8-2013

A force of nature: molecular mechanisms of mechanoperception in plants

Gabriele B. Monshausen
Pennsylvania State University - Main Campus

Elizabeth S. Haswell
Washington University in St Louis, ehaswell@wustl.edu

A Force of Nature: Molecular Mechanisms of Mechanoperception in Plants

Elizabeth S. Haswell[1] and Gabriele B. Monshausen[2]

[1]Department of Biology, Washington University in St. Louis, St. Louis, MO 63130, USA
[2]Biology Department, Pennsylvania State University, University Park, Pa 16802, USA

To whom correspondence should be addressed:
[1]ehaswell@wustl.edu; [2]gmonshausen@psu.edu

Abstract

The ability to sense and respond to a wide variety of mechanical stimuli—gravity, touch, osmotic pressure, or the resistance of the cell wall—is a critical feature of every plant cell, whether or not it is specialized for mechanotransduction. Mechanoperceptive events are an essential part of plant life, required for normal growth and development at the cell, tissue and whole-plant level and for the proper response to an array of biotic and abiotic stresses. One current challenge for plant mechanobiologists is to link these physiological responses to specific mechanoreceptors and signal transduction pathways. Here, we describe recent progress in the identification and characterization of two classes of putative mechanoreceptors, ion channels and receptor-like kinases. We also discuss how the second messenger Ca^{2+} operates at the center of many of these mechanical signal transduction pathways**.**

Introduction

Plant responses to mechanical stimulation have captured the imagination of biologists since Robert Hooke first described the touch-induced folding of leaves of the 'humble plant', *Mimosa pudica* (Hooke *et al.*, 1665). Rapid thigmonastic movements were subsequently discovered in a variety of carnivorous plants, such as *Dionaea muscipula* (Darwin, 1875; Ellis, 1770), *Aldrovanda vesiculosa* (Darwin, 1875), *Utricularia* (Darwin, 1875; Treat, 1875) and *Drosera* species (Darwin, 1875), whose leaves are modified to form complex snap, suction or sticky traps that capture and eventually digest prey. Traps are activated by mechanical deformation of specialized appendages such as trigger hairs (Adamec, 2012; Sibaoka, 1991), snap tentacles or adhesive emergences (Poppinga *et al.*, 2012). Typically, the deformation of mechanosensitive appendages triggers action potentials that propagate along symplastically connected, excitable cells and likely elicit turgor changes in responsive cells, resulting in fast nastic movements (Sibaoka, 1991).

Slower, but no less complex movements of mechanosensitive plant organs are found in many actively climbing plants. Unattached climbing plants exhibit exploratory movements to localize external support structures to which the plants then fasten themselves after making contact (reviewed by Isnard and Silk, 2009); continued growth along such vertical structures enables the climbing plants to optimize light capture without the costly investment of forming extensive support tissues. Intriguingly, stems and tendrils of many twining and tendril-coiling species contain layers of specialized fibers with a gelatinous cell wall layer that appears to be important for the tightening of coiling organs around a support (Bowling and Vaughn, 2009; Meloche *et al.*, 2007).

Further investigations revealed that not only specialized cells and organs are sensitive to mechanical perturbation. In fact, the ability to perceive mechanical stress appears fundamental to all plant cells. Protoplasts (Haley *et al.*, 1995; Haswell *et al.*, 2008; Lynch and Lintilhac, 1997; Wymer *et al.*, 1996), suspension-cultured cells (Gross *et al.*, 1993; Yahraus *et al.*, 1995), meristematic, expanding and fully differentiated cells of shoots and roots (e.g. Braam and Davis, 1990; Chehab *et al.*, 2009; Coutand, 2010; Ditengou *et al.*, 2008; Hamant *et al.*, 2008; Legue *et al.*, 1997; Lintilhac and Vesecky, 1981; Matsui *et al.*, 2000; Richter *et al.*, 2009; Wick *et al.*, 2003) all have been shown to undergo physiological or developmental changes upon mechanical stimulation. Many of these mechanical stresses are imposed by the environment in the form of wind, passing animals, the weight of climbing plants, or soil constraints such as compaction and other mechanical barriers. Plants typically acclimate to such disturbances via developmental responses that modulate the mechanical properties of load-bearing tissues and organs. Reduction of mechanical loads on the stem is achieved, for example, by a reduction in elongation, while stem thickening, increased production of support tissues and cell wall lignification promote stem flexural rigidity (Biddington, 1986; Braam, 2005; Chehab *et al.*, 2009; Coutand, 2010; Dejaegher and Boyer, 1987; Niklas 1998; Patterson, 1992; Porter *et al.*, 2009; Saidi *et al.*, 2009). Alternative strategies involve reducing the risk of stress-induced breakage by enhancing tissue flexibility, as observed in mechanically perturbed leaf petioles and the stems of some species (Anten *et al.*, 2010; Biddington, 1986; Liu *et al.*, 2007).

Mechanical stresses are not just exerted by the environment, however, but are intrinsic to plants at all levels of plant architecture. Woody plants experience progressive, gravity-dependent mechanical self-load as they increase in size and mass and this tends to be correlated with thickening of the stem and formation of supporting tissues. Plants also exhibit proprioceptive sensing whereby they appear to correct local organ curvature via autotropic straightening (Bastien *et al.*, 2013; Firn and Digby, 1979). Whether there is a causal link between self-load

and the extent of secondary growth is unclear, as there has been little opportunity to observe large woody plant species develop under microgravity conditions.

At the tissue level, mechanical stresses are generated when adjoining cells layers exhibit differential extensibility (reviewed by Kutschera, 1989; Nakamura *et al.*, 2012). Such stress patterns have been shown to inform the organization of cortical microtubule arrays in the epidermis of hypocotyls and at the shoot apical meristem (Hamant *et al.*, 2008; Hejnowicz *et al.*, 2000; Uyttewaal *et al.*, 2012) and even in protoplasts exposed to centrifugal forces (Wymer *et al.*, 1996). Root apical meristem architecture also appears sensitive to mechanical stresses in that external mechanical constriction of a root tip induces atypical periclinal cell divisions at the root pole and a switch from closed to open meristem organization (Potocka *et al.*, 2011). Excitingly, the development of lateral roots has recently been shown to also be receptive to the intrinsic mechanical constraints imposed by overlaying root tissues. The shape and emergence of lateral root primordia appears highly dependent not on a precise sequence of anticlinal and periclinal cell divisions, but on the mechanical resistance of the endodermis and its Casparian strip and the overlaying cortical and epidermal cell layers (Lucas *et al.*, 2013). Mechanical forces have long been postulated to orient cell division (Lynch and Lintilhac, 1997) and may play a key role not just in shaping the apical meristems, but in regulating cambial activity during secondary growth of stems and roots. As the vascular cambium forms secondary xylem to the interior of the stem/root by perclinal cell divisions, the cambium and all peripheral tissues are displaced outward. Compensatory anticlinal divisions in the vascular and cork cambium and, in some species, the ray cells of the secondary phloem increase the circumference of these tissues and prevent tearing. How the switch from periclinal to anticlinal division plane is regulated remains unclear. The frequent periclinal divisions of the lateral meristems are atypical in that they do not occur in the plane of minimal surface area (Chaffey, 2002), suggesting that patterning of cell divisions proceeds along other pathways, some of which are reminiscent of wound-induced cell division (Goodbody and Lloyd, 1990). Given that cells of the vascular cambium likely experience both compressive and tensile stresses as they are pushed outward against the bark (Hejnowicz, 1980), initiation of anticlinal divisions may be a response to a relative change in the ratio of these stresses (Lintilhac and Vesecky, 1981) in the course of a growing season.

The principal mechanical stress that is experienced by all living plant cells is turgor pressure. In mature cells of herbaceous plants, turgor is an important contributor to the structural stability of the plant. However, more fundamentally, turgor is the driving force for cell expansion and, in concert with tightly regulated cell-wall extensibility, a primary determinant of plant cell size and

shape. In the context of plant mechano-responses, this creates an interesting conundrum: mechanical forces drive cell elongation, creating local tensile strain, but a typical response to such mechanical strain is a reduction in growth (see above; Fig. 1). A complex feedback system involving mechanical stress both as motive and inhibitory force may account for the oscillatory growth patterns observed in root hairs and pollen tubes, where periods of rapid expansion alternate with periods of growth deceleration (Chebli and Geitmann, 2007; Monshausen *et al.*, 2008). Future studies should determine whether such mechanical feedback plays a universal role in growth control of all expanding cells.

Mechanisms of Mechanoperception

While it is very clear that plants tissues and cells sense and respond to mechanical signals, as summarized above, the various molecular mechanisms by which this is accomplished are still a major area of investigation. Below we describe current research into two major classes of molecules thought to serve as plant mechanoreceptors, and discuss the downstream role of calcium (Ca^{2+}) signaling and other ion flux events. These gene products and the pathways in which they are thought to act are summarized in Figure 2.

I. Mechanosensitive Ion Channels

A particularly well-studied mechanism for mechanoperception in bacterial and animal systems is the use of mechanosensitive (MS) ion channels (reviewed in Arnadottir and Chalfie, 2010; Haswell *et al.*, 2011; Sukharev and Sachs, 2012). Ion channels are membrane-embedded protein complexes that provide a pathway for the movement of ions from one side of the membrane to the other. Ligand- and voltage-gated ion channels have been studied for many years in plant systems, most notably in guard cell signal transduction (reviewed in Hedrich, 2012; Ward *et al.*, 2009). Though likely to be just as important and as abundant, mechanically-gated ion channels are much less well understood. Two (probably simplistic) two-state models for MS ion channels have long been proposed: In the “intrinsic” model (Fig. 3A), mechanical force is transmitted to the channel directly through the lipid bilayer in which the channel resides. Increased membrane tension leads to membrane thinning and an increase in the pulling forces exerted upon the channel by the bilayer lipids. These alterations induce a conformational change in the channel, favoring the open state. In contrast, in the “trapdoor” model (Fig. 3B), mechanical force is transmitted to a domain of the channel via links to other cellular structures such as the cell wall or cytoskeleton. Opening of the trapdoor allows ions to access the channel pore. The opening of a MS channel, once accomplished, could in principle lead to the release of

osmolytes, depolarization of the membrane, and/or the influx of the secondary messenger Ca^{2+} (see below).

As described above, the perception of mechanical signals, including gravity, touch, soil or media density, cell invasion by a pathogen, and rapid alteration in osmotic pressure, is integral to plant growth and development (many of which are mentioned in other chapters in this volume). As many of these mechanical stimuli are associated with ion fluxes within the plant cell (reviewed below, and in Fasano *et al.*, 2002; Kurusu *et al.*, 2013; Toyota and Gilroy, 2013; Trewavas and Knight, 1994), it is easy to see why MS channels are so frequently proposed to mediate plant mechanotransduction (see Fig. 2).

A classic example is the long-standing proposal that MS channels mediate the process of gravity perception (reviewed in Telewski, 2006; Toyota and Gilroy, 2013). It has been suggested that in the gravity-sensing columella cells of the root tip, the downward motion of starch-filled plastids (amyloplasts) could activate MS ion channels either in the amyloplast envelope, or in the ER upon which the amyloplasts settle (Boonsirichai *et al.*, 2002). In non-specialized cells of the root or in the shoot, MS ion channels embedded in the plasma membrane could be important for gravity perception if they were activated directly through asymmetric membrane tension produced by the weight of the protoplast (Wayne and Staves, 1997). Alternatively, plasma membrane channels could be activated indirectly—the weakened cell wall observed in plants grown in microgravity could lead to membrane stretch (Cowles *et al.*, 1984; Hamann, 2012), or downward-moving amyloplasts might impact the actin cytoskeleton network, pulling on the plasma membrane Multiple lines of evidence indicate that ionic flux occurs extremely rapidly after plants are exposed to a change in gravity vector (further described below and recently reviewed in (Toyota and Gilroy, 2013)), consistent with the involvement of MS ion channels. However, a direct role for MS channels in gravity perception or response still remains to be firmly established.

Broadly speaking, two main approaches have been used for the identification and characterization of MS channels in plant systems: (1) physiological analyses involving the use of patch-clamp, ion imaging, vibrating probes and other technologies to measure ion flux; and (2) Arabidopsis molecular genetics. Through these complementary approaches we have begun to gain insight into the abundance, distribution, channel characteristics, physiological functions, and molecular identity of plant MS ion channels.

A. Electrophysiological Studies

Pioneering studies of mechanotransduction measured the production of action and receptor potentials in giant algal cells such as *Chara* and *Nitella* (reviewed in Shimmen, 2006; Wayne, 1994). The large internodal cells of *Chara* allow researchers to observe the activation of Cl^- and Ca^{2+} fluxes both immediate to and at a distance from the site of initial mechanical stimulation (such as response to dropping a glass rod onto a cell) (Shimmen, 1997). A mechanosensitive Ca^{2+} channel may respond to touch, gravity, and osmotically induced membrane stretch in these "plant-like" cells (Iwabuchi *et al.*, 2005; Kaneko *et al.*, 2005; Kaneko *et al.*, 2009; Staves, 1997).

The advent of patch-clamp electrophysiology made possible the study of opening and closing of single mechanosensitive (or "stretch-activated") ion channels in plant membranes (Falke *et al.*, 1988; Schroeder and Hedrich, 1989). This technique is illustrated in Figure 4. Since then, over 18 distinct channel activities that can be elicited by suction or pressure introduced through the patch pipette have been described in land plants. These include channel activities found in the plasma membrane of *Arabidopsis thaliana* hypocotyl, leaf, and root cells (Haswell *et al.*, 2008; Lewis and Spalding, 1998; Qi *et al.*, 2004; Spalding and Goldsmith, 1993), *Lilium longiflorum* pollen grains and pollen tubes (Dutta and Robinson, 2004), cultured cells from *Nicotiana tabaccum* (Falke *et al.*, 1988), guard cells of *Commelina communis* and *Vicia faba* (Cosgrove and Hedrich, 1991; Liu and Luan, 1998; Schroeder and Hedrich, 1989; Zhang *et al.*, 2007), and epidermal cells of *Allium cepa* and the halophyte *Zostera muelleri* (Ding and Pickard, 1993; Garrill *et al.*, 1994). Similar activities were also recorded in the vacuolar membrane of *Beta vulgaris* (Alexandre and Lassalles, 1991). While these studies illustrate the ubiquity of MS channel activities among a wide variety of plants and cell types, they also demonstrate the substantial variation in channel character that is possible; ion preferences vary from non-selective to Cl^-, Ca^{2+} or K^+-selective channels with conductances that range over two orders of magnitude (these details are summarized in Table I of (Haswell, 2007)).

The physiological investigation of plant MS channel activities was further facilitated by the identification of pharmacological agents capable of inhibition or activation of stretch-activated ion channels. At low concentrations, the lanthanide gadolinium (Gd^{3+}) serves to block cation-selective mechanosensitive channels in animals and in plants (Alexandre and Lassalles, 1991; Ding and Pickard, 1993; Dutta and Robinson, 2004; Yang and Sachs, 1989). Gd^{3+} will also inhibit non-selective MS channels, albeit at higher concentrations (Berrier *et al.*, 1992), by promoting membrane stiffness, thereby favoring the closed state of the channels (Ermakov *et al.*, 2010). On the other hand, the amphipathic molecule trinitrophenol (TNP) can be used to activate MS channel activity by inducing membrane curvature and therefore membrane tension (Martinac *et al.*, 1990). Thus, MS channels may be involved in a particular physiological process

if the response is altered upon treatment with Gd^{3+} or TNP. For example, Gd^{3+} application relieves the root twisting phenotype of several *Arabidopsis thaliana* tubulin mutants (Matsumoto *et al.*, 2010) and guard cell opening in *Vicia faba* is inhibited by TNP application (Furuichi *et al.*, 2008), implicating MS channels in both signal transduction pathways.

B. Molecular Genetic Studies

Though the studies described above established that MS ion channels activities are pervasive in plant membranes—over eighteen different activities have been described in a variety of cell types (summarized in Haswell, 2007)—we still lack a molecular basis for most. However, the popularization of Arabidopsis molecular genetics has introduced a different suite of tools for the study of MS ion channels. To date, three genes or gene families have been implicated as providing 13 known or predicted MS ion channel activities, though many more likely await discovery. These three Arabidopsis genes or gene families have distinct characteristics and are at different stages of analysis, as outlined below and listed in Table 1.

1) The Mechanosensitive Channel of Small Conductance-Like (MSL) family. The Mechanosensitive Channel of Small Conductance (MscS) is a well-studied MS channel from *Escherichia coli* that provides the rapid release of osmolytes from cells in response to the increased membrane tension produced by hypoosmotic shock (reviewed in Booth and Blount, 2012)). MscS has served as an excellent model system for the study of MS channel structures, biophysical properties, and physiological functions (recently reviewed in Haswell *et al.*, 2011; Naismith and Booth, 2012), so the observation that genes encoding MscS homologs were not only found in the genomes of bacterial and archaeal species, but also in the recently sequenced *Arabidopsis thaliana* genome and in *Schizosaccharomyces pombe* (Kloda and Martinac, 2002; Pivetti *et al.*, 2003) provided a much-needed molecular clue to the entities that might underlie some MS channel activities in plant cells. The region of homology among MscS family members corresponds to the permeation pathway and the upper portion of the soluble cytoplasmic domain. Outside of this region, family members from Bacteria, Archaea, and plants vary considerably in the number of TM helices (ranging from 3-11) as well as the size of N- and C-terminal extensions and extracellular/cytoplasmic loops. Despite the low sequence conservation between MscS and its ten homologs in Arabidopsis (named MSL1-10), recent data indicate that the ability to assemble into mechanically gated channels in the membrane is evolutionarily conserved. *MSL9* and *MSL10* are required for an abundant MS channel activity located in the plasma membrane of root protoplasts (Haswell *et al.*, 2008), and single-channel patch clamp electrophysiology was used to show that MSL10 is capable of providing an ~100 pS anion-preferring MS channel activity when heterologously expressed in *Xenopus laevis* oocytes

(Maksaev and Haswell, 2012; Fig. 4D). Several sequence motifs conserved among MscS homologs (summarized in Balleza and Gomez-Lagunas, 2009) are required for normal MSL2 function (Jensen and Haswell, 2012), suggesting that bacterial and plant MscS homologs employ similar gating mechanisms. However, the structures, topologies, and oligomeric states of plant MscS homologs remain to be experimentally determined; this information should give us significant insight into the ways in which mechanosensitivity has evolved in the plant lineage.

In terms of physiological function, much is yet to be learned about plant MSL channels. The ten *MSL* genes in Arabidopsis exhibit a variety of tissue-specific expression patterns, and the proteins they encode exhibit distinct subcellular localizations and predicted topologies (Haswell, 2007). Reverse genetic approaches to determining the biological role of MSL proteins have had variable success. A quintuple mutant with lesions in *MSL4*, *MSL5*, *MSL6*, *MSL9* and *MSL10* has no discernable phenotype (Haswell *et al.*, 2008). However, some progress has been made studying the MscS homologs that localize to chloroplasts. MSL2 and MSL3 localize to the plastid envelope (Haswell and Meyerowitz, 2006), where they serve to relieve plastidic hypo-osmotic stress during normal plant growth and development (Veley *et al.*, 2012). MSL2 and MSL3 are partially redundantly required for normal size and shape of epidermal plastids, and for the proper regulation of FtsZ ring formation during chloroplast fission (Haswell and Meyerowitz, 2006; Wilson *et al.*, 2011). An MscS homolog from *Chlamydomonas*, MSC1, is also required for chloroplast integrity and provides a MS channel activity that closely resembles that of MSL10 when expressed in giant *E. coli* spheroplasts (Nakayama *et al.*, 2007). The two MscS homologs from *S. pombe*, Msy1 and Msy2, localize to the ER and are required for optimal survival of hypo-osmotic shock (Nakayama *et al.*, 2012).

Multiple genes encoding MscS-Like proteins are found in every plant genome so far inspected, and the proteins they encode fall into two general classes, one predicted to localize to chloroplasts and/or mitochondria and one predicted to localize to the plasma membrane (Haswell, 2007; Porter *et al.*, 2009). We speculate that the presence of paralogs with different topologies and subcellular localizations within a single plant genome reflects a multiplicity of functions for this class of channels. Several lines of evidence suggest that the ability of MscS homologs to release osmolytes in response to membrane tension may be modulated by additional signals. The gating of certain bacterial MscS family members is influenced by the extracellular ionic environment, by binding to small molecules, or by interaction with other proteins (Li *et al.*, 2002; Malcolm *et al.*, 2012; Osanai *et al.*, 2005). It is also possible that these channels have evolved a signaling function in addition to, or instead of, mediating ion flux. The preference of MSL10 for anions may indicate that it can both release osmolytes and depolarize

the membrane (Fig. 2), potentially leading to downstream signal transduction pathways, possibly even action potentials (Maksaev and Haswell, 2012). Experimentally testing this hypothesis will be an important future direction for the study of this family of proteins.

2) The Mid1-Complementing Activity (MCA) family. Arabidopsis MCA1 is the founding member of this plant-specific family of proteins (reviewed in Kurusu *et al.*, 2013). MCA1 was first identified as a cDNA capable of restoring the ability to take up Ca^{2+} ions in response to mating factor in a yeast strain lacking the stretch-activated Ca^{2+} channel Mid1 (Iida *et al.*, 1994; Kanzaki *et al.*, 1999; Nakagawa *et al.*, 2007). Unexpectedly, MCA proteins share no clear sequence similarity with Mid1, and indeed do not resemble ion channels previously characterized in any system. MCA1 and close homolog MCA2 form homomeric complexes localized to the plasma membrane and endomembranes of plant cells (Kurusu *et al.*, 2012a; Kurusu *et al.*, 2012b; Kurusu *et al.*, 2012c; Nakagawa *et al.*, 2007). Two-electrode voltage clamping experiments on *Xenopus laevis* ooctyes heterologously expressing MCA1 revealed increased whole-cell currents in response to hypoosmotic swelling, and 34 pS single channel events were occasionally observed in oocytes expressing MCA1 in response to increased membrane tension (Furuichi *et al.*, 2012). Together with the physiological data summarized below, these results support the hypothesis that MCA proteins assemble into mechanically gated Ca^{2+} channels, but their topology, structure and mechanism of mechanosensitivity will be both interesting and important to establish.

Overexpression of MCA1, MCA2, and/or related proteins from rice and tobacco is closely correlated with increased Ca^{2+} influx in response to hypoosmotic stress or mechanical stimulus in Arabidopsis protoplasts, Arabidopsis roots, cultured rice, tobacco, yeast and mammalian cells (Kurusu *et al.*, 2012b; Kurusu *et al.*, 2012c; Nakagawa *et al.*, 2007; Yamanaka *et al.*, 2010). In vivo, the two Arabidopsis MCA proteins have both redundant and unique functions: the *mca1* null mutant exhibits a marked loss of the ability for roots to grow from soft agar into hard agar, while the roots of *mca2* mutants show a defect in Ca^{2+} accumulation (Nakagawa *et al.*, 2007; Yamanaka *et al.*, 2010). Double *mca1 mca2* mutants show both of these defects, and are additionally small, early flowering, and hypersensitive to $MgCl_2$ (Yamanaka *et al.*, 2010).

Several recent reports from Hamann and colleagues implicate MCA1 in cell wall damage signaling pathway(s). MCA1 is required for the increased lignin production and altered transcript profile that result from treating seedlings with the cellulose synthesis inhibitor isoxaben (Denness *et al.*, 2011; Hamann *et al.*, 2009; Wormit *et al.*, 2012). Because isoxaben treatment results in cellular swelling (Lazzaro *et al.*, 2003) and the effects of isoxaben can be suppressed

by increased extracellular osmotic support (Hamann *et al.*, 2009), it is proposed that MCA1 may be involved in sensing membrane tension changes resulting from a rapid reduction in turgor upon cell wall loosening (Hamann, 2012). We anticipate that future studies will establish the mechanism by which MCA proteins contribute to Ca^{2+} influx in response to cell wall damage, hypoosmotic stress, and other mechanical stimuli.

3) Piezo proteins. There has been much excitement surrounding the identification of the Piezo channels, a family of mechanosensitive cation channels first identified in mouse cells (Coste *et al.*, 2010; Coste *et al.*, 2012) and implicated in pain perception in *Drosophila* larvae (Kim *et al.*, 2012), epithelial morphogenesis in Zebrafish (Eisenhoffer *et al.*, 2012) and disease in humans (McHugh *et al.*, 2012; Zarychanski *et al.*, 2012). Piezo proteins have as many as 36 TM helices per monomer, forming large homomeric complexes thought to underlie the long sought-after stretch-activated ion channels of the mammalian somatosensory system (Nilius, 2010). It has been noted a number of times that there is a single gene in the Arabidopsis genome predicted to encode a Piezo-like protein (Coste *et al.*, 2010; Hedrich, 2012; Kurusu *et al.*, 2013), but its characterization has not yet been reported.

II. Cell Wall Surveillance – the Role of Receptor-like Kinases

Since the discovery that cell wall fragments produced during plant cell wall degradation by pathogens serve as elicitors to trigger plant defense responses (Sequeira, 1983), it has become apparent that the cell wall is not only a target of cellular signaling but a vital source of information (Pennell, 1998; Wolf *et al.*, 2012). Removal of the cell wall by enzymatic digestion yields protoplasts that retain at least some mechanosensitivity (Haley *et al*., 1995; Wymer *et al*., 1996) but a precise evaluation of the contribution of the cell wall to mechanoperception is difficult as these assays are well known to alter membrane properties (Miedema et al., 1999). However, an elegant series of experiments by Hématy and coworkers showed that developmental defects in cell wall assembly are actively monitored by plants and lead to adjustments in growth and development (Hematy *et al.*, 2007). These findings have generated intense interest in the idea that changes in the mechanical status of cell walls, for example during cell wall loosening in expanding cells or upon deformation by external mechanical forces, are under continuous surveillance by plant mechanosensors (Cheung and Wu, 2011; Humphrey *et al.*, 2007; Monshausen and Gilroy, 2009). This idea was inspired by research on yeast, which established that monitoring cell wall integrity is essential for yeast survival under stress conditions and is achieved by a suite of five sensor proteins. The cell *w*all *s*tress response *c*omponent proteins Wsc1, 2 and 3, *m*ating-*i*nduced *d*eath 2 (Mid2) and *M*id*2-l*ike 1 (Mtl1) all

localize to the plasma membrane and consist of a small cytoplasmic domain, a single transmembrane domain and a highly *O*-mannosylated extracellular domain, which is thought to function as a molecular probe extending into the cell wall matrix. Activation of the sensors by mechanical stress leads to transcriptional responses via a GEF/Rho1 GTPase and MAPK-dependent pathway (Jendretzki *et al.*, 2011; Levin, 2011).

In plants, no orthologs of the yeast cell wall integrity sensors have been identified. However, plant genomes encode a very large family of membrane-localized receptor-like kinases (RLKs) harboring a cytosolic kinase domain, a single membrane-spanning domain and an extracellular domain; ligands have thus far only been identified for a small subset of these RLKs, but a significant number feature putative carbohydrate-binding domains (Gish and Clark, 2011; Hok *et al.*, 2011; Fig. 2). The most promising candidate RLK cell wall integrity sensors are listed in Table 1 and further described below.

1. WAK family. The wall-associated kinase (WAK) subfamily of RLKs contains five closely related members with high sequence identity. WAKs have been shown to tightly bind pectin and appear to function as receptors for oligogalacturonic acids, the degradation products of pectin which are produced during wounding or pathogen attack; WAKs thus likely play a key role in plant defense responses (Kohorn and Kohorn, 2012). Anti-sense RNA-mediated downregulation of *WAK* expression also results in dramatically reduced cell size in all plant organs, suggesting an important, but as yet unidentified, activity in growth control (Lally *et al.*, 2001).

2. CrRLK1L family. The most compelling evidence for an involvement of RLK in cell wall integrity sensing has been found for the *Catharanthus roseus* RLK subfamily. The CrRLK1L subfamily comprises 17 members, most of which harbor an extracellular malectin-like domain (Lindner *et al.*, 2012). Animal malectin proteins were shown to specifically bind Glc_2-high mannose N-glycans and are proposed to play a role in the quality control of glycoproteins in the ER (Qin *et al.*, 2012). It is conceivable that the CrRLK1L malectin-like domains bind polysaccharides or glycoproteins of plant cell walls, though no such interaction has yet been demonstrated. The CrRLK1L THESEUS1 was identified in a screen for suppressors of the cellulose deficient cellulose synthase CESA6 mutant *procuste1-1* (Hematy *et al.*, 2007). When grown in darkness, *procuste1-1* exhibits strongly reduced hypocotyl elongation, ectopic lignin accumulation in the root and hypocotyl, and significant deregulation of almost 900 genes. These defects were partially relieved in *prc1-1 the1* double mutants without restoring cellulose deficiency. Neither *the1* single mutants nor *THE1* overexpressors in a wild type background had detectable growth phenotypes, whereas *THE1* overexpression in the *prc1-1 the1* background

exacerbated some of the defects. The authors speculated that to be fully articulated, a subset of cellulose deficiency-associated phenotypes require active signaling via a THE1-dependent pathway, consistent with a role for THE1 as a sensor for cell wall damage (Denness *et al.*, 2011; Hematy *et al.*, 2007). Interestingly, THE1 also appears to be involved in modulating cell elongation in the absence of external stress. Triple mutants with genetic lesions in *THE1* and the closely related CrRLK1Ls *HERK1* and *HERK2* (*the1 herk1 herk2*) have significantly shorter petioles and hypocotyls than wild type Arabidopsis, suggesting that these RLKs function redundantly (Guo *et al.*, 2009a). These observations also support the idea that growth control and stress responses share some of the same signaling pathways.

Plants harboring lesions in the CrRLK1L *FERONIA* exhibit more dramatic growth and developmental phenotypes. The *feronia/sirène* allele was first identified in a screen for mutants defective in female gametophyte function (Huck *et al.*, 2003; Rotman *et al.*, 2003). Subsequent studies revealed that, in addition to impairing synergid signaling-dependent pollen reception (Escobar-Restrepo *et al.*, 2007), loss of FER activity inhibits leaf expansion, reduces the stature of the inflorescence, and, significantly, strongly disrupts root hair growth (Duan *et al.*, 2010; Guo *et al.*, 2009b). Root hairs of *fer* mutants typically form bulges or burst soon after transitioning to the tip-growing phase (Duan *et al.*, 2010) and, interestingly, bursting defects have also been observed in pollen tubes of *anx1 anx2*, a double mutant in CrRLK1Ls closely related to *FER* and expressed primarily in pollen tubes (Lindner *et al.*, 2012). Similar root hair bursting in the ATRBOHC mutant *rhd2* was proposed to reflect an imbalance between cell wall loosening and cell wall stabilizing processes (Monshausen *et al.*, 2007); it is therefore tempting to speculate that FER, ANX1 and ANX2 play an important role in sensing large changes in the mechanical equilibrium of cell walls und initiate compensatory processes to maintain cell wall stability. In addition to possible defects in cell wall integrity maintenance, *fer* mutants also exhibit strongly altered responsiveness to plant hormones such as ethylene, brassinosteroids, auxin and abscisic acid (Deslauriers and Larsen, 2010; Duan *et al.*, 2010; Yu *et al.*, 2012). As no evidence has thus far been uncovered to indicate that FER functions as–or directly interacts with—a hormone receptor, this suggests the exciting possibility that impaired cell wall integrity sensing modulates plant sensitivity to a plethora of other developmental and environmental cues. However, it is also conceivable that FER is not directly involved in cell wall integrity sensing, but acts as a hub where multiple signaling pathways (mechanical, hormone, compatible interaction with pollen tubes and fungal hyphae (Kessler *et al.*, 2010)) intersect.

Ca^{2+} and Friends - Early Events in Mechanical Signal Transduction

While the identification of mechanoreceptors is an ongoing challenge, common themes are emerging for the early stages of mechanical signal transduction. Rapid ion fluxes, typically involving the ubiquitous second messenger Ca^{2+}, are associated not only with mechanoperception, but also subsequent signal transduction processes. Propagating electrical signals (action potentials; APs) are ideally suited for transmitting information quickly and over long distances. They are observed in plants with fast thigmonastic movements, such as *Mimosa* and carnivorous plants, where they link spatially separated sites of mechano-perception and -response (Escalante-Perez *et al.*, 2011; Fromm and Lautner, 2007; Sibaoka, 1991). Action potentials are initially triggered by membrane depolarization (receptor potential) in specialized mechanoreceptor cells. While the ionic basis of receptor potentials in vascular plants is unknown, in characean algae the receptor potential is thought to be generated by mechanically gated Ca^{2+}- and Ca^{2+}-dependent Cl^- currents (described above). Action potentials in higher plants appear to have a similar charge composition, with Ca^{2+} and/or Cl^- carrying the depolarizing current (the subsequent repolarization of the plasma membrane being achieved by K^+ efflux) (Sibaoka, 1991).

Ca^{2+} influx into the cytosol, mediated directly by mechanosensitive ion channels or by channels activated downstream of mechanoreceptors, is also commonly observed in mechanically perturbed cells of non-specialized plants (reviewed by Chehab *et al.*, 2009; Toyota and Gilroy, 2013; Trewavas and Knight, 1994). While the subcellular stores from which Ca^{2+} is released have not been unequivocally identified, recent studies suggest that $[Ca^{2+}]_{cyt}$ elevation requires influx from the extracellular space across the plasma membrane (Kurusu *et al.*, 2012a; Monshausen *et al.*, 2009; Richter *et al.*, 2009); subsequent mobilization of Ca^{2+} from intracellular pools could play a role in amplifying Ca^{2+} signals (Chehab *et al.*, 2009; Toyota and Gilroy, 2013). Interestingly, mechanically triggered Ca^{2+} changes exhibit pronounced stimulus- and tissue specificity. Highly localized touch perturbation elicits Ca^{2+} signals with spatiotemporal characteristics (Ca^{2+} signatures) very different from those induced by bending of a plant organ (Monshausen *et al.*, 2009), adjoining tissues of mechanically stimulated roots show distinct Ca^{2+} response kinetics (Richter *et al.*, 2009), and tensile strain appears much more effective in activating Ca^{2+} fluxes than compressive strain (Monshausen *et al.*, 2009; Richter *et al.*, 2009; Fig. 5). Such distinct Ca^{2+} signatures may not only have functional significance in specifying particular response patterns, but reflect the activation of specific subsets of mechanoreceptors (Monshausen, 2012; Monshausen *et al.*, 2009; Fig. 2)

In recent years, evidence has accumulated that extracellular and cytosolic pH changes are intimately connected with cellular Ca^{2+} signaling. Stress responses to osmotic shock, salt, cold

shock, heat and elicitors, as well as root responses to auxin are all associated with rapid Ca^{2+} transients accompanied by extracellular alkalinization (Felix *et al.*, 2000; Fellbrich *et al.*, 2000; Felle and Zimmermann, 2007; McAinsh and Pittman, 2009; Monshausen *et al.*, 2011; Zimmermann and Felle, 2009; Monshausen, unpublished data). In mechanically stimulated roots, extracellular alkalinization closely mimics the dynamics of $[Ca^{2+}]_{cyt}$ changes, which are required and sufficient for eliciting the pH response (Monshausen *et al.*, 2009; Fig. 5). Similar Ca^{2+}-dependent pH changes may eventually be discovered in APs of *Mimosa* and carnivorous plants, as there is strong evidence linking apoplastic pH elevation to heat- and salt stress-induced APs (Felle and Zimmermann, 2007; Zimmermann and Felle, 2009). While the molecular mechanism(s) underlying the extracellular pH increase remain to be defined, the concurrence of extracellular alkalinization and cytosolic acidification (Felle and Zimmermann, 2007; Monshausen *et al.*, 2009) and the inhibition of extracellular alkalinization by the anion channel inhibitor NPPB (Zimmermann and Felle, 2009) suggest that H^+ and/or OH^-/weak acid transport processes across the plasma membrane are involved. Intriguingly, a transient deactivation of the plasma membrane H^+-ATPase has also been observed in mechanically stimulated *Bryonia* internodes (Bourgeade and Boyer, 1994). Collectively, these data support the idea that modulation of extra- and intracellular pH is a key component of plant mechanical signal transduction.

Perhaps surprisingly, given the wealth of data linking Ca^{2+} to mechanical signaling, we still have a very incomplete understanding of how Ca^{2+} signals are translated into growth and developmental responses (Fig. 2). Direct evidence linking Ca^{2+} to plant thigmomorphogenesis is sparse. Ca^{2+} signaling is required for mechanical induction of lateral root formation (Richter *et al.*, 2009), but no intermediate steps in the signal transduction pathway have been identified. A maize Ca^{2+}-dependent protein kinase, ZmCPK11, is quickly activated by touch stimulation (Szczegielniak *et al.*, 2012), but its physiological role is unclear. Mutations in the putative Ca^{2+} sensor protein, CML24 (TCH2), lead to abnormal skewing and barrier responses of Arabidopsis roots (Tsai *et al.*, 2007; Wang *et al.*, 2011), but while *CML24* is known to be up-regulated in response to mechanical (and other) stresses, a direct role for CML24 in relaying Ca^{2+} signals to downstream targets has yet to be established (Braam and Davis, 1990; Tsai *et al.*, 2013).
Whether Ca^{2+}-dependent pH changes plays an important role in plant thigmomorphogenesis, as opposed to contributing to a general stress response (Felle and Zimmermann, 2007), is entirely unknown; however, there is at least some evidence that Ca^{2+}-dependent pH signaling – in conjunction with production of reactive oxygen species (ROS; Monshausen *et al.*, 2009; Yahraus *et al.*, 1995) - modulates short-term plant mechanoresponses. In root hairs, oscillatory pH and ROS fluctuations appear to regulate the rate of growth by alternately restricting and

promoting cell expansion at the root hair apex (Monshausen *et al.*, 2007; Monshausen *et al.*, 2008). How precisely this is achieved is unclear, but pH-dependent cell wall loosening and oxidative cross-linking of cell wall components are attractive options (Monshausen and Gilroy, 2009).

Exciting insights linking jasmonic acid (JA) to thigmomorphogenesis now open a promising new line of investigation. The rapid kinetics of jasmonic acid responses to mechanical perturbation are consistent with a role for JA in the early phases of signal transduction: JA levels can rise over 10-fold within 60 s of mechanical wounding (Glauser *et al.*, 2009), *Dionaea* leaves show significantly elevated 12-oxo-phytodienoic acid (OPDA; JA precursor) levels within 30 min of insect capture (Escalante-Perez *et al.*, 2011) and a single touch stimulus triggers increased JA synthesis within 30 min in Arabidopsis leaves (Chehab *et al.*, 2012). Furthermore, an elegant study describing mechanoresponses of JA-biosynthesis and -receptor mutants provides very strong evidence that at least a subset of thigmomorphogenetic responses (reduction in leaf expansion, inflorescence stem elongation, and delay in flowering) requires JA-production and –signaling (Chehab *et al.*, 2012). While a potential link between mechanically triggered Ca^{2+} signaling and JA production is still tenuous and rests primarily on reports of Ca^{2+} dependent JA elevation in heat-stressed potato leaves (Fisahn *et al.*, 2004), future experiments should extend these initial assays to rigorously test a possible role for JA in converting Ca^{2+} signals into developmental mechanoresponses.

Concluding Remarks

Plant responses to mechanical perturbation occur in a variety of specialized and non-specialized tissues and span a wide range of developmental time. Linking such responses to specific perception and signal transduction events has been difficult in the absence of well-characterized molecular pathways. However, the recent progress in the three main areas described here should help to elucidate commonalities and specificities in the ways plants experience and adjust to their mechanical environment. First, mechanosensitive channel activities are abundant in plant membranes, as is evidence for their importance in a variety of biological roles. Furthermore, as three distinct genes or gene families have been identified that are likely to underlie some of these activities, it has become possible to match electrophysiological activities with the genes and proteins that produce them and we anticipate that further efforts to combine the toolkits of patch-clamp electrophysiology and Arabidopsis molecular genetics will begin to shed light on the long-proposed role played by MS channels in the perception of mechanical stimuli. Second, potential candidates for cell wall integrity sensing

are also abundant. Receptor-like kinases are ideally suited to transmitting information from the cell wall environment to the cell interior and future studies should provide a clear link to mechanical signal transduction pathways. Establishing whether candidate RLKs are genuine mechanoreceptors that are activated by conformational change in response to a mechanical force, or whether they monitor cell wall stress by binding cell wall-derived ligands is an important goal for future research. Finally, physiological studies have positioned the second messenger Ca^{2+} at the center of many mechanical signal transduction pathways. Identifying the transporters shaping Ca^{2+} signatures and mediating other downstream ion fluxes is essential to our understanding of how Ca^{2+} signals are generated and interpreted and may provide tools to manipulate Ca^{2+}-dependent mechanoresponses. In summary, the future will likely bring many exciting new discoveries regarding the molecular mechanisms of mechanotransduction.

Acknowledgements

This work was supported by National Science Foundation grants MCB-1121994 (GBM) and MCB-1253103 (ESH) and National Institutes of Health grant R01 GM084211-01 (ESH). The authors would also like to acknowledge the members of their labs, past and present, for their many contributions to the work described here.

References

Adamec L. 2012. Firing and resetting characteristics of carnivorous *Utricularia reflexa* Traps: Physiological or only physical regulation of trap triggering? *Phyton-Annales Rei Botanicae* **52**, 281-290.

Alexandre J, Lassalles JP. 1991. Hydrostatic and osmotic pressure activated channel in plant vacuole. *Biophysical Journal* **60**, 1326-1336.

Anten NPR, Alcala-Herrera R, Schieving F, Onoda Y. 2010. Wind and mechanical stimuli differentially affect leaf traits in *Plantago major*. *New Phytologist* **188**, 554-564.

Arnadottir J, Chalfie M. 2010. Eukaryotic mechanosensitive channels. *Annual Review of Biophysics* **39**, 111-137.

Balleza D, Gomez-Lagunas F. 2009. Conserved motifs in mechanosensitive channels MscL and MscS. *European Biophysical Journal* **38**, 1013-1027.

Bastien R, Bohr T, Moulia B, Douady S. 2013. Unifying model of shoot gravitropism reveals proprioception as a central feature of posture control in plants. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 755-760.

Berrier C, Coulombe A, Szabo I, Zoratti M, Ghazi A. 1992. Gadolinium ion inhibits loss of metabolites induced by osmotic shock and large stretch-activated channels in bacteria. *European Journal of Biochemistry* **206**, 559-565.

Biddington NL. 1986. The effects of mechanically-induced stress in plants – a review. *Plant Growth Regulation* **4**, 103-123.

Boisson-Dernier A, Roy S, Kritsas K, Grobei MA, Jaciubek M, Schroeder JI, Grossniklaus U. 2009. Disruption of the pollen-expressed *FERONIA* homologs *ANXUR1* and *ANXUR2* triggers pollen tube discharge. *Development* **136,** 3279-3288.

Boonsirichai K, Guan C, Chen R, Masson PH. 2002. Root gravitropism: an experimental tool to investigate basic cellular and molecular processes underlying mechanosensing and signal transmission in plants. *Annual Review of Plant Biology* **53**, 421-447.

Booth IR, Blount P. 2012. The MscS and MscL families of mechanosensitive channels act as microbial emergency release valves. *Journal of Bacteriology* **194**, 4802-4809.

Bourgeade P, Boyer N. 1994. Plasma-membrane H^+-ATPase activity in response to mechanical stimulation of *Bryonia dioica* internodes. *Plant Physiology and Biochemistry* **32**, 661-668.

Bowling AJ, Vaughn KC. 2009. Gelatinous fibers are widespread in coiling tendrils and twining vines. *American Journal of Botany* **96**, 719-727.

Boyer JS. 2009. Cell wall biosynthesis and the molecular mechanisms of plant enlargement. *Functional Plant Biology* **36**, 383-394.

Braam J. 2005. In touch: plant responses to mechanical stimuli. *New Phytologist* **165**, 373-389.

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

Chaffey N. 2002. Why is there so little research into the cell biology of the secondary vascular system of trees? *New Phytologist* **153,** 213-223.

Chebli Y, Geitmann A. 2007. Mechanical principles governing pollen tube growth. *Functional Plant Science and Biotechnology* **1**, 232-245

Chehab EW, Eich E, Braam J. 2009. Thigmomorphogenesis: a complex plant response to mechano-stimulation. *Journal of Experimental Botany* **60**, 43-56.

Chehab EW, Yao C, Henderson Z, Kim S, Braam J. 2012. *Arabidopsis* touch-induced morphogenesis is jasmonate mediated and protects against pests. *Current Biology* **22**, 701-706.

Cheung AY, Wu HM. 2011. THESEUS 1, FERONIA and relatives: a family of cell wall-sensing receptor kinases? *Current Opinion in Plant Biology* **14**, 632-641.

Cosgrove DJ, Hedrich R. 1991. Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of *Vicia faba* L. *Planta* **186**, 143-153.

Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A. 2010. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* **330**, 55-60.

Coste B, Xiao B, Santos JS, Syeda R, Grandl J, Spencer KS, Kim SE, Schmidt M, Mathur J, Dubin AE, Montal M, Patapoutian A. 2012. Piezo proteins are pore-forming subunits of mechanically activated channels. *Nature* **483**, 176-181.

Coutand C. 2010. Mechanosensing and thigmomorphogenesis, a physiological and biomechanical point of view. *Plant Science* **179**, 168-182.

Cowles JR, Scheld HW, Lemay R, Peterson C. 1984. Growth and lignification in seedlings exposed to eight days of microgravity. *Annals of Botany* **54**, 33-48.

Darwin CR. 1875. Insectivorous Plants. *London: John Murray*.

Dejaegher G, Boyer N. 1987. Specific inhibition of lignification in *Bryonia dioica* - effects on thigmomorphogenesis. *Plant Physiology* **84**, 10-11.

Denness L, McKenna JF, Segonzac C, Wormit A, Madhou P, Bennett M, Mansfield J, Zipfel C, Hamann T. 2011. Cell wall damage-induced lignin biosynthesis Is regulated by a reactive oxygen species- and jasmonic acid-dependent process in *Arabidopsis*. *Plant Physiology* **156**, 1364-1374.

Deslauriers SD, Larsen PB. 2010. FERONIA Is a key modulator of brassinosteroid and ethylene responsiveness in *Arabidopsis* hypocotyls. *Molecular Plant* **3**, 626-640.

Ding JP, Pickard BG. 1993. Mechanosensory calcium-selective cation channels in epidermal cells. *The Plant Journal* **3**, 83-110.

Ditengou FA, Tealea WD, Kochersperger P, Flittner KA, Kneuper I, van der Graaff E, Nziengui H, Pinosa F, Li X, Nitschke R, Laux T, Palme K. 2008. Mechanical induction of lateral root initiation in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 18818-18823.

Duan Q, Kita D, Li C, Cheung AY, Wu HM. 2010. FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 17821-17826.

Dutta R, Robinson KR. 2004. Identification and characterization of stretch-activated ion channels in pollen protoplasts. *Plant Physiology* **135**, 1398-1406.

Eisenhoffer GT, Loftus PD, Yoshigi M, Otsuna H, Chien CB, Morcos PA, Rosenblatt J. 2012. Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. *Nature* **484**, 546-549.

Ellis J. 1770. Directions for bringing over seeds and plants from the East-Indies and other distant countries, in a state of vegetation. *London: Printed and sold by L. Davis*.

Ermakov YA, Kamaraju K, Sengupta K, Sukharev S. 2010. Gadolinium ions block mechanosensitive channels by altering the packing and lateral pressure of anionic lipids. *Biophysical Journal* **98**, 1018-1027.

Escalante-Perez M, Krol E, Stange A, Geiger D, Al-Rasheid KAS, Hause B, Neher E, Hedrich R. 2011. A special pair of phytohormones controls excitability, slow closure, and external stomach formation in the Venus flytrap. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 15492-15497.

Escobar-Restrepo JM, Huck N, Kessler S, Gagliardini V, Gheyselinck J, Yang WC, Grossniklaus U. 2007. The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception. *Science* **317**, 656-660.

Falke LC, Edwards KL, Pickard BG, Misler S. 1988. A stretch-activated anion channel in tobacco protoplasts. *FEBS Letters* **237**, 141-144.

Fasano JM, Massa GD, Gilroy S. 2002. Ionic signaling in plant responses to gravity and touch. *Journal of Plant Growth Regulation* **21**, 71-88.

Felix G, Regenass M, Boller T. 2000. Sensing of osmotic pressure changes in tomato cells. *Plant Physiology* **124**, 1169-1179.

Fellbrich G, Blume B, Brunner F, Hirt H, Kroj T, Ligterink W, Romanski A, Nurnberger T. 2000. Phytophthora parasitica elicitor-induced reactions in cells of *Petroselinum crispum*. *Plant and Cell Physiology* **41**, 692-701.

Felle HH, Zimmermann MR. 2007. Systemic signalling in barley through action potentials. *Planta* **226**, 203-214.

Firn RD, Digby J. 1979. A study of the autotropic straightening reaction of a shoot previously curved during geotropism. *Plant Cell and Environment* **2**, 149-154.

Fisahn J, Herde O, Willmitzer L, Peña-Cortés H. 2004. Analysis of the transient increase in cytosolic Ca^{2+} during the action potential of higher plants with high temporal resolution: requirement of Ca^{2+} transients for induction of jasmonic acid biosynthesis and PINII gene expression. *Plant and Cell Physiology* **45**, 456-459.

Fromm J, Lautner S. 2007. Electrical signals and their physiological significance in plants. *Plant Cell and Environment* **30**, 249-257.

Furuichi T, Iida H, Sokabe M, Tatsumi H. 2012. Expression of *Arabidopsis* MCA1 enhanced mechanosensitive channel activity in the *Xenopus laevis* oocyte plasma membrane. *Plant Signaling and Behavior* **7**, 1022-1026.

Furuichi T, Tatsumi H, Sokabe M. 2008. Mechano-sensitive channels regulate the stomatal aperture in *Vicia faba*. *Biochemical and Biophysical Research Communication* **366**, 758-762.

Garrill A, Tyerman SD, Findlay GP. 1994. Ion channels in the plasma membrane of protoplasts from the halophytic angiosperm *Zostera muelleri*. *Journal of Membrane Biology* **142**, 381-393.

Gish LA, Clark SE. 2011. The RLK/Pelle family of kinases. *The Plant Journal* **66**, 117-127.

Glauser G, Dubugnon L, Mousavi SAR, Rudaz S, Wolfender JL, Farmer EE. 2009. Velocity estimates for signal propagation leading to systemic jasmonic acid accumulation in wounded *Arabidopsis*. *Journal of Biological Chemistry* **284**, 34506-34513.

Goodbody KC, Lloyd CW. 1990. Actin filaments line up across *Tradescantia* epidermal cells, anticipating wound-induced division planes. *Protoplasma* **157**, 92-101.

Gross P, Julius C, Schmelzer E, Hahlbrock K. 1993. Translocation of cytoplasm and nucleus to fungal penetration sites is associated with depolymerization of microtubules and defense gene activation in infected, cultured parsley cells. *EMBO Journal* **12**, 1735-1744.

Guo H, Ye H, Li L, Yin Y. 2009a. A family of receptor-like kinases are regulated by BES1 and involved in plant growth in *Arabidopsis thaliana*. *Plant Signaling and Behavior* **4**, 784 - 786.

Guo HQ, Li L, Ye HX, Yu XF, Algreen A, Yin YH. 2009b. Three related receptor-like kinases are required for optimal cell elongation in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 7648-7653.

Haley A, Russell AJ, Wood N, Allan AC, Knight M, Campbell AK, Trewavas AJ. 1995. Effects of mechanical signaling on plant cell cytosolic calcium. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 4124-4128.

Hamann T. 2012. Plant cell wall integrity maintenance as an essential component of biotic stress response mechanisms. *Frontiers in Plant Science* **3**, 77.

Hamann T, Bennett M, Mansfield J, Somerville C. 2009. Identification of cell-wall stress as a hexose-dependent and osmosensitive regulator of plant responses. *The Plant Journal* **57**, 1015-1026.

Hamant O, Heisler MG, Jonsson 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.

Hashimoto K, Kudla J. 2011. Calcium decoding mechanisms in plants. *Biochimie* **93**, 2054-2059.

Haswell ES. 2007. MscS-Like Proteins in Plants. In: Hamill OP, ed. *Mechanosensitive Ion Channels, Part A*, Vol. 58, 329-359.

Haswell ES, Meyerowitz EM. 2006. MscS-like proteins control plastid size and shape in *Arabidopsis thaliana*. *Current Biology* **16**, 1-11.

Haswell ES, Peyronnet R, Barbier-Brygoo H, Meyerowitz EM, Frachisse JM. 2008. Two MscS homologs provide mechanosensitive channel activities in the *Arabidopsis* root. *Current Biology* **18**, 730-734.

Haswell ES, Phillips R, Rees DC. 2011. Mechanosensitive channels: what can they do and how do they do it? *Structure* **19**, 1356-1369.

Hedrich R. 2012. Ion channels in plants. *Physiological Review* **92**, 1777-1811.

Hejnowicz Z. 1980. Tensional stress in the cambium and its developmental significance. *American Journal of Botany* **67**, 1-5.

Hejnowicz Z, Rusin A, Rusin T. 2000. Tensile tissue stress affects the orientation of cortical microtubules in the epidermis of sunflower hypocotyl. *Journal of Plant Growth Regulation* **19**, 31-44.

Hematy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, Balzergue S, Pelletier S, Renou JP, Hofte H. 2007. A receptor-like kinase mediates the response of *Arabidopsis* cells to the inhibition of cellulose synthesis. *Current Biology* **17**, 922-931.

Hok S, Danchin EGJ, Allasia V, Panabieres F, Attard A, Keller H. 2011. An *Arabidopsis* (malectin-like) leucine-rich repeat receptor-like kinase contributes to downy mildew disease. *Plant Cell and Environment* **34**, 1944-1957.

Hooke R, Martyn J, Allestry. J. 1665. *Micrographia, or, Some physiological descriptions of minute bodies made by magnifying glasses :with observations and inquiries thereupon*. London, Printed by Jo. Martyn and Ja. Allestry

Huck N, Moore JM, Federer M, Grossniklaus U. 2003. The *Arabidopsis* mutant *feronia* disrupts the female gametophytic control of pollen tube reception. *Development* **130**, 2149-2159.

Humphrey TV, Bonetta DT, Goring DR. 2007. Sentinels at the wall: cell wall receptors and sensors. *New Phytologist* **176**, 7-21.

Iida H, Nakamura H, Ono T, Okumura MS, Anraku Y. 1994. MID1, a novel *Saccharomyces cerevisiae* gene encoding a plasma membrane protein, is required for Ca^{2+} influx and mating. *Molecular and Cellular Biology* **14**, 8259-8271.

Ishikawa H, Evans ML. 1993. The role of the distal elongation zone in the response of maize roots to auxin and gravity. *Plant Physiology* **102,** 1203-1210

Isnard S, Silk WK. 2009. Moving with climbing plants from Charles Darwin's time into the 21st century. *American Journal of Botany* **96**, 1205-1221.

Iwabuchi K, Kaneko T, Kikuyama M. 2005. Ionic mechanism of mechano-perception in characeae. *Plant and Cell Physiology* **46**, 1863-1871.

Jendretzki A, Wittland J, Wilk S, Straede A, Heinisch JJ. 2011. How do I begin? Sensing extracellular stress to maintain yeast cell wall integrity. *European Journal of Cell Biology* **90**, 740-744.

Jensen GS, Haswell ES. 2012. Functional analysis of conserved motifs in the mechanosensitive channel homolog MscS-Like2 from *Arabidopsis thaliana*. *PLoS ONE* **7**, e40336.

Kaneko T, Saito C, Shimmen T, Kikuyama M. 2005. Possible involvement of mechanosensitive Ca^{2+} channels of plasma membrane in mechanoperception in *Chara*. *Plant and Cell Physiology* **46**, 130-135.

Kaneko T, Takahashi N, Kikuyama M. 2009. Membrane stretching triggers mechanosensitive Ca^{2+} channel activation in *Chara*. *Journal of Membrane Biology* **228**, 33-42.

Kanzaki M, Nagasawa M, Kojima I, Sato C, Naruse K, Sokabe M, Iida H. 1999. Molecular identification of a eukaryotic, stretch-activated nonselective cation channel. *Science* **285**, 882-886.

Kessler SA, Shimosato-Asano H, Keinath NF, Wuest SE, Ingram G, Panstruga R, Grossniklaus U. 2010. Conserved molecular components for pollen tube reception and fungal invasion. *Science* **330**, 968-971.

Kim SE, Coste B, Chadha A, Cook B, Patapoutian A. 2012. The role of *Drosophila* Piezo in mechanical nociception. *Nature* **483**, 209-212.

Kloda A, Martinac B. 2002. Common evolutionary origins of mechanosensitive ion channels in Archaea, Bacteria and cell-walled Eukarya. *Archaea* **1**, 35-44.

Kohorn BD, Johansen S, Shishido A, Todorova T, Martinez R, Defeo E, Obregon P. 2009. Pectin activation of MAP kinase and gene expression is WAK2 dependent. *Plant Journal* **60**, 974-982.

Kohorn BD, Kobayashi M, Johansen S, Riese J, Huang L-F, Koch K, Fu S, Dotson A, Byers N. 2006. An Arabidopsis cell wall-associated kinase requird for inveratse activity and cell growth. *Plant Journal* **46,** 307-316

Kohorn BD, Kohorn SL. 2012. The cell wall associated kinases, WAKs, as pectin receptors. *Frontiers in Plant Science* **3,** 88.

Kung C. 2005. A possible unifying principle for mechanosensation. *Nature* **436**, 647-654.

Kurusu T, Iida H, Kuchitsu K. 2012a. Roles of a putative mechanosensitive plasma membrane Ca^{2+}-permeable channel OsMCA1 in generation of reactive oxygen species and hypo-osmotic signaling in rice. *Plant Signaling and Behavior* **7**, 796-798.

Kurusu T, Kuchitsu K, Nakano M, Nakayama Y, Iida H. 2013. Plant mechanosensing and Ca^{2+} transport. *Trends in Plant Science* **18,** 227-233.

Kurusu T, Nishikawa D, Yamazaki Y, Gotoh M, Nakano M, Hamada H, Yamanaka T, Iida K, Nakagawa Y, Saji H, Shinozaki K, Iida H, Kuchitsu K. 2012b. Plasma membrane protein OsMCA1 is involved in regulation of hypo-osmotic shock-induced Ca^{2+} influx and modulates generation of reactive oxygen species in cultured rice cells. *BMC Plant Biology* **12**.

Kurusu T, Yamanaka T, Nakano M, Takiguchi A, Ogasawara Y, Hayashi T, Iida K, Hanamata S, Shinozaki K, Iida H, Kuchitsu K. 2012c. Involvement of the putative Ca^{2+}-permeable mechanosensitive channels, NtMCA1 and NtMCA2, in Ca^{2+} uptake, Ca^{2+}-dependent cell proliferation and mechanical stress-induced gene expression in tobacco (*Nicotiana tabacum*) BY-2 cells. *Journal of Plant Research* **125**, 555-568.

Kutschera U. 1989. Tissue stresses in growing plant organs. *Physiologia Plantarum* **77**, 157-163.

Lally D, Ingmire P, Tong HY, He ZH. 2001. Antisense expression of a cell wall-associated protein kinase, WAK4, inhibits cell elongation and alters morphology. *Plant Cell* **13**, 1317-1331.

Lazzaro MD, Donohue JM, Soodavar FM. 2003. Disruption of cellulose synthesis by isoxaben causes tip swelling and disorganizes cortical microtubules in elongating conifer pollen tubes. *Protoplasma* **220**, 201-207.

Legue V, Blancaflor E, Wymer C, Perbal G, Fantin D, Gilroy S. 1997. Cytoplasmic free Ca^{2+} in *Arabidopsis* roots changes in response to touch but not gravity. *Plant Physiology* **114**, 789-800.

Levin DE. 2011. Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: The cell wall integrity signaling pathway. *Genetics* **189**, 1145-1175.

Lewis BD, Spalding EP. 1998. Nonselective block by La^{3+} of *Arabidopsis* ion channels involved in signal transduction. *Journal of Membrane Biology* **162**, 81-90.

Li Y, Moe PC, Chandrasekaran S, Booth IR, Blount P. 2002. Ionic regulation of MscK, a mechanosensitive channel from *Escherichia coli*. *EMBO Journal* **21**, 5323-5330.

Lindner H, Muller LM, Boisson-Dernier A, Grossniklaus U. 2012. CrRLK1L receptor-like kinases: not just another brick in the wall. *Current Opinion in Plant Biology* **15**, 659-669.

Lintilhac PM, Vesecky TB. 1981. Mechanical stress and cell wall orientation in plants. 2. The application of controlled directional stress to growing plants - with a discussion on the nature of the wound reaction. *American Journal of Botany* **68**, 1222-1230.

Liu K, Luan S. 1998. Voltage-dependent K^+ channels as targets of osmosensing in guard cells. *Plant Cell* **10**, 1957-1970.

Liu Y, Schieving F, Stuefer JF, Anten NPR. 2007. The effects of mechanical stress and spectral shading on the growth and allocation of ten genotypes of a stoloniferous plant. *Annals of Botany* **99**, 121-130.

Lucas Ml, Kenobi K, von Wangenheim D, Voß U, Swarup K, De Smet I, Van Damme Dl, Lawrence T, Péret B, Moscardi E, Barbeau D, Godin C, Salt D, Guyomarc'h S, Stelzer EHK, Maizel A, Laplaze L, Bennett MJ. 2013. Lateral root morphogenesis is dependent on the mechanical properties of the overlaying tissues. *Proceedings of the National Academy of Sciences of the United States of America*, in press

Lynch TM, Lintilhac PM. 1997. Mechanical signals in plant development: A new method for single cell studies. *Developmental Biology* **181**, 246-256.

Maksaev G, Haswell ES. 2012. MscS-Like10 is a stretch-activated ion channel from *Arabidopsis thaliana* with a preference for anions. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 19015-19020.

Malcolm HR, Elmore DE, Maurer JA. 2012. Mechanosensitive behavior of bacterial cyclic nucleotide gated (bCNG) ion channels: Insights into the mechanism of channel gating in the mechanosensitive channel of small conductance superfamily. *Biochemical and Biophysical Research Communication* **417**, 972-976.

Martinac B, Adler J, Kung C. 1990. Mechanosensitive ion channels of *E. coli* activated by amphipaths. *Nature* **348**, 261-263.

Matsui T, Omasa K, Horie T. 2000. Anther dehiscence in two-rowed barley (Hordeum distichum) triggered by mechanical stimulation. *Journal of Experimental Botany* **51**, 1319-1321.

Matsumoto S, Kumasaki S, Soga K, Wakabayashi K, Hashimoto T, Hoson T. 2010. Gravity-induced modifications to development in hypocotyls of *Arabidopsis* tubulin mutants. *Plant Physiology* **152**, 918-926.

McAinsh MR, Pittman JK. 2009. Shaping the calcium signature. *New Phytologist* **181**, 275-294.

McHugh BJ, Murdoch A, Haslett C, Sethi T. 2012. Loss of the integrin-activating transmembrane protein Fam38A (Piezo1) promotes a switch to a reduced integrin-dependent mode of cell migration. *PLoS ONE* **7**, e40346.

Meloche CG, Knox JP, Vaughn KC. 2007. A cortical band of gelatinous fibers causes the coiling of redvine tendrils: a model based upon cytochemical and immunocytochemical studies. *Planta* **225**, 485-498.

Miedema H, Henriksen GH, Assmann SM. 1999. A laser microsurgical method of cell wall removal allows detection of large-conductance ion channels in the guard cell plasma membrane. *Protoplasma* **209,** 58-67.

Monshausen GB. 2012. Visualizing Ca^{2+} signatures in plants. *Current Opinion in Plant Biology* **15**, 677-682.

Monshausen GB, Bibikova TN, Messerli MA, Shi C, Gilroy S. 2007. Oscillations in extracellular pH and reactive oxygen species modulate tip growth of *Arabidopsis* root haris.

Proceedings of the National Academy of Sciences of the United States of America **104**, 20996-21001.

Monshausen GB, Bibikova TN, Weisenseel MH, Gilroy S. 2009. Ca^{2+} regulates reactive oxygen species production and pH during mechanosensing in *Arabidopsis* roots. *Plant Cell* **21**, 2341-2356.

Monshausen GB, Gilroy S. 2009. Feeling green: mechanosensing in plants. *Trends in Cell Biology* **19**, 228-235.

Monshausen GB, Messerli MA, Gilroy S. 2008. Imaging of the Yellow Cameleon 3.6 indicator reveals that elevations in cytosolic Ca^{2+} follow oscillating increases in growth in root hairs of arabidopsis. *Plant Physiology* **147**, 1690-1698.

Monshausen GB, Miller ND, Murphy AS, Gilroy S. 2011. Dynamics of auxin-dependent Ca^{2+} and pH signaling in root growth revealed by integrating high-resolution imaging with automated computer vision-based analysis. *Plant Journal* **65**, 309-318.

Naismith JH, Booth IR. 2012. Bacterial mechanosensitive channels--MscS: evolution's solution to creating sensitivity in function. *Annual Review of Biophysics* **41**, 157-177.

Nakagawa Y, Katagiri T, Shinozaki K, Qi Z, Tatsumi H, Furuichi T, Kishigami A, Sokabe M, Kojima I, Sato S, Kato T, Tabata S, Iida K, Terashima A, Nakano M, Ikeda M, Yamanaka T, Iida H. 2007. Arabidopsis plasma membrane protein crucial for Ca^{2+} influx and touch sensing in roots. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 3639-3644.

Nakamura M, Kiefer CS, Grebe M. 2012. Planar polarity, tissue polarity and planar morphogenesis in plants. *Current Opinion in Plant Biology* **15**, 593-600.

Nakayama Y, Fujiu K, Sokabe M, Yoshimura K. 2007. Molecular and electrophysiological characterization of a mechanosensitive channel expressed in the chloroplasts of *Chlamydomonas*. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 5883-5888.

Nakayama Y, Yoshimura K, Iida H. 2012. Organellar mechanosensitive channels in fission yeast regulate the hypo-osmotic shock response. *Nature Communication* **3**, 1020.

Niklas KJ. 1998. The influence of gravity and wind on land plant evolution. *Review of Palaeobotany and Palynology* **102,** 1-14.

Nilius B. 2010. Pressing and squeezing with Piezos. *EMBO Report* **11**, 902-903.

Osanai T, Sato S, Tabata S, Tanaka K. 2005. Identification of PamA as a PII-binding membrane protein important in nitrogen-related and sugar-catabolic gene expression in *Synechocystis* sp. PCC 6803. *Journal of Biological Chemistry* **280**, 34684-34690.

Patterson MR. 1992. Role of mechanical loading in growth of sunflower (*Helianthus annuus*) seedlings. *Journal of Experimental Botany* **43**, 933-939.

Pennell R. 1998. Cell walls: structures and signals. *Current Opinion in Plant Biology* **1**, 504-510.
Pivetti CD, Yen MR, Miller S, Busch W, Tseng YH, Booth IR, Saier MH, Jr. 2003. Two families of mechanosensitive channel proteins. *Microbiology and Molecular Biology Reviews* **67**, 66-85.
Poppinga S, Hartmeyer SRH, Seidel R, Masselter T, Hartmeyer I, Speck T. 2012. Catapulting tentacles in a sticky carnivorous plant. *PLoS ONE* **7,** e45735.
Porter BW, Zhu YJ, Webb DT, Christopher DA. 2009. Novel thigmomorphogenetic responses in *Carica papaya*: touch decreases anthocyanin levels and stimulates petiole cork outgrowths. *Annals of Botany* **103**, 847-858.
Potocka I, Szymanowska-Pulka J, Karczewski J, Nakielski J. 2011. Effect of mechanical stress on *Zea root* apex. I. Mechanical stress leads to the switch from closed to open meristem organization. *Journal of Experimental Botany* **62**, 4583-4593.
Qi Z, Kishigami A, Nakagawa Y, Iida H, Sokabe M. 2004. A mechanosensitive anion channel in *Arabidopsis thaliana* mesophyll cells. *Plant and Cell Physiology* **45**, 1704-1708.
Qin SY, Hu D, Matsumoto K, Takeda K, Matsumoto N, Yamaguchi Y, Yamamoto K. 2012. Malectin forms a complex with ribophorin I for enhanced association with nisfolded glycoproteins. *Journal of Biological Chemistry* **287**.
Richter GL, Monshausen GB, Krol A, Gilroy S. 2009. Mechanical stimuli modulate lateral root organogenesis. *Plant Physiology* **151**, 1855-1866.
Rotman N, Rozier F, Boavida L, Dumas C, Berger F, Faure JE. 2003. Female control of male gamete delivery during fertilization in *Arabidopsis thaliana*. *Current Biology* **13**, 432-436.
Saidi I, Ammar S, Demont-Caulet N, Thevenin J, Lapierre C, Bouzid S, Jouanin L. 2009. Thigmomorphogenesis in *Solanum lycopersicum*: Morphological and biochemical responses in stem after mechanical stimulation. *Plant Science* **177**, 1-6.
Schroeder JI, Hedrich R. 1989. Involvement of ion channels and active transport in osmoregulation and signaling of higher plant cells. *Trends in Biochemical Science* **14**, 187-192.
Sequeira L. 1983. Mechanisms of induced resistance in plants. *Annual Review of Microbiology* **37**, 51-79.
Shimmen T. 1997. Studies on mechano-perception in characeae: Effects of external Ca^{2+} and Cl^{-}. *Plant and Cell Physiology* **38**, 691-697.
Shimmen T. 2006. Electrophysiology in mechanosensing and wounding response. In: Volkov AG, ed. *Plant electrophysiology theory and methods*. Berlin: Springer-Verlag
Sibaoka T. 1991. Rapid plant movements triggered by action potentials. *Botanical Magazine-Tokyo* **104**, 73-95.
Spalding EP, Goldsmith M. 1993. Activation of K^{+} channels in the plasma membrane of *Arabidopsis* by ATP produced photosynthetically. *Plant Cell* **5**, 477-484.

Staves MP. 1997. Cytoplasmic streaming and gravity sensing in *Chara* internodal cells. *Planta* **203**, S79-84.

Sukharev S, Sachs F. 2012. Molecular force transduction by ion channels: diversity and unifying principles. *Journal of Cell Science* **125**, 3075-3083.

Szczegielniak J, Borkiewicz L, Szurmak B, Lewandowska-Gnatowska W, Statkiewicz M, Klimecka M, Ciesla J, Muszynska G. 2012. Maize calcium-dependent protein kinase (ZmCPK11): local and systemic response to wounding, regulation by touch and components of jasmonate signaling. *Physiologia Plantarum* **146**, 1-14.

Telewski FW. 2006. A unified hypothesis of mechanoperception in plants. *American Journal of Botany* **93**, 1466-1476.

Toyota M, Gilroy S. 2013. Gravitropism and mechanical signaling in plants. *American Journal of Botany* **100**, 111-125.

Treat M. 1875. Plants that eat animals. *The Gardeners' Chronicle* **62**, 303-304.

Trewavas A, Knight M. 1994. Mechanical signalling, calcium and plant form. *Plant Molecular Biology* **26**, 1329-1341.

Tsai Y, N. D, Chowdhury N, Braam J. 2007. *Arabidopsis* potential calcium sensors regulate nitric oxide levels and the transition to flowering. *Plant Signaling and Behavior* **2**, 446 - 454.

Tsai Y-C, Koo Y, Delk NA, Gehl B, Braam J. 2013. Calmodulin-related CML24 interacts with ATG4b and affects autophagy progression in *Arabidopsis*. *The Plant Journal* **73**, 325-335.

Uyttewaal M, Burian A, Alim K, Landrein B, Borowska-Wykret D, Dedieu A, Peaucelle A, Ludynia M, Traas J, Boudaoud A, Kwiatkowska D, Hamant O. 2012. Mechanical stress acts via katanin to amplify differences in growth rate between adjacent cells in *Arabidopsis*. *Cell* **149**, 439-451.

Veley KM, Marshburn S, Clure CE, Haswell ES. 2012. Mechanosensitive channels protect plastids from hypoosmotic stress during normal plant growth. *Current Biology* **22**, 408-413.

Vogel V, Sheetz M. 2006. Local force and geometry sensing regulate cell functions. *Nature Reviews Molecular Cell Biology* **7**, 265-275.

Wagner TA, Kohorn BD. 2001. Wall-associated kinases are exprssed throughout plant development and are required for cell expansion. *Plant Cell* **13,** 303-318.

Wang YC, Wang BC, Gilroy S, Chehab EW, Braam J. 2011. CML24 is involved in root mechanoresponses and cortical microtubule orientation in *Arabidopsis*. *Journal of Plant Growth Regulation* **30**, 467-479.

Ward JM, Maser P, Schroeder JI. 2009. Plant ion channels: gene families, physiology, and functional genomics analyses. *Annual Review of Physiology* **71**, 59-82.

Wayne R. 1994. The excitability of plant cells - with a special emphasis on characean internodal cells. *Botanical Review* **60**, 265-367.

Wayne R, Staves MP. 1997. A down-to-earth model of gravisensing. *Gravitational and Space Biology Bulletin* **10**, 57-64.

Wick P, Gansel X, Oulevey C, Page V, Studer I, Durst M, Sticher L. 2003. The expression of the t-SNARE AtSNAP33 is induced by pathogens and mechanical stimulation. *Plant Physiology* **132**, 343-351.

Wilson ME, Jensen GS, Haswell ES. 2011. Two mechanosensitive channel homologs influence division ring placement in *Arabidopsis* chloroplasts. *Plant Cell* **23**, 2939-2949.

Wolf S, Hematy K, Hofte H. 2012. Growth control and cell wall signaling in plants. *Annual Review of Plant Biology* **63**, 381-407.

Wormit A, Butt SM, Chairam I, McKenna JF, Nunes-Nesi A, Kjaer L, O'Donnelly K, Fernie AR, Woscholski R, Barter MC, Hamann T. 2012. Osmosensitive changes of carbohydrate metabolism in response to cellulose biosynthesis inhibition. *Plant Physiology* **159**, 105-117.

Wymer CL, Wymer SA, Cosgrove DJ, Cyr RJ. 1996. Plant cell growth responds to external forces and the response requires intact microtubules. *Plant Physiology* **110,** 425-430.

Yahraus T, Chandra S, Legendre L, Low PS. 1995. Evidence for a mechanically induced oxidative burst. *Plant Physiology* **109**, 1259-1266.

Yamanaka T, Nakagawa Y, Mori K, Nakano M, Imamura T, Kataoka H, Terashima A, Iida K, Kojima I, Katagiri T, Shinozaki K, Iida H. 2010. MCA1 and MCA2 that mediate Ca^{2+} uptake have distinct and overlapping roles in *Arabidopsis*. *Plant Physiology* **152**, 1284-1296.

Yang XC, Sachs F. 1989. Block of stretch-activated ion channels in *Xenopus* oocytes by gadolinium and calcium ions. *Science* **243**, 1068-1071.

Yu F, Qian L, Nibau C, Duan Q, Kita D, Levasseur K, Li X, Lu C, Li H, Hou C, Li L, Buchanan BB, Chen L, Cheung AY, Li D, Luan S. 2012. FERONIA receptor kinase pathway suppresses abscisic acid signaling in *Arabidopsis* by activating ABI2 phosphatase. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 14693-14698.

Zarychanski R, Schulz VP, Houston BL, Maksimova Y, Houston DS, Smith B, Rinehart J, Gallagher PG. 2012. Mutations in the mechanotransduction protein PIEZO1 are associated with hereditary xerocytosis. *Blood* **120**, 1908-1915.

Zhang W, Fan LM, Wu WH. 2007. Osmo-sensitive and stretch-activated calcium-permeable channels in *Vicia faba* guard cells are regulated by actin dynamics. *Plant Physiology* **143**, 1140-1151.

Zimmermann MR, Felle HH. 2009. Dissection of heat-induced systemic signals: superiority of ion fluxes to voltage changes in substomatal cavities. *Planta* **229**, 539-547.

Table 1. Arabidopsis genes described in this review. NR = not reported.

I. Mechanosensitive Ion Channels					
Family	**Gene Name**	**Gene Locus**	**Mutant Phenotype**	**Subcellular Localization**	**Single Channel Characteristics**
Mid-1 Comple-menting Activity	*MCA1*	At4g35920	*mca1* mutants are less able to penetrate hard agar (Nakagawa *et al*., 2007), hyper-produce lignin; *mca1 mca2* double mutants are hypersensitive to $MgCl_2$ and show developmental delays (Yamanaka *et al.*, 2010).	Plasma membrane (Nakagawa *et al*., 2007)	~15 or ~35 pS conductance in Xenopus ooctyes (Furuichi *et al*., 2012)
	MCA2	At2g17780	*mca2* mutants show a reduction in Ca^{2+} uptake (Yamanaka *et al*., 2010); see *MCA1* entry.	Plasma membrane (Yamanaka *et al*., 2010)	NR
MscS-Like	*MSL1*	At4g00290	NR	NR	NR
	MSL2	At5g10490	*msl2* null mutants show defective leaf shape (Jensen and Haswell, 2011)*; msl2 msl3* double mutants have enlarged chloroplasts and enlarged, round	Plastid envelope (Haswell and Meyerowitz, 2006)	NR

			non-green plastids (Haswell and Meyerowitz, 2006); *msl2 msl3* double mutant chloroplasts exhibit multiple division rings (Wilson *et al*., 2010)		
	MSL3	At1g58200	*See MSL2 entry*	Plastid envelope (Haswell and Meyerowitz, 2006)	NR
	MSL4	At1g53470	*msl4 msl5 msl6 msl9 msl10* quintuple lacks a predominant MS channel activity in root protoplasts (Haswell, Peyronnet *et al.*, 2008)	NR	NR
	MSL5	At3g14810	see *MSL4* entry	NR	NR
	MSL6	At1g78610	see *MSL4* entry	NR	NR
	MSL7	At2g17000	NR	NR	NR
	MSL8	At2g17010	NR	NR	NR
	MSL9	At5g19520	see *MSL4* entry	Plasma membrane (Haswell, Peyronnet *et*	~45 pS in root protoplasts (Haswell, Peyronnet *et*

				al., 2008)	*al.*, 2008)
	MSL10	At5g12080	see *MSL4* entry	Plasma membrane (Haswell, Peyronnet *et al*., 2008)	~137 pS conductance in root protoplasts (Haswell, Peyronnet *et al*., 2008); ~100 pS conductance in Xenopus oocytes and a moderate preference for anions (Maksaev and Haswell, 2012)
Piezo		At2g48060	NR	NR	NR

II. Candidate RLK mechanosensors					
Family	**Gene Name**	**Gene Locus**	**Mutant Phenotype**	**Subcellular Localization**	**Ligands**
Wall-Associa-ted Kinases	*WAK2*	At1g21270	*wak2* mutants show reduced cell expansion under sugar limited conditions (Kohorn *et al*., 2006), loss of pectin-induced MAPK3 activation and loss of pectin-induced differential gene expression (Kohorn *et al.*, 2009). 35S-driven antisense expression targeting all WAKS apparently lethal (Wagner and	Plasma membrane (Kohorn and Kohorn, 2012)	Pectin (de-esterified, charged galacturonic acid backbone; Kohorn *et al*., 2009)

			Kohorn, 2001)		
CrRLK1L	*THE1*	At5g54380	*the1* single mutants have no obvious growth phenotype; *the1* partially rescues *prc1-1* growth defects and ectopic lignification in *the1 prc1-1* double mutant (Hematy *et al*., 2007) and shows reduced ROS production and lignification in response to isoxaben treatment (Denness *et al.*, 2011); *the1 herk1 herk 2 triple mutants* have shortened petioles and hypocotyls (Guo *et al.*, 2009a,b)	Plasma membrane (Hematy *et al.*, 2007)	NR
	HERK 1	At3g46290	*the1 herk1* double mutants have shortened petioles, *the1 herk1 herk2* triple mutants also have shortened hypocotyls (Guo *et al.*, 2009a,b)	Likely plasma membrane (Guo *et al*., 2009b)	NR
	HERK 2	At1g30570	*the1 herk1 herk2* triple mutants have shortened hypocotyls (Guo *et al*., 2009a,b)	NR	NR
	FER	At3g51550	*fer* null mutant have reduced leaf expansion,	Plasma membrane (Duan *et al.*,	NR

			shorter inflorescences (Guo *et al*., 2009b), and bursting/bulging root hairs (Duan *et al.*, 2010)	2010)	
	ANX1	At3g04690	*anx1 anx2* double mutants show bursting pollen tubes, failure to reach female gametophyte (Boisson-Dernier *et al*., 2009)	Preferential localization to plasma membrane of pollen tube tip (Boisson-Dernier *et al*., 2009)	NR
	ANX2	At5g28680	see *ANX1* entry	see *ANX1* entry	NR

Table Legend

Table 1. Arabidopsis genes described in this review. NR = not reported.

Figure legends

Figure 1. External and internal mechanical forces cause deformation (strain) of plant cells. When a plant organ is bent (*top*), cells on the convex side are stretched (experience positive strain) while cells on the opposite, concave side are compressed (negative strain). During rapid, turgor-driven cell expansion (*bottom*), local positive strain rates as high as 50% h^{-1} have been measured in the elongation zone of maize and Arabidopsis roots (Ishikawa and Evans, 1993; G. Monshausen and N. Miller, unpublished data).

Figure 2. Model of mechanosensing and signal transduction. Mechanosensor proteins are activated when they undergo a conformational change in response to a mechanical force. Ion channels such as MSLs and putative stretch-activated Ca^{2+}-permeable channels (SACC) such as MCA or piezo are gated by changes in membrane tension. Other mechanosensory proteins may be linked to intra- and/or extracellular tethers such as the cytoskeleton or glycosylated proteins and polysaccharides of the cell wall; mechanical forces acting on sensors through

these linkages could cause conformational changes by breaking or stabilizing intra- and intermolecular bonds (e.g. protein unfolding, catch bonds; Vogel and Sheetz, 2006). Receptor-like kinases with (putative) carbohydrate-binding domains are found among the CrRLK1L, WAKs, S-domain and lectin-like RLK subfamilies (Gish and Clark, 2011) and may transmit information about deformation of the cell wall to the cell interior via kinase-dependent phosphorylation of target proteins. Downstream targets could include transcription factors (TF-P) to regulate the expression of mechanoresponsive genes, or Ca^{2+} permeable channels (CC), which, in conjunction with SACC, would shape the specific signature of mechanically triggered Ca^{2+} signals. $[Ca^{2+}]_{cyt}$ changes are typically interpreted by the Ca^{2+} sensors calmodulin (CaM) and calmodulin-like proteins, Ca^{2+}-dependent protein kinases (CDPK) and calcineurin B-like proteins (CBL; Hashimoto and Kudla, 2011) or directly by target proteins harboring Ca^{2+}-binding motifs. Ca^{2+} signaling regulates the expression of (some) mechanoresponsive genes and may be linked to the biosynthesis of jasmonic acid, a key reglulator of plant thigmomorphogenesis. Ca^{2+} signaling also activates plasma membrane transport processes (e.g. NADPH-oxidase mediated ROS production, H^+/OH^- transport to alter apoplastic and cytosolic pH) that could rapidly alter cell wall extensibility. Mechanical stress may also directly disrupt cell wall pectate structure and weaken Ca^{2+}-pectate cross-bridges to promote cell wall remodeling (Boyer, 2009).

Figure 3. Simplified two-state models for the gating of mechanosensitive ion channels. In the in intrinsic model (**A**), the open state (that which conducts ions) of a mechanosensitive channel, indicated in grey, is favored by increased membrane tension, which leads to membrane thinning and/or to changes in the force exerted on the protein-lipid interface. Alternatively (**B**), the open state is favored by the opening of a ‘trapdoor” domain that is tethered to an elastic component of the cytoskeleton or cell wall (indicated by a black bar).

Figure 4. Single-channel patch-clamp analysis of mechanosensitive channels expressed in Xenopus oocytes. A thin glass pipette is used to puncture a Xenopus oocyte (indicated by the dashed box in (**A**), capturing a patch of membrane in the tip, as shown in panel (**B**). Negative pressure (suction) introduced through the pipette deforms the patch of membrane, increasing membrane tension and gating intrinsically mechanosensitive ion channels (**C**). A step-wise increase in current can be observed as individual channels present in the patch pipette open upon application of suction (**D**).

Figure 5. Ion signaling in roots in response to mechanical bending. (**A)** Arabidopsis root expressing the FRET-based Ca^{2+} biosensor yellow cameleon (YC) 3.6 (Monshausen *et al.*, 2009) is bent to the side with the help of a glass capillary. The position of the root tip (not in the

field of view) is outlined in blue below the left panel. Roots exhibit low resting $[Ca^{2+}]_{cyt}$ prior to bending (left) and a rapid increase in $[Ca^{2+}]_{cyt}$ after bending on the stretched (convex) side, but not the compressed (concave) side of the roots (right). (**B)** Kinetics of mechanically triggered $[Ca^{2+}]_{cyt}$ changes in root epidermal cells are echoed by kinetics of changes in extracellular pH monitored using the fluorescent pH sensor fluorescein conjugated to dextran (Monshausen *et al*., 2009).

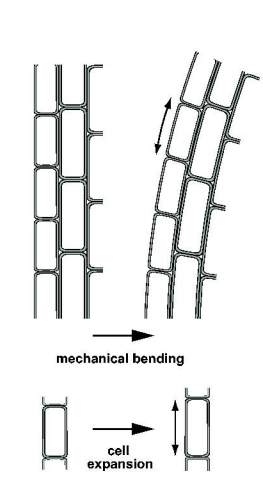

Figure 1. External and internal mechanical forces cause deformation (strain) of plant cells. When a plant organ is bent (*top*), cells on the convex side are stretched (experience positive strain) while cells on the opposite, concave side are compressed (negative strain). During rapid, turgor-driven cell expansion (*bottom*), local positive strain rates as high as 50% h-1 have been measured in the elongation zone of maize and Arabidopsis roots (Ishikawa and Evans, 1993; G. Monshausen and N. Miller, unpublished data)

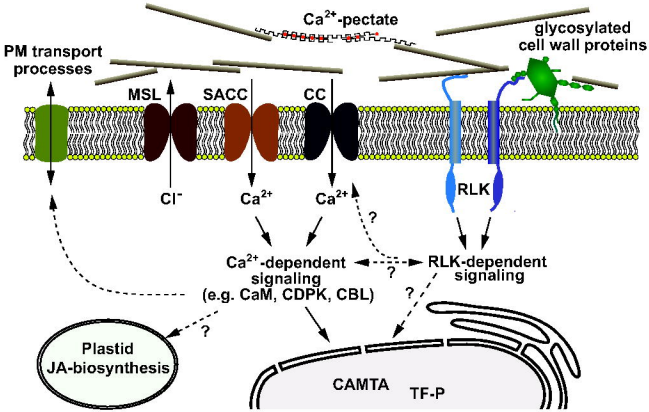

Figure 2. Model of mechanosensing and signal transduction. Mechanosensor proteins are activated when they undergo a conformational change in response to a mechanical force. Ion channels such as MSLs and putative stretch-activated Ca2+-permeable channels (SACC) such as MCA or piezo are gated by changes in membrane tension. Other mechanosensory proteins may be linked to intra- and/or extracellular tethers such as the cytoskeleton or glycosylated proteins and polysaccharides of the cell wall; mechanical forces acting on sensors through these linkages could cause conformational changes by breaking or stabilizing intra- and intermolecular bonds (e.g. protein unfolding, catch bonds; Vogel and Sheetz, 2006). Receptor-like kinases with (putative) carbohydrate-binding domains are found among the CrRLK1L, WAKs, S-domain and lectin-like RLK subfamilies (Gish and Clark, 2011) and may transmit information about deformation of the cell wall to the cell interior via kinase-dependent phosphorylation of target proteins. Downstream targets could include transcription factors (TF-P) to regulate the expression of mechanoresponsive genes, or Ca2+ permeable channels (CC), which, in conjunction with SACC, would shape the specific signature of mechanically triggered Ca2+ signals. [Ca2+]cyt changes are typically interpreted by the Ca2+ sensors calmodulin (CaM; and calmodulin-like proteins), Ca2+-dependent protein kinases (CDPK) and calcineurin B-like proteins (CBL; Hashimoto and Kudla, 2011) or directly by target proteins harboring Ca2+-binding motifs. Ca2+ signaling regulates the expression of (some) mechanoresponsive genes and may be linked to the biosynthesis of jasmonic acid, a key regulator of plant thigmomorphogenesis. Ca2+ signaling also activates plasma membrane transport processes (e.g. NADPH-oxidase mediated ROS production, H+/OH- transport to alter apoplastic and cytosolic pH) that could rapidly alter cell wall extensibility. Mechanical stress may also directly disrupt cell wall pectate structure and weaken Ca2+-pectate cross-bridges to promote cell wall remodeling (Boyer, 2009).

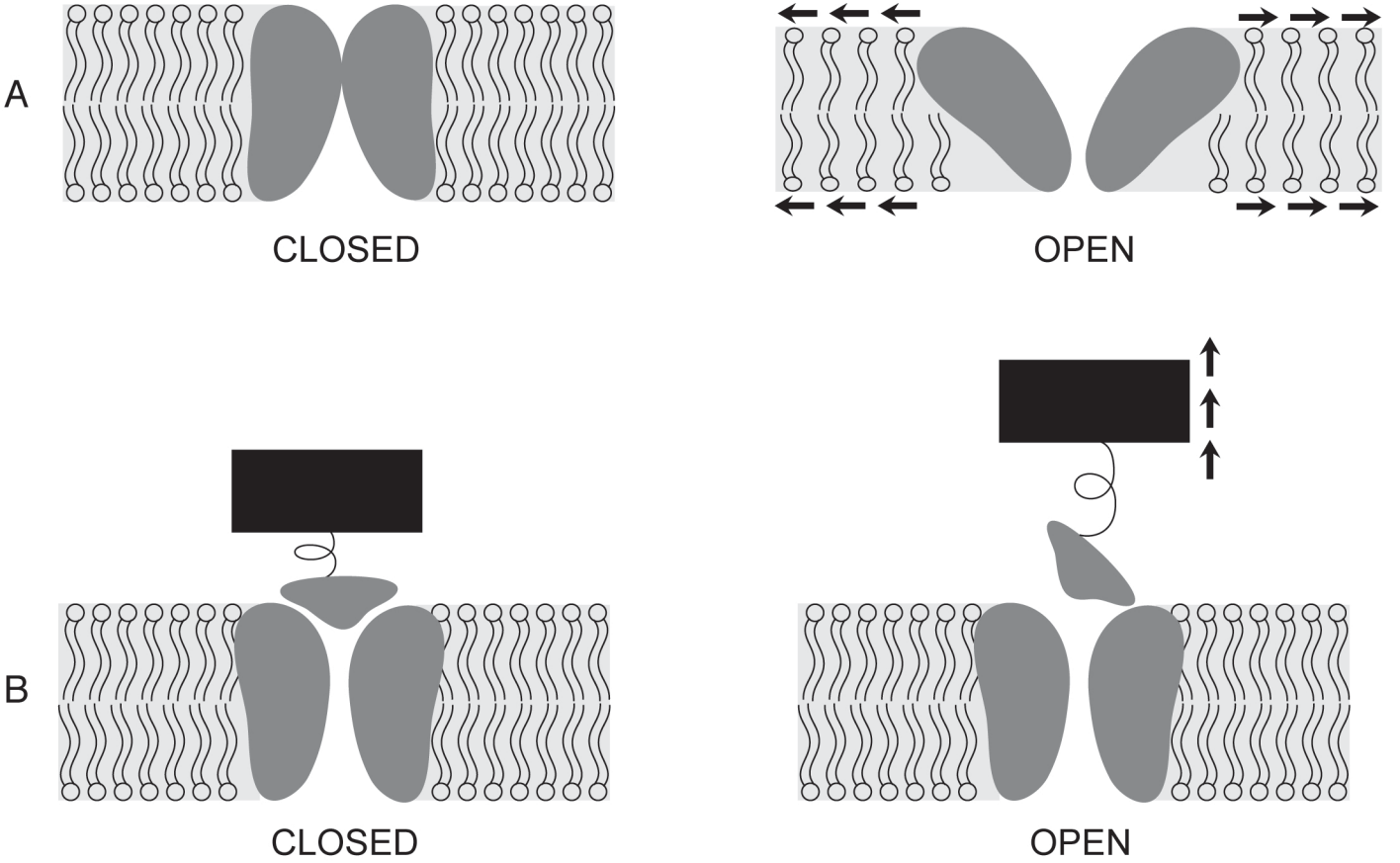

Figure 3. Simplified two-state models for the gating of mechanosensitive ion channels. In the in intrinsic model (**A**), the open state (that which conducts ions) of a mechanosensitive channel, indicated in grey, is favored by increased membrane tension, which leads to membrane thinning and/or to changes in the force exerted on the protein-lipid interface. Alternatively (**B**), the open state is favored by the opening of a 'trapdoor" domain that is tethered to an elastic component of the cytoskeleton or cell wall (indicated by a black bar).

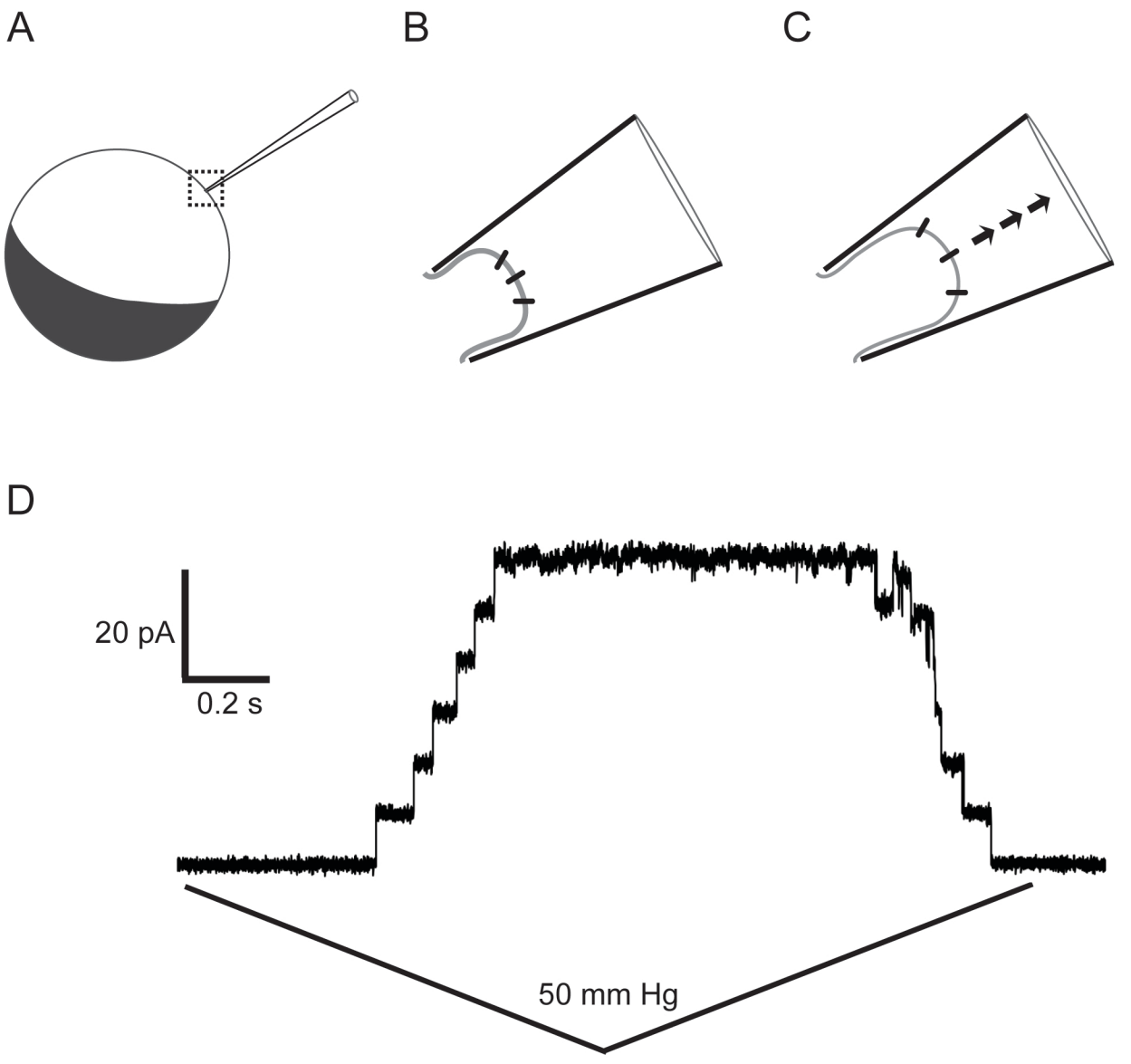

Figure 4. Single-channel patch-clamp analysis of mechanosensitive channels expressed in Xenopus oocytes. A thin glass pipette is used to puncture a Xenopus oocyte (indicated by the dashed box in (**A**)), capturing a patch of membrane in the tip, as shown in panel (**B**). Negative pressure (suction) introduced through the pipette deforms the patch of membrane, increasing membrane tension and gating intrinsically mechanosensitive ion channels (**C**). A step-wise increase in current can be observed as individual channels present in the patch pipette open upon application of suction (**D**).

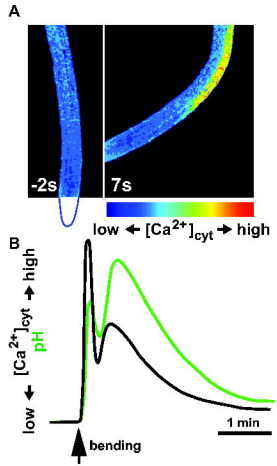

Figure 5. Ion signaling in roots in response to mechanical bending. **A**, Arabidopsis root expressing the FRET-based Ca2+ biosensor yellow cameleon (YC) 3.6 (Monshausen *et al*, 2009) was bent to the side with the help of a glass capillary. The position of the root tip (not in the field of view) is outlined in blue below the left panel. Roots exhibited low resting [Ca2+]cyt prior to bending (*left*) and a rapid increase in [Ca2+]cyt after bending on the stretched (convex) side, but not the compressed (concave) side of the roots (*right*). **B**, Kinetics of mechanically triggered [Ca2+]cyt changes in root epidermal cells are echoed by changes in extracellular pH monitored using the fluorescent pH sensor fluorescein conjugated to dextran (Monshausen *et al*, 2009).