Plant Gravitropism. Unraveling the Ups and Downs of a Complex Process[1]

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Wind and heavy rain can have a devastating effect on crop production if they occur late during the life cycle of the plants. They often flatten the crop on the ground, leaving seed and other harvestable products at the mercy of soil moisture and pathogens and inaccessible to mechanical harvesting machinery. However, farmers will not worry much if a storm of similar strength hits a crop earlier in its cycle. They know that younger plants are able to straighten up and resume upward growth. This example illustrates the significance of tropism, or the ability of plant organs to direct their growth along a path that is dictated by their surrounding environment. In nature, plant organs can use a variety of environmental cues to guide their growth, including gravity, touch, light, gradients in temperature, humidity, ions, chemicals, and oxygen. In the example illustrated above, the stems of younger crops were able to perceive a change in their orientation within the gravity field. The corresponding information was then translated into a complex growth response called gravitropism that allowed them to straighten up and to eventually resume growth at a defined angle from the gravity vector, the gravitational set point angle (GSPA).

The GSPA associated with specific plant organs is a function of the identity of the species and organ under consideration, its developmental phase, the physiological status of the plant, and a variety of environmental parameters to which the plant has been exposed. A plant organ is capable of detecting any deviation from its assigned GSPA and responds to the corresponding stimulus (gravitropic stimulus) by developing differential cellular elongation on opposite flanks, resulting in tip curvature and subsequent realignment with the GSPA (Firn and Digby, 1997).

Gravitropism has been observed and analyzed in a variety of plant organs, including roots, hypocotyls, and inflorescence stems of dicots; roots, coleoptiles, and pulvini of monocots; and rhizoids and protonemata of algae and moss. It also has been observed and studied in fungal fruiting bodies. In 1999, an *Update* discussing the status of our understanding of gravitropism in roots of higher plants was published (Chen et al., 1999). Since then, there have been several developments in the field that have provided new insights on the different issues related to gravitropism in multiple plant systems. These breakthroughs are discussed in this *Update*.

GRAVITY SENSING AND THE CURVATURE RESPONSE

For a plant organ to guide its growth along a defined GSPA, it must perceive any change in its orientation within the gravity field. The corresponding physical information must be transduced into a physiological and/or biochemical signal, which must be transported to the response site where differential growth generates a curvature, allowing the growing tip to regain its orientation along the GSPA. As discussed in our previous *Update* (Chen et al., 1999), different cells are specialized to carry out these successive phases of gravitropism in monocots and dicots. For instance, in roots, gravity is perceived mainly by the columella cells of the root cap, whereas the differential growth response associated with gravistimulation occurs in the elongation zone (EZ; Figs. 1A and 2). In shoots, cells located in specialized tissues at the periphery of the vasculature, including the endodermis of hypocotyls (Fig. 1B), and the bundle sheath parenchyma in inflorescence stems and cereal pulvini perceive gravity and generate a signal that is transported laterally to the more peripheral tissues. There, the signal promotes the differential growth responsible for gravitropic curvature (Fig. 1B). On the other hand, in lower plants and algae exhibiting single-cell tip growth (i.e. rhizoids and protonemata), gravity perception and curvature response occur in the same cell (Fig. 1C). Hence, different species and organs adopt different molecular, cytological, and physiological strategies to guide their growth along specified GSPAs.
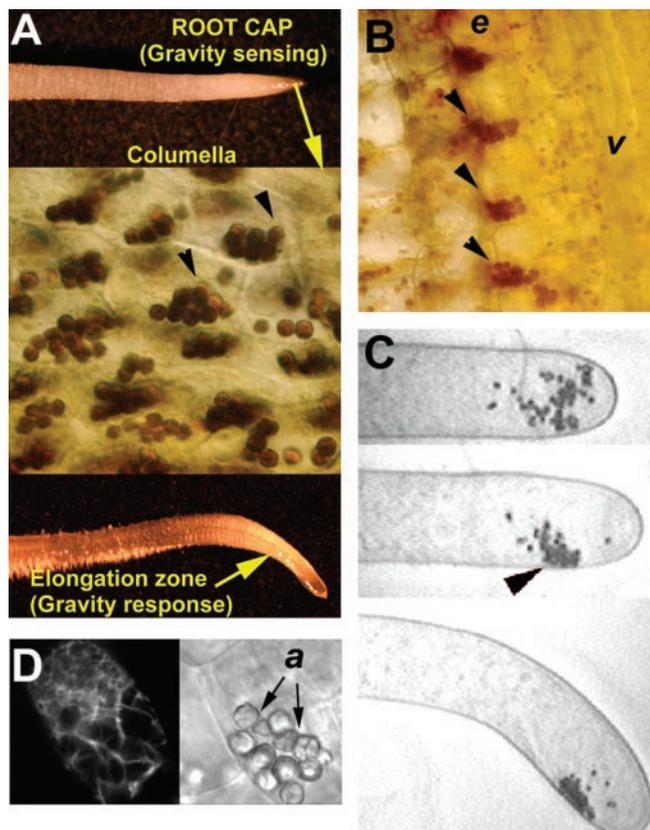
Figure 1. Gravitropism in higher plants and algae. A, In roots of higher plants, the site of gravity sensing (i.e. the root cap) is spatially distinct from the site of the response (the EZ). Amyloplasts present in root columella cells settle to the bottom side of the cell (arrowheads) when the root is reoriented. There is overwhelming support that this process (starch-statolith hypothesis) constitutes one of the primary mechanisms of gravity sensing in plants. Depending on various environmental and endogenous factors, the reoriented root attains a specific GSPA with respect to the vertical. B, In shoots (e.g. hypocotyls), sedimentable amyloplasts (arrows) are located in the endodermal (e) cell layer adjacent to the vasculature (v). C, In tip-growing rhizoids of the green alga *Chara* sp., mineral-rich particles function as statoliths (red arrowhead). Reorientation of the rhizoid results in sedimentation of mineral rich statoliths, which triggers downward curvature. In contrast to higher plants, the sensing, transduction, and response phases of gravitropism all occur in the same cell. D, Alexafluor phalloidin labeling of a single root columella cell shows the relationship between amyloplasts (a) and the actin cytoskeleton. It has been proposed that the actin network in the statocytes is involved in transducing the physical information of sedimenting statoliths to a physiological signal that leads to the gravity response.

PLASTID-BASED GRAVITY SENSING GAINS INCREASING SUPPORT

How do plants sense gravity? One answer to this question, the starch-statolith hypothesis, has remained virtually unchanged for over a century. Its proponents agree that the sedimentation of starch-filled amyloplasts (statoliths) within the columella cells of the root cap and endodermal cells or bundle sheath parenchyma in shoots (statocytes) constitute one of the initial events in gravity perception (for

review, see Sack, 1991; Fig. 1, A and B). Since the lively debates culminating from work with the starchless Arabidopsis and *Nicotiana* mutants in the 1980s, the starch-statolith hypothesis has continued to gain support from a number of molecular and cell biological studies. For example, surgical removal of the root cap, which was typically used in studies of root gravitropism, has been refined with laser ablation techniques. Laser ablation of the central columella cells of Arabidopsis, which had the most readily sedimentable amyloplasts, caused the strongest inhibitory effect on root bending (Blancaflor et al., 1998). More recently, heavy ion microbeam destruction of root cap cells demonstrated that lateral cap cells located on the lower side of horizontally gravistimulated roots, unlike those located on the upper side, also may play an important role in gravitropism (Tanaka et al., 2002). Hence, the lateral root cap could serve as a conduit for the movement of a signal from the columella to the responding tissues of the EZ (Tanaka et al., 2002).

Genetic ablation studies and the analysis of Arabidopsis mutants that are unable to develop an endodermis, lack amyloplasts, or have impaired amyloplast sedimentation in their endodermis have confirmed the laser ablation work. These plants are defective in their ability to bend in response to a
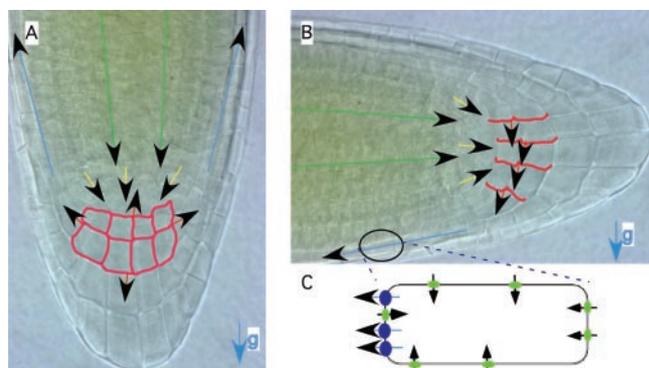
Figure 2. The fountain model of auxin transport in roots. In vertically grown roots (A), auxin is transported through the vasculature into the root tip, where it accumulates in the first layers of columella. There, it is transported laterally to more peripheral tissues, then basipetally to the EZs where it inhibits cell elongation. Upon gravistimulation (B), the lateral redistribution of auxin occurs preferentially to the bottom side, resulting in a lateral auxin gradient that is transported to the EZs where it promotes a downward tip curvature. Polar auxin transport within cell files is mediated by auxin influx and efflux carriers (C). Typically, the efflux carrier is localized polarly within the transporting cells, thereby mediating the polarity of transport. In A to C, the direction of auxin transport is indicated by arrows with black arrowheads. In A and B, the line's color in each arrow represents the involvement of a specific PIN protein in the corresponding efflux carrier: green, PIN1; yellow, PIN4; red, PIN3; and blue, AGR1/PIN2. Location of the PIN3 protein in upper layers of the columella is also represented in red. PIN3 is distributed at the periphery of these cells in vertically grown roots (A), but quickly redistributes to accumulate in the lower membrane upon gravistimulation (B). The gravity vector (g) is indicated by a blue arrow in A and B. This figure was inspired by Friml (2003).

horizontal reorientation (for review, see Boonsirichai et al., 2002). Interestingly, the *alh1* (*ACC-related long hypocotyl 1*) mutant of Arabidopsis contains extra cells in the columella region and exhibits faster root gravitropic responses. Although it is tempting to argue that the additional cells in the columella could enhance gravity sensing, leading to a stronger bending response (Vandenbussche et al., 2003), it is not known whether these extra cells contain amyloplasts that sediment with respect to gravity.

In addition to mutant analysis, high-gradient magnetic fields (HGMFs), which are capable of displacing amyloplasts to mimic the gravitational pull of the earth, have been used to induce gravitropic-like curvature in a variety of plant organs. Interestingly, HGMFs applied in the vicinity of starchless *pgm-1* mutant organs of Arabidopsis could not trigger such a response, whereas in wild-type inflorescence stems, curvature could only be induced if the HGMF was applied to a region of the stem that is permissive to graviresponse (Weise et al., 2000). Taken together, these studies further establish the importance of cells with sedimentable plastids for gravity sensing in higher plants and suggest that amyloplast displacement in the statocytes is sufficient to trigger a curvature that mimics the gravitropic response.

Despite growing experimental support for plastid-based gravity susception, the alternative gravitational pressure model, which proposes that it is the pressure exerted by the entire cell that mediates gravity perception, has not been discounted (Staves et al., 1997). This model was tested recently in single-cell protonemata of the moss *Ceratodon purpureus*, which exhibit upward bending when reoriented horizontally. Like the columella and endodermal cells of higher plants, *C. purpureus* protonemata contain amyloplasts that sediment with respect to the gravity vector. If gravity sensing were dependent on the mass of the cell as proposed by the gravitational pressure model, then media with higher density should either prevent gravitropism or reverse its direction. The fact that *C. purpureus* protonemata continued to exhibit strong upward curvature when positioned horizontally in high-density media (Schwuchow et al., 2002), indicates that plastid-based intracellular gravity sensing operates in this moss system. Moreover, the identification of additional moss genera that have gravitropic protonemata with sedimentable plastids suggests that plastid sedimentation serves a conserved and specialized function in gravitropism (Schwuchow et al., 2002).

Thus, experimental evidence gathered during the past 5 years suggests that the gravitational pressure model may only be applicable to gravity-induced changes in the pattern of cytoplasmic streaming in giant internodal cells of the green alga *Chara* sp. and to gravitaxis (i.e. directed motility) in unicellular flagellates such as *Euglena gracilis* (Hemmersbach et al., 1999). Interestingly, in *Chara* sp., the upward and

downward bending of protonemata and rhizoids, respectively, also rely on the mass of sedimenting barium sulfate-containing vesicles for gravity sensing (Braun, 2002; Fig. 1C). Furthermore, sporangiophores of the unicellular fungus *Phycomyces blakesleeanus* sense gravity via sedimenting protein bodies and depend on the buoyancy of lipid globules to transmit a signal when tilted horizontally (Schimek et al., 1999). Therefore, *Chara* sp. are examples of organisms that could display more than one type of gravity-sensing systems, whereas certain species of *Phycomyces* depend on both sedimentation and buoyancy to trigger differential growth.

The possibility that dual gravity sensors also may exist in higher plants was again raised by a recent study of root gravitropism using novel growth-analysis software linked to a rotating stage (called "ROTATO"). This study showed that there is continued curvature generation in maize (*Zea mays*) roots as long as a region within the distal EZ (DEZ) is maintained at some angle from the vertical, even after the cap region has reached a vertical orientation. Hence, a gravity signal may originate in regions outside the root cap, at the DEZ, where cells do not contain sedimenting amyloplasts (Wolverton et al., 2002). Although this observation deserves further investigation, much experimental evidence continues to favor the hypothesis of intracellular gravity susception based on sedimenting plastids.

IS THE CYTOSKELETON INVOLVED IN GRAVITY SIGNAL TRANSDUCTION?

Most Models Attempting to Explain Gravity Sensing Hypothesize a Role for Actin in This Process

The plant cytoskeleton, which consists primarily of actin filaments and microtubules (MTs), is known to play a key role in a wide range of fundamental cellular processes including cell division, cell signaling, and cell expansion. With the completed sequence of the Arabidopsis genome supplementing an arsenal of biochemical, cell biological, and molecular genetic tools, we now know more about the numerous binding and motor proteins that regulate or work in tandem with the plant cytoskeleton to regulate these important cellular functions (Blancaflor, 2002). Because gravitropism often has been described as a process that consists of perception, signaling, transmission, and growth response phases (Boonsirichai et al., 2002), a role for the cytoskeleton has been ascribed for each of these stages. In gravity sensing and signal transduction, for example, it has been proposed that sedimenting amyloplasts interact with the actin network in the statocytes to trigger downstream signaling events (Fig. 1D).

The most recent model is based on the concept of cellular tensegrity (Ingber, 2003) and was adopted for plant gravitropism based on a detailed analysis of amyloplast sedimentation kinetics in maize colu-

mella cells. It proposes that sedimenting amyloplasts locally disrupt a dense actin network that infuses the central columella cytoplasm and is linked to the plasma membrane. Network disruption would modify the tension of actin filaments within the statocytes, thereby activating mechanosensitive ion channels at the plasma membrane (Yoder et al., 2001). In turn, channel activation could be responsible, directly or indirectly, for the rapid transients in Ca^{2+}, H^+, and inositol 1,4,5-trisphosphate ($InsP_3$) observed during the early stages of gravitropism (Scott and Allen, 1999; Fasano et al., 2001; Perera et al., 2001; Plieth and Trewavas, 2002). In agreement with this model, ground-based and microgravity experiments have demonstrated that the actomyosin system mediates the movement of amyloplasts in higher plant roots (for review, see Volkmann and Baluska, 1999). However, it should be cautioned that alternative models have also been proposed, which implicate mechanosensitive ion channels located within the endoplasmic reticulum (ER; Sack, 1991) or vacuolar membranes (see below) as potential gravity receptors. Such models are based on observations of tight associations between sedimenting amyloplasts and the ER (root statocytes: Sack, 1991) or vacuolar membranes (shoot statocytes; Yano et al., 2003; see below).

The multilayered tissue organization of higher plant organs often has confounded attempts at analyzing the involvement of cytoskeletal structures in the various phases of gravitropism. To circumvent these problems, some investigators have resorted to systems where gravity sensing, signaling, and curvature are confined to the same cell. A popular model system for the study of cytoskeletal function during gravity sensing and signaling has been the single-cell rhizoid and protonemata of the green algae *Chara* sp., which elongate by tip growth (i.e. expansion is confined to the very apical region of these cells). In these cells, sedimentable statoliths located at the apex function in gravity perception (Fig. 1D), and the actin cytoskeleton is involved in their positioning and movement within the cells (for review, see Braun and Wasteneys, 1998). Using laser tweezers and a slowly rotating centrifuge microscope, researchers demonstrated that statolith sedimentation per se is not sufficient to trigger tip bending in *Chara* sp. The mineral-rich statoliths have to settle onto specific regions of the plasma membrane for gravitropism to proceed. These regions are confined to the apical 0 to 10 μm in upward bending protonemata and 10 to 35 μm in downward bending rhizoids, and the actin cytoskeleton is directly involved in directing statoliths to these specific sites in the plasma membrane (Braun, 2002). Although this is the first demonstration, to our knowledge, that statoliths need to contact specific regions of the cell for gravitropism to occur, different mechanisms may exist in higher plants. This assumption is based on numerous physiological analyses both in space and on Earth showing that the percep-

tion time and presentation time (i.e. parameters to estimate gravity sensitivity) can occur within seconds in these systems (for review, see Perbal and Driss-Ecole, 2002). Therefore, instead of long-distance sedimentation as in *Chara* sp., short-distance movements or the simple weight of amyloplasts in statocytes of higher plants may be sufficient to alter the tension of an interlinked actin filament system and trigger the signaling cascades leading to organ bending (Volkmann and Baluska, 1999).

Pharmacological Approaches to Probe the Function of the Cytoskeleton in Gravity Signal Transduction. New Headlines or the Same Old Story?

In higher plants, it is still difficult to reconcile the models of an actomyosin-mediated gravity-sensing machinery with the divergent results from pharmacological studies. Although such studies have been riddled with controversy (for review, see Blancaflor, 2002), investigators continue to employ them in studies of plant gravitropism. In one surprising study, it was shown that the sensitivity of maize roots to gravity is enhanced by disrupting actin filaments with latrunculin B. The enhanced gravity response, manifested as exaggerated root curling in the direction of the original gravity vector, was more pronounced when roots were given a brief period of horizontal stimulation followed by extended rotation on a clinostat (i.e. a mechanical device that rotates the root axially to eliminate unilateral gravistimulation). This suggests that disrupting actin may be affecting the reset mechanisms that prevent the root from overshooting the vertical or attain a specific GSPA (Hou, et al., 2003). The promotive effect of latrunculin B on gravitropism also was observed in hypocotyls and inflorescence shoots of Arabidopsis (Yamamoto and Kiss, 2002), suggesting that similar mechanisms for actin regulation of organ bending exist in shoots.

Molecular genetic approaches using knockouts to actin provides an alternative to pharmacological studies. A recent report shows that a genetic knockout of the actin isoform ACT7 in Arabidopsis has no effect on gravitropism despite disrupting other aspects of root development (Gilliland et al., 2003). Although this observation confirms many of the results from pharmacological studies, it remains possible that different actin isoforms may be involved in gravitropism. The enhanced root curvature responses elicited by disrupting actin with latrunculin B is an intriguing observation (Hou et al., 2003) and requires additional work to fully understand its implications. Perhaps utilizing Arabidopsis knockouts of multiple actin isoforms and actin regulatory proteins (Gilliland et al., 2003) in combination with conventional measurements of gravitropic sensitivity (Hou et al., 2003) could prove vital toward achieving this goal.

Interestingly, knockouts of *MDR* (multidrug resistance)-like genes in Arabidopsis display enhanced hypocotyl gravitropism, severely reduced polar auxin transport, and mislocalization of the putative auxin efflux carrier PIN1 (Noh et al., 2001, 2003). Disrupting the actin cytoskeleton with drugs has been shown to reduce polar auxin transport (Muday and Murphy, 2002) and alter the cycling of PIN1 (Geldner et al., 2001). It is possible that the enhanced gravitropic responses induced by actin disruption (Yamamoto and Kiss, 2002; Hou et al., 2003) could be explained by altered targeting of auxin transporters (see below), leading to a larger lateral auxin conductance as is the case in the *MDR* mutants (Noh et al., 2003).

DO PLANTS UTILIZE IONS TO TRANDUCE THE GRAVITY SIGNAL?

Cytoplasmic Calcium. Messenger or Pretender?

With the wide acceptance of the starch-statolith hypothesis, there is now a more focused effort toward deciphering the mechanisms by which the physical signal resulting from amyloplast sedimentation is translated into a physiological and/or biochemical signal to initiate the gravity response. As noted above, sedimenting amyloplasts could activate mechanosensitive ion channels in the plasma and/or intracellular membranes, resulting in transient increases in cytoplasmic ion levels that then trigger the signal transduction pathway leading to organ bending (for review, see Boonsirichai et al., 2002). Plants exhibit transient elevations in cytoplasmic Ca^{2+} ($[Ca^{2+}]_{cyt}$) in response to a variety of environmental and endogenous signals. These $[Ca^{2+}]_{cyt}$ transients activate a multitude of Ca^{2+}-interacting proteins that directly or indirectly influence a specific cellular process (for review, see Sanders et al., 2002). Given the variety of plant responses regulated by Ca^{2+}, it is not surprising that its role in gravitropism has been a favorite topic of research for many years. Although its involvement in gravitropism has not been defined clearly, there have been some interesting developments since the last *Update* (Chen et al., 1999), which we will discuss next.

The ability to reliably monitor $[Ca^{2+}]_{cyt}$ levels is an important prerequisite toward establishing its signaling role during plant gravity responses. This has not been an easy task as reflected by the limited number of studies to date that have directly measured $[Ca^{2+}]_{cyt}$ during the gravitropic response. Although previous attempts to directly measure $[Ca^{2+}]_{cyt}$ have been contradictory (for review see, Fasano et al., 2002), a recent study on Arabidopsis seedlings expressing the luminescent Ca^{2+} reporter aequorin demonstrated transient increases in $[Ca^{2+}]_{cyt}$ associated with the gravity response (Plieth and Trewavas, 2002). Ca^{2+} triggers light emission from aequorin with the intensity of luminescence being propor-

tional to the $[Ca^{2+}]_{cyt}$ concentration. By measuring luminescence from hundreds of pooled Arabidopsis seedlings after a 135° rotation, a burst of luminescence lasting 25 s followed by a longer 15-min shoulder was detected. The kinetics and intensity of $[Ca^{2+}]_{cyt}$ spiking in response to gravistimulation was different from $[Ca^{2+}]_{cyt}$ changes induced by other mechanical stimuli (Plieth and Trewavas, 2002). The higher signal-to-background ratio of aequorin over fluorescent indicator dyes and the fact that measurements of $[Ca^{2+}]_{cyt}$ were obtained from the integrated signal of hundreds of seedlings in contrast to individual plants or cells could explain this recent success in measuring $[Ca^{2+}]_{cyt}$ during the graviresponse (Plieth and Trewavas, 2002).

Although the report by Plieth and Trewavas (2002) supports the notion that small and highly localized changes in $[Ca^{2+}]_{cyt}$ are associated with gravitropism, the question as to whether the observed $[Ca^{2+}]_{cyt}$ changes are directly linked to amyloplast sedimentation remains unanswered. It will be crucial to identify the origin of $[Ca^{2+}]_{cyt}$ changes at the cellular and/or subcellular level by targeting aequorin to specific tissues (e.g. columella and endodermis) and cell compartments (Fasano et al., 2002; Plieth and Trewavas, 2002). Furthermore, it will be important to characterize the $[Ca^{2+}]_{cyt}$ transients on selected gravitropic mutants (e.g. starchless or intermediate starch mutants) to verify whether these $[Ca^{2+}]_{cyt}$ changes are directly related to amyloplast sedimentation or early gravitropic signaling events.

Despite the uncertainties that beset Ca^{2+} imaging experiments, there is a wealth of indirect evidence supporting calcium's role in plant gravitropism. For example, earlier studies have shown that one can inhibit the gravitropic bending of a variety of plant organs by altering the function of Ca^{2+} regulatory proteins (e.g. calmodulin and Ca^{2+}-ATPase) with inhibitors or by applying Ca^{2+} channel blockers or Ca^{2+} chelators (for review, see Fasano et al., 2002). More recently, a model system that has provided additional information on the role of Ca^{2+} in gravity signaling has been the pulvinus of cereal grasses. This system offers several advantages over other models, one of which is the greater amount of tissue that can be collected for subsequent biochemical analysis. Also, the internodes located between successive pulvini are non-gravitropic, constituting excellent negative controls for this system. One significant finding in relation to Ca^{2+} signaling using the oat (*Avena sativa*) pulvinus system is that $InsP_3$, which is known to trigger the release of Ca^{2+} from intracellular stores, increased 3-fold over vertical controls within 15 s of gravistimulation. Elevated $InsP_3$ levels fluctuated between upper and lower halves during the first few minutes of stimulation. Then, $InsP_3$ levels remained elevated in the lower one-half of the pulvini between 10 and 30 min of gravistimulation before returning to basal level 60

min after gravistimulation. Interestingly, gravitropic bending and the sustained increases in InsP$_3$ were inhibited by a phospholipase C antagonist, suggesting that phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate into InsP$_3$ may regulate differential growth in cereal pulvini (Perera et al., 2001).

Although these findings implicate components of the phosphoinositide signaling pathway in higher plant gravitropism, the downstream events that occur after InsP$_3$ induction are not known. The next obvious question is whether the increases in InsP$_3$ trigger a corresponding intracellular Ca^{2+} increase. Although this question may take time to answer considering the problems associated with Ca^{2+} imaging, it is worth noting that transcripts of Ca^{2+}-interacting proteins such as calreticulin and calmodulin were elevated within 15 min of gravistimulation in maize pulvini. These transcripts were recruited onto polyribosomes along the lower side of the pulvini, thereby opening up the possibility that increased synthesis of these Ca^{2+}-interacting proteins may alter Ca^{2+} homeostasis during early stages of pulvinus bending (Heilmann et al., 2001). These findings could be tied in nicely with earlier reports showing that calmodulin and calmodulin-like proteins are more abundant in statocytes, making these cells more sensitive to localized changes in Ca^{2+} that may not be detected by conventional imaging methods (Fasano et al., 2002).

Protons as Second Messengers in Plant Gravity Signal Transduction

Unlike Ca^{2+}, the emergence of protons (H$^+$) as signaling molecules in plant gravitropism has not been as controversial because reports of H$^+$ fluxes and pH changes in graviresponding plant organs have been more consistent. In Arabidopsis roots, alkalinization of the columella cytoplasm has been shown to occur within minutes of gravistimulation (Scott and Allen, 1999; Fasano et al., 2001), concurrently with the acidification of the columella apoplast (Fasano et al., 2001). Such changes were significantly reduced in the starchless Arabidopsis mutants, which are less sensitive to gravity (Fasano et al., 2001). Significantly, the pulvinus of maize also exhibited rapid cytoplasmic alkalinization when repositioned with respect to the gravity vector. In this system, the cytoplasmic pH changes were confined to amyloplast-containing bundle sheath parenchyma cells (Johannes et al., 2001).

Although the above studies demonstrate a strong link between amyloplast sedimentation and cytoplasmic pH changes, the downstream cellular events regulated by pH during gravitropism are not known. Recent studies, however, are providing some important clues as to how changes in pH may initiate organ bending. For instance, by employing multiple

proton-selective microelectrodes, Monshausen and Sievers (2002) observed the development of gravity-induced asymmetric pH changes at the surface of *Lepidium sativum* roots. The pH asymmetry was first detected in the root cap and progressed to the meristematic zones and EZs. Interestingly, the rate of differential surface acidification from the cap to the EZ was comparable with known auxin transport rates (Monshausen and Sievers, 2002), suggesting a possible link between both processes. Hence, it is possible that the activity or distribution of auxin transporters might be partly regulated by the early gravity-induced pH changes in the root columella (Scott and Allen, 1999; Fasano et al., 2001).

ARE AUXIN TRANSPORTERS TARGETED BY THE GRAVITY SIGNAL TRANSDUCTION PATHWAY?

Cholodny-Went Theory with a Face Lift?

In 1928, Cholodny and Went independently proposed that auxin is transported laterally across gravistimulated plant organs, accumulating in their bottom one-half. The resulting lateral auxin gradient would promote differential cell elongation on opposite flanks of the stimulated organ, resulting in a gravitropic curvature. This hypothesis has been heavily debated since its inception. However, recent experiments support at least some of its basic features. Using auxin reporters based on auxin-inducible promoters, several researchers have gathered indirect evidence supporting the formation of lateral auxin gradients across hypocotyls and root tips in response to gravistimulation (Rashotte et al., 2001; Boonsirichai et al., 2003; Ottenschläger et al., 2003). In each case, auxin was shown to accumulate in the bottom flank of the stimulated organ. In roots, the lateral auxin gradient was generated across the root cap and then progressively transmitted from the cap to the EZ, where the curvature response develops (Ottenschläger et al., 2003). Because auxin promotes cell elongation in roots and inhibits it in hypocotyls and inflorescence stems, the gravity-induced auxin gradients described in these reports may be responsible for the opposite gravitropic curvatures displayed by these organs.

Auxin Transport and Gravitropism. Arabidopsis Root as a Model

How is lateral auxin transport controlled by gravity? In higher plants, auxins are synthesized mainly in young shoot tissues and transported by a passive system through the phloem into the root and by a polar transport system through multiple shoot and root tissues. Polar auxin transport from cell to cell is mediated by transmembrane transporters. Auxin influx carriers mediate auxin uptake by the transporting cells, whereas efflux carriers mediate its export. The asymmetric distribution of these carriers medi-

ates the polarity of transport within specific cell files or tissues.

This process has been most thoroughly investigated in Arabidopsis, where the genes encoding both influx and efflux carriers were first identified and characterized (for review, see Friml, 2003). In this plant, members of the AUX1 family mediate auxin influx, whereas members of the AGR/PIN family and specific MDR-like proteins contribute to its efflux (for review, see Friml, 2003; Noh et al., 2001, 2003). In roots (Fig. 2, A and C), AUX1 and PIN1 contribute to transporting auxin from the vasculature into the root tip through protophloem cells. In these cells, these two transporters are located in the plasma membrane at the basal and apical sides, respectively, thereby mediating an acropetal auxin transport from the differentiating vasculature toward the root cap. In the tip, the PIN4 protein appears to target auxin toward a center of auxin maximum, located in the upper layers of columella cells within the cap. There, both *AUX1* and *PIN3* are expressed. AUX1 ensures auxin uptake by these columella cells, whereas PIN3 ensures its efflux. Interestingly, PIN3 protein distribution within the columella cells appears to depend upon the root's orientation within the gravity field. When a root is oriented vertically downward, the PIN3 protein is located symmetrically at the plasma membrane of the columella cells (Fig. 2A). However, upon gravistimulation, PIN3 relocalizes within 2 min, accumulating in the plasma membrane at a lateral side of the cells, believed to correspond to the new physical bottom (Fig. 2B; Friml, 2002, 2003). Hence, a relocalization of PIN3 and/or other auxin efflux carriers within the columella cells of the root cap may be the first step toward the establishment of a lateral auxin gradient upon gravistimulation.

After being transported downward across the columella region of a gravistimulated root, auxin is taken up by the peripheral cap cells, which also express AUX1. The corresponding gradient then can be transported basipetally through the epidermal and cortical cells of the root tip, which express the AGR1/EIR1/PIN2/WAV6 protein, toward the DEZ and central EZs (CEZs), where it triggers the differential growth responsible for root tip curvature. Consistent with this model of auxin transport during gravitropism, mutations in *AUX1* and *AGR1/EIR1/PIN2/WAV6* result in dramatic defects in root gravitropism (for review, see Chen et al., 1999). However, the gravitropic phenotype of *pin3* mutant roots is somewhat subtle (Friml et al., 2002), suggesting that other PIN proteins might also contribute to the establishment of a lateral gradient across the root cap in response to gravistimulation.

Hence, lateral auxin transport in response to gravistimulation is accompanied by rapid relocalization of at least one putative component of the auxin efflux carrier complex within the root statocytes. This important result suggests that the gravity signal transduction pathway may control the cellular trafficking of this protein between endosome and plasma membrane. Interestingly, pharmacological studies have suggested that actin filaments might be needed for the recycling of PIN proteins between endosomal compartments and the plasma membrane (Friml et al., 2002). Moreover, the Arabidopsis *GNOM* gene, which encodes an ADP ribosylation factor-GTP exchange factor, mediates cycling of PIN1 between endosomes and plasma membrane (Geldner et al., 2003). It is possible that the distribution of auxin transporters in the root columella cells might be directly regulated by the changes in the actin network that are brought about by sedimenting amyloplasts upon gravistimulation (Friml et al., 2002). Alternatively, the distribution and/or activity of auxin transporters could be regulated by early gravity-induced pH and/or Ca^{2+} changes in the root columella or by more complex signal transduction processes, such as protein phosphorylation (Scott and Allen, 1999; Fasano et al., 2001; Muday and Murphy, 2002; Plieth and Trewavas, 2002; Clore et al., 2003).

Several proteins that may contribute to gravity signal transduction in gravistimulated plant organs may already have been isolated through forward genetic screens. For example, mutations in the Arabidopsis *ARG1* and *ARL2* genes appear to affect early stages of gravity signal transduction in roots and hypocotyls (Sedbrook et al., 1999; Guan et al., 2003). Such mutations affect gravitropism without altering starch accumulation in the statocytes, root growth response to phytohormones or to polar auxin transport inhibitors, and without affecting phototropism. Furthermore, the gravitropic response of *arg1-2* roots and hypocotyls can be rescued by expressing *ARG1* in the root cap or endodermis, respectively, suggesting that its function in gravitropism occurs within the statocytes (Boonsirichai et al., 2003). Interestingly, *arg1-2 arl2-1* double mutants display phenotypes that are similar to those of single mutants, suggesting that both genes function in the same pathway (Guan et al., 2003).

ARG1 and *ARL2* encode paralogous J-domain proteins that are expressed ubiquitously in plants. ARG1 is associated with cellular organelles that contribute to the secretory pathway and is needed for gravity-induced cytoplasmic alkalinization of the statocytes, at least in roots (Boonsirichai et al., 2003). Interestingly, analyses of expression of auxin-responsive reporter constructs suggested that vertical *arg1-2* mutant root caps accumulate more auxin in an extended domain of the columella than wild type. Upon gravistimulation, *arg1-2* mutant roots did not display detectable lateral auxin gradient compared with wild type, supporting a role for this protein in the regulation of lateral auxin transport (Boonsirichai et al., 2003). Considering ARG1's association with components of the secretory pathway and its colocalization with AGR1/EIR1/PIN2/WAV6 upon treatment with

an inhibitor of vesicular trafficking (brefeldin A), it has been hypothesized that this J-domain protein might regulate the activity or recycling of PIN3 and/or other auxin transporters at the plasma membrane of statocytes (Boonsirichai et al., 2003).

How about Hypocotyls and Inflorescence Stems?

The *PIN3* gene is also expressed in the gravity-sensing endodermal cells of hypocotyls. There, the protein localizes near the inner periclinal membrane of the cells, an ideal position to promote the lateral transport of auxin within this system (Friml et al., 2002). *pin3* mutant hypocotyls also display altered phototropic and gravitropic phenotypes. Hence, it is likely that this protein also regulates the lateral transport of auxin in hypocotyls in response to tropic stimulation, as it does in root cap columella cells. It should be noted, however, that the PIN3 protein is located on the inner side of endodermal cells, implying that efflux will result in auxin transport toward the vasculature rather than toward more peripheral tissues. It remains to be determined if PIN3 relocalizes to the outer membrane in response to gravistimulation or if its activity is differentially regulated in the upper and lower flanks of gravistimulated hypocotyls.

Several genes also have been suggested to function in early phases of gravity signal transduction in shoots. For example, mutations in *SGR2*, *SGR3*, and *ZIG/SGR4*, which affect inflorescence stem gravitropism, have been characterized. The *SGR2* gene was shown to encode a novel protein similar to the bovine phosphatidic acid-preferring phospholipase A1 that localizes to the vacuole and small organelles and may affect vacuolar function (Kato et al., 2002). *SGR3*, on the other hand, encodes a syntaxin-like protein (AtVAM3) that is localized to the prevacuolar and vacuolar compartments (Yano et al., 2003), whereas *ZIG/SGR4* encodes a v-SNARE-like protein (AtVTI11) that is homologous to a yeast (*Saccharomyces cerevisiae*) protein involved in the transport of vesicles to the vacuole (Kato et al., 2002). AtVAM3 and AtVTI11 were localized to the prevacuolar and vacuolar compartments (Yano et al., 2003), and were shown to form a SNARE complex that may contribute to vesicular trafficking to these organelles (Sanderfoot et al., 2001; Yano et al., 2003).

The structure of the vacuoles was altered in *sgr2*, *sgr3*, and *zig/sgr4* mutants, suggesting a role for these proteins in vacuolar biogenesis and/or function. Furthermore, although amyloplasts were surrounded by flexible vacuolar membranes in wild-type statocytes, they were located in the cytoplasm and did not sediment in mutant statocytes (Kato et al., 2002; Yano et al., 2003). Therefore, it was concluded that the large vacuoles present in shoot statocytes may be involved in gravity perception or signal transduction (Morita et al., 2002).

How vacuoles function in shoot gravitropism remains uncharacterized. However, we can propose possible explanations for these intriguing observations. For instance, it is interesting to note that a vacuolar transient receptor potential channel was shown recently to be mechanosensitive in yeast (Zhou et al., 2003). If such mechanosensitive ion channels exist in the vacuolar membranes of plants, they could serve as gravity receptors in shoot statocytes, where sedimenting amyloplasts are likely to generate tensions at the vacuolar membranes that surround them. Alternatively, it is also possible that some function associated with the vacuole is needed for gravity signal transduction or lateral auxin transport within these cells. For instance, vacuolar proton pumps could contribute to cytoplasmic alkalinization in response to gravistimulation, as discussed above. On the other hand, vacuoles could only play indirect roles in gravitropism, serving as a system that allows proper positioning of amyloplasts for gravity perception within the statocytes. Hence, it appears that more work is needed to elucidate the mechanism(s) that contribute to vacuolar function in gravity signal transduction within the shoot statocytes.

Another set of potential gravity signal transducers in shoots was identified in a clever genetic screen (Wyatt et al., 2002), taking advantage of the fact that Arabidopsis inflorescence stems can perceive a gravistimulus in the cold but cannot develop a curvature response under these conditions. Amazingly, Arabidopsis inflorescence stems that have been gravistimulated at 4°C can "remember" the signal for up to 1 h in the cold, such that a curvature response will be initiated if the stem is returned to room temperature during this time period (Fukaki et al., 1996). The *gps* (*gravity persistence signal*) mutants were isolated for a defect in their ability to develop a curvature when returned to room temperature after they were gravistimulated at 4°C. The interesting properties of these mutants suggest that the *GPS* loci contribute to gravity perception or signal transduction at a step that follows amyloplast sedimentation but precedes lateral auxin transport (Wyatt et al., 2002). Molecular analysis of these mutations should provide important insights into the mechanisms that control gravity signal transduction in shoot statocytes.

GRAVICURVATURE INVOLVES A COMPLEX CELLULAR RESPONSE TO AUXIN

Reactive Oxygen Species (ROS) and Calcium May Contribute to the Auxin-Dependent Curvature Response to Gravistimulation

As discussed in previous sections, the auxin gradient generated across gravistimulated organs appears to modulate differential growth at the EZ, responsible for gravitropic curvature. In roots, the curvature is downward, whereas it develops upward in shoots. This opposite curvature response reflects the oppo-

site effect that auxin has on cell elongation in these two systems: positive in shoots and negative in roots. How does auxin regulate cell expansion?

It appears that auxin effects cell elongation by regulating multiple cellular processes, including gene regulation, ion homeostasis, cytoskeletal organization, and wall extensibility (for review, see Leyser, 2002). Yet, little is known about the molecular mechanisms that control these processes. For instance, although several auxin-binding proteins (ABPs) have been identified over the years, only a few of them are good candidates to function as auxin receptors. ABP1, first isolated from maize, is mainly found in the ER, where the pH is too high to allow strong auxin binding. However, a small fraction of ABP1 has also been found at the plasma membrane, where it may function as an auxin receptor modulating various activities, including cellular hyperpolarization and the balance between cell division and cell expansion in transgenic plants (for review, see Leyser, 2002). On the other hand, an intracellular 57-kD ABP from rice that appears to interact with the plasma membrane proton ATPase may serve as an auxin receptor modulating proton pumping, wall acidification, and cell expansion (Kim et al., 2001). Finally, it is also possible that components of the auxin influx or efflux carrier complexes might serve as receptors by "counting" the number of auxin molecules flowing through them (Sachs, 1981).

One of the most characterized cellular responses to auxin involves the regulation of expression of a set of auxin-responsive genes, which encode multiple proteins that contribute directly or indirectly to the cellular responses to this hormone. Such auxin-responsive genes are subjected to transcriptional control by transcription factors, named auxin response factors (ARFs). Arabidopsis contains 10 *ARF* genes showing variation in expression patterns, half-lives, dimerization affinities, and effects on transcription. The ARF proteins dimerize among themselves and with another set of regulators: the Aux/IAA proteins. The latter proteins are believed to function as negative regulators of auxin-responsive gene expression. When a cell is exposed to higher concentrations of auxin, a pathway is activated that results in the ubiquitination of Aux/IAA proteins by ubiquitin ligase (E3) named SCF^{TIR1} complex. Aux/IAA protein ubiquitination targets them to the proteasome for degradation. Hence, upon auxin activation, Aux/IAA proteins are degraded. ARFs can dimerize among themselves and activate the expression of specific subsets of auxin-responsive genes, depending on the set of ARF factors present in the responding cells (for review, see Leyser, 2002). Interestingly, mutations in some of the genes that encode proteins involved in the SCF^{TIR1} complex and in the rubination of the cullin component of that complex resulted in altered gravitropism. Similarly, mutations in some ARF and Aux/IAA genes resulted in gravitropism

defects, supporting a role for this pathway in the gravitropic response (for review, see Leyser, 2002).

Auxin may also regulate cell expansion more directly by controlling cell polarization, wall acidification (see above), and the formation of ROS (Joo et al., 2001; Leyser, 2002). Gravistimulation or unilateral application of auxin to vertical roots resulted in a transient increase in ROS concentration in the convex endodermis. Furthermore, unilateral application of hydrogen peroxide to the EZ of vertically positioned roots induced curvature, whereas ROS scavenging by antioxidants inhibited root gravitropism (Joo et al., 2001). Interestingly, there is accumulating evidence that Ca^{2+} regulates hydrogen peroxide homeostasis in plants (Neill et al., 2002). Conversely, free oxygen radicals were also shown to activate K^+- and Ca^{2+}-permeable channels and to promote a large Ca^{2+} influx in the EZ of Arabidopsis roots (Demidchik et al., 2003). Hence, it appears likely that a cross talk between ROS and Ca^{2+} contributes to regulating the auxin-induced differential growth responsible for gravicurvature in roots. With recent transcription profiling work showing that genes involved in oxidative bursts form the largest functional category of gravity-regulated genes (Moseyko et al., 2002), research along this area should be worth pursuing in the future.

Secreted low-M_r phospholipase A2, an extracellular enzyme that hydrolyzes membrane glycerophospholipids at the sn-2 position to yield fatty acids and lysophospholipids, may also contribute to auxin-mediated cell elongation. Overexpression of the corresponding *AtsPLA$_2$* gene in Arabidopsis resulted in longer leaf petioles and inflorescence stems, whereas its silencing resulted in shorter organs. These alterations in organs' sizes were mediated by changes in cell elongation and were associated with alterations in inflorescence stem and hypocotyl gravitropism. Interestingly, treatments of plant organs with PLA$_2$ or with some of its enzymatic products was shown previously to stimulate the activity of enzymes that have been implicated in the control of cell elongation, such as the proton pump and NADH oxidase. Furthermore, the Arabidopsis *AtsPLA$_2$* gene was strongly up-regulated in auxin-treated tissues and in the curving region of gravistimulated inflorescence stems. Taken together, these results strongly support a role for secreted phospholipase A2 in auxin-mediated cell elongation and the differential growth that accompanies shoot gravitropic curvature (Lee et al., 2003).

Is Gravicurvature a Simple Cellular Response to Auxin Redistribution?

Hence, a better understanding of the molecular mechanisms governing the cellular responses to gravity-induced auxin gradients emerges from multiple studies in a variety of model systems, even

though several questions remain unanswered at this time. However, it should be cautioned that detailed studies of root gravicurvature in *Lepidium sativum*, maize, and Arabidopsis revealed a level of sophistication that was not anticipated in a model postulating the simple involvement of an auxin gradient generated across the root cap and transmitted to the EZs. For instance, in Arabidopsis, early signs of graviresponse include an inhibition of cellular elongation on both the upper and lower sides of the EZs. This initial phase precedes the initiation of a differential cell elongation on opposite flanks of the DEZ and does not comply with the simple Cholodny-Went model described above. This complication potentially may derive from the fact that a subapical region of the root tip, other than the cap, appears to contribute significantly to graviperception in roots (Wolverton et al., 2002). Alternatively, it is possible that this original phase might have nothing to do with gravitropism, instead deriving from the mechanostimulus that is typically associated with organ reorientation within the gravity field (Moseyko et al., 2002; Wolverton et al., 2002). Careful reevaluation of this information under conditions of gravistimulation that minimize mechanoperturbation is needed before one can resolve this important and outstanding question in our understanding of gravitropism.

INFLUENCE OF ENVIRONMENTAL AND ENDOGENOUS CUES ON THE GRAVITROPIC RESPONSE

Thigmotropism and Hydrotropism Influence Gravitropism by Affecting the Gravity-Sensing Apparatus

In addition to gravity, other environmental factors induce tropistic responses in plants, with light (phototropism), moisture (hydrotropism), and touch (thigmotropism) among the best characterized. Under natural situations, all these environmental stimuli interact with each other and with gravity to influence plant growth, justifying a succinct description of such interactions in a review on gravitropism. Among them, light has been the most extensively studied, and outlines of the interactions between phototropism and gravitropism are discussed in detail in a recent review (Correll and Kiss, 2002). Hence, they will not be covered in this *Update*.

One outmost important function of roots is to take up water from the soil and deliver it to the rest of the plant. Unfortunately, steep moisture gradients develop in the soil. Therefore, it is not surprising that, through evolution, roots have acquired ways to sense water gradients and use them to guide their growth toward soil areas with higher water potential (positive hydrotropism). Studies on the mechanisms underlying root hydrotropism have lagged behind those on gravitropism because of the influence that gravity, which is continuously present, has on the

direction of root growth. To circumvent this problem, investigators have employed mutants that do not respond to gravity or clinostats that randomize the plant's orientation within the gravity field (Mizuno et al., 2002; Takahashi et al., 2002, 2003) to show that hydrotropism and gravitropism share some transducing steps, whereas other steps are pathway specific. For example, the expression pattern of an auxin-inducible gene in hydrotropically stimulated cucumber (*Cucumis sativus*) roots grown on a clinostat was similar to its expression pattern in gravi-stimulated roots (Mizuno et al., 2002). On the other hand, the auxin-insensitive *axr* mutants of Arabidopsis, which show reduced root gravitropism, were strongly hydrotropic (Takahashi et al., 2002). These studies indicated that auxin may regulate gravitropism and hydrotropism in different ways, even though both tropisms rely on the development of an asymmetric auxin gradient for differential growth.

Hydrotropism and gravitropism both utilize the root cap for sensing moisture gradients and gravity, respectively. However, it appears that different sensing mechanisms might be involved. This can be inferred from studies on both Arabidopsis root gravitropism and hydrotropism mutants. Roots of the starchless (*pgm-1*) mutant are less sensitive to gravity but show normal or slightly enhanced hydrotropic responses (Takahashi et al., 2002). Conversely, the *nhr1* (*no hydrotropic response1*) semidominant mutant of Arabidopsis has roots that, unlike wild type, grow toward regions of low water potential within a moisture gradient. Yet, such roots display strong gravitropic responses (Eapen et al., 2003).

A recent study demonstrated that water stress and the subsequent development of a hydrotropic response are accompanied by the degradation of amyloplasts in the columella and reduced gravirespon-siveness of Arabidopsis roots (Takahashi et al., 2003). This implies that hydrotropism influences gravitropism by affecting the gravity sensing capability of the root. Although there is ample evidence that moisture sensing in roots also resides in the root cap, it appears to be independent of amyloplasts because the *pgm-1* mutant and roots with degraded columella amyloplasts are strongly hydrotropic (Takahashi et al., 2002, 2003). The processes underlying hydrotropic sensing and signal transduction remain unknown. However, analyses of the *Arabidopsis nhr1*, *aba1-1*, and *abi2-1* mutants suggest that abscisic acid might be implicated (Takahashi et al., 2002; Eapen et al., 2003). The identification of hydrotropic mutants in Arabidopsis paves the way toward better understanding the mechanisms of hydrotropic stimulus perception and its interaction with other tropisms (Eapen et al., 2003).

As in hydrotropism, there is evidence that plant touch responses may influence gravitropism. In a recent study of Arabidopsis roots that were touch stimulated by allowing them to grow onto the sur-

face of a horizontal glass barrier, it was shown that touch also may modulate gravity responses by influencing the gravity-sensing apparatus in the root cap. When the root encountered the glass barrier, it produced a step-like growth habit with curvature occurring in the DEZ and CEZ. This allowed the root cap to remain in contact with the physical obstacle (Massa and Gilroy, 2003). Laser ablation of the columella cells altered this growth habit such that the tip angle with respect to the barrier became more horizontal. The involvement of a gravitropic signaling component in the touch response also was demonstrated by the variability in the growth behavior of the *pgm-1* mutant as it encountered the barrier. Interestingly, transient touch stimulation in the cap inhibited both gravitropic bending and amyloplast sedimentation (Massa and Gilroy, 2003). Thus, like moisture gradients, touch could modulate root gravity responses by transiently affecting gravity sensing within the cap. It is tempting to speculate that this complex interaction between the gravitropic and thigmotropic responses may facilitate obstacle avoidance in roots.

Complex Growth Behaviors Emerge from Interactions between Gravitropism and Multiple Environmental and Endogenous Cues

When subjected to multiple directional cues, roots follow complex growth behaviors, as illustrated by Arabidopsis roots growing on tilted hard-agar surfaces that they cannot penetrate. Under these conditions, the roots display a wavy growth pattern that appears to result from a combined response to gravity, touch, and other surface-derived stimuli (for review, see Migliaccio and Piconese, 2001). For most Arabidopsis ecotypes, they also tend to skew toward the right of gravity (when viewed from the back of the plate, through the medium; Migliaccio and Piconese, 2001).

Amazingly, the mechanisms underlying root waving and skewing on solid agar surfaces vary depending on the growth conditions. When seedlings are grown on a medium that does not contain nutrients or Suc, root waving and skewing appear to result from differential cellular growth on opposite flanks, without root twisting (Buer et al., 2003). However, on an agar-based medium containing nutrients and Suc, root waving appears to involve a succession of left- and right-handed circumnutation-like movements that are accompanied by a twisting of the root tip about its axis, manifested as a rotation of epidermal cell files (cell file rotation; Migliaccio and Piconese, 2001; Buer et al., 2003).

The asymmetrical touch stimulus associated with growth on agar surfaces may contribute to root waving (Okada and Shimura, 1990). However, mutational analysis in Arabidopsis also suggests that other environmental stimuli and endogenous pro-

cesses regulating cellular expansion also control this behavior. For instance, gravitropism appears essential for waving. All root gravitropism mutants isolated to date display altered root waving phenotypes on tilted hard-agar surfaces (for review, see Migliaccio and Piconese, 2001). Similarly, an analysis of the *wav2-1* and *wav3-1* mutants of Arabidopsis, which display compressed root waves on tilted hard surfaces, suggests an involvement of hydrotropism in root waving (Takahashi et al., 2002). This conclusion is not surprising because humidity gradients are likely to develop at the surface of agar-based media. Finally, an analysis of wave frequency for roots grown on agar surfaces under different nutritional and/or Suc conditions suggested a possible involvement of the circadian clock in the regulation of waving (Buer et al., 2003).

The differential flank growth that accompanies root waving on agar surfaces in the absence of nutrients and Suc has not been characterized. However, the twisting that assists bending in waving and/or skewing roots exposed to nutrients and Suc initiates in a region of the CEZ (Okada and Shimura, 1990) that also appears to be the initial site of curvature response to horizontal glass barriers (Massa and Gilroy, 2003). This interesting observation suggests possible analogies between these two responses to somewhat different obstacles.

What are the molecular and cellular bases for root twisting? Although this process remains poorly understood, observations of MT arrays in cells of spiraling wild-type and mutant Arabidopsis roots led Furutani et al. (2000) to propose that the helical arrangement of cortical MTs in basal CEZ cells might control the formation of a parallel helical network of cellulose microfibrils (MFs) in the wall, which would be responsible for organ twisting and its direction. However, research performed on *sku5* and on the temperature-sensitive *mor1-1*, *rsw4*, and *rsw7* mutants of Arabidopsis suggested that the correlation between cortical MTs and either cellulose MF alignment or direction of organ twisting can be disturbed (Sedbrook et al., 2002; Buer et al., 2003; Sugimoto et al., 2003). Therefore, it is possible that root spiraling might be mediated by unbalanced mechanical properties of cell walls within elongating cells (Sugimoto et al., 2003). Under some circumstances, the helical arrangement of cortical MTs might control the establishment of such wall properties (Thitamadee et al., 2002). However, under other circumstances, helical growth may be a consequence of subtle changes in the patterns of cellular expansion allowed by structural modifications in the cell wall, which would be brought about by other, yet undefined mechanisms (Sugimoto et al., 2003).

In most of these studies, cortical MTs and newly deposited cellulose MFs were analyzed within the epidermal cells of the root EZ for technical reasons. However, it should be cautioned that spiral growth

might also find its origin in cellular processes that occur deeper in the root. In this regard, it is interesting to note that an analysis of cell expansion and cell size in the *spr1* mutant of Arabidopsis suggested that root twisting might derive from differential rates of cell elongation between adjacent tissues, such as epidermis and cortex. According to this model, the longer epidermal cells would have to elongate at some angle from the subtending, shorter cortical cells in order for the root to maintain its integrity (Furutani et al., 2000). An analysis of epidermal and cortical cell sizes in the *wvd2-1* mutant of Arabidopsis was consistent with this model (Yuen et al., 2003), although it is impossible, at this time, to determine if the differential growth of epidermal and cortical cells is a cause or consequence of organ twisting.

To conclude, it appears that a number of environmental parameters, including light, humidity, touch, nutrients, Suc, and phytohormones, interact with gravity and endogenous regulatory processes to determine complex growth patterns that optimize plant's survival under stressful conditions. A very exciting challenge in the future will be to determine how these changes are orchestrated at the biochemical and cell biological levels.

CONCLUDING REMARKS

Since our last *Update* on gravitropism, several important breakthroughs have improved our understanding of the mechanisms that allow plant organs to use gravity as a guide for growth. Several recent reports provide important support for the starch-statolith hypothesis. A role for protons, cytosolic Ca^{2+}, and IP_3 in gravity signal transduction within the gravity-sensing statocytes has been more thoroughly documented, and a molecular mechanism underlying gravity-induced lateral auxin transport across the statocytes has been proposed. Similarly, the mechanisms underlying the differential growth response to gravistimulation have been better documented, and the influence that other environmental and endogenous cues may have on the gravitropic response is under intense scrutiny (Fig. 3).

In the future, we anticipate that the major advances recently made in genetics, genomics, proteomics, cell biology, biochemistry, physiology, and bioinformatics will allow us to develop the tools that are needed to efficiently investigate the role played by the cytoskeleton in the various phases of gravitropism, to identify possible gravity receptors and additional signal transducers that contribute to gravity signal transduction within the statocytes, and to better understand the molecular mechanisms that allow plant organs with seemingly simple cellular organizations to integrate the information coming from multiple environmental cues and translate it into very complex growth behaviors, such as those displayed by roots growing on tilted hard surfaces. These are promising and very exciting times in a research field

Figure 3. Model of root and shoot gravitropism summarizing the identity of molecules, organelles, and processes functioning in all phases of the pathway and illustrating the environmental factors that influence the process.
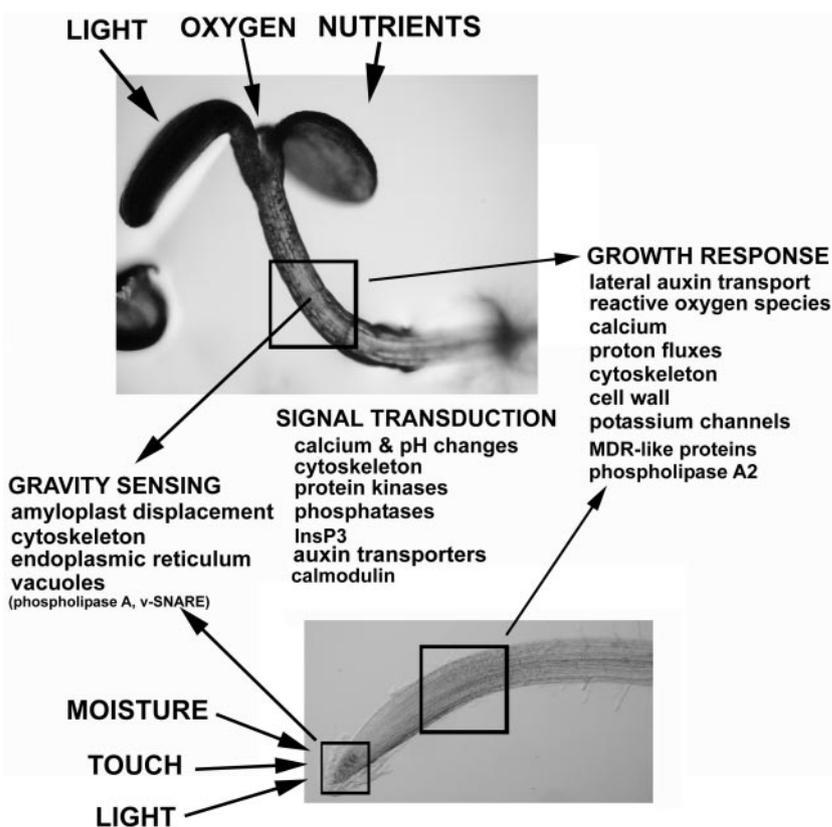
that has long been underpopulated despite the importance and complexity of the biological processes that contribute to these fascinating growth behaviors!

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