Feeling green: mechanosensing in plants

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Owing to the sessile nature of their lifestyle, plants have to respond to a wide range of signals, such as the force of the wind or the impedance of the soil, to entrain their development to prevailing environmental conditions. Indeed, mechanically responsive growth has been documented in plants for many years but new work on lateral root formation strongly supports the idea that biophysical forces can elicit complete de novo developmental programs. In addition, only recently have molecular candidates for plant mechanosensors emerged. Such advances in understanding plant mechanosensory development have relied heavily on comparison with mechanosensors characterized in organisms such as *Saccharomyces cerevisiae* and *Escherichia coli*, but key questions remain about the cellular basis of the plant mechanosensory system.

Mechanical forces and morphogenesis

Mechanical forces have a crucial role in plant morphogenesis, whether it be the sculpting of a tree by the wind, the twining of a tendril as a vine grows up a support, or the development of the root system as it navigates past rocks in the soil. In all these cases, mechanical sensing and response have dramatic effects on the final form a plant will adopt. Although remarkably detailed descriptions of these mechanical responses have been available for >100 years (e.g. in Darwin’s classic treatise on *The Power of Movement in Plants* from 1880 [1]), the molecular mechanisms behind these processes have begun to be unraveled only recently.

Plants have evolved two broad classes of mechanical response. The first is characterized by extremely rapid movements of organs in response to touch stimulation, as exemplified by the closing of the trap of the Venus flytrap (*Dionaea muscipula*) or the rapid folding of the leaflets of the sensitive fern (*Mimosa pudica*; Box 1). In both of these cases, rapid organ movements are linked to a highly specialized mechanosensory apparatus [2,3]. By contrast, a second broad class of mechanical response occurs in all plants over developmental time. Thus, plants subjected to mechanical stimulation tend to be shorter and more robust, develop more support tissues, and entrain their overall architecture to the prevailing mechanical forces in their vicinity.

Mechanical forces as morphogenetic factors

Although mechanical forces can clearly shape plant form through alterations in the growth habit of existing organs, they have also long been proposed to act as plant morphogenetic factors [4,5]. However, evidence for mechanical forces reprogramming development has been largely indirect. For example, microactivation of an inducible transgene was used to drive local production of the expansin proteins that support cell enlargement in the apical meristem of the plant. This treatment was shown to induce a developmental program that leads to leaf formation [6]. Such observations imply that alterations in the biomechanics of the apex might induce new organ formation. However, recent work on lateral root production provides evidence for a direct role of mechanical stimuli in such developmental reprogramming.

Lateral root formation in plants represents postembryonic organ formation whereby cells in the pericycle of the root are directed to a lateral root founder cell fate, undergo divisions and form a primordium that will develop into an emergent lateral root [7] (Figure 1a). Although periodic maxima in the levels of the hormone auxin could well pre-pattern the distribution of these founder cells, and although auxin is known to have a crucial role in subsequent lateral root development [8,9], the positioning of founder events is also highly entrained to the environment [10–12]. As early as 1900, Noll recognized that a major determinant of the positioning of the lateral roots was the physical architecture of the main root, with laterals emerging on the convex side of curves in the root system [13]. One explanation of this bend-related phenomenon [14] is that lateral root induction is being triggered by the redistribution of auxin that occurs as roots undergo directional (tropic) growth responses as they make the curves [15]. However, recent evidence indicates that biomechanics are actually responsible for reprogramming a pericycle cell to a founder cell fate. Thus, manually bending the root can elicit lateral root formation [16,17] (G.L. Richter, G.A.B., A. Krol and S.G., unpublished). However, it is important to note that manual bending represents an extreme stimulus that has the potential to elicit responses unrelated to normal plant development. Crucially, therefore, this same phenomenon can be generated by means of the endogenous forces of the root growing through soil. Thus, when a root encounters a barrier to growth such as a rock or a hardpan layer of soil, it adopts an avoidance response to circumnavigate the obstacle [18] (Figure 1b). The initial bending of the root to grow round the object is dominated by mechanical responses, and the curve that is elicited by the biophysical forces at play leads to lateral root formation to the convex side (i.e. the endogenous forces of growth can elicit bend-related organ formation) (Figure 1c). In this

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Mechanoreceptors in the leaf of *M. pudica* elicit an electrical, chemical or, possibly, hydraulic signal that moves to the leaflet base. Specialized motor organs (pulvini) release ions from their cells, causing a loss of internal hydrostatic pressure (turgor; for a review, see Ref. [2]). Within 1 s, water equivalent to up to 26% of the cell volume is lost via osmosis. Such rapid water movements are probably sustained by aquaporins or other water transporters in the plasma membrane of the pulvinar cells. The loss of turgor in these cells at the base of each leaflet causes the leaflet to very rapidly fold inwards (Figure 1). The larger the mechanical stimulus, the further the signal travels, leading to a progressive wave of leaflet movement. With sufficiently intense stimuli, the entire leaf can fold downwards. These movements are reversible, with recovery occurring as ions are pumped back into the pulvinar cells, with an accompanying uptake of water and reinstatement of turgor pressure. Pulvini have abundant H+ATPase activity, probably to sustain these ion fluxes. These rapid movements are thought to either startle potential herbivores or to make the plant seem less appetizing (for reviews, see Refs [2,3]).

Mechanical sensing and ion fluxes

There is much evidence from patch clamp analyses that the plasma membranes of plant cells contain a wide diversity of mechanosensitive ion channels [3]. However, none of these mechanosensitive conductances characterized electrophysiologically have to date been identified to the molecular level. There is also a wealth of evidence linking changes in ion fluxes (principally Ca2+ fluxes) to mechanoresponses at the whole plant level, with mechanical stimulation ranging from touch to wind disturbance (for a review, see Ref. [3]; Figure 2a,b), and possibly even to responses to gravity [19,20], leading to a transient increase in cytosolic Ca2+ levels.

Recent work has also highlighted a role for proton fluxes and reactive oxygen species (ROS) production in mechanosensation, both of which might contribute to linking Ca2+ signals to downstream developmental responses. Thus, suspension cells of *Taxus* (yew), parsley and soybean respond to mechanical stimulation with an oxidative burst and an alkalinization of the culture medium within 2–10 min [21–23], although the temporal resolution of these measurements could not precisely determine the onset of these ROS and pH changes at a cellular level. Intriguingly, similar changes have been observed in tip-growing cells such as pollen tubes and root hairs [24–26]. During tip growth, highly localized wall loosening processes are carefully balanced with restriction of turgor-driven expansion to sustain cell elongation. During peak expansion, relaxation of the cell wall will be accompanied by stretching of the tightly appressed plasma membrane; this is similar to the stretching that occurs during external mechanical deformation of the cell. Thus, it is not surprising that a burst in...
Ca²⁺, ROS and pH could all contribute to signal transduction, modulating the activity of membrane transporters and regulating gene transcription, the accompanying alterations in extracellular ROS and alkalinization are likely to directly impact cell wall rigidity and, therefore, growth by promoting the formation of intermolecular cross-links (e.g. see Refs [29,30]; Figure 2c).

The mechanoreceptor in plants
Despite such evidence for a central role of Ca²⁺-dependent mechanical signaling in plants, there are at present no molecularly identified mechanoreceptive receptors or channels supporting these changes. Indeed, a paucity of obvious molecular candidates for a mechanically sensitive Ca²⁺ channel in plant genome sequences has meant that the search for the plant mechanoreceptive receptor has largely revolved around models of mechanoreceptive elements defined in other kingdoms.

Models for plant mechanosensation I: the role of channels
Although a variety of mechanosensitive channels have been identified across a broad range of organisms, only the mechanosensitive channels of bacteria have so far proven to be a useful model for potential plant mechanoperception. For example, the transient receptor potential (TRP) channels of animal cells (and the yeast TRPY homolog) [31,32], the DEG/ENaC voltage-independent Na⁺ channel family [31,33], and the TREK K⁺ channel family [32] are all strong candidates for mechanosensitive channels in a range of cell types. However, there seem to be no clear homologs of these mechanosensitive channels in any of the currently sequenced plant genomes. Fortunately, the bacterial mechanoreceptive channel of small conductance (MscS) has proven an important starting point for identifying at least one family of plant mechanosensor candidates.

The MSL gene family
The mechanosensitive channels of small (MscS) and large (MscL) conductance represent osmotic safety valves for bacteria [34]. When the bacterium encounters a sudden drop in the osmotic strength of its environment, the channels open, enabling the efflux of solutes to prevent cellular bursting. The channels are formed of multimers with an iris-like pore, which is opened by the increasing tension in the membrane as the cell begins to swell (Figure 3a,b). There are six homologs of the MscS channels in the rice genome and ten MscS-like (MSL) genes in Arabidopsis [35] but, to date, no clear mechanoresponsive whole plant phenotype has been found in MSL knockout mutants generated in Arabidopsis. However, MSL2 and MSL3 are localized to the plastids where they have a redundant role in plastid division, as does their ortholog MSc in Chlamydomonas [35]. These observations are consistent with a conserved role for MscS in the division of the endosymbiotic progenitors of the chloroplast.

Of the other eight Arabidopsis MSL genes [35], MSL9 and MSL10 have received most attention to date. The proteins are found mainly in the plasma membrane of root cells. They are expressed in an overlapping but not identical pattern, with both appearing in cortical cells [36]. An
electrophysiological analysis of protoplasts derived from the root cortex showed that the major mechanoresponsive channel activity in wild-type plants was most probably a Cl⁻ conductance [36]. This activity was largely lost in the msl9/msl10 double mutant background. Residual mechanosensitive channel activity was attributed to MSL4–6, which are also expressed in roots and show ~40% amino acid identity to MSL9 and MSL10 (which themselves are 75% identical). A quintuple knockout of all these MSL genes abolished all mechanosensitive channel activity [36]. MSL9 and MSL10 are thought to form the core of a multimeric channel, as inferred from the effects of knock-out mutants in each gene on mechanosensitive channel conductance [36] (Figure 3c), consistent with the known subunit structure of MscS [34].

The MSL gene family provides some very strong candidates for mechanoresponse elements in plants. However, elucidating whether or not these proteins represent mechanosensitive channels or accessory proteins that are required for mechanoresponse will require an analysis of heterologously expressed protein. At present, we can say they are required to facilitate mechanosensitive gating of a probably Cl⁻-permeable channel in the plasma membrane [36]. The physiological function of these proteins also remains enigmatic. Apart from the intriguing role in plastid division of msl2 and msl3 mutants, knockouts in the other MSL genes have no overt phenotype. Even the quintuple mutant of all root-expressed MSL genes (msl4/msl5/msl6/msl9/msl10) shows no obvious disruption of development and seems to respond as much as the wild type to osmotic, salt, mechanical, dehydration and rehydration stresses [36].

**MCA1 – a MID1 homolog from plants?**

Another candidate for a component of a plant mechanosensory channel has come from functional complementation of the *Saccharomyces cerevisiae* MID1 mutant with plant cDNAs. *MID1* mutants lack a component of a yeast stretch-activated, Ca²⁺-permeable channel complex. Nakagawa and colleagues obtained an *Arabidopsis* clone named MCA1 that partially complemented the *mid1* phenotype [37]. The predicted protein shares only 10% identity and 41% similarity to MID1. It also has no obvious homology to known channel components and seems unlikely to be acting in the same fashion as the yeast protein. However, MCA1 was shown to increase Ca²⁺ uptake in yeast, thereby indicating that it does have a link to Ca²⁺ homeostasis and potentially explaining its partial complementation of the *mid1* mutant.

A knockout mutant of MCA1 showed a reduced ability of its roots to penetrate a layer of hard agar, suggesting some defect in either growth or mechanical responsiveness. However, constitutive MCA1 overexpressing lines exhibited more obvious defects in development, with short stems, small rosettes, no petals and shrunken seed pods. These plants also showed an increased basal Ca²⁺ uptake and an elevated cytosolic Ca²⁺ level in response to osmotic shock that was not evident in wild-type plants. Similarly, when MCA1 was heterologously expressed in Chinese hamster ovary cells, a novel Ca²⁺ increase could be elicited upon stretching the cells [37].

Thus, MCA1 seems to provide a possible link between Ca²⁺ fluxes and mechanical response in *Arabidopsis*, although its precise role remains enigmatic. It might be a regulatory component of a mechanosensitive channel complex conserved between yeast and plants. The evidence for a link to mechanoresponse is tantalizing. For example, the gene *TCH3* (*CML11*) encodes a Ca²⁺-dependent protein that has been closely linked to touch response in *Arabidopsis* [38]. This gene is upregulated in *MCA1*-overexpressing lines, indicating that touch sensing is constitutively activated in these lines. However, as a note of caution, *TCH3* expression is also responsive to environmental stimuli such as darkness and to developmental regulation and so its altered levels could simply reflect the disruption of growth that accompanies MCA1 overexpression.

**Models for plant mechanosensation II: the role of the wall**

Although the plasma membrane is the primary interface between the living protoplast and the external environment and transduces many environmental cues into phys-
iological responses, an external mechanical perturbation will first act on the plant cell wall encasing the protoplast and cause cell-wall deformation. Because the large hydrostatic pressure of 2–50 atmospheres exerted by the plant protoplast (turgor) presses the plasma membrane against the wall, any such deformation will immediately be conveyed to the plasma membrane. The relaxation in the wall that occurs as plant cells expand will lead to similar wall stresses and probably activate similar monitoring systems as external mechanical stimulation. Such cell-wall surveillance systems might monitor wall polymer status directly or act through the accompanying secondary deformation of the plasma membrane and associated cytoskeleton. No clear candidate sensor for monitoring cell-wall deformation has yet been identified in plants, but mechanical signaling via the extracellular matrix (ECM) in other kingdoms again provides insight into some of the general mechanisms likely to underlie such processes.

In the ECM of animal cells, large multimodular proteins such as the ECM adhesion glycoprotein fibronectin are thought to unravel stepwise upon exposure to mechanical forces, revealing previously hidden catalytic domains or recognition sites. The number and type of newly exposed sites could thus encode information about the magnitude and localization of mechanical load acting on the protein [39,40]. No proteins of similar architecture have been identified in proteomic analyses of the plant cell wall, but the intercalated polysaccharide and glycoprotein networks, which form the structural composite of the cell wall, could potentially serve a similar function by presenting formerly occupied binding sites to cell surface receptors upon mechanical disruption.

In animals, integrins have a vital role as surface adhesion receptors by transmitting mechanical deformation of the ECM to the cytoskeleton and/or cell interior [41]. The idea that integrin-like proteins also mediate mechanosensation in plants arose from the observation that treatment with the integrin-binding tripeptide RGD altered growth of soybean root suspension cells and inhibited mechanical signaling in internodal cells of the green alga Chara [42,43]. Seemingly substantiating evidence was provided by reports that a polyclonal antibody against the avian β1 integrin subunit recognized a protein in Arabidopsis and Chara membranes [44,45], but neither the Arabidopsis nor rice genomes seem to contain a true integrin homolog. However, intriguingly, further studies continued to accumulate support for the involvement of an RGD-like recognition system in physically connecting the plasma membrane to the cell wall and in sensing mechanical stress. Thus, during plasmolysis of Arabidopsis suspension or onion epidermal cells, formation of cytoplasmic Hechtian strands linking the plasma membrane of the shrinking protoplast to wall attachment sites was abolished by RGD treatment [46,47]. In addition, regenerating protoplasts normally show oriented division in response to unidirectional compressive forces [48] but division became randomized after pre-treatment with RGD [49]. RGD also interfered with induction of defense responses elicited by fungal penetration [47] and with mechanical signaling of Taxus suspension cells exposed to shear stress [23]. Although the plant RGD-recognition system(s) remains unknown, an exciting recent investigation identified eight receptor-like kinases (RLKs) as potential RGD-binding proteins [50]. One of the RLKs contained an extracellular lectin-like domain that bound RGD-containing peptides in vitro [50]. Although the function of this lectin RLK remains to be elucidated, the cytosolic kinase domain of the protein was predicted to be active, making the protein an attractive candidate for signaling changes in cell wall status to the cell interior.

The receptor-like wall-associated kinases (WAKs) and WAK-like kinases (WAKLs) are similarly positioned to communicate cell wall perturbations to the cytoplasm [51]. WAKs consist of a highly conserved cytoplasmic kinase domain, which is linked to the more variable extracellular domain via a single transmembrane region. The external domain interacts with glycan-rich proteins of the cell wall and binds tightly to pectins [52,53]. Intriguingly, the affinity of the recombinant extracellular subdomain WAK147–254 for pectins (polygalacturonic acid) was greatly enhanced by desterification of pectins and the presence of Ca²⁺, both of which promote the formation of intermolecular Ca²⁺ bridges to cross-link pectins in the cell wall [54]. If, as Decreux and Messiaen suggest [54], subtle changes in pectin architecture result in differential WAK binding, this would provide a potential mechanism for monitoring cell wall perturbations. WAKs are upregulated upon exposure to various biotic and abiotic stresses such as pathogens, wounding and Al³⁺ toxicity [55], but have thus far not been implicated in mechanical stress responses. However, WAKs are expressed in cells undergoing expansion, and a reduction in WAK levels leads to a reduction in leaf size and to shorter roots [51,55]. Because cell expansion requires a constant remodeling of the cell wall [56], it will be interesting to elucidate the relationship between growth-associated (and mechanically triggered) changes in pectin organization and WAK-dependent signaling.

In S. cerevisiae, the two sensors WISC1 and MID2 monitor cell wall status during vegetative cellular expansion or the formation of a mating projection, respectively. Structurally, MID2 and WISC1 share many features: their C-terminal cytoplasmic domains both interact with the ROM2 guanosine-nucleotide-exchange factor to elicit Rho1 and protein-kinase-C-dependent signal transduction events, both possess a single transmembrane domain and both extend O-glycosylated rod-like extracellular domains into the periplasmic space. This extracellular domain presumably interacts with the cell wall via an N-terminal glycan (MID2) or cysteine-rich region (WISC1) [57,58].

Although no homologs of the yeast WISC and MID2 sensors have been found in the Arabidopsis genome, THE-SEUS1 (THE1) has recently emerged as an exciting candidate for an analogous cell wall integrity sensor in plants [59,60]. THE1, a RLK expressed in elongating cells and the vasculature, was identified in a suppressor screen of the cellulose synthase mutant cesA6prc1-1, which shows short hypocotyls and accumulates ectopic lignin when grown in the dark [60]. These phenotypes were partially rescued in the cesA6prc1-1/the1 double mutant, although, importantly, the cellulose biosynthesis deficiency of cesA6prc1-1 was not restored. These results indicate that the reduced cell expansion of some cellulose-deficient mutants is not simply
caused by an inability to elongate, but is the result of active inhibition in response to damaged wall structure, triggered by potential sensors such as THE1 [59]. Interestingly, several members belonging to the same RLK subfamily (CrRLK) as THE1 are expressed in cells exhibiting polar growth such as trichomes and conical cells of Antirrhinum petal epidermis as well as tip growing cells of Arabidopsis [59]. Because root hairs and pollen tubes show highly dynamic expansion, balanced on the brink of cell rupture (see earlier), the presence of such RLKs is particularly intriguing.

Continuing with this theme of kinases as possible mechanosensors, AtHK1, an Arabidopsis plasma-membrane-localized histidine kinase, can complement yeast lines containing a deletion in their own putative osmosensing histidine kinase SLN1 [61]. In the plant, lesions in AtHK1 cause an increased sensitivity to osmotic stress [62], leading to the speculation that this protein might directly respond to changes in cellular pressure, perhaps transmitted through tension in the plasma membrane, as proposed for SLN1 [63]. However, whether AtHK1 could in fact be responding secondarily to alterations induced by a mechanosensitive channel, such as an influx of Ca²⁺, remains to be defined.

Two major themes arise from the spectrum of putative plant mechanosensors discussed here. The MSL family and possibly AtHK1 seem likely to sense mechanical forces through monitoring tension in the plasma and, possibly, in other membranes (Figure 4). By contrast, by potentially linking the ECM to the plasma membrane and cell interior, RLKs are ideally placed to monitor mechanical strain while it affects cell wall integrity or the interaction between the cell wall and the plasma membrane. There are >600 RLKs in the Arabidopsis genome that are thought to

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**Figure 4.** Action of possible mechanoresponsive elements. Plant mechanosensors probably fall into two broad classes: those activated by tension in the membrane, as exemplified by the MSL family of CI⁺ permeable channels, and those monitoring wall status and/or shear between the wall and the plasma membrane. SAC represents the stretch-activated Ca²⁺-permeable conductance identified through electrophysiology but yet to be cloned [3]. AtHK1 operates in an osmosensing pathway that potentially functions through a phosphorelay cascade that uses the antagonistic response regulators ARR3/4 and ARR 8/9 [60]. The RLKs such as WAKs [51–53], THE1 [59,60] and the lectin domain containing RLK [50] provide models for how a wall sensor might operate. They are likely to elicit protein-kinase-dependent signals that could relay mechanical information directly to mechanoresponsive gene expression for example, or interact with the Ca²⁺-dependent signaling cascade that, to date, remains the best characterized mechanically induced signal transduction event in plants.
be involved in processes as varied as hormone response, pathogen recognition and cell differentiation [64]. A subset of this superfamily could well represent a suite of mechanosensitive elements. The potential functional redundancy within this large group of proteins might help to explain why, at present, none of the mutants in the candidate mechanosensing WAKS, lectin-like RLK or THE1 have been directly linked to mechanical sensing. Defining the direct substrates and the wider signaling networks they elicit should greatly aid in understanding the degree to which these RLKs share overlapping function.

Concluding remarks and future perspectives

Despite the key role mechanical forces have in plant growth and development, molecular candidates such as the MSL gene family have only recently been identified as potential mechano-receptors. However, a major question still remains as to the role of the MSL genes outside of plastid division. The lack of a root growth phenotype of the quintuple msl4/msl5/msl6/msl9/msl10 knockout clearly shows that there are other mechanosensors active in the plant and their identification remains a pressing goal for the field. In addition, whether or not the Ca\(^{2+}\) increase seen upon mechanostimulation represents direct gating of a mechanosensitive, Ca\(^{2+}\)-permeable channel or a downstream event triggered by an alternate primary mechanosensor remains an unanswered question. Although several important studies have identified RLKs with the potential of signaling cell wall status to the cytoplasm, future work will need to determine whether or not these or related proteins have roles as true mechanosensors. Because none of these RLKs are predicted to possess ion channel function, it will also be crucial to elucidate their relationship to the Ca\(^{2+}\) signaling cascade, which at present is the most firmly established rapid response pathway triggered by mechanical stimulation.

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