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REVIEW PAPER

A force of nature: molecular mechanisms of mechanoperception in plants

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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 secondary messenger Ca^{2+} operates at the centre of many of these mechanical signal transduction pathways.

Key words: Calcium; cell-wall integrity; mechanoperception; mechanosensitive ion channels; receptor-like kinases; thigmomorphogenesis.

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* (Ellis, 1770; Darwin, 1875), *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 (Sibaoka, 1991; Adamec, 2012), snap tentacles, or adhesive emergences (Poppinga *et al.*, 2012). Typically, the deformation of mechanosensitive (MS) appendages triggers action potentials that propagate along symplastically connected, excitable cells and probably elicit turgor changes in responsive cells, resulting in fast nastic movements (Sibaoka, 1991).

Slower but no less complex movements of MS 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 fibres with a gelatinous cell-wall layer that appears to be important for the tightening of coiling organs around a support (Meloche *et al.*, 2007; Bowling and Vaughn, 2009).

Further investigations have revealed that it is not only specialized cells and organs that are sensitive to mechanical

Abbreviations: JA, jasmonic acid; MS, mechanosensitive; RLK, receptor-like kinase; TNP, trinitrophenol.

perturbation. In fact, the ability to perceive mechanical stress appears to be fundamental to all plant cells. Protoplasts (Haley *et al.*, 1995; Wymer *et al.*, 1996; Lynch and Lintilhac, 1997; Haswell *et al.*, 2008), suspension-cultured cells (Gross *et al.*, 1993; Yahraus *et al.*, 1995), meristematic, expanding, and fully differentiated cells of shoots and roots (e.g. Lintilhac and Vesecky, 1981; Braam and Davis, 1990; Legue *et al.*, 1997; Matsui *et al.*, 2000; Wick *et al.*, 2003; Ditengou *et al.*, 2008; Hamant *et al.*, 2008; Chehab *et al.*, 2009; Richter *et al.*, 2009; Coutand, 2010) have all 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; Dejaegher and Boyer, 1987; Niklas 1998; Patterson, 1992; Braam, 2005; Chehab *et al.*, 2009; Porter *et al.*, 2009; Saidi *et al.*, 2009; Coutand, 2010). 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 (Biddington, 1986; Liu *et al.*, 2007; Anten *et al.*, 2010).

Mechanical stresses are not just exerted by the environment 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 (Firn and Digby, 1979; Bastien *et al.*, 2013). 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 cell 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, at the shoot apical meristem (Hejnowicz *et al.*, 2000; Hamant *et al.*, 2008; 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 also to be receptive to the intrinsic mechanical constraints imposed by overlaying root tissues. The shape and emergence of lateral root primordia appears to be 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 overlying 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 also 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 periclinal cell divisions, the cambium and all peripheral tissues are displaced outwards. 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 this 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 probably experience both compressive and tensile stresses as they are pushed outwards 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 the growth control of all expanding cells.

Mechanisms of mechanoperception

While it is very clear that plant 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+}) signalling and other ion flux events. These gene products and the pathways in which they are thought to act are summarized in Fig. 2.

MS ion channels

A particularly well-studied mechanism for mechanoperception in bacterial and animal systems is the use of MS ion

Key terms

Action potential: a short-time event in which the transmembrane voltage of a cell rapidly depolarizes and repolarizes, following a consistent all-or-nothing pattern. Voltage changes are caused by ion fluxes across the membrane.

Deformation and conformational changes: forces are capable of deforming, or changing the configuration of, a body (e.g. a protein complex or a cell). This deformation can be the stretching of bonds and/or sliding/shearing of internal elements until a new internal and external mechanical equilibrium is achieved. The amount of deformation generated in response to a particular force is measured by strains (and strain rates), and the density of reaction forces (per unit area) by stresses. These internal reaction stresses are transmitted to the sites of external constraints, where they generate external reaction forces. If the body is stiff or rigid, then little strain will be necessary before a new configuration allowing equilibrium with the applied loads is achieved. However, if it is compliant or flexible, large strains are necessary.

Ion channel: a membrane-embedded protein complex that provides a pathway for the passive movement of ions from one side of a membrane to the other along their electrochemical gradient. A channel typically has two or more states. In the open state, ions to which the channel is permeable pass through the channel pore; in the closed state, ion flux ceases. The transition between the closed and open states is called gating. MS (also called stretch-activated or stretch-gated) ion channels are ion channels in which the open state is favoured in the presence of a mechanical load. MS ion channels thus transduce a mechanical signal into cellular ion flux.

Kinase: an enzyme that phosphorylates a substrate by transferring a phosphate group from a donor to an acceptor (substrate).

Orthologue: a gene arising from a common ancestral gene by speciation.

Paralogue: a gene arising from a gene duplication event.

Patch-clamp electrophysiology: a laboratory technique that records the current across a patch of membrane harbouring ion channels while the voltage across the same membrane is kept constant. A pressure clamp can be used to apply a controlled hydrostatic pressure to the membrane of the patch, increasing membrane tension and gating any resident mechanosensitive ion channels.

Strain: a measure of the relative deformation of a body. The overall deformation can be broken down into two basic types of strain: longitudinal strain, which is a change in length, and shear strain, which is a change in angle. Longitudinal strain is the relative change in length of a body, i.e. the ratio of lengthening or shortening displacement to the original length. Shear strain is the amount of angular deformation; it can be estimated as the ratio between the displacement and the perpendicular original length. Because it is always a ratio, strain is dimensionless, but is often stated as a percentage.

Strain rate: the rate of change of strain. The unit is s^{-1} but is often stated as percentage per time unit. Growth-induced strain rates are used to quantify expansion growth and its spatial distribution.

Stress: density of force per unit of cross-sectional area that develops within a structure in response to applied loads (unit: Pascal, Pa =N m^{-2}). The stress may be normal (changing the length in a structure) or shear (changing the angle in a structure). Normal stresses can be tensile or compressive. At a given location, stresses are usually acting over several directions at the same time, but all together they balance.

Thigmonasty: active motion of an organ or cell in response to physical contact. The direction of this motion is stereotypical and independent of the direction of the stimulus.

Transduction (from the Latin 'bring across'): signal transduction starts when an extracellular stimulus activates a cell receptor (e.g. a mechanosensitive channel). The signal is then transduced into a physiological response, typically via a cascade of intracellular events (with possible amplification, regulation, and cross-talk).

channels (reviewed by 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 by Ward *et al.*, 2009; Hedrich, 2012). Although 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, favouring 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 an 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, density of the medium, cell invasion by a pathogen, and rapid alteration in osmotic pressure, is integral to plant growth and development (many of these are mentioned in other papers in this volume). As many of these mechanical

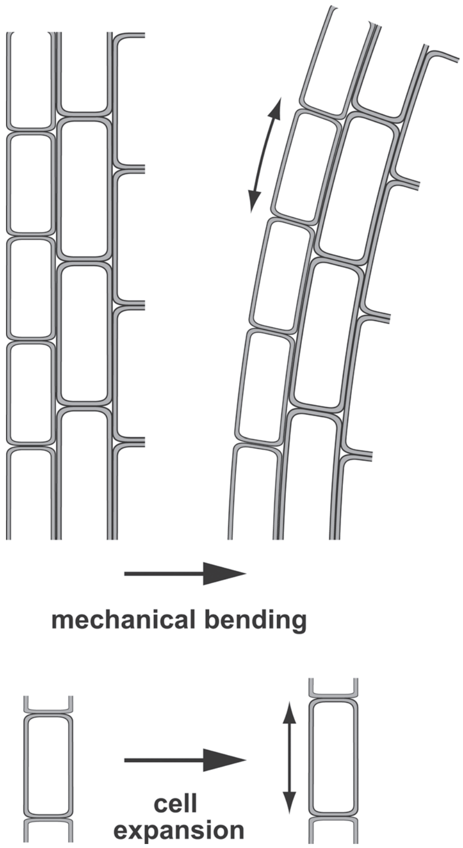

Fig. 1. External and internal mechanical forces cause deformation (strain) of plant cells. When a plant organ is bent (top), cells on the convex side are stretched (experience positive strain) while cells on the opposite, concave side are compressed (negative strain). During rapid turgor-driven cell expansion (bottom), local positive strain rates as high as 50–70% 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).

stimuli are associated with ion fluxes within the plant cell (reviewed below and by Trewavas and Knight, 1994; Fasano *et al.*, 2002; Kurusu *et al.*, 2013; Toyota and Gilroy, 2013), 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 by 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 endoplasmic reticulum 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 by 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: (i) physiological analyses involving the use of patch-clamp, ion imaging, vibrating probes, and other technologies to measure ion flux; and (ii) *Arabidopsis* molecular genetics. Through these complementary approaches, we have begun to gain insight into the abundance, distribution, channel characteristics, physiological function, and molecular identity of plant MS ion channels.

Electrophysiological studies

Pioneering studies of mechanotransduction measured the production of action and receptor potentials in giant algal cells such as *Chara* and *Nitella* (reviewed by Wayne, 1994; Shimmen, 2006). 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 dropping a glass rod onto a cell) (Shimmen, 1997). An MS Ca^{2+} channel may respond to touch, gravity, and osmotically induced membrane stretch in these 'plant-like' cells (Staves, 1997; Iwabuchi *et al.*, 2005; Kaneko *et al.*, 2005, 2009).

The advent of patch-clamp electrophysiology made possible the study of opening and closing of single MS (or 'stretch-activated') ion channels in plant membranes (Falke *et al.*, 1988; Schroeder and Hedrich, 1989). This technique is illustrated in Fig. 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 (Spalding and Goldsmith, 1993; Lewis and Spalding, 1998; Qi *et al.*, 2004; Haswell *et al.*, 2008), *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* (Schroeder and Hedrich, 1989; Cosgrove and Hedrich, 1991; Liu and Luan, 1998; 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

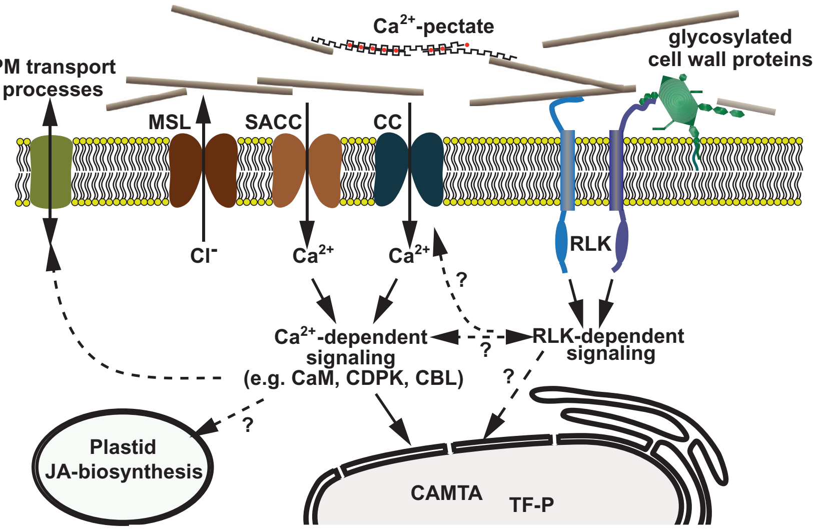

Fig. 2. Model of mechanosensing and signal transduction. Mechanosensor proteins are activated when they undergo a conformational change in response to a mechanical force. Ion channels such as MSLs and putative stretch-activated Ca^{2+}-permeable channels (SACC) such as MCA or Piezo are gated by changes 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) that, 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 (CDPKs), and calcineurin B-like proteins (CBLs) (Hashimoto and Kudla, 2011) or directly by target proteins harbouring Ca^{2+}-binding motifs. Ca^{2+} signalling 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+} signalling also activates plasma membrane transport processes (e.g. NADPH-oxidase mediated reactive oxygen species production or 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 remodelling (Boyer, 2009).

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 MS channels in animals and plants (Yang and Sachs, 1989; Alexandre and Lassalles, 1991; Ding and Pickard, 1993; Dutta and Robinson, 2004). Gd^{3+} will also inhibit non-selective MS channels, albeit at higher concentrations (Berrier *et al.*, 1992), by promoting membrane stiffness, thereby favouring 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 *A. thaliana* tubulin mutants (Matsumoto *et al.*, 2010), and guard-cell opening in *V. faba* is inhibited by TNP application (Furuichi *et al.*, 2008), implicating MS channels in both signal transduction pathways.

Molecular genetic studies

Although the studies described above established that MS ion channel activities are pervasive in plant membranes 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, although many more probably 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.

The MS channel of small conductance-like (MscS) family

The *m*echano*s*ensitive *c*hannel of *s*mall 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 hypo-osmotic shock (reviewed by Booth and Blount, 2012). MscS has served as an excellent model system for the study of MS channel structures, biophysical properties, and physiological

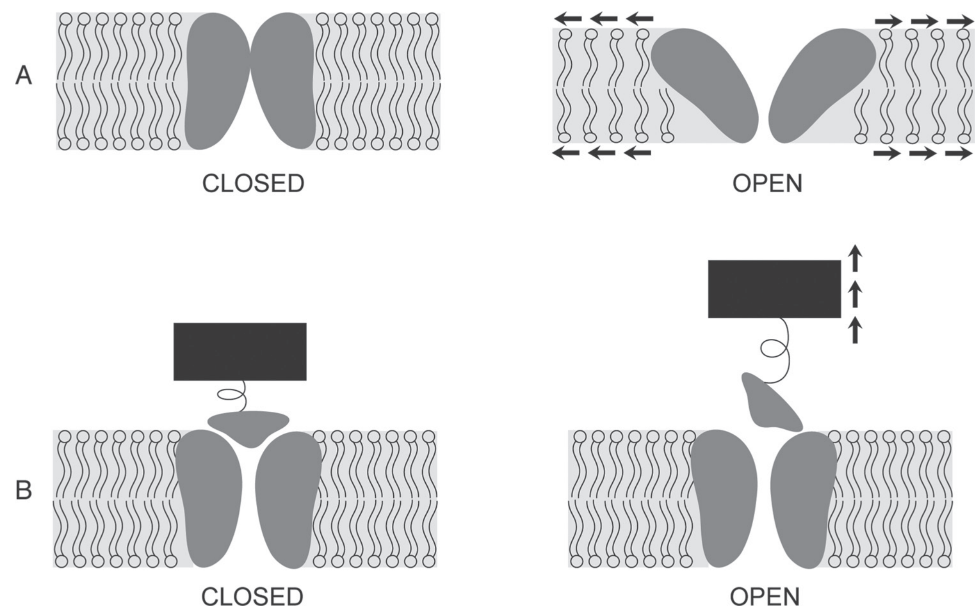

Fig. 3. Simplified two-state models for the gating of MS ion channels. In the intrinsic model (A), the open state (that which conducts ions) of an MS channel, shaded in grey, is favoured by increased membrane tension, which leads to membrane thinning and/or to changes in the force exerted on the protein–lipid interface. Alternatively (B), the open state is favoured 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).

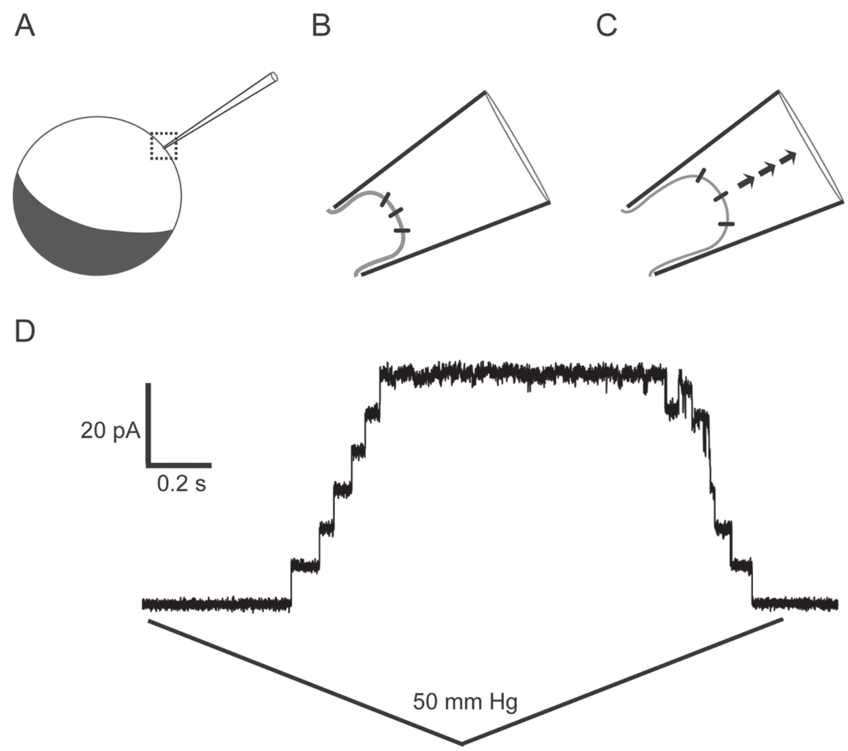

Fig. 4. Single-channel patch-clamp analysis of MS channels expressed in *Xenopus* oocytes. A thin glass pipette is used to puncture a *Xenopus* oocyte, indicated by the dashed box in (A), capturing a patch of membrane in the tip, as shown in (B). Negative pressure (suction) introduced through the pipette deforms the patch of membrane, increasing membrane tension and gating intrinsically MS 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) (E. Haswell and G. Maksaev, unpublished data).

functions (recently reviewed by Haswell *et al.*, 2011; Naismith and Booth, 2012), so the observation that genes encoding MscS homologues were not only found in the genomes of bacterial and archaeal species but also in the recently sequenced *A. thaliana* genome and in *Schizosaccharomyces pombe* (Kloda and Martinac, 2002; Pivetti *et al.*, 2003)

Table 1. Arabidopsis *genes described in this review*

I. Mechanosensitive ion channels					
Family	**Gene name**	**Gene locus**	**Mutant phenotype**	**Subcellular localization**	**Single-channel characteristics**
Mid-1 complementing activity	*MCA1*	At4g35920	*mca1* mutants are less able to penetrate hard agar (Nakagawa *et al.*, 2007), hyperproduce 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, 2012); *msl2 msl3* double mutants have enlarged chloroplasts and enlarged, round non-green plastids (Haswell and Meyerowitz, 2006); *msl2 msl3* double mutant chloroplasts exhibit multiple division rings (Wilson *et al.*, 2011)	Plastid envelope (Haswell and Meyerowitz, 2006)	NR
	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 al.*, 2008)	~45 pS in root protoplasts (Haswell, Peyronnet *et 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-associated 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 Kohorn, 2001)	Plasma membrane (Kohorn and Kohorn, 2012)	Pectin (de-esterified, charged galacturonic acid backbone; Kohorn *et al.*, 2009)

Table 1. Continued

II. Candidate RLK mechanosensors					
Family	**Gene name**	**Gene locus**	**Mutant phenotype**	**Subcellular localization**	**Ligands**
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 reactive oxygen species production and lignification in response to isoxaben treatment (Denness *et al.*, 2011); *the1 herk1 herk2* triple mutants have shortened petioles and hypocotyls (Guo *et al.*, 2009*a*,*b*)	Plasma membrane (Hematy *et al.*, 2007)	NR
	HERK1	At3g46290	*the1 herk1* double mutants have shortened petioles, *the1 herk1 herk2* triple mutants also have shortened hypocotyls (Guo *et al.*, 2009*a*,*b*)	Probably plasma membrane (Guo *et al.*, 2009*b*)	NR
	HERK2	At1g30570	*the1 herk1 herk2* triple mutants have shortened hypocotyls (Guo *et al.*, 2009*a*,*b*)	NR	NR
	FER	At3g51550	*fer* null mutant have reduced leaf expansion, shorter inflorescences (Guo *et al.*, 2009*b*), and bursting/ bulging root hairs (Duan *et al.*, 2010)	Plasma membrane (Duan *et al.*, 2010)	NR
	ANX1	At3g04690	*anx1 anx2* double mutants show bursting pollen tubes and 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

NR, not reported.

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 transmembrane helices (ranging from three to 12) 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 homologues in *Arabidopsis* (named MSL1–10), recent data indicate that the ability to assemble into mechanically gated channels 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 expressed heterologously in *Xenopus laevis* oocytes (Maksaev and Haswell, 2012; Fig. 4D). Several sequence motifs conserved among MscS homologues (summarized by Balleza and Gomez-Lagunas, 2009) are required for normal MSL2 function (Jensen and Haswell, 2012), suggesting that bacterial and plant MscS homologues employ similar gating mechanisms. However, the structures, topologies, and oligomeric states of plant MscS homologues remain to be determined experimentally; 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 genetics 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 homologues 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 homologue from *Chlamydomonas*, MSC1, is also required for chloroplast integrity and provides an MS channel activity that closely resembles that of MSL10 when expressed in giant *E. coli* spheroplasts (Nakayama *et al.*, 2007). The two MscS homologues from *S. pombe*, Msy1 and Msy2, localize to the endoplasmic reticulum 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 paralogues 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 homologues 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; Osanai *et al.*, 2005; Malcolm *et al.*, 2012). It is also possible that these channels have evolved a signalling 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.

The Mid1-complementing activity (MCA) family

Arabidopsis MCA1 is the founding member of the plant-specific *M*id1-*c*omplementing *a*ctivity (MCA) family of proteins (reviewed by 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 characterized previously in any system. MCA1 and close homologue MCA2 form homomeric complexes localized to the plasma membrane and endomembranes of plant cells (Nakagawa *et al.*, 2007; Kurusu *et al.*, 2012*a*,*b*,*c*). Two-electrode voltage clamping experiments on *X. laevis* ooctyes heterologously expressing MCA1 revealed increased whole-cell currents in response to hypo-osmotic 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 hypo-osmotic stress or mechanical stimulus in *Arabidopsis* protoplasts, *Arabidopsis* roots, cultured rice, tobacco, yeast, and mammalian cells (Nakagawa *et al.*, 2007; Yamanaka *et al.*, 2010; Kurusu *et al.*, 2012*b*,*c*). *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 signalling 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 (Hamann *et al.*, 2009; Denness *et al.*, 2011; 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, hypo-osmotic stress, and other mechanical stimuli.

Piezo proteins

There has been much excitement surrounding the identification of the Piezo channels, a family of MS cation channels first identified in mouse cells (Coste *et al.*, 2010, 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 transmembrane 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.

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 defence responses (Sequeira, 1983), it has become apparent that the cell wall is not only a target of cellular signalling but is also 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 known to alter membrane properties (Miedema *et al.*, 1999). However, an elegant series of experiments by Hématy and co-workers 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 (Humphrey *et al.*, 2007; Monshausen and Gilroy, 2009; Cheung and Wu, 2011). This idea was inspired by research on yeast, which established that monitoring cell-wall integrity is essential for 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*id2-*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 orthologues 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) harbouring 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.

WAK family

The *w*all-*a*ssociated *k*inase (WAK) subfamily of RLKs contains five closely related members with high sequence identity. WAKs have been shown to bind pectin tightly and appear to function as receptors for oligogalacturonic acids, the degradation products of pectin produced during wounding or pathogen attack; WAKs thus probably play a key role in plant defence responses (Kohorn and Kohorn, 2012). Antisense 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).

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 harbour 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 endoplasmic reticulum (Qin *et al.*, 2012). It is conceivable that the CrRLK1L malectin-like domains bind polysaccharides or glycoproteins of plant cell walls, although no such interaction has yet been demonstrated. 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 requires active signalling via a THE1-dependent pathway, consistent with a role for THE1 as a sensor for cell-wall damage (Hematy *et al.*, 2007; Denness *et al.*, 2011). 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.*, 2009*a*). These observations also support the idea that growth control and stress responses share some of the same signalling pathways.

Plants harbouring lesions in CrRLK1L *FERONIA* (*fer*) 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 signalling-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 (Guo *et al.*, 2009*b*; Duan *et al.*, 2010). 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 *Arabidopsis thaliana* respiratory burst oxidase homologue C (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 and 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 signalling 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 secondary messenger Ca^{2+}, are associated not only with mechanoperception but also with subsequent signal transduction processes. Propagating electrical signals (action potentials) 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 (Sibaoka, 1991; Fromm and Lautner, 2007; Escalante-Perez *et al.*, 2011). 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 MS ion channels or by channels activated downstream of mechanoreceptors, is also commonly observed in mechanically perturbed cells of non-specialized plants (reviewed by Trewavas and Knight, 1994; Chehab *et al.*, 2009; Toyota and Gilroy, 2013). While the subcellular stores from which Ca^{2+} is released have not been identified unequivocally, recent studies suggest that $[Ca^{2+}]_{cyt}$ elevation requires influx from the extracellular space across the plasma membrane (Monshausen *et al.*, 2009; Richter *et al.*, 2009; Kurusu *et al.*, 2012*a*); 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 to be 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 may also reflect the activation of specific subsets of mechanoreceptors (Monshausen *et al.*, 2009; Monshausen, 2012; Fig. 2)

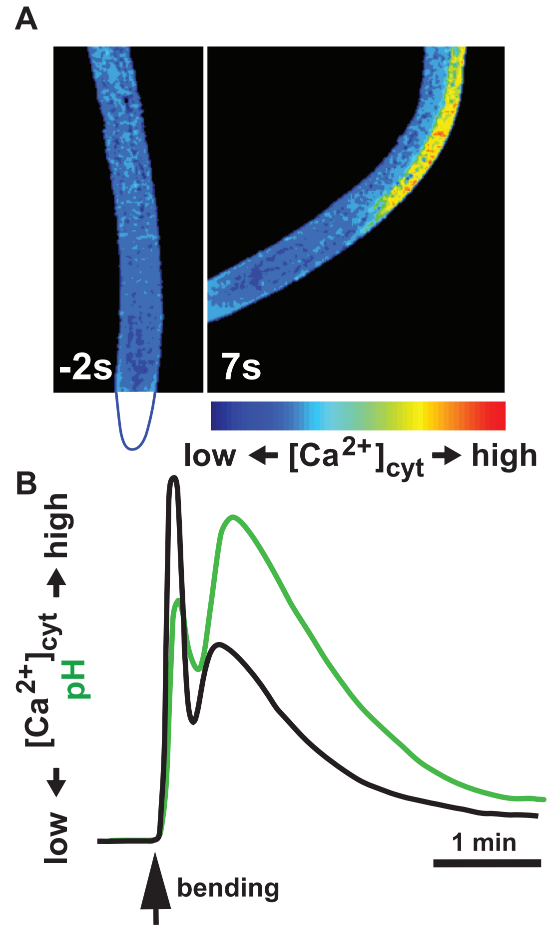

Fig. 5. Ion signalling in roots in response to mechanical bending. (A) *Arabidopsis* root expressing the FRET-based Ca^{2+} biosensor yellow cameleon 3.6 (Monshausen *et al.*, 2009) is bent to the side with the help of a glass capillary. The position of the root tip (not in the field of view) is outlined 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 the kinetics of changes in extracellular pH monitored using the fluorescent pH sensor fluorescein conjugated to dextran (based on Monshausen *et al.*, 2009).

In recent years, evidence has accumulated that extracellular and cytosolic pH changes are intimately connected with cellular Ca^{2+} signalling. 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; Zimmermann and Felle, 2009; Monshausen *et al.*, 2011; G.B. 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 action potentials of *Mimosa* and carnivorous plants, as there is strong evidence linking apoplastic pH elevation to heat and salt stress-induced action potentials (Felle and Zimmermann, 2007; Zimmermann and Felle, 2009). While the molecular mechanism(s) underlying the extracellular pH increase remains 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 5-nitro-2-(3-phenylpropyl-amino) benzoic acid (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 signalling, 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+} signalling 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 upregulated 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 play 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 signalling—in conjunction with production of reactive oxygen species (Yahraus *et al.*, 1995; Monshausen *et al.*, 2009)—modulates short-term plant mechanoresponses. In root hairs, oscillatory pH and reactive oxygen species fluctuations appear to regulate the rate of growth by alternately restricting and promoting cell expansion at the root hair apex (Monshausen *et al.*, 2007, 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 JA responses to mechanical perturbation are consistent with a role for JA in the early phases of signal transduction: JA levels can rise over tenfold within 60 s of mechanical wounding (Glauser *et al.*, 2009), *Dionaea* leaves show significantly elevated 12-oxo-phytodienoic acid (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 signalling (Chehab *et al.*, 2012). While a potential link between mechanically triggered Ca^{2+} signalling 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, 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, MS 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. Secondl, potential candidates for cell-wall integrity sensing are also abundant. RLKs 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 monitor cell-wall stress by binding cell-wall-derived ligands, is an important goal for future research. Finally, physiological studies have positioned the secondary messenger Ca^{2+} at the centre 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 probably bring many exciting new discoveries regarding the molecular mechanisms of mechanotransduction.

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