J Plant Growth Regul (2002) 21:71–88
DOI: 10.1007/s003440010049

J P G R
Journal of Plant Growth Regulation

Ionic Signaling in Plant Responses to Gravity and Touch

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Abstract

Touch and gravity are two of the many stimuli that plants must integrate to generate an appropriate growth response. Due to the mechanical nature of both of these signals, shared signal transduction elements could well form the basis of the cross-talk between these two sensory systems. However, touch stimulation must elicit signaling events across the plasma membrane whereas gravity sensing is thought to represent transformation of an internal force, amyloplast sedimentation, to signal transduction events. In addition, factors such as turgor pressure and presence of the cell wall may also place unique constraints on these plant mechanosensory systems. Even so, the candidate signal transduction elements in both plant touch and gravity sensing, changes in Ca^{2+}, pH and membrane potential, do mirror the known ionic basis of signaling in animal mechanosensory cells. Distinct spatial and temporal signatures of Ca^{2+} ions may encode information about the different mechanosignaling stimuli. Signals such as Ca^{2+} waves or action potentials may also rapidly transfer information perceived in one cell throughout a tissue or organ leading to the systemic reactions characteristic of plant touch and gravity responses. Longer-term growth responses are likely sustained via changes in gene expression and asymmetries in compounds such as inositol-1,4,5-triphosphate (IP_3) and calmodulin. Thus, it seems likely that plant mechanoperception involves both spatial and temporal encoding of information at all levels, from the cell to the whole plant. Defining this patterning will be a critical step towards understanding how plants integrate information from multiple mechanical stimuli to an appropriate growth response.

Key words: Aequorin; Auxin; Calcium; Cytoskeleton; Gravitropism; Membrane potential; pH; Roots; Signal transduction; Thigmotropism

Introduction

Plants are capable of perceiving a wide range of internal and environmental stimuli. After processing this information, they respond with highly appropriate, typically growth-related responses. In nature, multiple stimuli are always present and each may modify the response to others. Thus, the plant must possess signaling mechanisms permitting extensive cross-talk between stimulus/response pathways. Figure 1A shows the range of stimuli a root must respond to as it explores the soil. Many of these perception events involve chemical transactions between the plant and environment, such as pathogen elicitor binding to a receptor to initiate a defense response, or root cell responses to the presence of nutrients or toxic heavy metals (Eissenstat and others 2000; Kochian 1995; Nuernberger 1999; Sattelmacher 2001). However, in two root

Received: 23 October 2001/Accepted: 12 December 2001/
Online publication: 20 May 2002
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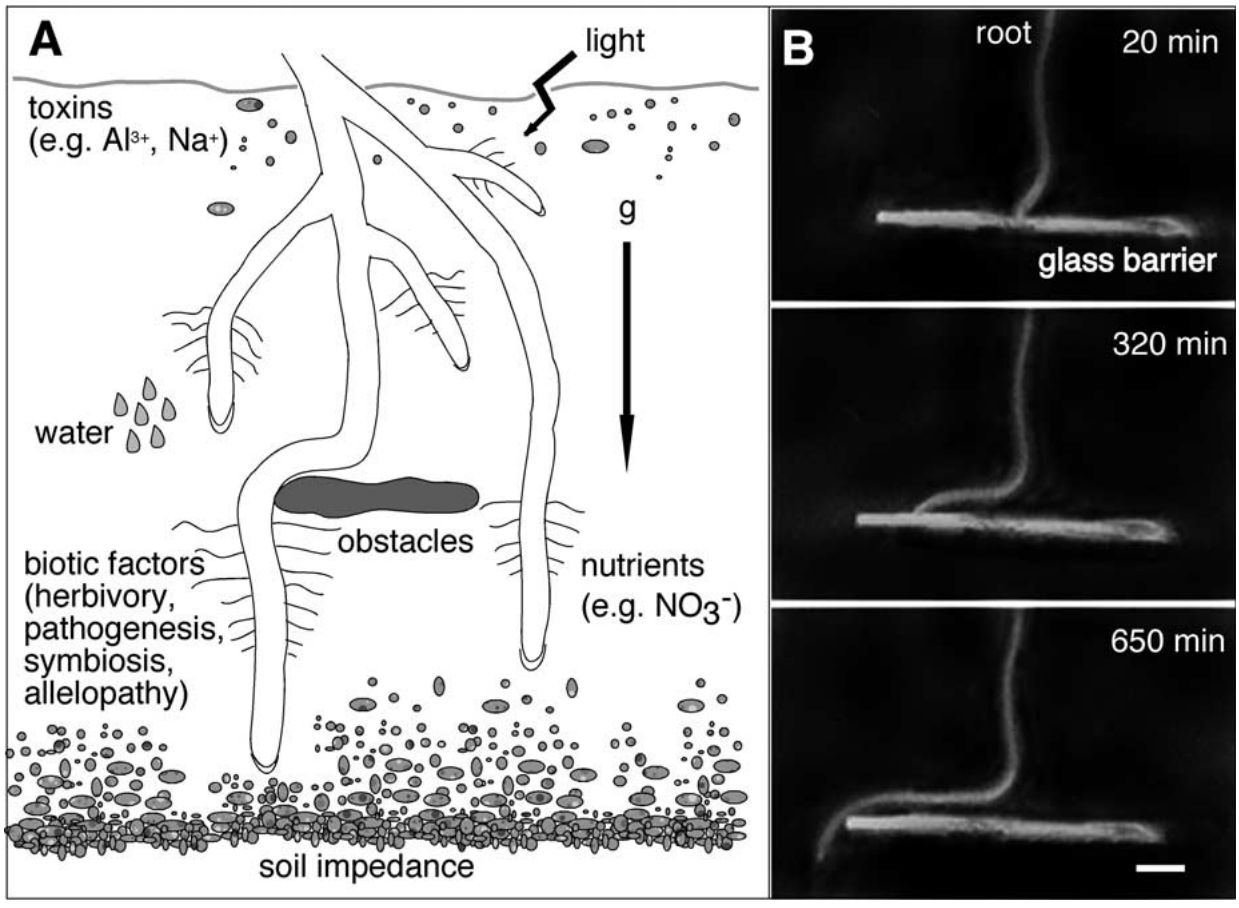


Figure 1. **(A)** Abiotic and biotic stimuli encountered by a root growing through the soil. This complex mix of signals must be sensed and integrated to an appropriate developmental response. **(B)** The interaction of touch and gravitropism in the primary root of *Arabidopsis thaliana.* The roots were grown in phytagel and a horizontal glass barrier was placed across their path of growth. On encountering the barrier (0 minutes) the roots reoriented growth, grew sideways until the barrier was cleared, and then resumed normal gravitropically directed growth. During the passage across the barrier, however, the root tip maintained a set angle intermediate between horizontal and vertical. The lateral growth across the glass barrier appears to represent an integrated response between downward gravitropic growth and information from touch sensors in the root cap. The lateral growth thus represents an adaptive compromise where touch and gravity sensing interact to efficiently direct obstacle avoidance responses in the root (Massa and Gilroy unpublished). Scale bar = 1 mm.

responses, touch sensing and gravity perception, it is a physical force that must be transformed into biochemical information.

There is evidence that touch and gravity sensing/response systems interact (Figure 1B) (Mullen and others 2000; Massa and Gilroy unpublished) and considering the physical nature of both stimuli, it seems likely that they could share common mechanotransduction elements. It has even been proposed that gravity sensing is derived from an ancestral touch perception apparatus (Trewavas and Knight 1994). Shared signal transduction elements provide obvious points where cross-talk between these two signaling systems could occur. However, there are important differences between the touch and gravity stimulus. For example, the forces applied to a cell during touch stimulation arrive from the extracellular environment, whereas gravistimulation is thought to be generated by the action of masses within the cell. These different sites of initial signal generation could be coupled to very different subsequent transduction events. Therefore, in this review, we will compare how these different mechanical sensors might operate and how their respective signaling systems may lead to response integration. The theme to emerge from such comparisons is that temporal and spatial signatures of ionic signaling are likely to underlie signal processing in plant mechanoperception.

Models of Mechanosensing

Before reviewing the specific ionic elements of the plant touch and gravity perception systems, it is useful to first define how any mechanical force could be transduced into a biochemical signal. The resulting models not only suggest the spatial and temporal characteristics we might expect from touch and gravity signaling elements but also emphasize that a rigid cell wall and substantial turgor pressure place significant constraints on such signaling systems in plants.

One of the simplest models of mechanotransduction involves plasma membrane deformation, which then can modulate the activity of mechanosensitive ion channels (often Ca^{2+} channels; Gillespie and Walker 2001; Figure 2A). In animal and bacterial cells, there has been extensive characterization of such mechanosensitive channels gated by membrane flexion (Gillespie and Walker 2001). Artificially applied local membrane deformation, for example, through use of a patch clamp pipette, also alters channel gating in isolated plant membrane patches (Cosgrove and Hedrich 1991; Ding and Pickard 1993). On the other hand, the rigid cell wall and the 8–10 atmospheres of internal turgor pressure of the intact plant cell suggest that substantial deformation of the plasma membrane would be a less effective and sensitive method of mechanosensing than in animals. In *Chara* internodal cells, sensitivity to mechanical stimulus increases as turgor decreases, implying that here at least touch sensing is constrained by cell turgor (Shepherd and others 2001). However, in these *Chara* experiments the mechanical stimulus (impact of a falling glass rod) was great. The rapid and substantial changes in membrane flexion likely induced by this class of stimulus may well model biologically relevant signaling of severe mechanical strain or perhaps changes in turgor, such as occur in wounding or during the massive changes in osmotic environment experienced by an intertidal alga (Goddard and others 2000).

A distinct system may exist to detect less extreme mechanical stimuli. Force exerted on the wall may be transmitted through the plasma membrane to the cytoskeleton, which would then amplify and retransmit the force to the plasma membrane or, perhaps more likely, to a responsive protein such as an ion channel embedded in the plasma membrane (Figure 2B). Alternatively, mechanical stimuli may pass from wall, to cytoskeleton, to internal membranes such as ER or tonoplast. Such an internal site of transduction would circumvent the constraints imposed by turgor pressure at the plasma membrane and allow substantial membrane deformation with, for example, associated gating of ion channels (Figure 2B). Thus, in plants, membrane deformation as a direct mechano-gating system may be localized to internal membrane sites rather than occur at the plasma membrane as seen in mammalian cell mechanoperception (Gillespie and Walker 2001).

In contrast to direct effects on membrane curvature, force perception at the plasma membrane would likely be perceived by a complex transmembrane sensor attached to both the wall and the cytoskeleton (Gillespie and Walker 2001). Such 'integrin-like' linkages (Swatzell and others 1999) could effectively amplify the force exerted on the plasma membrane because the rigid wall should experience most of the stress from any external force and, through mechanisms such as a tensegrity-based network, the cytoskeleton should effectively transmit and amplify force throughout the cytoplasm (Figure 2C; Chicurel and others 1998). The membrane-based portion of the sensor may involve complex assemblages of proteins in a lipid microdomain or membrane 'raft' (Brown and Jacobson 2001 and references therein) and could include mechanosensitive ion channels. The rapid gating and large signal amplification seen from individual channels passing thousands to millions of ions per second make them especially strong candidates for transmembrane mechano-transduction amplifiers. Integrating these channels into a lipid raft-based sensor complex should result in a system more responsive to smaller forces than an isolated channel due to amplification generated by the associated proteins (Viola 2001; Galbiati and others 2001). Indeed, a Ca^{2+}-channel-based signal transduction complex at the plasma membrane was proposed for plants in the early 1990's (the plasmalemmal control center model, or PCC; Pickard and Ding 1993) and proposed to be a major nexus for mechanotransduction activity.

Although the previous examples of mechanical force transduction describe an essentially direct effect of physical force on channel gating, a range of less direct mechanotransduction mechanisms could also occur. For example, allosteric regulators could be brought into close proximity to a channel in response to mechanical deformation of the cytoskeleton (Figure 2D), or channel gating might be a secondary response to a mechanical perturbation in a global regulator such as cytosolic pH or membrane potential (Figure 2E). At present there is little information either supporting or refuting these alternative mechanisms of force transduction although, as described below, pH and membrane potential changes have been linked to mechanotransduction events.

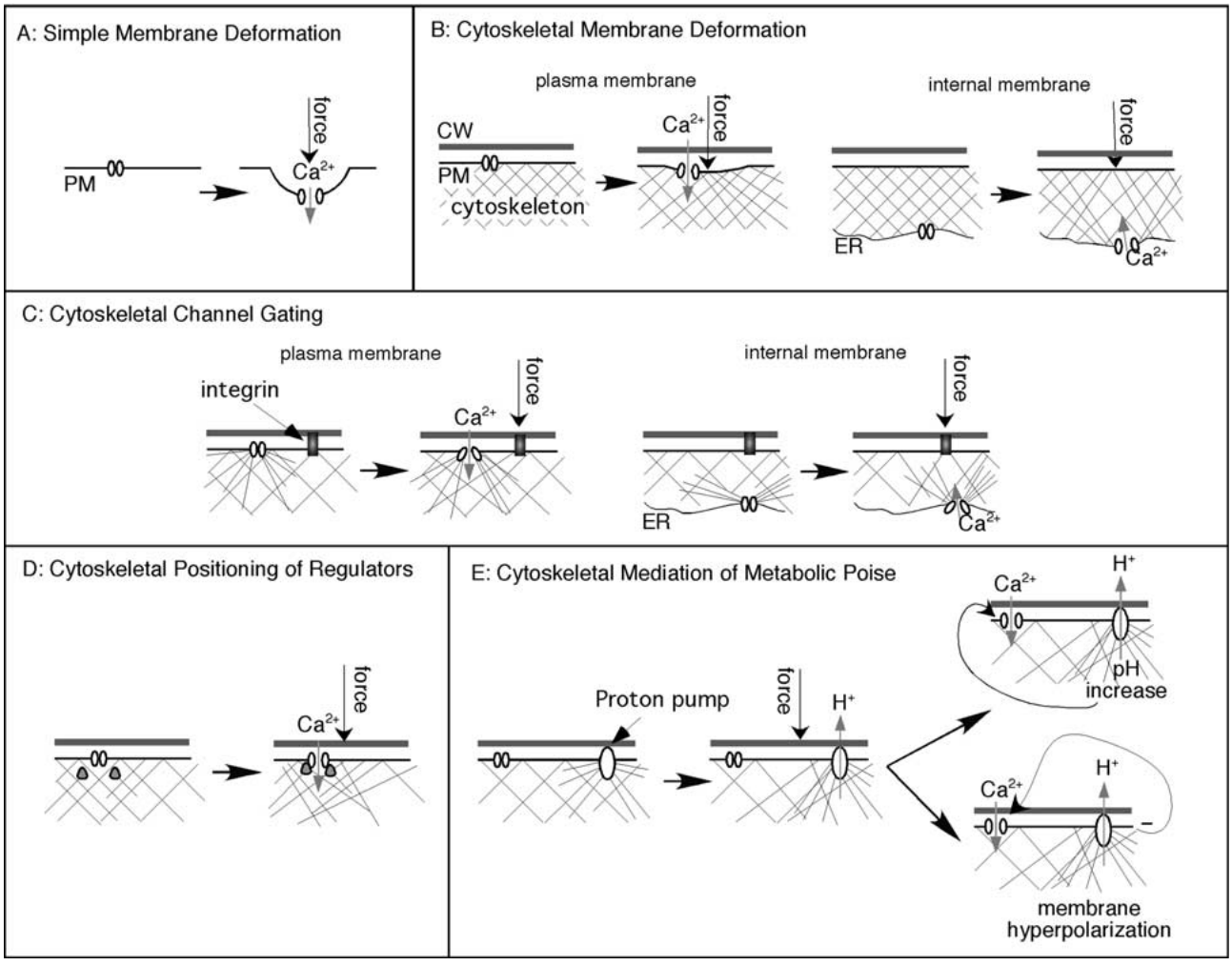


Figure 2. Models for mechanoperception. Note that although the initial force is shown as being imposed externally, the models are also relevant for internally generated forces transmitted through the cytoskeleton, such as is likely to occur during gravity perception. **(A)** An applied force directly deforms the plasma membrane leading to gating of a mechanosensitive Ca^{2+} channel, causing calcium influx. **(B)** An applied force is integrated by the cytoskeleton such that it is focused on the plasma membrane or transmitted to internal membranes. This model may be most directly applicable to plant cells where turgor may limit the degree to which the plasma membrane may be directly deformed by a force. The cytoskeleton movements lead to membrane deformation and channel gating. **(C)** The cytoskeleton is linked directly to channels at either the plasma membrane or internal membranes, avoiding the need for membrane deformation by directly transmitting applied force to the channel. **(D)** Force transmitted through the cytoskeleton causes shifts in the position of cytoskeleton-linked regulatory proteins. Regulators are therefore brought into proximity to a channel and so modulate its activity. **(E)** The cytoskeleton may connect applied force to membrane proteins that alter the 'poise' of the cell, indirectly affecting channel activity. For example, activation of the H^+-ATPase in the plasma membrane could result in membrane hyperpolarization and altered cytosolic and apoplastic pH. Ion channels responsive to these types of changes have been characterized in plants.

From the above discussion it seems likely that the initial transduction events elicited by a mechanical stimuli, be it touch or gravity, will likely involve changes, probably ionic fluxes, localized to the surface of a membrane system such as the plasma membrane or ER. In addition, we can expect the cytoskeleton to act as a transduction/amplification system.

Defining Mechanosensing: Gravity vs Generalized Touch Sensing

Vibration, flexion, point pressure, and sliding contact all induce physiological changes in plant organs (Takahashi and Jaffe 1990; reviewed in Mitchell and Myers 1995). These kinds of applied forces often induce responses characteristic of ethylene

production: reduced elongation, radial swelling, and tissue hardening (Biro and Jaffe 1984; Mitchell 1996 and references therein). In addition, if point pressure or limited sliding contact is applied asymmetrically in certain regions of the root or shoot, it can induce tropic curvature (negative in roots, positive in shoots). Thus, while having a significant effect on plant architecture, this general rnechanosensory system does not seem to rely upon highly specialized sensory cells.

In contrast, higher plant gravity sensing is confined to specialized sensory cells called 'statocytes' (Kiss 2000; Sack 1991). These cells are found in the columella of the root cap and in the endodermis of the shoot elongation zone and contain mobile starch-filled plastids ('statoliths') that are thought to exert a directional force that changes with the orientation of the organ. In the aerial parts of the plant, gravity perception and the tropic growth response occur in the same cells, although of course other non-statocyte stem cells such as those in the cortex and epidermis must also exhibit the growth response. In contrast, in roots, the statocytes are in the root cap, spatially separated from the growth response in the elongation zone (reviewed in Sack 1991). Although alternative gravity perception mechanisms have been proposed (such as the protoplast pressure model; Wayne and Staves 1996) and it is possible that multiple gravity perception systems have appeared successively or in parallel during plant evolution (Barlow 1995), the starch-statolith model is supported by most current data as the mechanism for gravity perception in both roots and shoots of higher plants (Kiss 2000).

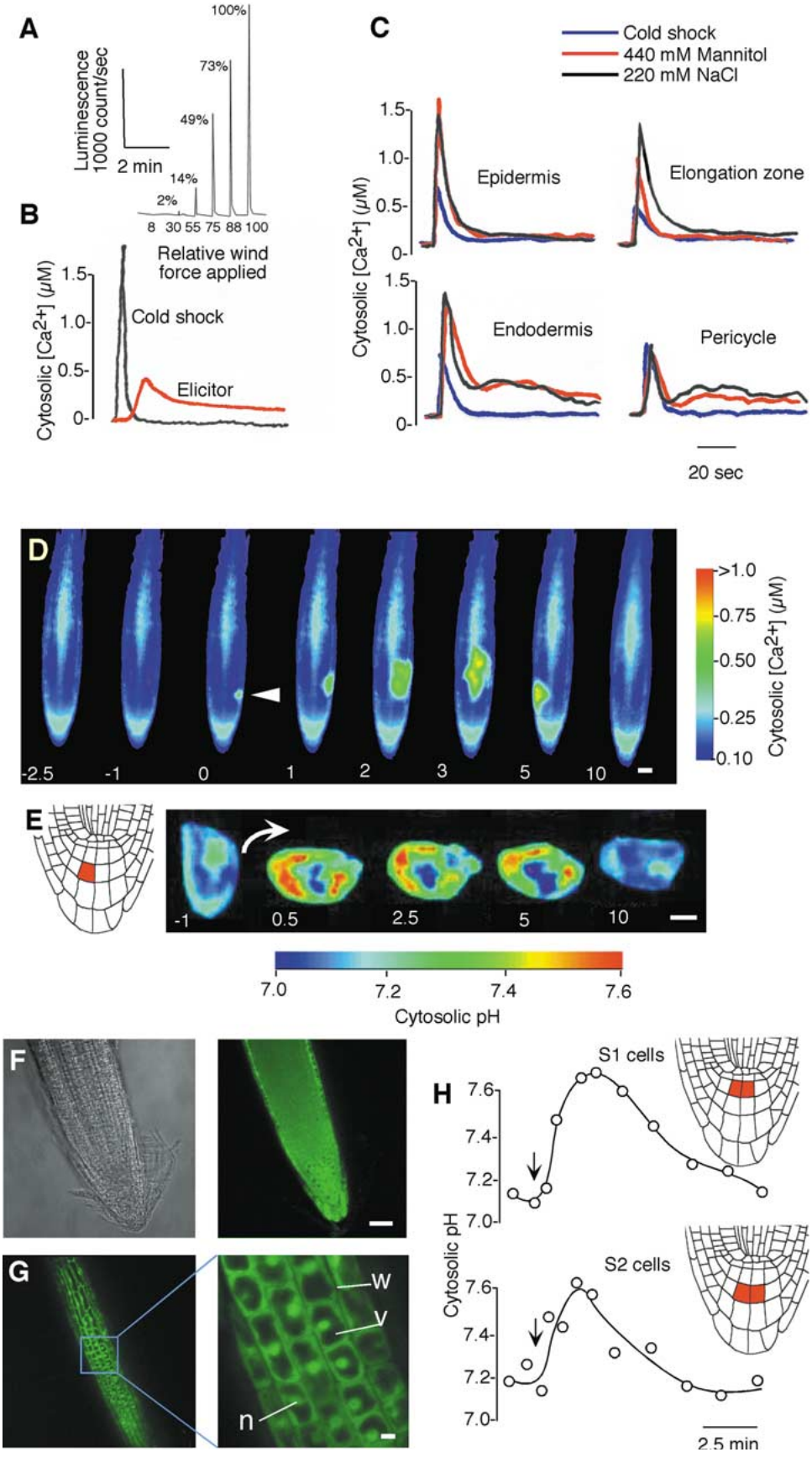
In the starch statolith model, the mechanical force sensed by cellular transduction machinery is generated internally by sedimenting amyloplasts rather than externally as in touch perception. However, the mechanisms for force transmission/transduction outlined in Figure 2 apply equally well to sensing both external and internal forces, although the wall is unlikely to be used directly as a strain gauge in gravity perception as it would be in touch sensing. Thus, the models of mechanosensing outlined in Figure 2 are consistent with similar or even shared features of transduction between touch and gravity sensing.

The Same Only Different: Ionic Signaling in Touch and Gravity Perception

Animal touch and gravity signaling is inextricably linked to the rapid gating of mechanosensitive ion channels (Gillespie and others 2001). The rapid gating of ion channels and their inherent signal amplification make them ideal signal transduction elements and changes in ion concentration (for example, Ca^{2+}, pH) and membrane potential are often involved in rapid signaling events in plants (Blume and others 2000; Guern and others 1992; Johannes and others 1998; Malho and others 1998; Stankovic and Davies 1997; Trebacz and Sievers 1998). Not surprisingly therefore, ionic signaling has been proposed to transduce both touch and gravity perception in plants (see for example, Fasano and others 2001; Haley and others 1995; Perera and others 1999; Scott and Allen 1999; Sievers and others 1995). The picture emerging from studies of such ionic signaling is that while touch and gravity do indeed seem to share some common elements of a Ca^{2+} based signal transduction system, specificity becomes evident in their spatial and temporal signaling signatures and in the dynamics of other regulators such as the pH signaling system.

Calcium

Changes in the concentration of cytosolic free Ca^{2+} are recognized as part of a ubiquitous Ca^{2+}-dependent second messenger system (Sanders and others 1999 and references therein). Transient elevations in Ca^{2+} have been reported to occur in response to a wide variety of stimuli ranging from pathogen attack to gibberellin action during seed germination (Bush 1995; Trewavas and Knight 1994; Sanders and others 1999) and both touch and gravity signaling have been proposed to utilize Ca^{2+}-dependent signaling systems (for example, see Hepler and Wayne 1985; Trewavas and Knight 1994). If all these signals are linked by similar Ca^{2+}-dependent signal transduction processes, the obvious question is how can the plant distinguish between stimuli in general, and touch and gravitational stimuli in particular? This question is especially pertinent if both touch and gravity signaling systems are present in the same cell. The specificity of a cellular response to Ca^{2+} is thought to lie in part in the timing, duration, and magnitude of the Ca^{2+} spike or pulse elicited, the so-called 'Ca^{2+} signature' of the stimulus (Blume and others 2000; Wood and others 2000). Alternatively, a specialized cellular or subcellular environment (microdomain) may exist where factors such as pH, cytoskeletal interactions, the availability of Ca^{2+}-dependent regulatory proteins or a host of other compounds could also impose specificity on spatially distinct Ca^{2+} signals.


A
Luminescence 1000 count/sec
2 min
100%
73%
49%
14%
2%
8 30 55 75 88 100
B
Relative wind force applied
Cytosolic [Ca2+] (μM)
Cold shock
Elicitor
C
Cold shock
440 mM Mannitol
220 mM NaCl
Epidermis
Elongation zone
Endodermis
Pericycle
20 sec
D
-2.5 -1 0 1 2 3 5 10
>1.0
0.75
0.50
0.25
0.10
Cytosolic [Ca2+] (μM)
E
-1 0.5 2.5 5 10
7.0 7.2 7.4 7.6
Cytosolic pH
F
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S1 cells
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Cytosolic pH
7.6
7.4
7.2
7.0
2.5 min

Calcium Changes During the Touch Response

Cytoplasmic Ca^{2+} changes have been directly observed following mechanical perturbation in plants. Most of these reports have relied upon measurements made with the Ca^{2+}-sensitive photoprotein aequorin. Light emission from aequorin increases with Ca^{2+} concentration, and thus the protein can serve as a reporter of Ca^{2+} levels. The gene for the aequorin apoprotein has been cloned and introduced into a range of plants including *Arabidopsis*, tobacco, and *Physcomitrella patens* (Knight and others 1991, 1992, 1993; Russell and others 1996). When aequorin's luminophore cofactor, coelenterazine, is added to the plant, active aequorin is reconstituted *in vivo*. Thus, luminescence can be measured from the transformed plants and the amount of light related to changes in cytoplasmic Ca^{2+}. The luminescence from these transgenic plants is generally too low for single cell imaging. Therefore, most of the insights from this approach relate to either whole plants or, using highly sensitive imaging equipment, to organ level responses (Knight and others 1993).

In tobacco, aequorin has helped reveal transient increases in Ca^{2+} in response to either point pressure or wind flexion (Figure 3A; Knight and others 1991, 1992, 1993). However, luminescence peaks were also seen in response to cold shock and fungal elicitors (Knight and others 1992). Similarly, in the moss *P. patens*, both cold shock and touch, elicited transient Ca^{2+} increases (Russell and others 1996). Indeed Ca^{2+} increases have been reported in response to many stimuli other than touch or cold. For example, pathogen elicitors (Figure 3B; Blume and others 2000) and salt and osmotic stress (Figure 3C; Kiegle and others 2000) all elicit transient elevations in Ca^{2+}. One possibility is that these increases may reflect the role of Ca^{2+} in a general cellular activation that sets the stage for the plant to respond, but where specificity about the stimulus eliciting the response is carried by a separate signaling system. Alternatively, specificity might be encoded in the temporal signature or subcellular location of the Ca^{2+} transient. Indeed, variation in spatial and temporal Ca^{2+}signature between different stimuli or cell types has been reported in several plant systems using the aequorin measurement system (for example, see Blume and others 2000; Sedbrook and others 1996; Wood and others 2000). Thus for example, when cold shock and fungal elicitor are applied to parsley suspension culture cells, they yielded a very different temporal pattern of Ca^{2+} increase (Figure 3B; Blume and others 2000). In addition, by targeting aequorin to different cell types in the root, Kiegle and others (2000) have shown that each tissue may respond to stimuli with specific Ca^{2+} signatures and that these Ca^{2+} changes can be different between different stimuli (Figure 3C). However, at present there is little data

Figure 3. Signal-related changes in cytoplasmic Ca^{2+} and pH. **(A)** Cytoplasmic Ca^{2+} increases induced by wind flexion of tobacco seedlings expressing recombinant aequorin. Note that the amplitude of the Ca^{2+} change correlates with the intensity of the mechanical stimulation and so may encode this information for the plant (redrawn from Knight and others 1992). **(B)** Ca^{2+} changes elicited by cold or elicitor in parsley cell cultures expressing recombinant aequorin. Note the kinetics of the Ca^{2+} change is different for each signal and so may encode information about each stimulus for the cell (redrawn from Blume and others 2000). **(C)** Cold shock, osmotic and salt stress elicit different Ca^{2+} signatures in different root cell types. Aequorin was targeted to specific root cells using the Gal4 enhancer trap system and aequorin luminescence monitored after application of the indicated stress. Note that each stress can have a unique temporal profile of Ca^{2+} signal but these signatures are different among different cell types (redrawn from Kiegle and others 2000). **(D)** Ca^{2+} wave elicited by transient touch stimulation (arrowhead) of an *Arabidopsis* root. Plants expressing the transgenic fluorescent GFP Ca^{2+} reporter Cameleon YC2.1 (Allen and others 1999) were stimulated at 0 minutes by a 2-second pulse of growth medium expelled from a micropipette positioned adjacent to the root (arrowhead). The change in Cameleon sensor's Ca^{2+}-dependent fluorescence signal was monitored using a Zeiss LSM 410 confocal microsocope (Gilroy, unpublished). Note the wave of Ca^{2+} change elicited by transient touch stimulation. **(E)** pH increase in a root cap columella cell elicited by gravitstimulation. An S2 columella cell (position indicated in inset diagram of the root cap) was microinjected with the fluorecent pH sensor BCECF-dextran and its cytoplasmic pH monitored according to Fasano and others (2001) as the root was rotated to gravistimulate the cap. Note the transient increase in cytoplasmic pH upon gravistimulation. **(F)** Bright field and fluorescence images of a root expressing the pH-sensitive GFP-H148D driven by the CMV 35S promoter. **(G)** Confocal images showing a tangential (surface) plane of an *Arabidopsis* root expressing cytoplasmic pH-sensitive GFP. Note that single cells can be identified and that fluorescence is localized to the cytoplasm, being excluded from cell wall and vacuole. **(H)** Gravistimulated changes in pH in S1 and S2 columella cells monitored using this GFP technology. Note the pH-sensitive GFP reveals similar transient pH increases upon reorientation of the root (arrow) as seen in **E** with microinjected BCECF (Swanson and Gilroy, unpublished; see also Fasano and others 2001). Scale bars in **D** and **F** = 50 μm; **E** and **G** = 10 μm. n, nucleus; v, vacuole; w, wall.

defining a clear temporal Ca^{2+} signaling signature for the touch response of the plant.

Although aequorin's signal strength in transgenic plants does not allow direct subcellular imaging of Ca^{2+} release sites, studies with inhibitors of Ca^{2+} transporters have been used to test the idea that specificity might be encoded via microdomains or localized subcellular Ca^{2+} release sites. Ruthenium red, a compound believed to disrupt Ca^{2+} release from internal stores (Knight and others 1992), inhibits Ca^{2+} transients induced by mechanical stimuli such as wind flexion and point pressure. Significantly, these transients are not affected by La^{3+} (thought to block plasma membrane-bound calcium channels) or EGTA (a calcium chelator which lowers available apoplastic calcium; Haley and others 1995). Thus, for touch stimuli, the Ca^{2+} transients may be mediated by transporters located on internal membranes such as the ER, rather than influx at the plasma membrane.

Ca^{2+}-sensitive fluorescent dyes, such as Indo-1, have also been used to measure cytoplasmic Ca^{2+} increases in response to touch stimuli. Legué and others (1997) loaded Indo-1 into *Arabidopsis* roots and then applied pulses of growth medium from a micropipette near the root surface. Ca^{2+} transients lasting for several minutes were seen in stimulated cells, and after a lag period, in adjacent cells. This suggests that a Ca^{2+} wave was propagating through root cells in response to touch (Figure 3D; Legué and others 1997). Cells in all regions of the root showed a response to the pulses of media, but the root cap cells appeared most sensitive (Legué and others 1997). The root cap might be expected to be the first region of the root to encounter obstacles in the soil as the root elongates and thus the higher sensitivity of cap cells to touch might reflect specialization towards more effectively directing root obstacle avoidance. In addition, ruthenium red inhibited the touch-induced transients, consistent with the aequorin-based observations. Thus, the current evidence suggests that touch operates via mechanically gated channels localized to internal membranes.

The Ca^{2+} transients induced in response to the pressure pulses reported by Legué and others (1997) were similar in amplitude though longer in duration (approximately 5 minutes versus 10–20 seconds) relative to those described using aequorin (compare Figures 3A and 3D). These differences in kinetics might reflect differences in the measurement techniques, for example, differences in the cellular resolution or buffering capacity of the respective sensors. Alternatively, the differences in kinetics may be hinting that cells can encode the differences in how each touch stimulus was applied, single cell stimulation (Legué and others 1997) versus whole organ deflection/measurement (Knight and others 1991, 1992). Aequorin measurements clearly show that the amplitude of the Ca^{2+} signal can reflect intensity of touch stimulus (Figure 3A). However, the possibility that the Ca^{2+} signature may indeed encode such subtle information about the mechanical stimulus that evoked the change is an area yet to be systematically investigated.

Calcium-based Manipulation of the Touch Response

Clearly, observation of touch induced Ca^{2+} changes does not indicate that such changes are functionally significant in touch sensing. However, treatments that alter Ca^{2+} dynamics have been shown to affect touch responses such as radial swelling and reduced elongation (thigmomorphogenesis), tendril coiling, and thigmotropic curvature, providing evidence for such a functional role. For example, the mechanical stress response of soybean seedling hypocotyls (reduction of elongation) was simulated by treating with Ca^{2+} and the Ca^{2+} ionophore A23187 (Jones and Mitchell 1989). This could simply reflect a Ca^{2+}-related 'stress' response impairing growth, but treatment with the Ca^{2+} chelator EGTA or calmodulin antagonists was also able to partially block the mechanically induced reduction of growth (Jones and Mitchell 1989), consistent with a calcium-mediated pathway acting somewhere in touch signaling or response.

Touch-responsive tendril coiling in *Bryonia dioica* can be blocked by Gd^{3+} and erythrosin B, both putative inhibitors of calcium transport (Klüsener and others 1995; Liß and others 1998). Gd^{3+} is thought to block stretch-activated Ca^{2+} channels, while erythrosin B reportedly inhibited both these channels and an ER localized Ca^{2+}-ATPase. An ER localized voltage-dependent Ca^{2+}-permeable channel (BCC1; Klüsener and others 1995) and ER-localized Ca^{2+}-ATPase have been isolated from tendril tissue and do appear to be sensitive to Gd^{3+} and erythrosin B *in vitro* (Liß and others 1998). Thus, both calcium transporters may be functionally significant for tendril mechanoperception, reinforcing the idea that Ca^{2+} transporters are required during plant touch signaling.

Studies on the effects of adding inhibitors of Ca^{2+} action to roots illustrate just how several potential sites of inhibitor action and the inherently complex, organ wide nature of tropic signaling and response may complicate interpretation of Ca^{2+} antagonist action *in vivo*. For example, asymmetric application of

agar blocks to the root cap of *Zea mays* roots induces a negative thigmotropic response which is enhanced when Ca^{2+} (20 mM) is present in the block (Ishikawa and Evans 1992; Takahashi and others 1992). However, asymmetric application of agar blocks containing Ca^{2+} to the distal elongation zone of the root induces inhibition of growth on the side of application, whereas agar blocks alone are ineffective (Ishikawa and Evans 1992, but see Takahashi and others 1992 for an alternative view). One possible confounding factor in these experiments is the pH of the agar block, which could potentially have altered the wall pH of the cells of the cap or elongation zone. Thus, alterations in growth would be a pH rather than Ca^{2+}-based event. However, Fasano and others (2001) have noted that 50–100 mM pH buffer is required to alter the pH of wall of cells in the cap and elongation zone of *Arabidopsis* roots. The buffer strength present in the agar used in the block experiments was 10-fold lower than this level, making these potential wall pH-related effects on growth less likely. These results therefore suggest that Ca^{2+} may play a role in tropic curvature both at the level of mechanoperception and in signaling events unrelated to the touch stimulus. Thus, Ca^{2+} application to the cap might be affecting cytoplasmic signaling events, perhaps related to gravity perception, by changing root cap apoplastic Ca^{2+} levels, or possibly be affecting auxin redistribution (Young and Evans 1994). Applying Ca^{2+} to the elongation zone might be modulating growth through changing cytoplasmic signaling events (perhaps related to touch, Figure 3D; Legué and others 1997) or through direct effects on wall elasticity, for example, through affecting pectin crosslinking, or perhaps even altering cellular sensitivity to growth regulators such as auxin. These experiments clearly highlight a functional role for Ca^{2+} in the overall tropic response, and perhaps directly in touch perception. The challenge now is to determine exactly where and how such manipulations of Ca^{2+} are acting at the cellular level.

Touch Induction of Calmodulin

Calmodulin and calmodulin-like proteins comprise a widespread component of Ca^{2+}-based signal transduction in eukaryotes (Snedden and From 2001; Zielinski 1998). Ca^{2+}-activated calmodulin has been shown to regulate the activity of certain plant proteins ranging from NAD kinase to calmodulin-dependent protein kinases (Lu and Feldman 1997). Plant calmodulins and calmodulin-like proteins form a large multigene family (Zielinski 1998). Yet, despite their highly conserved nature, different calmodulins can have very different enzyme activation characteristics (Lee and others 2000; Liao and others 1996).

Induction of a specific subset of calmodulin and calmodulin-like genes by mechanical stimulation has been reported in several plants, including *Arabidopsis* (for example, see Braam and Davis 1990; Ito and others 1995), mung bean (Botella and Arteca 1994), and potato (Takezawa and others 1995). Thus, changes in the abundance or localization of specific calmodulins may point to the activation of highly specific calcium-mediated touch response pathways. However, there is currently little data revealing the functional significance of these changes in calmodulin and calmodulin-like gene expression. It is unlikely that the relatively slow touch responsive gene induction is part of the primary mechanoperception machinery but may instead reflect an isoform-specific 'fine-tuning' of Ca^{2+}-mediated signaling processes. Thus, modulation of calmodulin isoform expression levels might constitute a change in the capacity of the cell to process subsequent mechanical signals, that is, a sort of cellular memory of previous touch history of the plant. Alternatively, the change in gene expression might reflect induction of a sustained response to the initial stimulus.

In addition to changing the relative proportions of calmodulin isoforms, changes in calmodulin's placement within the cell may have significant effects on its signaling activities. In animal cells, microdomains of activated calmodulin have been reported that do not simply reflect sites of elevated Ca^{2+} levels (Torok and others 1998) but likely indicate a role of locally increased levels of calmodulin to activate low affinity calmodulin response elements (Persechini and Cronk 1999). Calmodulin activation *in vivo* may also be modulated by the proteins it interacts with (Zieleinski 1998). Thus, localized features of the cellular microenvironment appear able to alter calmodulin's responsiveness to Ca^{2+}. Defining which of the spectrum of plant calmodulins show microdomain targeting and activation and whether this is altered by touch stimulation may be an important step to assigning function to the touch-induced caimodulins.

It is also important to note that while many of the mechanically induced genes appear to be related to calmodulin and Ca^{2+} signaling, seemingly unrelated genes are also upregulated. *TCH4*, one of the touch-responsive genes, encodes a xyloglucanendotransglycosylase (XET; Xu and others 1995). This protein acts to modify cell walls, possibly by incorporating xyloglucan to facilitate growth or wall reinforcement (Braam and others 1996). The activity of *TCH4* and similar proteins may help provide the long-term

growth responses observed following mechanical perturbation. Clearly, mechanotransduction and response inevitably involve a complex interaction of initial signaling and long-term regulation and response elements.

Calcium and Gravitropism

Given the prominence of Ca^{2+} in cellular signaling (Bush 1995; Hepler and Wayne 1985; Sanders and others 1999) and the apparent role of Ca^{2+} signaling in touch sensing, it is not surprising to find that much attention has been devoted to the potential involvement of Ca^{2+} in gravity perception (for example, Sinclair and Trewavas 1997). To date, the work of Gehring and others (1990) in maize coleoptiles represents the only report of cytosolic Ca^{2+} changes in response to gravistimulation. However, technical limitations inherent in the imaging approach that had to be adopted in this study make it difficult to clearly attribute the changes reported to gravity-related alterations in cytosolic Ca^{2+}, especially as there was likely significant touch stimulation in the experimental manipulations. In contrast, imaging of cytosolic Ca^{2+} in root statocytes failed to reveal any changes upon gravistimulation (Legué and others 1997). The study of Legué and others (1997) suggests that sustained, steady state changes in Ca^{2+} are unlikely to mediate gravity signaling events in roots. However, highly localized changes in Ca^{2+}, especially if these changes occur in membrane delimited microdomains, are beyond the resolution of the techniques that have been applied. In addition, the elevated levels of calmodulin (Dauwalder and others 1986) and calmodulin-like proteins (for example, TCH3, Antosiewicz and others 1995) reported in statocytes should sensitize these cells to small changes in Ca^{2+}, perhaps compounding problems of detection. The abundance of TCH3 in the columella is particularly interesting as it is induced elsewhere in the plant upon touch stimulation (Antosiewicz and others 1995), providing a possible common element for Ca^{2+} action in both touch and gravity sensing. However, the question of whether Ca^{2+} changes trigger gravisignaling events in statocytes of higher plants remains open.

Asymmetric Calcium Transport after Gravistimulation

Although direct observations of gravisignaling-related changes in cytosolic Ca^{2+} have remained elusive, polar transport of Ca^{2+} in the apoplast has been monitored in maize and pea root caps following gravistimulation. These apoplastic changes also appear to be functional parts of the tropic response (Lee and others 1983a, 1984). For example, Björkman and Cleland (1991) reported that a 3-fold difference in apoplastic Ca^{2+} concentrations appeared across the cap within 10 min after gravistimulation. Application of Ca^{2+} chelators such as EDTA, EGTA and BAPTA to maize root caps abolished gravitropic curvature (Björkman and Cleland 1991; Lee and others 1983b), possibly due to suppression of the apoplastic Ca^{2+} gradient. Interestingly, treatment of maize and pea roots with auxin transport inhibitors not only reduced gravitropic bending but also blocked cap-based polar Ca^{2+} transport (Lee and others 1984). This observation implies a potential functional link between auxin action and Ca^{2+} transport.

Gravity and Calmodulin

There is substantial evidence that calmodulin is involved in root gravity perception. Calmodulin protein levels are higher in the root cap and meristem than elsewhere in the root (Allan and Trewavas 1985; Stinemetz and others 1987) and its expression is induced upon gravistimulation of the *Arabidopsis* root (Sinclair and others 1996). In addition, calmodulin levels were found to be low in the apex of dark grown Merit roots (a maize cultivar requiring light to become gravitropic), but upon illumination, increased to that of light-grown seedlings. The roots gained sensitivity to gravity with a time course paralleling the increase in apical calmodulin levels (Stinemetz and others 1987). Thus, calmodulin is unusually abundant in gravisensing-competent root tips. It may also be significant that MCK1, a calcium/calmodulin-dependent kinase homologue, is expressed in maize root caps (Lu and Feldman 1997) providing a possible response element for a Ca^{2+}/calmodulin-dependent gravisignaling system.

Work with calmodulin antagonists suggests that calmodulin is also functionally important for gravity perception. Low dosages of calmodulin antagonists that do not inhibit root elongation still inhibit gravitropic curvature (Sinclair and others 1996; Stinemetz and others 1992). These antagonists also affect rapid events in the gravistimulated root cap, such as the polar transport of Ca^{2+} (Lee and others 1983a, 1984) and the development of an asymmetric proton current (Björkman and Leopold 1987). The proton current shift appears to precede polar Ca^{2+} transport, as it is not blocked by auxin transport inhibitors (Björkman and Leopold 1987).

Therefore, calmodulin antagonists may interfere with an early step in perception which involves altered proton effluxes rather than either apoplastic Ca^{2+} transport in the root cap or downstream events involving the control of cell elongation.

Calcium Appears to be Important for Auxin Activity

One theme that emerges from the above discussion is that treatments that perturb Ca^{2+} homeostasis appear to affect the action of auxin in growth regulation. For example, Young and Evans (1994) showed that the Ca^{2+} chelator EGTA inhibited gravity-induced redistribution of auxin and gravitropic bending in intact maize roots, and this was reversed when Ca^{2+} was applied. Hasenstein and Evans (1986) germinated seedlings in solutions containing Ca^{2+} versus in water depleted of Ca^{2+} using EGTA and showed that the reversible, auxin-induced inhibition of root growth is dependent on extracellular Ca^{2+}. Work on oat pulvini (gravireresponsive stem nodes) revealed an apparent role for Ca^{2+} in auxin action although the interactions were complex and may reflect auxin switching between different signaling pathways dependent on the background stimulus (Brock and others 1992). However, the work of Brock and others (1992) does imply that early gravitropic sensory events depend on Ca^{2+} release from internal stores, while later events involving auxin transport/action are modulated by influx via the plasma membrane. Cyclopiazonic acid, an inhibitor of ER Ca^{2+}-ATPases, blocked gravicurvature but not elongation in cress roots (Sievers and Busch 1992), also providing tentative support for an internal calcium source involved in gravity perception.

Involvement of Inositol-1,4,5-Triphosphate

Recent work has implicated inositol-1,4,5-triphosphate (IP_3) in the gravitropic response of grass pulvini. IP_3 has been shown to trigger release of Ca^{2+} from intracellular stores, most likely the vacuole, in plants (Munnik and others 1998) and constitutes an important component of many Ca^{2+}-based signaling pathways (Berridge and others 1998; Munnik and others 1998). Short-term increases in IP_3 were observed in maize pulvinal cells within minutes of gravistimulation, fluctuating rapidly with no clear asymmetry developing across the organ (Perera and others 1999). Longer term, sustained increases in IP_3 levels appeared on the lower side of the pulvinus several minutes following gravistimulation. Phosphatidyl-inositol-phosphate (PIP) kinase activity also increased in the same locations, suggesting that the asymmetry in IP_3 was due to elevated PIP_2 biosynthesis and turnover to IP_3 via phospholipase C (PLC) action. Whether there is coordinated gravity-related stimulation of PIP kinase and PLC or whether PIP kinase activation is just providing increased substrate for a constitutively active PLC remains to be determined. In the oat pulvinus at least, PLC does seem to mediate the IP_3 increase. Similar to maize, a sustained asymmetric increase in IP_3 was detected after 10 minutes of gravistimulation in oat pulvini (Perera and others 2001). Application of U73122, a permeant phospholipase C inhibitor, blocked this long-term increase in IP_3 and significantly reduced the graviresponse.

Thus, in pulvini there is a sustained elevation in an IP_3-related signaling pathway that appears to be functionally important for the eventual graviresponse, but a role for IP_3 in gravisignaling in other organs remains to be demonstrated. However, given the Ca^{2+} wave seen in touch-stimulated plants (Figure 3D) and the widespread role of IP_3 in mediating such waves in animal cells (Trewavas 1999), IP_3 is a promising candidate regulator acting in both gravity and touch mechanosignaling.

pH as a Second Messenger in Touch and Gravity Perception

Calcium is perhaps the most widely recognized ionic second messenger in plants but changes in cytoplasmic pH are also known to have widespread, regulatory effects on cell function. The cytosolic concentration of protons is tightly controlled at low levels relative to external concentration, and the measured columella cell cytoplasmic pH of 7.2 (63 nM; Fasano and others 2001; Moseyko and Feldman 2001; Scott and Allen 1999) is close to that of Ca^{2+} (100–200 nM; Legué and others 1997). Thus, as for Ca^{2+}, the low resting H^+ concentration should constitute a good background for distinguishing transient, signaling-related changes in pH within the cell. However, unlike Ca^{2+} (Hodgkin and Keynes 1957), protons diffuse rapidly within the cell. Therefore, without some significant cytoplasmic architecture to slow their movement, protons are unlikely to regulate localized changes in cellular microdomains. Instead, signal-related changes in pH could serve as a global regulatory system for the cell. For example, pH has been implicated as a signaling element in cellular hormonal responses (Beffagna and others 1994; Felle 1988), pathogen defense (Mathieu and others 1996; Roos and others 1998; Zhou and others 2000), bacterial symbiosis (Felle

and others 1996), metabolic regulation (Paterson and Nimmo 2000), and turgor control (Grabov and Blatt 1998; Johannes and others 1998; Schulz-Lessdorf and others 1996). Specific targets for pH regulation have also been identified, such as pH-dependent changes in the activation of ion channels (Grabov and Blatt 1997; Hoth and Hedrich 1999; Hoth and others 1997; Lacombe and others 2000). Thus, pH may be a significant intracellular second messenger acting to generate a global redirection of the suite of signal transduction pathways operating in a cell.

To date, there have been no reports of cytoplasmic pH changes in plant touch response. However, there is some support for the involvement of the H^+-ATPase in mechanoperception. Touch elicited a brief inhibition of the proton pump followed by a longer period of enhanced activity in the internodes of *Bryonia dioica* and *Bidens pilosa* (Bonnin and others 1996; Bourgeade and Boyer 1994). Although the functional significance of these changes in proton pumping remains to be determined, the activity of a voltage-sensitive ER Ca^{2+} channel implicated in *Bryonia* tendril coiling (Klüsener and others 1995) can be modulated by cytoplasmic pH (Klüsener and others 1997), providing hints to a possible mechanism whereby pH and Ca^{2+}-related touch signal transduction may interact. On the other hand, Shimmen (1997) has argued that at least in *Chara*, touch does not require modulation of the activity of the H^+-ATPase as application of proton pump inhibitors failed to disrupt the receptor potential triggered by touch. Clearly, investigations of pH and mechanoperception in plants are in their infancy, and we must await a systematic study of touch and pH signaling to draw firm conclusions as to how pH may modulate the touch response.

In contrast, there is much evidence that pH changes constitute an early, functional element of the gravity signaling apparatus. Several researchers have reported that proton flux from the root cap, generally considered to be the site of root graviperception, shifts after gravistimulation. Prior to gravistimulation, fluxes are low and variable, but after reorientation, substantial proton efflux can be detected from the upper flank of the cap (for example, Collings and others 1992; Zieschang and others 1993). These elevated and asymmetric proton fluxes appear to be associated with both a sustained apoplastic acidification and transient (10 minute) cytoplasmic pH changes in the columella (Figure 3 E–H; Fasano and others 2001; Scott and Allen 1999).

The cytoplasmic changes observed in columella cells appear to be a functional element in graviperception because disturbing the pH dynamics of the cap (Scott and Allen 1999) or specifically the columella cells (Fasano and others 2001) changes the capacity of the root to undergo tropic curvature. Interestingly, acidification of the columella apoplast is induced upon reorientation but the wall pH only returns to prestimulation levels as the root reorients itself to the vertical. This suggests a qualitative difference in columella signaling activity between vertical and non-vertical orientations, and is consistent with the idea that vertical roots are not continuously generating gravistimulated pH signaling events, that is, are in the off position as far as pH-related gravisignaling is concerned (Figure 3E). In addition to these findings in roots, work on the graviresponsive maize pulvinus indicates that pH may also change upon gravistimulation in this organ and the pH changes may even encode subcellular information about the orientation of the cell (Johannes and others 2001). Thus, pH fluxes appear to be a widespread feature of gravisignaling.

In addition to signaling, pH also appears to have a distinct regulatory role in the tropic growth response. Upon gravistimulation, proton efflux and apoplastic acidification can be detected in the elongation zone of the root (Fasano and others 2001; Monshausen and others 1996; Mulkey and Evans 1981; Mulkey and others 1982; Pilet and others 1983; Taylor and others 1996; Versel and Pilet 1986; Zieschang and others 1993). In contrast to the root cap, no gravistimulated cytoplasmic pH changes could be detected in the elongation zone (Fasano and others 2001). The pH changes in the wall occurred after 10–20 minutes and correlated with the initiation of asymmetrical growth. These changes are consistent with an acid growth mechanism of tropic growth response (Büntemeyer and others 1998; Collings and others 1992; Edwards and Scott 1974; Evans 1976; Felle 1998; O'Neill and Scott 1983; Peters and Felle 1999; Taylor and others 1996) where asymmetrical elongation is facilitated by reduction in wall pH across the organ altering the activity of wall-loosening factors such as expansions (Cosgrove 2000) and promoting localized growth. The dual role of pH in growth control and signaling in the root highlights one of the complexities of analyzing tropic signaling, trying to dissect growth control from primary signal generation. In the root, the spatial separation of gravity sensor (cap) and response region (elongation zone) helps place events in either signaling or response. In the shoot or pulvinus, the growth response and signaling occur in the same cell. In these cases the temporal separation of rapid signaling and extended growth control may help determine which events are signal transduction related.

Membrane Potential Changes as a Signaling Element in Touch and Gravity

In addition to Ca^{2+} and pH, changes in membrane potential have also been proposed as signaling events in both touch and gravitropism (Behrens and others 1985; Sievers and others 1995; Shimmen 2001; Wayne and Staves 1993). Membrane potential changes can directly modulate further changes in membrane transport activity by opening voltage-gated channels (Grabov and Blatt 1998, 1999; Liu and Luan 1996; Schroeder and Keller 1992), changing protein associations with the membrane, altering membrane protein conformations (Gilroy and Trewavas 2001), and even through changes in the curvature of the plasma membrane itself (Zhang and Sachs 2001). Therefore, membrane potential may act as a global regulator of plasma membrane components much as pH could globally regulate cytoplasmic components. Membrane potential-related transduction chains have been observed in guard cells and in pathogen-challenged cells (Grabov and Blatt 1999; Roelfsema and Prins 1998; Thain and others 1995; Zimmermann and others 1998). In addition, fluctuations in membrane potential appear important for transmitting information in the form of action potentials; self-propagating waves of depolarization-induced ion exchange across the membrane. These waves have been observed in several plant species such as *Dionaea muscipula* (Hodick and Sievers 1988), *Mimosa pudica* (Shimmen 2001), and *Aldrovanda vesiculosa* (Iijima and Sibaoka 1982) and may also be involved in systemic wound signaling (Rhodes and others 1996; Stankovic and others 1998).

Membrane Potential and Touch

A two-stage touch-stimulated perception system has been characterized in *Chara* involving both local and propagating membrane potential changes (Shepherd and others 2001; Shimmen 2001). The initial stage appears to involve mechanosensitive calcium channels that in turn activate calcium-sensitive chloride channels, resulting in a localized membrane depolarization at the point of touch stimulation (a receptor potential). If the amplitude of the depolarization is large enough, it recruits voltage-gated chloride and potassium channels that propagate a wave of depolarization (action potential) along the internode. Thus, membrane potential appears to be an important element in touch perception and signal transmission in *Chara.* A similar system appears to exist in specialized mechanosensory tissues in plants such as *Mimosa pudica, Dionaea,* and *Aldrovanda vesiculosa* (Shimmen 2001). However, the steps prior to and following the action potential have not been well-characterized in these systems. Even though events such as induction of calmodulin gene expression have been proposed to involve regulation by membrane potential (Vian and others 1996), in general the events triggered by the propagated change in membrane potential have yet to be deciphered.

Although such propagating action potentials typically involve membrane depolarization, Monshausen and Sievers (1998) reported membrane hyperpolarization in response to very weak mechanostimulation (a stream of medium impinging on the plant). It is unclear whether this represents an alternative mechanism of touch perception that operates in response to stimuli with a different signature for example, having different rise time kinetics, amplitude, duration, and decline). Even so, this finding provides an intriguing hint that plants may possess multiple modes of touch perception potentially mediated by different mechanisms (see Figure 2).

Membrane Potential and Gravity Sensing

Membrane hyperpolarization has also been detected in the gravisensing columella cells of the root cap upon gravistimulation (Sievers and others 1995). This membrane potential change appears to be associated with graviperception because it was abolished by treatment with cytochalasin D, an actin depolymerizing agent, that also abolished gravitropic curvature (Monshausen and others 1996). The functional significance of the observed hyperpolarization, which occurs over a 15-second period approximately 60 seconds after gravistimulation, has not been demonstrated. However, it is consistent with the activation of the plasma membrane H^+-ATPase implicit in the pH changes reported in the columella upon gravistimulation (Fasano and others 2001; Scott and Allen 1999).

In addition to membrane potential changes in the columella cells of the root cap, gravistimulation has been reported to elicit a small but sustained hyperpolarization and depolarization on the upper and lower flanks of the elongation zone of mung bean roots (Ishikawa and Evans 1990). These changes occur within 30 seconds of reorientation, and may therefore represent either rapid transmission of information from the sensory cells of the root cap to the elongation zone or very early changes in control of cell elongation. An alternative possibility to

explain this rapid response is the presence of a novel gravity sensor actually within the elongation zone cells. It is interesting to note that touch-related membrane potential changes and gravity-related membrane potential changes in the columella are transient, while the potential changes observed in gravistimulated cortical cells of the elongation zone were sustained. This difference in duration may indicate that the latter changes are a very early component of the long-term growth response rather than perception/signaling elements.

Conclusion and Perspectives

It is clear that we are far from a comprehensive view of the ionic machinery of plant mechanoperception or a description of the molecular basis of signal cross-talk in the touch and gravity response. However, current evidence strongly suggests that ionic signals represent some of the earliest transduction events of mechanoperception and response, with Ca^{2+} emerging as a significant element in both touch and gravity signaling. Either apoplastic or cytoplasmic Ca^{2+} changes occur following both stimuli, and Ca^{2+} response elements such as calmodulin are present and appear functionally significant for both. Even so, the results from the studies of Ca^{2+}-dependent regulation of touch and gravity illustrate that we need to clearly understand the potential complexity of these responses in order to interpret the changes we see.

The ionic signaling events monitored during mechanical and gravitropic responses seem to be a compound of multiphasic signaling, with an initial signal sensing event and then generation of longer-term asymmetries leading to a sustained growth response. Only in the case of root gravitropism does the architecture of the organ aid in separating perception events (in the cap) from the growth response (in the elongation zone) and even in the gravistimulated root cap, complex multiphasic events of signal processing may be occurring. The kinetics of the gravity-related pH changes in the root cap suggest complex temporal dynamics with a transient cytoplasmic increase (perhaps related to initial perception events) and a sustained apoplastic decrease, possibly reflecting generation of the asymmetrical export of regulators (Fasano and others 2001). Ca^{2+} may also be involved at two stages in gravity perception. Pharmacological studies suggest that Ca^{2+} is involved in an initial, transient signaling event that triggers the perception machinery (Lee and others 1983b; Björkman and Leopold 1987; Stinemetz and others 1992; Sinclair and others 1996). Although such a change has yet to be detected, this may reflect limitations on current Ca^{2+} imaging technology. Ca^{2+}may also be involved in a second stage of transduction relating to sustained transmission of vectorial information. This second phase might explain the interrelationship of asymmetrical Ca^{2+} flux through the root cap apoplast and auxin transport. Gene regulation (for example, upregulation of calmodulin expression; Sinclair and others 1996) and large-scale sustained asymmetries of molecules like the second phase of IP_3 elevation seen upon gravistimulation of pulvini (Perera and others 1999, 2001) might fall into this latter category. Analysis of these secondary events, particularly through approaches such as global expression profiling using micro array technology, may eventually help define the upstream signaling agents that are active in different mechanosensing events.

A further explanation of the complex and sometimes contradictory nature of current ionic signaling observations may be due to responses resulting from an integration or interaction of multiple signaling processes. There are a growing number of examples of the interactions of stimulus/response systems such as gravitropism and phototropism (Okada and Shimura 1992; Ruppel and others 2001; Vitha and others 2000), or touch, gravitropism, and obstacle avoidance (Figure 1; Massa and Gilroy unpublished; Mullen and others 1998; Okada and Shimura 1990; Simmons and others 1995). It is clear that the stimulus environment of the plant as a whole may alter the output of each specific response system. For example, the temperature of the plant's growth regime has been proposed to switch stomatal guard cell ABA signaling between Ca^{2+}-dependent and -independent pathways (Allan and others 1994) and prior oxidative stress can inhibit Ca^{2+}-dependent signaling in response to subsequent drought stress (Knight and others 1998). Thus, it may be that the stimulus background under which an experiment is performed might partly determine the suite of signaling events monitored.

Ionic signaling also highlights some potential future directions to help dissect this complexity in the mechanoperception machinery. The presence of lipid raft and caveolae structures in animal sensing systems support the idea of a sensitive signal-response complex integrating the extracellular space, plasma membrane, and cytoskeleton (Brown and Jacobson 2001). The models of touch perception shown in Figure 2 reinforce this idea that the microstructure of the cell will be a critical determinant of signaling events. The specificity inherent in such a

structural basis for signal processing is clearly shown in neuronal signal processing. Despite myriad voltage-gated Ca^{2+} channels in the neuronal plasma membrane, only Ca^{2+} flowing through a specific subclass of voltage-regulated Ca^{2+} channels can elicit activation of the CREB transcription factor and so cause changes in nuclear gene transcription (Dolmetsch and others 2001). This specificity is mediated via a sub-pool of calmodulin specifically bound to that particular L-type channel. Thus, neither voltage-gated influx of Ca^{2+} through N-type channels, general elevations in cytoplasmic Ca^{2+}, nor altering the bulk cytoplasmic pool of neuronal calmodulin affect CREB activity. Specificity is hard wired into the microdomain structure of the signaling system.

At present our understanding of the subcellular location of ionic second messengers and response elements activated by either touch or gravity in plants is based almost entirely on pharmacological inhibitor studies. However, an array of new sensors based on green fluorescent proteins (GFPs) should greatly help us to more directly approach these kinds of questions. Modified GFPs that can detect concentrations of both Ca^{2+} and protons have been successfully used in plant cells (Figure 3F–H; Allen and others 1999; Fasano and others 2001). Targeting such GFP-based sensors to different cellular compartments such as the ER, vacuole, and plasma membrane might help resolve the extent to which microdomains and structural specificity underpin the touch and gravity signaling systems in plants.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of NASA and NSF (SG and GDM). JMF was funded through the Plant Responses to the Environment training grant from NSF. We are indebted to Dr. Sarah Swanson for critical reading of the manuscript.

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