CHAPTER 14

Delivering Force and Amplifying Signals in Plant Mechanosensing

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I. OVERVIEW

Mechanosensitive multimodally modulated Ca^{2+}-selective ion channels of certain plant cells integratively transduce several kinds of stimuli. There may be many similar kinds of Ca^{2+} channels, but pending their detailed description it is useful to consider the possible significance of these channels. Outcomes of channel activation depend on the cell type involved. There is evidence that the channels are associated with a recently discovered form of cytoskeleton that may be differentiated to help focus force and control the outcome of transduction. Roles of the channels and the cytoskeleton are examined for two of the most-studied mechanoresponses: gravitropism and thigmotropism by root tips. Specificity of these responses is in part due to separation of receptor tissues. Speculatively, specificity is in part due to whether force is experienced perpendicular or tangential to the channels, to how much the cytoskeleton helps cluster the channels, and to how the cytoskeleton participates in cloistering signal translation proteins and the membrane-adhering regions of the cortical ER where the Ca^{2+} exits the channels. It is suggested that gravitropism has both immediate and sustained phases of cellular Ca^{2+} elevation that result either from two subsequently participating substates of the channels or from two different mechanosensory channels. A definitive test of the model will probably await molecular identification of the channels and many of the associated proteins.

II. INTRODUCTION

Plants are suggested to depend on mechanosensitive Ca^{2+}-selective cation channels in a wide range of mechanosensory processes. Curiously, it is far from evident that activation of the channels is accomplished in the identical way for all these processes. This is all the more interesting because plant mechanoresponses can be extraordinarily sensitive, and the literature witnesses that sorting out the mechanisms for achieving sensitivity is not an easy problem. Viewing the forest scene of Fig. 1, gravity and wind come prominently to mind as important stimuli above the ground, and gravity and soil resistance are particularly important below ground. For practical reasons, many plant physiologists have narrowed their investigations to swiftly growing little seedlings and to two specific kinds of reception with quickly seen outcomes: gravitropism and thigmotropism ("touch" tropism). Although over the years, researchers have felt less puzzled about the thigmotropic mechanism because laboratory testing has often involved fairly large and obvious stimuli, probably touch reception and gravity reception can be equally sensitive. Early evidences that mechanosensory Ca^{2+} channels transduce both stimuli are that each depends

FIGURE 1 Plants exist in a world of mechanical forces and respond in a myriad ways. Here, a toppled tree is growing asymmetrically as it lies on its side; the branches on its upper side have turned straight up, in effect each assuming the role of the primary trunk. In the background, branches maintain their more characteristic angles, but tend to point down a little more with each year of growth (perhaps due to winter sag). The play of gravity, wind, and rain- and ice-loads on this heterogeneous architecture results in constant elaborate force focusing within the internal tissue (Fig. 3). Picture by William F. Pickard.

on availability of Ca^{2+} outside the sensing cells, and each is strongly poisoned by the Gd^{3+} ion so often used as an inhibitor of mechanosensitive Ca^{2+}-permeable channels. When it was shown that plants with cells containing the luminescing Ca^{2+}-reporter system aequorin release a pulse of light following flexure or horizontal placement, acceptance of mechanosensory cation channel participation increased markedly; unfortunately, as will be discussed, these data are critically ambiguous. Touch is now shown in two cases to elicit response by the newer but still slightly ambiguous cameleon reporters, strengthening the case for Ca^{2+} channel participation in both tropisms. Evidence keeps building for the thesis that the sensitivity of each tropism is achieved by utilizing the same or closely comparable mechanosensitive Ca^{2+}-selective channels and a special form of plant cell cytoskeleton—but using the cytoskeleton in contrasting ways.

The electrophysiology of plant mechanosensitive channels has not been extensively investigated at the single channel level. In particular, they have been studied in only a few kinds of plant cells, and the infant subject of molecular biology of plant mechanosensitive channels is even more limited. However, multimodally modulated Ca^{2+}-selective channels have been studied by patch-clamping "model" cells to justify speculations that these channels

could transduce not only stimuli such as gravity, friction, and flexure but also voltage shifts, temperature drops, and gradients of moisture and minerals. A very quick summary of the most pertinent properties of the channels is reproduced in Fig. 2. Plants, more than animals, depend on distributive, elaborately integrational, sensing capability because in general they cannot move about and their organs must make responses to the summed vicissitudes of environment. The multimodally modulated mechanosensory Ca^{2+}-selective cation channels or MCaCs suggests that they may indeed have widespread and

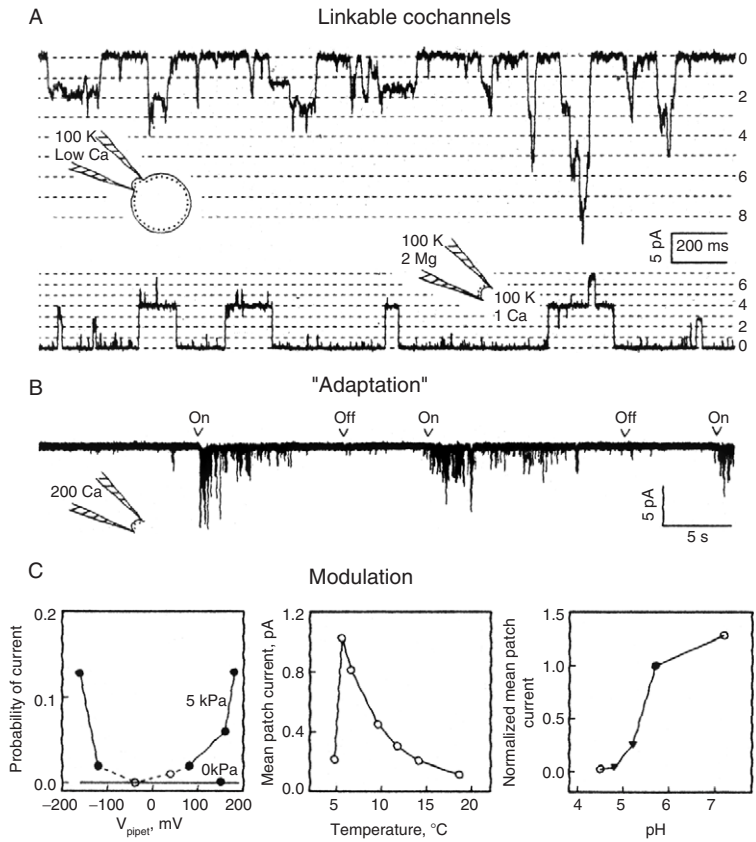

FIGURE 2 Some properties of MCaC activity important for sensory function. (A) Channel clustering is suggested by two representations of linked channel opening. (B) Channels are inactivated, which has the physiological function of "adaptation." (C) Three ways in which activity of channels already under stretch can be modulated: shifting transmembrane potential, temperature, and pH at the extracellular surface. From Ding and Pickard (1993a,b).

diverse functions, and this justifies their selection as a candidate for transducer of multiple kinds of tropism.

All the nonmechanical tropisms except phototropisms probably require some basic level of membrane stretch before more specialized triggering can occur, but membrane stretch is itself the immediate trigger of mechanotropisms. Nonetheless, given the apparent though poorly quantified sensitivity of mechanotropism, it seems unlikely that one could gain full insight into mechanoreception simply by understanding how membrane stretch leads to channel opening. We will develop a model suggesting that separable features of a cytoskeletal channel regulation system may prove specially suited for amplifying response to forces applied in different ways and in different cells.

The choice of root tips for concentrated study is convenient because, to the first approximation, the sensing region is separated from the region in which bending away from—or in some cases toward—the stimulus occurs.[1] However, we will be required to combine mechanoresponse data from a variety of plant species and plant parts because experimentation has not been as restricted in scope as our model.

III. FOCUSING FORCE

A. Force Experienced by a Plant Is Chiefly Borne by the Heterogeneous Wall System

Analysis of thigmotropism and closely related mechanical responses begins by realizing that the plant is an elaborate force-focusing structure, operating at several hierarchical levels. An oblique branch in Fig. 1, for example, is cantilevered out from the trunk. Along the whole length of the branch, there is tension and compression. The branch is made up of heterogeneously arranged tissues, somewhat like those of the zinnia stem in Fig. 3. The tissues are in turn made of heterogeneous cells, with different kinds of relatively rigid walls delimiting different sizes and shapes of cells. In general, these cells are three-dimensional polygons; bound together in a unit, they form very elaborate polygonal arrays. The arrays are not mechanically

[1]Interest has reawakened in the usually much more limited response said to occur well back from the tip. Most seedling organs have strongest sensing at the tip and weaker sensing behind it; and it has been argued that mechanisms of reception are in some cases quite different. In general, kinetics of vigorous seedling roots rigorously maintained under constant light and humidity on the bench top suggest that the tip dominates; it shows enough complexity to suffice for initial modeling.

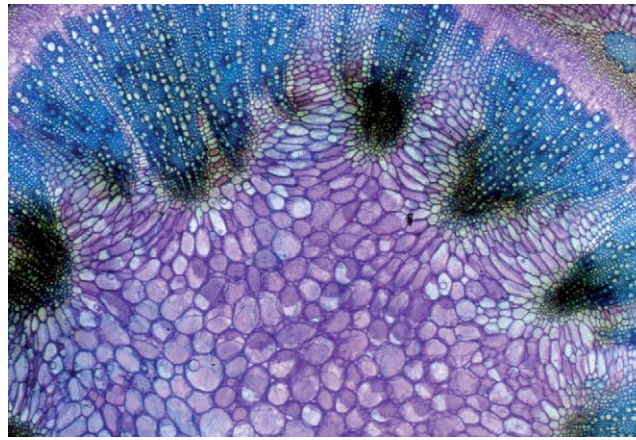

FIGURE 3 A stem from a mature zinnia, sectioned live and stained with a metachromatic die in order to emphasize chemical and derived mechanical differences among the cell walls, illustrates the heterogeneity of plant tissue into which the forces experienced at the level of the whole plant (Fig. 1) are directed. When younger, the cell shapes, sizes, and walls would have been more uniform, but stresses have helped to guide their development in heterogeneous ways that both recall their cellular history and poise them to heterogeneously distribute the forces they experience. As a particularly striking example, note how accommodation to stress has the contorted formerly approximately isodiametric cells between the compact vascular bundles of small, thick-walled cells so that they have elongate and bean-shaped cross-sectional contours. Picture by Joseph E. Varner.

simple, for the cells are not all compactly arranged but in some tissues form hydrophobic and hydrophilic channels. The next contribution to heterogeneity is the gross nature of the cell wall, which is clearly illustrated (though at low resolution) in the zinnia cross section of Fig. 3. All this heterogeneity is a prescription for force focusing within the plant, and the specifics of the architecture control how the mechanical force received by the plant is experienced at a relatively local level.

B. The Plasmalemmal Reticulum Carries Force to the Channels

If a tissue is poked, membranes separating the walls from the cytoplasm can be directly deflected and surely this can activate stretch-sensitive channels. However, cells must be able to more subtly detect when neighbors grow, when microbes invade, or when the organ makes contact with a physical obstruction. If MCaCs in the plasma membrane are to maximally utilize the signal value available in the walls, they must be connected to it.

A number of molecules are now known to connect the wall to the membrane. Just as the MCaCs were identified to help explain highly evolved mechanotransduction, a cytoskeletal structure made up in part of such linker molecules has been discovered to help explain force transmission to the channels (Gens *et al.*, 2000). This structure, named the plasmalemmal reticulum (PR), is shown in Fig. 4. The figure represents one image of a set in which pairs of fluorescently tagged antibodies were applied to the outer face of the plasmalemma of a cultured tobacco cell from which the wall had been enzymatically removed. The image shows what mechanical engineers call a ''polygonal design plan''; it is more simply called a polygonal array or mesh.

Experiments represented by Fig. 4 indicated the presence of a fairly large number of proteins at the vertices of the reticulum, and one conspicuous glycoprotein that was present throughout. For present purposes, three of

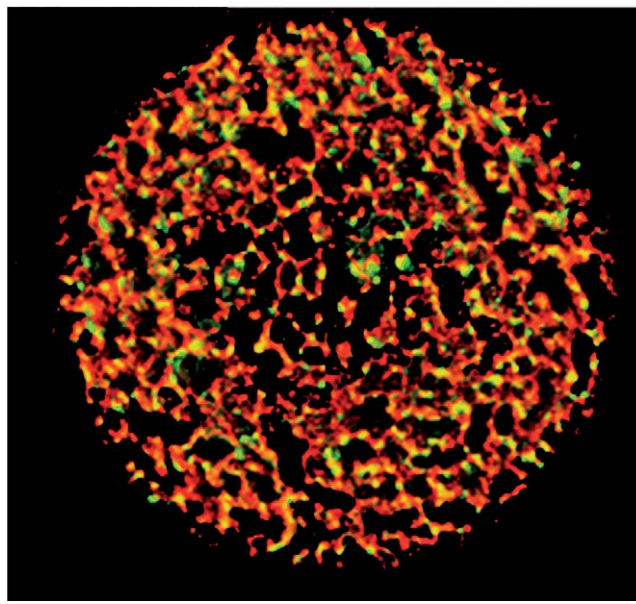

FIGURE 4 The central topic of this chapter, the PR, is proposed to distribute and link channels such as MCaCs and to focus force to them. It is here seen spread over the surface of a tobacco cell which is spherical because its wall has been enzymatically removed. The visible diameter is about 33 μm. Red vs green represent fluorescent antibodies thought to indicate positions of AGP vs proteins such as a β1-integrin ortholog and wall-associated kinase; yellow shows overlapped distribution. The fluorescence image was projected onto a single plane from 3D data represented in Gens *et al.* (2000); it has been shown in a supplemental publication by Pickard and Fujiki (2005), in Functional Plant Biology and is presented with journal permission.

these will suggest how the reticulum operates, albeit in a slightly different way, for both gravitropism and thigmotropism.

Specifically at the vertices of the PR is an ortholog of β1-integrin (Canut *et al.*, 1998; Laval *et al.*, 1999; Gens *et al.*, 2000), a protein famous for its participation in many animal adhesion sites. Its action in a variety of types of animal mechanoreception suggests a similar role in plants, and its participation in clustering a variety of signal processing proteins at both sides of the membrane and in organizing cytoskeletal proteins is also evocative. Accompanying the integrin ortholog are proteins which at the very least have antigenicity similar to the vitronectin and fibronectin often found in the company of animal integrins (Gens *et al.*, 1996).

Conspicuous at the vertices but also present at lesser levels throughout is wall-associated kinase, a transmembrane protein considered essential for growth (Anderson *et al.*, 2001; Verica *et al.*, 2003; Kohorn *et al.*, 2006). Its abundance suggests that regulatory activities sensitive to the local level of Ca^{2+} may be important.

Throughout the reticulum, at the outer side of the plasmalemma, is fasciculin-like arabinogalactan protein (AGP). AGP has for several years been suspected to form an electrostatic cushion between the relatively rigid cellulosic part of the wall and the membrane (Serpe and Nothnagel, 1999; Lamport *et al.*, 2006). In the present context, it is more descriptive to call it an electromechanical cushion. It is now understood that fasciculin-like AGP is attached to the membrane by glycosylphosphatidylinositol (GPI) linkages, which are broken rapidly to free the glycoprotein to move into and through the wall (review Lamport *et al.*, 2006). Meanwhile, the GPI-bound AGP is continually replaced. The distribution of both linked and unlinked AGP doubtless varies with numerous conditions, as does the geometry of the PR. However, we will restrict our view to the simplest known conditions.

The glycan portion of fasciculin-like AGP is highly repetitive, and is suggested (Lamport *et al.*, 2006) to be aligned (Fig. 5). Such alignment would be consistent with interactions along the lengths of the sides of the polygons that would give a certain amount of rigidity. A polygonal array such as the PR is inherently a good force-focusing structure.

These wall- and membrane-associated molecules of the PR offer hints that the structure might focus force from wall to membrane and, indeed, most effectively to the vertices. Particularly if MCaCs cluster at the vertices, the PR might function to capture and focus force for them.

Can the integrity and rigidity suggested by the more-or-less intact reticulum seen in Fig. 4 be evaluated? One experiment that bears on this was a bonus of discovering the reticulum (Gens *et al.*, 2000). More strenuous treatment of the cultured cells with preparations of commercial hydrolases, which often contain contaminant enzymes, yielded apparently fragmented

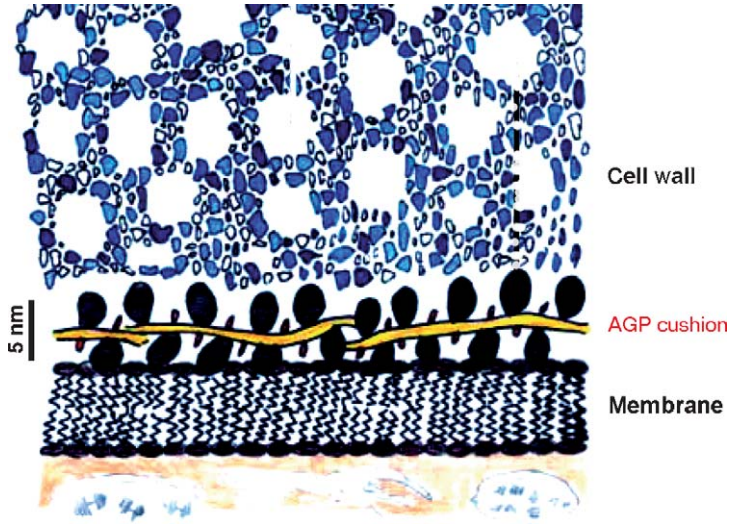

FIGURE 5 According to a model by Lamport *et al.* (2006), a layer of AGP is anchored to the plasmalemma and cushions it from the wall. This chapter develops the idea that the AGP layer may be important for how force is experienced by the mechanosensory channels in the membrane; indeed, they might enable discrimination between tangentially and perpendicularly applied force. Consistent with expectations of the PR model, the protein chains of the Lamport *et al.* model tend to be aligned in parallel. The diagram is not to perfect scale, but a size bar indicates the diameter of the AGPs. The wall drawn from Lamport *et al.* emphasizes pores in the pectin component of the wall rather than polymers, these may not be relevant for the PR model, but are left in this modified excerpt to provoke thought about their proposal that AGPs can fit into such pores end-on under some circumstances and anchor the PR to the wall.

meshes. These fragments, even though often very small, retained characteristic angles between short linear segments of fasciculin-like AGP with the integrin at the vertices. The characteristic geometry of the mesh never dissipated into unrecognizable splotches or diffusely dispersed areas even though during extensive digestion sides might shorten further when only one, two, or three remained linked together. The integrin ortholog never dissociated from AGP.

A remarkable set of so-called Yariv compounds allows a direct test whether the AGP and by implication the PR exerts control over the channels (Pickard and Fujiki, 2005). Some stereoisomers of the set bind AGP very specifically, while others do not bind at all. In solution, the binding isomers precipitate the AGP. The results of applying binding and nonbinding Yariv agent to BY-2 cells expressing a ratiometric cameleon Ca^{2+} indicator make reasonably clear that binding causes Ca^{2+} elevation (Fig. 6). [Controls showed that the effect was not due to acidification, but new evidence from

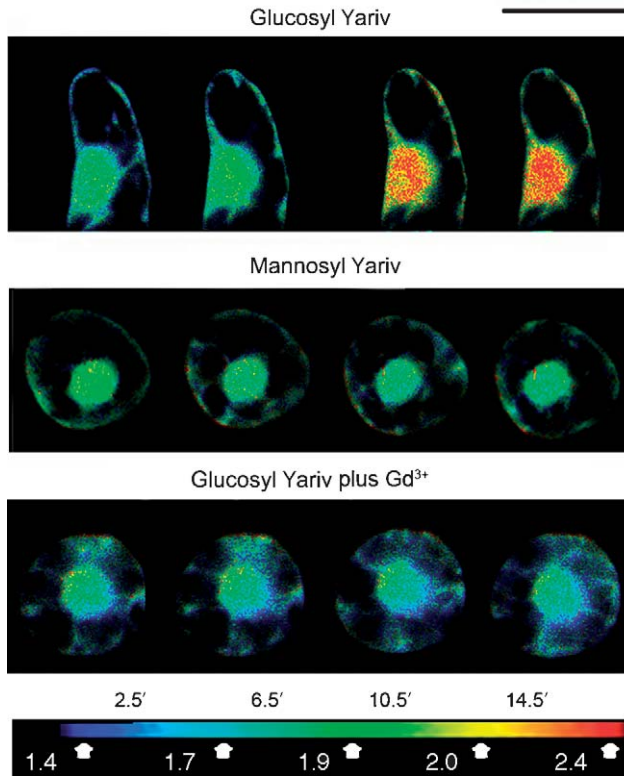

FIGURE 6 Evidence that AGP, shown in Fig. 5, to be a constituent of the PR controls elevation of Ca^{2+} in cytosol and nucleoplasm of tobacco cells. Ca^{2+} level is indexed ratiometrically (see rainbow scale) with a cameleon reporter; frames are selected from movies by Pickard and Fujiki (2005) with permission from Functional Plant Biology. Application of glucosyl Yariv compound, which binds specifically to AGP, leads to elevation, the inactive stereoisomeric mannosyl Yariv compound has no effect, and mechanosensory channel inhibitor Gd^{3+} nullifies the effect of the glucosyl compound. Bar 50 μm.

Plieth (2005) suggests that Cl^- must also be evaluated for its role in cameleon responses.]

Further experiments tended to implicate MCaCs in the rise because it could be inhibited by Gd^{3+}. However, the visualized rise (which extended into the nucleus) appeared to be due to release from the ER.

Elevation of Ca^{2+} by Yariv agent had already been seen in lily pollen tubes, which have abundant AGP of unknown distribution and walls of composition different from those of root tip cells (Majewska-Sawka and Nothnagel, 2000).

Mechanosensitive Ca^{2+}-selective channels are now described by patch clamp of pollen tube protoplasts and apparently have a simpler behavior than the MCaCs of the present model (Dutta and Robinson, 2004). Ca^{2+} plays a critical role in guiding the direction of pollen tube growth as well as in the basic growth process itself. All this is important corroborative evidence for a role for AGP in regulating mechanosensitive Ca^{2+}-selective channels. [However, while the fundamental control mechanisms for pollen tubes and root tips are doubtless similar, they have many superficial dissimilarities and their cell-growth patterns are strikingly different. In order to maintain linear arguments, most authors consider the two kinds of cells separately, and we will follow suit. Fortunately, the excellent review of Majewska-Sawka and Nothnagel (2000) provides good access to the pollen tube mode of development.]

While the Yariv agent experiments suggest that the PR is important for focusing force to the channels, they do not address whether force is carried to the channels by direct molecular linkage or merely carried to the vicinity of the membrane, relying either on lipids or other molecules for the final tug on the channel protein. Since force is often transmitted to mechanosensitive channels by lipids in animal cells (Hamill and Martinac, 2001; Kung, 2005), this seems a likely possibility, though direct transmission might seem more efficient for subtle sensory activities. Lacking evidential basis for argument, it is fortunate that this issue is not absolutely critical for evaluation of the model. In fact, a much more important issue to be sorted out is where the channels are grossly localized within the plasma membrane.

It would seem that efficiency of force collection would require the MCaCs to associate with the PR. But furthermore, for at least six reasons the most probable distribution for sensitive detection of local occurrence of force around the periphery of the cell would seem to be at the PR vertices.

1. For this kind of sensing, it is desirable to spread out numerous channels with relatively low Ca^{2+} conductance, and it is desirable that the local dynamic range of channel activation be large. This is well accomplished by clustering channels of the MCaC kind.
2. In a polygonal array such as the PR, the force is best focused at the corners.
3. There are centers of cytoplasmic activity just internal to the vertices, and thus this might be an area rich with proteins in reaction cascades initiated by Ca^{2+}.
4. The vertices are binding sites for the reticulum of cortical ER. In the experiments of Fig. 6 above, there was evidence that the ER was the internal store responsible for Ca^{2+} elevation into the cytosol. Unpublished experiments done in association with those of Fig. 6 show that local mechanical

probing causes local elevation of Ca^{2+}, with evident local biochemical consequences—consistent with close local association of ER.

5. In patch clamp, the channels appear to cluster. One quarter of the patches of onion epidermal cells made by Ding and Pickard as cited above evidenced no channels, whereas three quarters had so much activity that the channels could not be counted. Four other kinds of channels in cells prepared and patched in much the same way showed no unusual grouping.

6. The channels evidence physical linkage that appears to vary rapidly (Fig. 2A). In cell-attached patch clamp, they can open or close simultaneously in groups ranging from two to at least nine. Linkage can be strikingly altered by changing the ionic composition at the cytosolic face of excised patches.

For the ultimate determination of channel distribution, it will be necessary to identify the channel proteins and visualize the cells after tagging channels with a fluorescent label. On the basis of the persuasive indirect evidence of the six-point list, however, the working hypothesis is that the channels are situated in the membrane covered by the PR, and may in some cells cluster at the PR vertices.

C. Implication of Heterogeneous Walls for Thigmotropic Reception

It is easy to imagine how thigmotropic reception happens. When a tissue is flexed, poked, or rubbed, force is distributed within the wall system and transmitted to the PR, where it is expressed tangentially. At the vertices of the PR (or maybe along the sides), the tangential tug on the membrane and perhaps even directly on the channel complex causes channels to open. Ca^{2+} enters the cytoplasm, particularly in regions subjacent to the adhesion sites, and elicits release from the ER anchored there. Depending on the magnitude of the stimulus and the type of cell, various reaction cascades follow.

D. Walls Are Only Half the Mechanical Story: Gravitropism, Like Plant Form, Depends on Force Generated Inside Cells

As compared with thigmotropism, the basis of gravitropic reception is less obvious. It seems useful to return to very basic considerations. The trees of Fig. 1 and the cells of Fig. 3 would not be experiencing much force in their wall systems were it not for the force of turgor pressure, maintaining all the living (as opposed to dead, woody) cells in expanded and fairly rigid geometries. In turgor sensing, the force experienced by a cell is not transmitted through the wall system, but is generated internally due to its own osmotic potential. Of course, the forces generated by the cytoplasm and in the restraining wall

act together and in opposition, but in terms of mechanoreception they may have a different kind of impact on the MCaCs.

Whereas thigmotropism depends on force external to the cell borne by the wall, gravitropism may depend on a little asymmetric hydrostatic boost to normal turgor pressure. Expressed another way, we might imagine (1) that the more effective the gravitropic receptor cells, the more specialized their turgor sensing mechanism; (2) that the cells tend to be somewhat protected from the effects of turgor vicissitudes so often experienced by the "ground tissue" or parenchymal cells; and (3) that the cells are sensitive enough to react to the *differential* pressure across them when displaced. It is only the differential that matters: a change that occurs on both sides of the cell will not have a tropic effect. It seems unlikely that the membrane on the lower side of the cell compresses enough to rapidly push significantly more membrane into the upper side. Rather, it seems likely that the increased signal force experienced by the channels will be applied *locally and perpendicularly*. It cannot be readily imagined that the PR deflects much force tangentially in the sensory membrane, and the net extra force cannot be large. Considering that turgor of a typical cell is comparable to the pressure in an automobile tire—say, 1 to 4 atmospheres which is the pressure exerted by a column of water 10 to 40 $\times$ 10^6 μm high—how can a differential hydrostatic pressure due to laying a 10 to 40 μm cell on its side be detected? (Moreover, plants undergo gravitropism when suddenly tipped only two or three degrees.)

How does ordinary turgor sensing work? Can a highly sensitive variant of the mechanism of turgor sensing sufficient to detect gravitropic displacement be imagined? Could the PR play one or more roles in it?

1. An Electromechanical Pillow for the Channels

When turgor pressure is steady, the high-resolution geometry of the membrane-wall interface must accommodate to the opposing forces in some way. If during gravitropic displacement the MCaCs (as well as a variety of other membrane proteins) were to be pressed harder directly against the rigid cellulosic portion of the cell wall, one imagines that opening movement could be inhibited and molecular damage might occur. However, the AGP suggested to be arranged somehow to help provide PR rigidity (refer back to Fig. 3) also may provide a springy periplasmic electromechanical cushion, as first suggested by Serpe and Nothnagel (1999). (They applied the term electrostatic, but electromechanical seems more descriptive.) According to Lamport *et al.* (2006), part of the cushioning is created by the GPI-linked AGP, and part is thought to be freed molecules slowly tending to work their way through pores in the wall, destined for the intercellular boundary layer. Depending on the state of differentiation of the cell and on its environmental stressors, sometimes the linked AGP might putatively blanket the entire

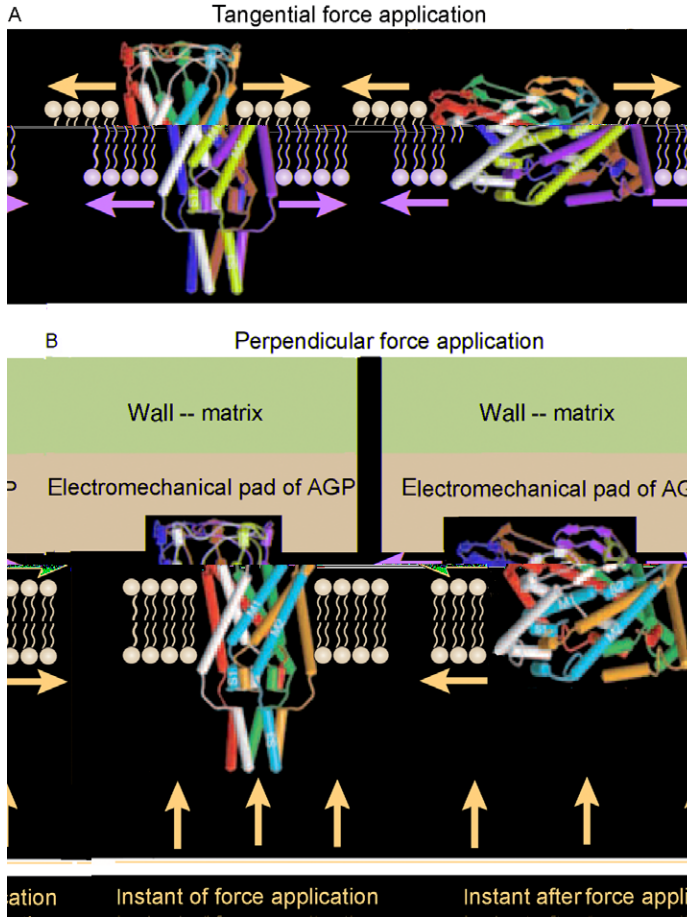

FIGURE 7 A minimalist diagram suggests how force applied perpendicular to the plasmalemma might have consequences different from force applied tangentially. (A) Encapsulation of transduction of laterally applied force; this part of the diagram is excerpted with only modest modification from a large panel by Vogel and Sheetz (2006) and indicates how stretch-sensitive channels are opened in animal cells. Such a mechanism would not necessarily be strongly influenced by the plant cell wall when force is applied tangential to the membrane via the PR. (B) The interface between cellulosic wall and channels might be rough and rigid were it not for padding by the AGP-rich PR. The AGP cushion might be beneficial in response to tangential force, but more critically it could optimize transduction of a tiny increase in turgor pressure (perpendicularly applied force) which tends to press the channels ever more tightly against the wall. (This action of turgor at the cellular level is to be distinguished from how shifts of turgor in a heterogeneous tissue might set up a complex pattern of tangential forces among the numerous cells.) While the AGP of the PR contributes importantly to the pad, there is also a layer of AGP freed from the membrane into the periplasmic space (cf. Lamport *et al.*, 2006). AGP has for some years been suggested to provide an electromechanically spongy interface (cf. Serpe and Nothnagel,

membrane. However, for our thigmotropism and gravitropism modeling, it is important that it is a component of the PR. The cushion might allow MCaCs and neighbor molecules to shift a little both tangential and perpendicular to the membrane during normal motions. Indeed, it is presumably the cushion that allows the interfacial molecules to accommodate to each other in the first place.

2. Radial Reinforcement by the PR

In turgor and gravitropic sensing, perhaps one role for the plump clusters of wall-associated proteins and glycoproteins at the vertices of the PR–ER mesh may be to act as rods which tend to stabilize the adjacent membrane radially under perpendicular force increase. At the same time, there might be some differential expression of stress between the "rods" and adjacent membrane, resulting in torquing. And all the while, during the push toward the wall, the electrostatic cushion of AGP might prevent mashing the channels against an unyielding cellulosic surface, preserving their organization and integrity.[2] Figure 7 crudely simplifies the idea to aid in visualization: adequately large and rapid shifts in differential pressure across the cell might result in slight yielding and reshaping by the springy cushion and permit a small, perhaps well-controlled, momentary opening deformation of the MCaCs; at the last stage of force transmission, during the controlled squash

[2]Conceivably such a push might be more likely to move proteins that lever against integrin than to open channels; in some systems, integrins can have a mechanotransductive effect either with or without the participation of channels (Vogel and Sheetz, 2006). Participation of integrin is hinted not only by presence of β1-integrin ortholog at the vertices but also by the inhibitory effect of the binding peptide RGD on mechanically controlled cytoplasmic streaming in a certain giant-celled alga (Staves *et al.*, 1992, 1996; Wayne *et al.*, 1992).

1999; Lamport *et al.*, 2006 on "electrostatic cushioning"; cf. Pickard *et al.*, 2006, for more ideas on how to think about the capabilities of such electromechanical action). According to this concept, the pad could yield a little while permitting the channel to deform/open also. Wall-to-membrane-to-cytoplasm linker proteins—not shown—are putatively colocalized with the channels, and while no specific role in responding to perpendicular force is postulated, it is possible that they might also contribute as springs or as levers. In both (A) and (B), tawny arrows on the left side indicate forces at the moment of application. On the right, the channel systems have just responded to the force by opening. Not shown: the membrane system relaxes rapidly. It cannot be squeezed hard, and adjusts to increased pressure in various ways including endocytosis. This may explain in part why many sustained physical stimuli result in transient channel opening, although some kinds of stimuli result in persistent channel activity.

when the channels open, there might be an effective transformation of some perpendicular force into transverse force.

E. Not Just Any Displacement Triggers Gravitropism

A feature of gravitropism usually ignored by casual observers but arresting to those watching closely is: once gravitropic curvature is underway, the angle of stimulation is constantly shifting at a slow rate. Yet, the plant does not react to the changing angle, but keeps on responding in accord with the original placement stimulus. The fact that very slow small shifts of gravitropic position are often without result, whereas comparatively large or, especially, sudden shifts of gravitropic position elicit tropism, *suggests that the initiation of gravitropic reception and its steady-state continuation must be somewhat different*. There is stunning evidence, if still incompletely worked out, that favors this idea.

However, before considering this evidence, and before asking whether the proposed reception of perpendicularly directed force due to differential pressure provides the required level of signal amplification, it is useful to consider some geometric features of the root cap in which reception occurs and to further examine what happens in the apparently simpler case of thigmotropism after force has been directed to the MCaCs.

F. Map of Mechanotropic Cells in the Root Cap

The morphology of our selected model organ, the root cap (Fig. 8A), is not only well suited for guiding the growing root but also for helping us to sort out the differences between the two mechanotropisms we are considering. Not only is the downstream tropic curvature response zone reasonably well separated from the cap, but also the gravity and touch sensing regions are apparently separated from each other into central and peripheral parts of the cap. Within the core, the cells are tightly packed and have relatively thin walls. The ER of the cells is specially differentiated (Zheng and Staehelin, 2001). Unsurprisingly, the zone that detects pushing and rubbing of the root as it grows through the soil is more superficial. It contains and tightly restrains the central zone. This outer region has an extraordinary specialization that is simply called the root cap net (Guinel and McCully, 1987; Guinel *et al*., 1987) (Fig. 8A and B). This polygonal net consists of reinforcing material along the three-wall junctional edges and at the corners of the cells; its toughness is evident because enzymatic isolation does not collapse the structure flat. A major function is to hold the cells together as the root

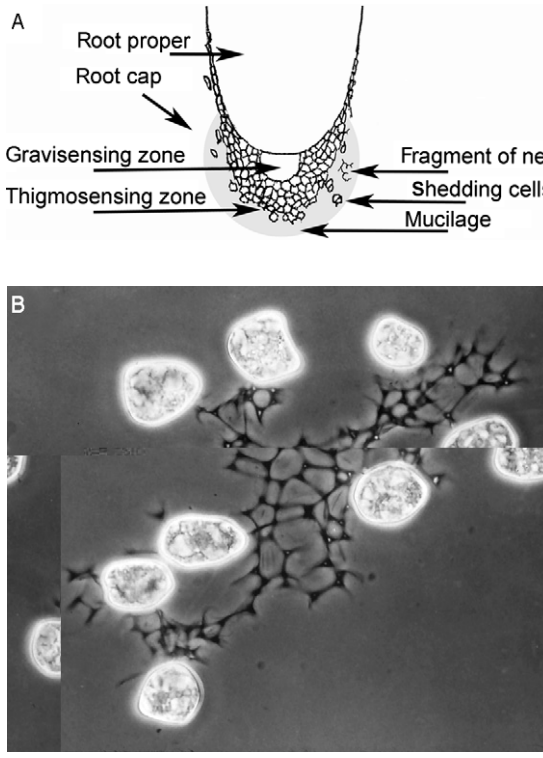

FIGURE 8 (A) The tip of the root, a center for mechanosensing. Root tips of different species display a great deal of variety; this rough schematic gives only a general idea of features relevant for our model. The zone of gravitropic sensing cells is compacted inside the zone of thigmotropic sensing cells, which are encased in a tough polygonal net. It is the outline of the net which is sketched in this cross-sectional diagram. There is no sharp internal delimitation of the net zone such as has been drawn here for simplicity. The net reinforces the outer part of the cap as it forges through the soil; it is continually developing external to the core of the cap, and being enzymatically digested at the outer surface and shedding cells into the soil environment. These cells help condition the soil, interacting with soil microbes. The outer cells of the thigmotropic zone secrete mucilage, which is thought to have several functions. A pertinent one is to ease the root through the soil without excessive thigmoresponse. When grown in moist air, as shown here, the mucilage and shedding cells remain in place along with bits of the partially digested reticulum. None of the fairly complicated details are shown for the root proper, into which hormone moves asymmetrically to initiate differential growth. (B) Photograph of released fragments of the root cap net of maize, plus released cells, from unpublished work of Frederique C. Guinel.

probes through the particles of the soil. It appears that by holding them tightly together, it helps compress the central net-free core, a function that should not be ignored when thinking about why gravitropic reception is localized in that region. By so tightly packing the fairly uniformly shaped

central cells, it probably helps to minimize thigmotropic-type tangential stimulation. For the tissues within the net, it exaggerates the heterogeneity of the cell wall system and its force-focusing capability for thigmotropism.

IV. TRANSDUCTION AND ENSUING EVENTS IN THIGMOTROPISM

The first putative rise of cytosolic Ca^{2+} was assessed with aequorin as a reporter. Observing the response with a variety of plants stimulated with a pulse of wind, an immediate spike of luminescence peaks in about half a minute and decays in a couple of minutes. A slower and more sustained rise may occur before the first has dropped to baseline. These data are intriguing, but not entirely convincing because the coelenterazine component of the aequorin reporting system detects reactive oxygen species (ROS) (Plieth, 2005). Luminescence is also elevated during responses to a wide variety of stimuli and stresses, and data distinguishing between Ca^{2+} and ROS are often lacking! Many experiments should be repeated with coelenterazine-only controls unless independent evidence for Ca^{2+} kinetics can be provided.

Of more reassuring relevance, then, are unpublished higher-resolution experiments that Simon Gilroy and associates have carried out on root tips expressing a cameleon that reports Ca^{2+} (personal communication). Criticisms of Plieth (2004) that cameleons may also report Cl^- need to be considered, but it is hard to imagine that they are responsible for the dramatic results of Gilroy and associates: by pressing on the cap they can cause an increase in apparent cytosolic Ca^{2+} in cells in the stimulated area followed by a spread through the superficial zone—but not the central zone. A little later, a Ca^{2+} wave spreads into the root proper. The ultimate symmetry of this response is consistent with the idea that the system must have a large dynamic range: for a slight tropic response, a small and asymmetric initial Ca^{2+} entry would be required, and it would probably lead in one way or another to asymmetric transport of the growth hormone auxin: large responses leading to symmetric Ca^{2+} elevation would tend to bring about morphogenetic rather than tropic behaviors.

The wave of Ca^{2+} initiated by a sizable mechanical poke illustrates in an important way how even a small signal can be substantially amplified posttransduction. This spread probably occurs in four main steps as follows:

1. Ca^{2+} entering through the MCaCs or related channels is cloistered in a space between the PR vertices and the ER vertices.
2. Ca^{2+}-stimulated release at the ER vertices sweeps along the connected ER, elevating Ca^{2+} throughout the cytoplasm. The Ca^{2+} elevation is accompanied by a depolarization of the plasmalemma.

3. The Ca^{2+} and depolarization propagate through the symplast, which is defined as a tissue in which cells are connected at least electrotonically via open or readily openable plasmodesmata.
4. The Ca^{2+} and depolarization amplify iteratively in the neighbor cells by repeating release from the ER and subsequent propagation through the symplast.

V. EARLY EVENTS IN GRAVITROPISM

A. Direct Evidence for Pulsed Ca^{2+} Elevation

Part of the reason gravitropism remains mysterious is that it has proved hard to reliably visualize a rise of cellular Ca^{2+} following displacement of the plant. For gravitropism as well as for thigmotropism, it is possible to express the apoaequorin, add the coelenterazine required to form aequorin, and then to see spikes of luminescence instantly following stimulation (Plieth and Trewavas, 2002) (Fig. 9).

Since our model suggests that immediate and prolonged gravitropic response have slightly different features, this might be viewed as encouraging. On the other hand, it is discouraging to realize that this reporter system is

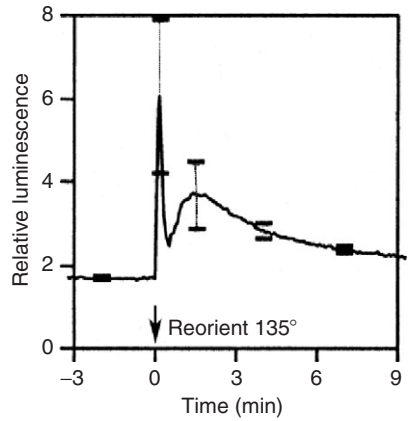

FIGURE 9 The first sign of gravitropic response is likely a fast transient elevation of cytosolic Ca^{2+}. Entire *Arabidopsis* seedlings expressing aequorin and provided with cp-coelenterazine were rotated 135° out of their equilibrium orientation to administer optimal gravitropic stimulation. Unfortunately, the reporter also responds to ROS. More controls are awaited. From Plieth and Trewavas (2002) with permission of Plant Physiology.

nonspecific, and coelenterazine controls have not been carried out yet. Unfortunately, in contrast to the situation for thigmotropism, for gravitropism the nonspecific studies with the luminescent reporter are all we have available so far. Despite the unsatisfactory nature of such evidence, many aspects of curvature kinetics and information about some kind of early Ca^{2+} involvement encourage us to proceed with modeling gravitropic reception based on MCaCs and Ca^{2+}.

B. Curvature Kinetics Are Consistent with MCaCs as Gravitropic Transducers

The curvature kinetics of gravitropism constrain modeling of reception in five important ways, each of which is consistent with a transductive role for MCaCs.

1. The laboratory induction plot for seedling gravitropism can extrapolate to almost the moment the plant is turned on its side [an early discussion was provided by Johnsson and Pickard (1979) and many authors have provided further evidence] and induction is best fit by a power law (unpublished data with *Arabidopsis* wild type and mutant roots).
2. Estimating the induction plot as linear during the first half hour (only under selected fixed lighting conditions) or taking into account the equation for the time-course of induction, induction is roughly proportional to the perpendicular component of gravity (when the quadrant of stimulation lies on either side of the vertical).[3] A relatively early, statistically tidy, experiment using oat seedlings was reported by Pickard (1973) along with historical credits. Several more experiments have followed, even in the reduced gravity of low rocket orbit.
3. Gravity reception has dual component processes, at least for maize roots (LaMotte and Pickard, 2004a,b). Naturally both processes are responses to the vector of gravity, and must for some time run in parallel. But while the outcome of the second kind is vectorial, that of the first is nonvectorial and simply serves to enable or facilitate both initial and ongoing activity of the second kind.
4. Consistent with dependence of each of the processes on the perpendicular vector, the full-response data (within a quadrant) are slightly better

[3]Suddenly shifting quadrants above or below the horizontal has large but possibly readily explained effects; see LaMotte and Pickard (2004b).

matched by a cosine-of-stimulus-squared plot than by a simple cosine plot (LaMotte and Pickard, 2004a).

5. Finally, the state achieved by facilitative reception for dimly lit (but not brightly lit) seedling maize roots can decay, sometimes rapidly. This decay can save the plant from over-responding and in general can enable roots to establish oblique (plagiogravitropic) orientations that may be more advantageous than vertical growth. The establishment of induction probably has multiple steps which are not separated in curvature kinetics, but the observable consequence of decay is fast enough that after a lag of some minutes any sudden subsequent reorientation reawakes gravitropic sensitivity (LaMotte and Pickard, 2004a,b). Decay rate relative to secondary, vectorial, induction rate would necessarily vary with organ and environment in order to explain the varied kinetics so abundantly lodged in the literature.

The dependence of curvature response on the perpendicular gravitropic stimulus vector and the rapid onset of induction are formally consistent with initiation of gravifacilitative reception and of vectorial gravitropic reception by establishing a differential distribution of force across the cell. (Given the forward location of the root cap receptor cells, it is unlikely to be a flexure response due to cantilevering by the weight of the more apical cells.)[4] Next, it must be considered whether signal transduction by MCaCs initiates the two reception processes.

C. Ca^{2+} Kinetics and Xenobiotic Effects Are Consistent with MCaCs as Gravitropic Transducers

If MCaCs serve as gravitropic transducers, there should be diverse evidences pointing toward early shifts in Ca^{2+} distribution during stimulation. There are at least seven such evidences, including some suggesting that MCaC inhibitors block induction.

[4] They are also consistent with abstract features of some, though far from all statolith models. It is well demonstrated that some plant responses to gravity depend on statoliths. However, the huge and rapidly growing, readily accessible statolith literature for seedlings is ignored in the present chapter because models lack firm and direct (rather than correlative) supporting evidence and suggestions for how statoliths might act are sometimes vague, sometimes support predictions that contradict data in the literature, and do not seem to inspire explicit ideas at the molecular and near-molecular levels. There are a number of ways statoliths (in seedlings, the amyloplasts) might play indirect but important supporting roles, but if these are secondary it may be profitable to study putative primary reception as a basis for understanding statolith action.

1. Apoplastic Ca^{2+} is essential for gravitropism of root tips; removing it destroys sensitivity and replacing it restores sensitivity. This was shown for maize roots by soaking their tips in Ca^{2+} chelator or simply water and testing with or without restoration of Ca^{2+} (Lee *et al.*, 1983; Millet and Pickard, 1988a, respectively), and was shown by Masatsugu Toyota and Masahiro Sokabe (personal communication) for *Arabidopsis* seedling stems (hypocotyls) by soaking them in Ca^{2+} chelator.

2. Figure 9 showed that cytosolic Ca^{2+} as judged by aequorin luminescence is elevated immediately on gravitropic stimulation of entire *Arabidopsis* seedlings (Plieth and Trewavas, 2002; but see Plieth, 2005). As shown in the original publication, the initial rise peaks within a very few seconds, and the magnitude of the first peak depends on the perpendicular stimulus vector in at least roughly the same way as curvature induction. (However, since if "sag" occurred it would have the same dependence—experiments to exclude this would be desirable, because these results are for whole seedlings and not root caps alone.) Because of the novelty and delicacy of the experiments, it is important that they have been repeated and extended by Masatsugu Toyota and Masahiro Sokabe (personal communication) for the hypocotyl region of *Arabidopsis*, with reinforcing results. Controls for specificity of the reporter response are still being undertaken.

3. The decay kinetics of facilitative induction (LaMotte and Pickard, 2004a,b) and cytosolic Ca^{2+} elevation indexed by aequorin luminescence (Plieth and Trewavas, 2002; Toyota and Sokabe as above) are plausibly consistent—given the differences in the experimental systems. The first peak of the whole-tissue response undergoes 50% decay in about half a minute, during which a second, slower, and lower rise has begun. This second peak begins to fall more or less exponentially in a minute and a half; and Plieth and Trewavas state that it ultimately returns to baseline in about half an hour. It remains to be firmly established, even for the measurement restricted to hypocotyls, that both peaks occur in individual cells, as there are clearly disparate types of cells in the populations. However, while the presence of a low second peak may indeed influence interpretation of secondary steps in signal reception, it does not negate the possible significance of the primary elevation in the present context. For subsequent purposes, it will be hypothesized that both peaks occur in the same cells and represent true gravitropic response. (Fortunately, although this evidence inspires the model, with slight modification the model can stand without it.)

4. Mechanosensory Ca^{2+} channels proposed to serve as gravity sensors exist in some gravitropically sensitive tissue, though the gravitropic receptor cells per se were not identified (Ding and Pickard, 1993a). Predicted inhibition of MCaCs by Gd^{3+} was dramatic; of course, this is the inhibitor specifically worked out with gravitropic maize roots by Millet and Pickard (1988b) to

enable correlating patch-clamped channels with mechanotropic capability. Just as gravitropic curvature is less sensitive to the similar ion La^{3+}, channel response is in some sense less sensitive; at least, it is less definitive.[5] Elevation of cytosolic Ca^{2+} in BY-2 tobacco cells can be inhibited by Gd^{3+}, as already evidenced in Fig. 6. Such information is unfortunately not directly applicable to Ca^{2+} kinetics during gravitropism, but has suggestive value nonetheless.

6. Asymmetric application of $AlCl_3$ to maize root tips has been shown by Hasenstein *et al.* (1988) to cause bending away; significantly, "Al^{3+}" is as strong an inhibitor of onion MCaCs as is Gd^{3+} (Ding *et al.*, 1993; Pickard and Ding, 1993).
7. Amphipathic membrane-inserting molecules that open channels inhibit gravitropism, putatively by flooding cells with Ca^{2+} (Pickard and Ding, 1993).

All this evidence is consistent with a role for MCaCs in detection of perpendicularly directed force due to differential increase of pressure from the cell contents. Therefore, it is worth speculating on how sensitivity of the channel system can be further enhanced, for it is intuitively clear that no satisfactory model for MCaC participation can be built unless it includes further amplification.

D. Ramping Sensitivity Up and Down Again: Voltage and pH Modulation of MCaCs

The properties of MCaCs per se offer two potential mechanisms for sensitizing response to weak signals: channel opening is strongly modulated by transmembrane voltage and periplasmic pH (Fig. 2C; Ding *et al.*, 1993). Control of the range of apparent sensitivity is dramatic. Cells can typically control steady-state voltage, thus differentiating their sensory capabilities. Shifts of pH are more likely transient. They almost inevitably participate in feedbacks with the channels in some situations, because plasmalemmal proton pumps are often activated by elevation of cytosolic Ca^{2+} and when this promotes acidification of periplasmic pH, MCaC activity is inhibited (Fig. 2C). In the most general case, activation of the proton pump by MCaC action and inhibition of the MCaCs by results of pump action might alternate,

[5]Sensitivity is difficult to appraise because La^{3+} causes fast promotion of opening, followed by oscillating inhibition and promotion. Gd^{3+} also very briefly promotes initial opening, but inhibition develops rapidly (Ding and Pickard, 1993a). With extremely long observation, however, Gd^{3+} action can appear to oscillate; data of Jiu-Ping Ding and Barbara G. Pickard after publication of the 1993 papers.

in oscillatory fashion, to modulate response to shifting mechanical forces and permit delicate temporospatial control of cell growth.

E. Variable Linkage: A ''Nonmechanical'' Role for the PR

The scheme for setting channel sensitivity by changing its ionic environment makes some progress in accounting for a systematic response to gravity, but still it does not seem to provide enough amplification to explain its sensitivity. Fortunately, more powerful mechanisms can be envisioned by returning to the PR itself. To view the PR only as a device for focusing force on MCaCs is to miss some of its potentially most stunning ways of enhancing mechanosensitivity. Given the supposition that MCaCs cluster at the vertices of the PR, it is a small step to postulate that it is the PR that regulates their cooperative behavior. The possible impact of the variable linkage shown in Fig. 2B is made clear by revolutionary new experiments on neurons that show how surface density of channels can encode response.

Naundorf *et al.* (2006) [see also commentary by Gutkin and Ermentrout (2006) and see Århem *et al.* (2006)] have shown that while the Hodgkin-Huxley theory of action potentials can predict the behavior of sparsely distributed voltage-activated cation channels in at least certain nerve cells, it cannot predict behavior when the channels are closely spaced. High surface density of channels promotes cooperative behavior. The opening of any particular channel shifts the activation curve of each channel to which it is coupled toward more hyperpolarized values, thus increasing its probability of opening (sensitivity to signal input). The ultimate consequence of greater surface density for neurons is that action potentials of a train recur at a greater rate. Indeed, the rate of firing can be controlled over one or two orders of magnitude.

Given that the plant cell membrane is in general squeezed between the cell wall and the cytoplasm with its remarkably negative water potential, and given that patch-clamped MCaCs are modulated by transmembrane voltage (Fig. 2C), for practical purposes MCaCs seem to perform much like voltage-activated channels. Could the MCaCs be especially tightly clustered in gravitropic cells? Though it is not obviously necessary for thigmotropism, such clustering could facilitate that and other thigmoresponses as well.

F. Cloistering Ca^{2+}

The definition of adhesion sites by the vertices of the PR and the often-close association with the ER at those vertices offers an advantageous possibility for clustering other molecules in addition to channels—molecules

critical for the gravitropic signal transmission cascade. These could cloister Ca^{2+} in a small niche so that it would not likely escape without activating appropriate biochemical amplification mechanisms. The adhesion site and auxiliary Ca^{2+}-impounding proteins and lipids might be more vividly described as a special kind of signalosome—an architectural device analogous to the metabolome, but designed for signal rather than metabolite processing. Metabolomes minimize diffusion lags and losses by direct handing forward of reactants and products in a reaction series. By analogy to some alleged metabolomic efficiencies, such gravitropic assemblies might increase the encounter rates of reaction participants a good order of magnitude over those of widely separated participants. However, in the metabolic channeling literature, the question of rate enhancement is not frequently raised—it is instead asked whether the direction of a reaction sequence can be controlled or whether a reaction sequence can be caused to go forward at all (cf. Anderson, 1999). Special inspiration may derive from considering the extreme case of tryptophan synthase (Miles, 2001), in which a substrate passes into a protein barrel containing successive sites of enzyme activity. Such temporospatial guidance and intensity of reaction events might perhaps help push the response to Ca^{2+} entering through MCaCs over the threshold for promoting the next step of the gravitropic reaction cascade.

VI. FROM PRIMARY TRANSDUCTION PULSE FORWARD: FACILITATIVE AND VECTORIAL GRAVITROPIC RECEPTION

We have now set out enough facts to round out a model, however speculative. The basis of any model must be the knowledge that there are both facilitative and vectorial kinds of reception. The concept that dual paths are required to effect gravitropism in representative seedling plants (LaMotte and Pickard, 2004a,b) may at first seem counterintuitive, but this conclusion is forced by careful kinetic experiments. For present purposes, it can either be accepted or studied carefully in the rather long original references.[6]

[6]Some subtle definitions may be helpful. Gravifacilitative reception, which is nondirectional, may be conveniently described as starting just after pulsed transduction. Vectorial gravitropic reception is enabled when processes leading to sustained transduction are met with the results of gravifacilitative reception. Gravitropic induction is the process ending when the responses to gravitropic stimulus are well enough locked in that they will produce gravitropic curvature even if the gravitropic stimulus ceases.

A. Facilitative Gravitropic Reception

The following proposal for the facilitative part of reception is not especially bold.

1. As a hydrostatic pressure differential is imposed across the cell, the small increase on the lower side leads to local opening of MCaCs and local entry of Ca^{2+}.
2. Ca^{2+} activates proton pumps in the lower plasmalemma (cf. Fasano *et al.*, 2001).
3. Extruded H^+ accumulates and inactivates MCaCs (cf. Fig. 2C).
4. As H^+ is pumped out, H^+ from elsewhere in the cell diffuses and greatly reduces the pH gradient. The presence of OH^- then becomes more prominent—that is, the cytoplasm becomes more alkaline. (Because H^+ and OH^- diffuse more rapidly than most ions, it requires only milliseconds to restore pH uniformity across a gravitropic receptor cell of 10- to 40-μm width.)
5. The Ca^{2+} spike or pulse meanwhile has initiated Ca^{2+}-regulated Ca^{2+} release from the cortical ER. This might possibly be evidenced (probably to varying degrees in different situations) by a second, broad, low peak in Fig. 9.
6. Alkaline pH, perhaps coupled with the Ca^{2+} spike and signals triggered by it, leads to enhanced synthesis of a set of relevant proteins, or perhaps to some synthesis of new proteins.

B. Vectorial Gravitropic Reception

The following proposal for sustained induction and vectorial reception is definitely bold.

7. (Continuing from preceding section.) The initial transductive burst of Ca^{2+} and the Ca^{2+}-regulated Ca^{2+} release it promotes (second peak of elevated Ca^{2+}) lead to phosphorylations and other regulatory reactions on the lower side of the cell, perhaps in the cloisters proposed to exist where MCaC clusters can empty Ca^{2+} into them. IP3 is likely involved in the reaction cascade (cf. Perera *et al.*, 2006). The second peak of Fig. 9 does not seem to mark the definitive and exclusive vectorial response. In fact, it is unclear whether the second peak of Fig. 9 is associated with facilitative response, vectorial response, or both; but it certainly does not seem to have the linear relation to exposure that would be expected of the basic vectorial transduction. More definitive experiments are of course needed. In particular,

the crowded seedlings measured by Plieth and Trewavas (2002) may have been under some kind of stress in their experimental chamber, and their gravitropic induction may have been very nonlinear as a function of time. In fact, different types of cells may have contributed different behaviors. Kinetic data on separated cell types, with controls, would be desirable.

8. For vectorial transduction to proceed, low-level and undamped opening of MCaCs or other mechanosensory Ca^{2+} channels must somehow be promoted in the context of the PR–ER cloisters. It is equivocal whether the low amount of immediately sequestered Ca^{2+} postulated to enter during this period of sustained transduction has been visualized using aequorin, and in any case if it binds almost immediately to special receptor proteins it would not activate reporters of "free Ca^{2+}." Somewhat analogous ligand-stimulated localized spikes of Ca^{2+} entering through a kind of high-conductance channel, binding, and ER-controlled entry through abundant low-conductance plasmalemmal channels is believed to occur in some animal cells (Gill *et al.*, 2006; Parekh, 2006). There is mostly unpublished precedent (B. G. Pickard and M. Fujiki) for membrane "memorization" of mechanically induced pulsed Ca^{2+} entry in a nongravitropic epidermal onion cell that can influence the Ca^{2+} physiology of the membrane for a good part of an hour. The apparently extremely low-amplitude mechanically controlled cation conductance in patch-clamped onion epidermal cell membranes with such abundant activity that it could almost be mistaken for noise (Ding and Pickard, 1993a) now seems worthy of further examination. The activity may represent MCaC substate behavior, since it seems to precede the regular MCaC behavior when application of suction is gradual, but may possibly indicate activity of other mechanosensory Ca^{2+}-selective channels.

9. As long as Ca^{2+} is entering the cloistered space, key proteins in the region are activated (as for example by phosphorylation).

10. At the activated lowermost inner surface of the cell membrane, proteins responsible for the active transport of the growth hormone indoleacetic acid (IAA) fasten down. These include one or more members of both the PIN family and the PGP family (Geisler *et al.*, 2005; Geisler and Murphy, 2006; Petrášek *et al.*, 2006).

11. Gravitropic induction has occurred as soon as the PGP–PIN system is asymmetrically organized for transport. Lateral IAA transport can proceed, and IAA will move by axial transport into the rest of the organ. If stimulation ceases, for sometime development of asymmetry will continue and longitudinal transport will propagate it, resulting in differential growth curvature. If gravitropic induction continues, larger amounts of IAA will be moved laterally and the final gravitropic response will be proportionately bigger.

12. Of uncertain but possible future place in the model, the alkaline pH of the cell might, while it persists, chemically alter activity of some of the participants in vectorial reception and induction; for example, it can reduce the affinity of the flavonoid kaempferol for the nucleotide binding fold 1 of PGPs, which might lead to effects on creation of hormone asymmetry following induction. (Thanks to Wendy Peer for a personal review of flavonoid behavior.)

En passant, when gravitational and more general mechanical stimuli are supplied simultaneously, thigmotropism tends to win out over gravitropism—which makes sense because there is usually more benefit to a root by trying to move around a stone than by trying to push it aside. In systems in which gravitropic and thigmotropic cells might be less isolated from each other, maybe thigmotropic Ca^{2+} just overwhelms everything. In the root cap, isolation of the two systems may prevent that. Rather, the excess Ca^{2+} in the path along which IAA must travel is likely inhibitory when touch is excessive.

C. *Decay of Facilitative Reception*

Finally, in a broad sense, the decay of facilitative reception can be as important as its rise. As suggested by the forest scene of Fig. 1, most plant stems, trunks, and branches do not orient vertically—they find oblique positions which help them deploy their leaves in optimum patterns for catching sunlight. This happens in different ways. For corn roots, the phenomenon has been studied in some detail, and it is believed that exponential decay of facilitative reception can make it possible for vectorial induction to halt before it has carried the root to a vertical orientation (LaMotte and Pickard, 2004a,b). Since the orientation of roots is critical for plant anchorage and uptake of water and minerals, decay as well as facilitation is probably controlled by several environmental factors. (Other important mechanisms for establishing plagiotropic angles—likely applicable for the trees of Fig. 1—are also discussed in the references.) Thus, the scheme has one more step, as follows.

13. (Continued from preceding section.) The potential duration of the facilitative process is not indefinite and not fixed; indeed, it depends on factors such as developmental state and position of the organ and especially on illumination. The mechanistic nature of decay is unclear. Perhaps it begins with the return of cytoplasmic alkalinity toward normal (Boonsirichai *et al.*, 2003; Hou *et al.*, 2004) as proton pumps lose activation and H^+ ions pass back into the cytoplasm. Perhaps, it is a result of decline of the early pulse of Ca^{2+} entry.

What brings about such alleged early inactivation of Ca^{2+} channels is not established, but it is seen for other mechanosensitive channels as well. There may be multiple reasons, and this is not the place to evaluate them all. Suffice it to note that the once-common expectation that readjustment of plasmalemmal lipids to stretch-deformation would terminate mechanosensitive channel activity is formally consistent with the rapid turnover of plasmalemmal lipids and vesicular lipid exchanges in plants. A general discussion of lipid adjustment is provided by Hamill and Martinac (2001). For patch-clamped astrocytes, however, Suchyna *et al.* (2004) have measured membrane capacitance to show that this does not occur on a pertinent timescale. Instead, channel inactivation is brought about by a positive action of cytoskeletal elements. When an activating stimulus is applied, the standard conductance states of the channels are preceded by an inconspicuous subconductance—something that, curiously, abstractly compares with the activity seen many times for MCaCs of onion but which seemed at the time too small to characterize for publication (mentioned above). The activation–inactivation plots for major conductance state shown by Suchyna *et al.* (2004) are remindful of the envelope of channel activity seen for MCaCs stimulated on the cell-attached patch (cf. Fig. 4B). As to relevance of the submembrane cytoskeleton model, there is abundant evidence for existence of the spectrin-actin-band4 type skeleton in plants but a paucity of data on what its functions may be; there is certainly no evidence that it controls stretch-sensitive channels.

In any case, regardless of the mechanism of inactivation during patch clamp, it is consistent with the expectation of adjustment of membrane molecules at the interface with the AGP cushion in the intact cell.

VII. WHAT COMES NEXT

It is clear that mechanosensitive Ca^{2+} channels, whether precisely like MCaCs, play a role in thigmotropism, and it is highly plausible that they play a role in gravitropism. Even if the putatively participating channels turn out to be somewhat dissimilar to MCaCs, properties such as cooperativity, pH sensitivity, temperature sensitivity, and voltage sensitivity seem likely to be shared. These properties would help explain the existence of the less-studied root tropisms such as thermotropism, hydrotropism, and electrotropism.

Models such as this must be tested at the molecular level, and a major problem for current studies is that MCaCs and similar plasmalemmal channels have not been characterized as genes or proteins. One of the most important questions to answer is how the channels are distributed in the membrane, which could best be answered with fluorescence-tagged proteins.

The importance of channel identification and characterization is huge: tropisms represent only a tiny area of plant regulatory biology in which MCaCs are suspected to be integrative sensors.

But according to our model, channel importance for the plant cannot be understood until we know how they are associated with other molecules. Ca^{2+} signaling involves not only the temporospatial distribution of Ca^{2+}, but equally the distribution of its receptors and the molecules of the reaction cascade that Ca^{2+} activates.

Mechanoreception by plants is obviously somewhat neglected despite wide acceptance that it is important. In part, this may be because it has not yielded easily to those who have attempted to understand it. A scholarly wit has called gravitropism, for example, "the black hole of plant biology." But optimistically we may presume that many of the unsolved problems have simply been awaiting the modern era of genomic and visualization technologies. Now that we have so many tools to work with, we may hope to see rapid progress—lots of meaningful data coming out the far side of the black hole.

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