for building new cell wall during cytokinesis (Müller et al. 2007). Pectins cross-linked with boron and calcium are crucial for mechanical strength and spatial organization of plant cell walls. Therefore, actin-dependent endocytosis and secretion of these molecules are pivotal for modulating mechanical properties of plant cells. In consequence, endocytosis might regulate plant growth and morphogenesis.

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