

## Update on Development

# Gravitropism in Higher Plants<sup>1</sup>

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Since 1806, we have known that plant organs use gravity as a guide for growth (Knight, 1806). The gravity-directed growth process, called gravitropism, dictates upward shoot growth to ensure a proper positioning of the leaves for efficient photosynthesis and gas exchange. It also directs roots to grow downward in soil, where they can reach out to take up the water and mineral ions required for plant growth and development.

Gravitropism has an important impact on agriculture. It allows plants to compete for the limited resources available in their immediate environment and ensures that crop shoots resume upward growth after prostration by the action of wind and rain (Fig. 1). Consequently, plants can keep their seeds away from soil moisture and pathogens and are more amenable to mechanical harvesting.

At the end of the 19th century, Ciesielski (1872) and Darwin (1880) demonstrated that a structure at the tip of the roots, the cap, is essential for root gravitropism. They postulated that the root cap could perceive a change in root-tip orientation within the gravitational field (gravistimulus). Gravi-perception would then produce a physiological signal that, upon transmission to the elongation zone, would promote a differential cellular elongation on opposite flanks, which is responsible for the development of a curvature. The resulting curvature would allow the root tip to resume growth along a gravitropically more acceptable vector.

These important early observations along with the proposed model for gravity perception marked the beginning of numerous studies that extended throughout the entire 20th century and helped us to gain a better understanding of the various physiological and molecular processes underlying gravitropism. In this *Update* we discuss our current knowledge of the gravitropic response of higher plants, with a special emphasis on roots.

### AMYLOPLAST DISPLACEMENT IN SPECIALIZED CELLS APPEARS TO BE THE PRIMARY GRAVITY-SENSING MECHANISM

In physical terms, the force of gravity can deform or displace objects of specific mass. Hence, a biological

gravity-sensing device would contain a molecular receptor that perceives the physical information generated by the deformation or displacement of specific objects, known as susceptors. In higher plants the gravity susceptors, or statoliths, are believed to be dense amyloplasts that sediment in specialized cells, or statocytes (Haberlandt, 1900; Nemeček, 1900). In shoots and grass pulvini, amyloplast-containing statocytes are located in the starch parenchyma cells that surround vascular tissues (Sack, 1991). In roots they are located in the columella of the cap (Figs. 2 and 3, a and b; Sack, 1991).

The statocytes are highly polarized cells that contain a peripheral ER, a nucleus positioned in the middle or at the top, and dense amyloplasts sedimented at the physical bottom (Fig. 3b). When a plant organ is tilted within the field of gravity, amyloplasts sediment to the new physical bottom of the statocytes (Fig. 3, b and c). Amyloplast sedimentation is believed to activate receptors that trigger a signal transduction pathway leading to the formation of a physiological signal, which is responsible for organ-tip curvature (Evans and Ishikawa, 1997).

Both direct and indirect evidence support a role for amyloplast sedimentation in gravitropic sensing. First, decapped roots do not respond to gravistimulation despite wild-type rates of growth (Darwin, 1880). Hence, the root cap appears to be essential for root gravitropism (Sack, 1997). Second, centrifugation experiments in which lateral acceleration forces were applied to different regions of a plant positioned along the general radius of a centrifuge demonstrated that the primary site of gravity sensing in roots overlaps with the root cap (for review, see Poff and Martin, 1989). Third, a good correlation exists between amyloplast density and gravitropic sensitivity in plants carrying amyloplasts, the starch content of which was reduced by physiological treatments or by mutations in genes required for its synthesis or accumulation (Sack, 1991; Kiss et al., 1996). Fourth, developmental mutants lacking a differentiated endodermis in their shoots and roots are shoot agravitropic. Because the shoot endodermis contains statocytes and the root counterpart does not, this observation is also compatible with the proposed model (Fukaki et al., 1998).

Amyloplast displacement in statocytes is sufficient to promote organ-tip curvature. The application of high-gradient magnetic fields to Arabidopsis root tips promoted a lateral displacement of root-cap amyloplasts and a sub-

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Abbreviations: CEZ, central elongation zone; DEZ, distal elongation zone.

**Figure 1.** Gravitropism dictates upward shoot growth, as illustrated by the trees shown at the end of the corn field. The corn plants, on the other hand, were recently prostrated by a heavy storm (a). However, in a few hours, their shoots curved upward, as a result of gravitropism (b).



sequent development of root-tip curvatures in the direction of statolith displacement (Kuznetsov and Hasenstein, 1996). The presence of starch in the plastids was necessary for amyloplast displacement and root-tip curvature in this system (Kuznetsov and Hasenstein, 1996). Furthermore, the displacement of amyloplasts in the starch parenchyma of barley coleoptiles and tomato hypocotyls promoted the development of a curvature in the direction opposite to that of statolith displacement, as expected for organs subject to negative gravitropism (Kuznetsov and Hasenstein, 1997).

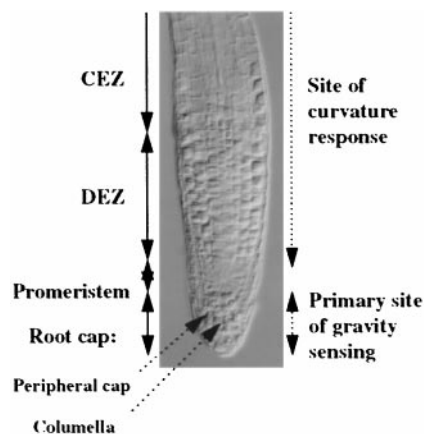
The role of amyloplast sedimentation in gravitropic sensing is also supported by recent laser-ablation experiments, which showed that *Arabidopsis* roots lose most of their gravitropic abilities when the central amyloplast-containing columella cells are ablated (Blancaflor et al., 1998). We should caution, however, that amyloplast sedimentation might not be the sole mechanism of gravity sensing. In fact, the laser-ablation experiments discussed above revealed that the decapped root tips are still able to respond to gravistimulation, albeit with altered kinetics and increased threshold stimulation times (Blancaflor et al., 1998). In addition, electrophysiological studies demonstrated that fast changes in proton flux occur on the physical topside of the DEZ upon gravistimulation. Such changes are too fast to derive from the transmission of a chemical signal from the root cap to the DEZ, suggesting that DEZ cells can directly sense the gravistimulation or that a fast electrical signal is transmitted from the root cap to the DEZ (Behrens et al., 1982; Monshausen et al., 1996).

Other gravity-sensing mechanisms may also produce these interesting secondary responses. The gravitational pressure model proposes that plant cells perceive gravity by sensing their buoyancy within the surrounding medium (Staves, 1997). Hence, gravity may tend to displace the protoplast within the cell wall, exerting a tension between the plasma membrane and the extracellular matrix on the topside and compression at the bottom. The tension and compression may result in the activation of specific stretch-activated channels that trigger signal transduction pathways leading to the final cellular responses (Staves, 1997). According to that model, the dense amyloplasts contribute to gravity sensing by increasing the total weight of the cell, thus increasing the differential tension/compression exerted by the cell on its extracellular matrix. The gravitational pressure model seems adequate to explain the gravity sensing that directs cytoplasmic streaming in the large internodal cells of *Chara corallina* (Staves et al., 1997) and

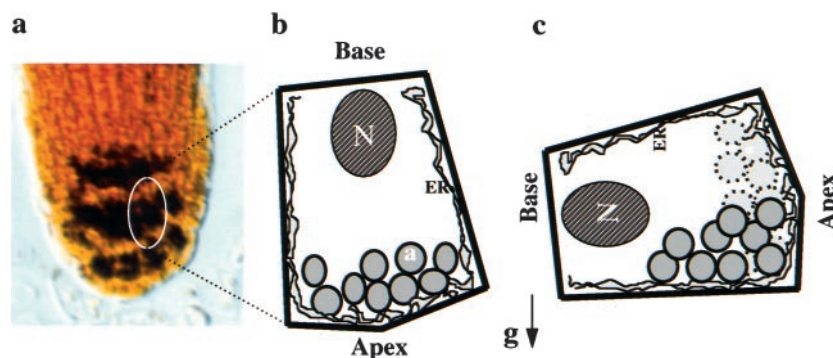
causes gravitaxis in *Euglena* cells (Konings, 1995). However, its involvement in gravitropic sensing by much smaller cells such as the statocytes in higher plants remains controversial (Sack, 1997).

#### DO $\text{Ca}^{2+}$ AND PHOSPHOINOSITIDES ACT AS SECOND MESSENGERS IN THE GRAVITY SIGNAL TRANSDUCTION PATHWAY?

How amyloplast sedimentation is transduced into a physiological signal in the statocytes remains an enigma. Indirect evidence suggests that the sedimentable amyloplasts are enmeshed in a dense network of short and dynamic actin microfilaments connected to a region of the statocyte cortex rich in microtubules, ER, and membrane-bound cytoskeleton elements. This network appears to restrain the movement of amyloplasts at the statocyte periphery (Volkmann et al., 1991; Baluska and Hasenstein, 1997). The association of statoliths with microfilaments may be mediated by myosin-like motor proteins found in the vi-



**Figure 2.** Different root-tip cells are involved in the sensing of and response to a gravistimulus (reorientation within the gravity field). The root tip of a 5-d-old *Arabidopsis* seedling contains a root cap made of columella and peripheral cells, a promeristem, a DEZ composed of cells that expand both longitudinally and radially, and a CEZ consisting of cells that preferentially elongate. Gravity sensing occurs primarily in the columella cells, called statocytes, of the root cap, whereas the curvature response to a gravistimulus occurs in the DEZ and CEZ. The root shown in this picture was viewed under a light microscope using Nomarski optics. Root width = 160  $\mu\text{m}$ .



**Figure 3.** The primary mechanism for gravity sensing in roots involves the sedimentation of amyloplasts in the root-cap columella cells. a, In this light micrograph starch-filled amyloplasts (dark brown) are revealed in the root-cap columella cells of a vertically grown *Arabidopsis* seedling by staining with an iodine/potassium iodide solution (Kiss et al., 1996). One of these columella cells, chosen as an example, is identified by a white circle in a and is schematized in b. The columella cell contains a nucleus (N) at the top, a peripheral ER, and sedimentable amyloplasts (a) at the physical bottom. c, Upon gravistimulation (reorientation of the root to the horizontal), the statocyte amyloplasts sediment from their original position (punctuated, light gray circles) to the new physical bottom of the cell (darker gray circles). According to the amyloplast-statolith hypothesis, amyloplast sedimentation is the primary mechanism by which gravity sensing occurs. In all of the panels, the root tip and statocytes were drawn according to their orientation relative to the gravity vector, *g*, represented by an arrow.

cinity of sedimenting amyloplasts in root-cap statocytes (Wunsch and Volkmann, 1993). Unfortunately, direct evidence for interaction between amyloplasts and microfilaments in statocytes is not available. Because the sedimentable amyloplasts lie on top of the peripheral ER in the statocytes, it is also possible that the peripheral ER restrains amyloplast movement (Sack, 1997).

The molecules transducing the gravity signals in statocytes have not yet been characterized. The pulling action generated by amyloplast sedimentation could stretch specific plasma and/or ER membranes to activate mechanosensitive channels. The opening of these channels could then allow a local transient increase in cytoplasmic  $Ca^{2+}$  levels that would trigger a signal transduction pathway, leading to the production of physiological signals (Sievers et al., 1991).

The involvement of cytosolic  $Ca^{2+}$  as a second messenger in the transduction of gravity signals in the statocytes has long been hypothesized. Gravitropism is eliminated by both blockers of stretch-activated channels (e.g.  $Gd^{3+}$  and  $La^{3+}$ ) and inhibitors of calmodulin or  $Ca^{2+}$ -ATPase activities (Biro et al., 1982; Björkman and Leopold, 1987; Stinemetz et al., 1987; Sievers and Busch, 1992). Furthermore, high concentrations of  $Ca^{2+}$  were detected in statocyte amyloplasts (Chandra et al., 1982), and statocytes were found to contain higher levels of calmodulin than other cell types (Allan and Trewavas, 1985). Also, a  $Ca^{2+}$ /calmodulin-dependent protein kinase may be involved in the light-dependent orthogravitropic response of roots in several corn cultivars (Lu and Feldman, 1997). Unfortunately, recent experiments with  $Ca^{2+}$  reporter systems failed to identify gravity-induced transient changes in cytosolic  $Ca^{2+}$  levels (Legue et al., 1997).

The phosphoinositide pathway may also be involved in gravitropism. The phosphatidylinositol-4-phosphate-5-kinase activity responsible for the production of phosphatidyl-

inositol-4,5-bisphosphate was found to increase in the lower side of grass pulvini within 10 min after gravistimulation and in the upper side in approximately 30 min (Perera et al., 1998). Phosphatidylinositol-4,5-bisphosphate is a biosynthetic precursor of inositol-1,4,5-trisphosphate, an intracellular second messenger that activates  $Ca^{2+}$  release from internal stores. This suggests that the phosphoinositide pathway is an important mediator of gravity signal transduction, at least in grass pulvini.

#### A GENETIC APPROACH IMPLICATES A DnaJ-LIKE PROTEIN IN THE GRAVITY SIGNAL TRANSDUCTION PATHWAY

Little is known about the molecular mechanisms involved in gravity signal perception and transduction. In fact, although several gravitropism genes have been identified, only *ARG1* has been implicated in that phase (Sedbrook et al., 1999). This role for *ARG1* was proposed based on genetic and physiological studies of *Arabidopsis*, which indicated that *arg1* mutations affect root and hypocotyl gravitropism but display no pleiotropic phenotypes. Molecular analysis indicates that the *ARG1* gene encodes a DnaJ-like protein, which carries a coiled coil domain at the carboxy terminus and a putative transmembrane domain in the middle (Sedbrook et al., 1999).

DnaJ-like proteins are encoded by large gene families in all of the species that were analyzed. They are reported to function in protein folding, protein trafficking, and the facilitation of multiple signal transduction pathways (Miyata and Yahara, 1991; Xu and Lindquist, 1993; Kimura et al., 1995). The J domains of several DnaJ-like proteins were found to interact with a conserved subdomain of HSP70, modulating its ATPase activity (Langer et al., 1992; Tsai and Douglas, 1996). It is interesting that some of these proteins can form large hetero-oligomeric complexes,



which bind to actin filaments in a calmodulin-dependent fashion, and mediate specific signal transduction pathways (Nishida et al., 1986; Pickard et al., 1990; Miyata and Yahara, 1991; Xu and Lindquist, 1993; Kimura et al., 1995).

The putative coiled coil domain found at the carboxy terminus of ARG1 is structurally similar to that found in a number of proteins that bind to cytoskeleton elements (Sedbrook et al., 1999). Taken together, these data suggest that the ARG1 protein may connect some components of the gravity signal transduction pathway to the cytoskeleton or connect the cytoskeleton to specific plasma or organelle membranes in the statocytes, mediating the reception of gravity signals. Alternatively, ARG1 may target proteins involved in gravity signal transduction to specific compartments within the statocytes (Sedbrook et al., 1999). Detailed analysis of ARG1 protein localization and function will shed more light on the molecular mechanisms underlying gravity signal transduction.

#### DO AUXIN AND APOPLASTIC $\text{Ca}^{2+}$ GRADIENTS PLAY A ROLE IN THE DIFFERENTIAL GROWTH RESPONSE TO GRAVISTIMULATION?

Gravitropic curvature is a consequence of differential cell elongation on opposite sides of the organ (root or stem); it is believed to be mediated by an auxin gradient, as originally proposed in the Cholodny-Went theory (for review, see Lomax, 1997). This model is supported by experiments that revealed a correlation between the gravitropic response and the redistribution patterns of exogenously applied radiolabeled IAA across gravistimulated organs (Lee et al., 1983; Young et al., 1990; Lomax, 1997) and by the observation that several auxin-responsive genes are asymmetrically activated upon gravistimulation (Li et al., 1991; Luschnig et al., 1998).

The statocyte-containing endodermal tissue in shoots transports auxin from its site of synthesis in the shoot apex to its site of action (Lomax et al., 1995; Gälweiler et al., 1998). Hence, it is plausible that amyloplast sedimentation upon gravistimulation activates membrane-associated, auxin-efflux carriers in cells on the bottom side, promoting the lateral transport of auxin to adjacent cortical and epidermal tissues. Auxin accumulation at the bottom side would promote a differential cellular elongation between upper and lower flanks, leading to upward shoot curvature (Lomax, 1997). Accordingly, a change in the polarity of lateral auxin transport across gravistimulated shoots was shown to correlate with the changes in the direction of gravitropic curvature induced in *lazy-2* tomato shoots by nondirectional red-light treatment (Lomax, 1997).

In roots the physiological signal(s) generated upon gravity-receptor activation in the root-cap statocytes must be transmitted to the DEZ and the CEZ for a curvature response to develop (Evans and Ishikawa, 1997). Careful time-lapse video analyses of graviresponding roots suggest that the curvature response is rather complex. Soon after stimulation, there is a transient cessation in cell expansion on both sides (upper and lower) of the CEZ. Simulta-

neously, a small group of cells on the upper side of the DEZ elongate more rapidly than they would in the absence of a gravistimulus, resulting in root-tip curvature. Then cellular elongation proceeds on the upper side of the CEZ, whereas cellular elongation remains inhibited on the lower side. As a result, the root tip reorients, tending to return to its original growth vector, and the site of curvature moves basipetally toward the mature zone. When the root tip reaches a gravitropically acceptable growth direction, the rate of curving decreases and then reverses. An oscillation of the root tip around that vector occurs with decreasing amplitude at each cycle and continues until the root resumes straight growth (Zieschang and Sievers, 1991; Ishikawa and Evans, 1993).

The application of exogenous auxin to roots at levels sufficient to completely inhibit growth does not eliminate the root graviresponse. In fact, a robust graviresponse involving a small group of DEZ cells occurred under these conditions (Ishikawa and Evans, 1993). Therefore, the phase of graviresponse that involves an increased rate of cellular elongation at the topside of the DEZ appears to be auxin insensitive.

A gradient of apoplastic  $\text{Ca}^{2+}$  is generated across the root cap in response to gravistimulation (Lee et al., 1984; Björkman and Cleland, 1991). Although no experimental data have thus far demonstrated that this gradient is transmitted to the DEZ, it is possible that it promotes the auxin-insensitive phase of the graviresponse. Chelation of extracellular  $\text{Ca}^{2+}$  results in inhibition of root gravitropism. Furthermore, asymmetric application of  $\text{Ca}^{2+}$  to one side of the DEZ results in the development of a curvature toward the site of application (for review, see Evans and Ishikawa, 1997).  $\text{Ca}^{2+}$  is believed to play an important role in the regulation of cell wall rigidity by cross-linking pectin molecules (Rayle and Cleland, 1992). Hence, the increased  $\text{Ca}^{2+}$  concentration in the cell walls may increase wall rigidity and consequently inhibit cellular elongation. It is also possible that changes in apoplastic  $\text{Ca}^{2+}$  concentrations are responsible for changes in intracellular  $\text{Ca}^{2+}$  levels (Sinclair and Trewavas, 1997). The  $\text{Ca}^{2+}$  gradient may regulate auxin transport during gravitropism, and apoplastic  $\text{Ca}^{2+}$  may modulate the sensitivity of root cells to auxin action (for review, see Evans and Ishikawa, 1997).

Even though the first phase of root graviresponse appears to be insensitive to inhibitory concentrations of exogenous auxin, it was abolished in auxin-transport and auxin-response mutants (Evans and Ishikawa, 1997). This may suggest a role for auxin that is independent of growth regulation. In that regard, it is interesting to note that the development of an apoplastic  $\text{Ca}^{2+}$  gradient across the root cap requires active auxin transport (Lee et al., 1984; Björkman and Cleland, 1991).

There is compelling evidence supporting the involvement of auxin in the second phase of root graviresponse, in which a differential cellular elongation occurs on opposite flanks of the CEZ. Not only is the second phase sensitive to inhibitory concentrations of auxin but inhibitors of polar auxin transport also abolish root gravitropism (Muday and

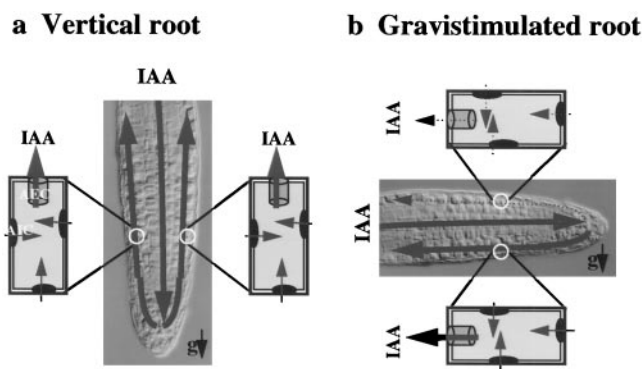
Haworth, 1994). Accordingly, mutations in genes involved in auxin transport or response affect this process.

#### HOW IS AUXIN TRANSPORTED FROM THE SITE OF GRAVITY SENSING TO THE SITE OF CURVATURE RESPONSE?

It is believed that auxin is transported through the vasculature from the shoot apex into the root tip. There it is redistributed to peripheral tissues (cortex and epidermis) and transported back into more basal regions of the root where it regulates cell division and elongation, as well as root-hair formation. Auxin transport occurs through cell files by an active mechanism that involves cellular influx and efflux carriers (Fig. 4). Auxin influx carriers allow cells to take up the protonated form of IAA from the apoplast. In *Arabidopsis*, the *AUX1* gene appears to encode a root-specific auxin-influx carrier (Yamamoto and Yamamoto, 1998). Mutations in that gene resulted in decreased root growth sensitivity to auxin, ethylene, and cytokinin, as well as altered root gravitropism (Bennett et al., 1996). The ionic form of IAA is transported out of the cells by auxin-efflux carriers. Recently, the *Arabidopsis AGR1/EIR1/PIN2* locus was cloned and shown to be essential for root gravitropism. It encodes a component of the auxin-efflux carrier predominantly expressed in roots (Chen et al., 1998;

Luschnig et al., 1998; Müller et al., 1998; Sedbrook et al., 1998; Utsuno et al., 1998). The basipetal polarity of transport is believed to be mediated by the predominant localization of functional efflux carriers in the basal membranes of the cells (Lomax et al., 1995; Bennett et al., 1996; Müller et al., 1998).

The patterns of *AUX1* and *AGR1/EIR1/PIN2* expression are consistent with their involvement in polar auxin transport and in gravitropic signal transmission in roots. Both genes are expressed in the DEZ and CEZ of *Arabidopsis* roots (Chen et al., 1998), and the *AGR1/EIR1/PIN2* protein is localized in the basal membranes of DEZ and CEZ epidermal cells and in cortical cells (Müller et al., 1998). However, neither of these genes is expressed in the root cap, where an auxin gradient is believed to be generated in response to gravistimulation. Hence, we must speculate that other gene products mediate the lateral distribution of auxin in the cap. This could be the function of other members of the large *AGR1/EIR1/PIN2* gene family (Chen et al., 1998). Alternatively, the electrical signals discussed earlier could directly regulate the activity of auxin-efflux carriers in the DEZ and CEZ (Sachs, 1981). Development of sensitive techniques allowing the measurement of free auxin concentrations in different regions of the root tip during gravistimulation and functional analyses of all members of the *AGR1/EIR1/PIN2* gene family are needed to answer these critical questions.



**Figure 4.** Gravistimulation results in the redistribution of auxin in the root tip. a, The fountain model of auxin transport in vertical roots postulates that auxin synthesized in the shoot apex is transported to the root tip through the vasculature (Evans and Ishikawa, 1997). From there it is redistributed laterally to more peripheral tissues and is transported basipetally back to the DEZ and CEZ, where it regulates cellular elongation. The polar transport of auxin through epidermal cells (black upward arrows) involves auxin-influx and -efflux carriers (AIC, represented by a cylinder, and AEC, represented by a black oval, respectively). Two epidermal cells of the CEZ are schematized on each side of the root. They demonstrate a basal localization of the auxin-efflux carrier complex, which is responsible for the basal polarity of transport in the epidermis. b, When a root is reoriented within the gravity field ( $g$ , indicated by an arrow), activation of the gravity signal transduction pathway in the root cap results in the formation of an auxin gradient across the root tip. Consequently, more auxin accumulates at the bottom than at the top of the root, as illustrated by the width of the black arrows. The resulting auxin gradient is transmitted from the root cap into the DEZ and CEZ, where it promotes a differential rate of cellular elongation on opposite flanks, which is responsible for the curvature. Root width = 160  $\mu\text{m}$ .

#### HOW DOES AUXIN REGULATE THE RATE OF CELLULAR ELONGATION IN THE GRAVIRESPONDING ZONE?

As emphasized earlier in this review, auxin promotes cell elongation in shoots and inhibits it in roots. Consequently, the increased auxin concentration observed on the bottom side of gravistimulated organs promotes an upward curvature in shoots and a downward curvature in roots. But how does auxin regulate cellular elongation in plants?

It appears that auxin regulates cellular elongation by modulating the activity of the plasma membrane proton pump, by affecting cell wall extensibility and cellular exocytosis, and by regulating the expression of a number of auxin-responsive genes (Jones, 1994). Although a number of cell wall, plasma membrane, cytoplasmic, and nuclear proteins have been found to bind auxin at physiologically relevant concentrations, only one, termed ABP1, has been proposed to play the role of auxin receptor in the control of cellular expansion (Hobbie, 1998; Jones et al., 1998). ABP1 is an auxin-binding protein found predominantly in the ER, although some of it was also found outside the cell. It appears to modulate the activity of the plasma membrane proton pump (Ephritikhine et al., 1987) and to promote cell expansion when overexpressed in tobacco plants and maize cell lines (Jones et al., 1998). Although these properties strongly suggest that ABP1 functions as an auxin receptor in cellular expansion, it remains possible that other auxin-binding proteins play similar roles.

In addition to activating the plasma membrane proton pumps and exocytosis, a process essential for the secretion

of new cell wall components (Jones, 1994), auxin also modulates intracellular signal transduction pathways that result in changes in gene expression. Some components of the auxin signal transduction pathway have recently been characterized. The *Arabidopsis* *AXR1* gene, essential for root gravitropism and auxin perception, encodes a nuclear protein that interacts with ECR1 to activate members of the RUB/NEDD8 family of ubiquitin-related proteins (del-Pozo et al., 1998). Similarly, the *Arabidopsis* *TIR1* gene encodes an F-box protein, which has also been proposed to function in ubiquitin-mediated processes (Ruegger et al., 1998). These results suggest that auxin activates the ubiquitin-dependent destruction of repressor proteins, resulting in the up-regulation of several auxin-responsive genes. Among them, *AXR3* was also shown to be essential for root gravitropism (Rowse et al., 1998).

### CONCLUSIONS

Our understanding of the molecular processes that control the various phases of gravitropism in higher plants has improved in the past few years. The involvement of amyloplasts as primary gravitropic susceptors has been confirmed, and various components of the gravity signal transduction pathway have been identified and are being characterized. The role of auxin in the graviresponse has also been confirmed, and several molecules involved in its transport and action have been identified and are being characterized. However, we still know very little about the molecular nature and function of the putative gravity receptors and how receptor activation results in the formation of a physiological signal. Similarly, the composition of that physiological signal has not been completely elucidated, although auxin is probably one of its components.

Several other hormones are involved in the gravitropic response. For instance, ethylene and cytokinin are believed to modulate root and shoot gravitropism by regulating auxin transport (Lomax, 1997; Chen et al., 1998). Their modes of action and involvement in the coordination of the overall response in different plant organs have yet to be determined.

The gravitropic response is only one of several tropic responses that determine the direction of plant growth in heterogeneous environments. For instance, touch, light, gradients in temperature, humidity, ions, chemicals, and O<sub>2</sub> also regulate the patterns of growth (Masson, 1995). The simultaneous exposure to multiple and competing environmental cues may result in complex growth patterns, such as the wavy growth of roots subjected to a combination of gravity and touch stimulation (Okada and Shimura, 1990). The graviresponse may also be modified by other environmental parameters. For instance, the roots of several maize cultivars become orthogravitropic when red light is perceived by the phytochrome photoreceptor and a Ca<sup>2+</sup>/calmodulin-dependent protein kinase is activated (Lu and Feldman, 1997). Similarly, the gravitropic response of *Arabidopsis* hypocotyls is altered when seedlings are exposed to continuous red light. This response is mediated by phy-

tochromes A and B and is inhibited by exogenous application of cytokinin acting through ethylene (Golan et al., 1996). Again, the molecular mechanisms responsible for the integration of these complex regulatory processes have yet to be elucidated.

An amazing battery of new tools based on forward and reverse genetics, structural and functional genomics, physiology, biochemistry, and structural biology is now available and can be applied to the development of an integrated approach to the study of the complex processes we have briefly discussed. They promise to bring important and exciting breakthroughs in our understanding of the molecular and physiological processes that govern plant growth responses to environmental cues.

### NOTE ADDED IN PROOF

Transient and sustained increases in inositol-1,4,5-trisphosphate have been detected at the bottom side of gravistimulated maize pulvini, supporting a role for that molecule in gravity signal transduction (Perera et al., 1999).

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