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Abbreviations: GSPA: gravitational set point angle; IAA: indole-3-acetic acid; NAA: 1- naphthalene acetic acid; NPA: 1-N-naphthylphthalamic acid; 2,4-D: 2,4-dichlorphenoxy acetic acid; TIBA: 2,3,5-triiodobenzoic acid

Abstract

For most plants, shoots grow upward and roots grow downward. These growth patterns illustrate the ability for plant organs to guide their growth at a specified angle from the gravity vector (gravitropism). They allow shoots to grow upward toward light, where they can photosynthesize, and roots to grow downward into the soil, where they can anchor the plant as well as take up water and mineral ions.

Gravitropism involves several steps organized in a specific response pathway. These include the perception of a gravistimulus (reorientation within the gravity field), the transduction of this mechanical stimulus into a physiological signal, the transmission of this signal from the site of sensing to the site of response, and a curvature-response which allows the organ tip to resume growth at a predefined set angle from the gravity vector.

The primary sites for gravity sensing are located in the cap for roots, and in the endodermis for shoots. The curvature response occurs in the elongation zones for each organ. Upon gravistimulation, a gradient of auxin appears to be generated across the stimulated organ, and be transmitted to the site of response where it promotes a differential growth response. Therefore, while the gravity-induced auxin gradient has to be transmitted from the cap to the elongation zones in roots, there is no need for a longitudinal transport in shoots, as sites for gravity sensing and response overlap in this organ.

A combination of molecular genetics, physiology, biochemistry and cell biology, coupled with the utilization of *Arabidopsis thaliana* as a model system, have recently allowed the identification of a number of molecules involved in the regulation of each phase of gravitropism in shoots and roots of higher plants. In this review, we attempt to summarize the results of these experiments, and we conclude by comparing the molecular and physiological mechanisms that underlie gravitropism in these organs.

INTRODUCTION

Most plants are sessile organisms that spend their entire life cycle at the site of seed germination. Roots anchor them to the ground, and take up the water and mineral ions that are needed for plant growth and development. Shoots, on the other hand, grow above ground. There, they harvest light, which provides energy for photosynthesis. Hence, at germination and thereafter, shoots have to grow upward, and roots downward, independently of the original orientation of the seed on the ground. In fact, both organs are endowed with sophisticated machinery that allows them to sense the direction of gravity and guide their growth at a specific angle from the corresponding vector. This process, called gravitropism, has received a great deal of attention since its recognition two centuries

ago (Knight, 1806). Yet, most of the processes that underlie each phase of the gravitropic response remain poorly understood. This is because most of the classic analytical methods were insufficient to dissect such sophisticated machinery. Recent advances in the disciplines of genetics, physiology, cell biology and biochemistry, associated with the adoption of *Arabidopsis thaliana* as a model, have allowed major breakthroughs in our understanding of both root and shoot gravitropism. Additionally, technological breakthroughs in the disciplines of genomics and proteomics, made possible by the recent completion of the *Arabidopsis thaliana* genome sequencing effort, have provided increased power and sophistication in our analysis of this complex physiological process (Bevan et al., 2001). It is anticipated that the next few years will allow us to better understand each phase of gravitropism. In this review, we discuss our current state of knowledge of the molecular processes that underlie gravitropism of shoots and roots of *Arabidopsis thaliana*.

ORGAN GROWTH IN PLANTS

Plant organs grow using a combination of cell divisions in apical meristems and cell elongation in subapical elongation zones (Figure 1). While expanding, cells differentiate until they reach a final stage of differentiation in the mature zone. In roots, the apical meristem is covered at the tip by the cap, which protects it from mechanical damage. Cells in the root cap are constantly replaced by new cells deriving from the division of distal initials in the promeristem. External cell layers are progressively sloughed off and replaced by newer cells, as divisions proceed in the promeristem. On the basal side of the promeristem, new cells deriving from the division of proximal initials expand in all 3 dimensions, until they reach a transition zone where expansion switches from quasi-isotropic to an anisotropic process (elongation along the root axis). The region of the tip where quasi-isotropic cell expansion occurs and coexists with cell division is referred to as the distal elongation zone (DEZ). The region basal to the DEZ, where cells undergo anisotropic expansion with rates of cellular elongation higher than 30% of their maximal value, constitutes the central elongation zone (CEZ) (Figure 1A) (Ishikawa and Evans, 1997). While expanding, cells progressively differentiate into the cell types that constitute the tissue layers present in the mature zone: epidermis, cortex, endodermis, pericycle and vasculature (Figure 1A).

Inflorescence stems are also made of several tissue types arranged in concentric circles. From the outside inward, these tissues include one epidermal layer, three layers of cortex, one layer of endodermis, and the stele containing vascular tissues (Figure 1B). Stems also grow using a combination of cell divisions in apical meristems and expansion in subapical elongation zones. In young Arabidopsis inflorescence stems, cell elongation occurs throughout most of the length while, in older stems, cellular elongation capacity decreases in more basal regions, where cells have matured and have synthesized stiffer walls, often accumulating lignin (Weise et al., 2000).

Hypocotyls have a very similar tissue organization compared to inflorescence stems, except for the fact that they contain only two layers of cortical cells instead of three in shoots. The hypocotyl is an embryonic structure that grows only by cell elongation, at least in etiolated seedlings. No cell division occurs in this organ, even though cell ploidy increases over time (Gendreau et al., 1997). The pattern of cell elongation in the hypocotyl differs significantly between light-grown and dark-grown seedlings. In the former case, all epidermal cells elongate continuously during the entire growth period while, in dark-grown seedlings, elongation occurs along a steep acropetal spatial and temporal gradient (Gendreau et al., 1997).

GRAVITROPIC CURVATURE IN ROOTS, INFLORESCENCE STEMS AND HYPOCOTYLS

Gravitropism affects the direction of organ growth by regulating the rate of cellular elongation on opposite flanks of the stimulated organ. When Arabidopsis seedlings are placed horizontally (gravistimulated), their roots respond by developing a downward tip curvature (Figure 2). This curvature originates at the DEZ, and results from a cessation of cell elongation in the central elongation zone and at the bottom side of the distal elongation zone, while cells at the topside of the DEZ elongate faster. Then, the curvature is propagated through the central elongation zone, and reaches the mature zone where it becomes fixed in space (Ishikawa and Evans, 1997) (Figure 2B). When wild type WS seedlings are grown under the conditions defined in figure-2 legend, the kinetics of graviresponse are such that the tip of the root resumes vertical downward growth after approximately 12 hours following a gravistimulus (Figure 2C) (Chen et al., 1998).

In hypocotyls and shoots, gravitropism also involves a differential elongation at opposite flanks of the elongation zone: enhancement at the bottom side and decrease at the topside. Consequently, shoot and hypocotyl gravitropic curvatures are upward (Fukaki et al., 1996; Weise and Kiss, 1999; Weise et al., 2000) (Figure 3A, B). When placed hor-
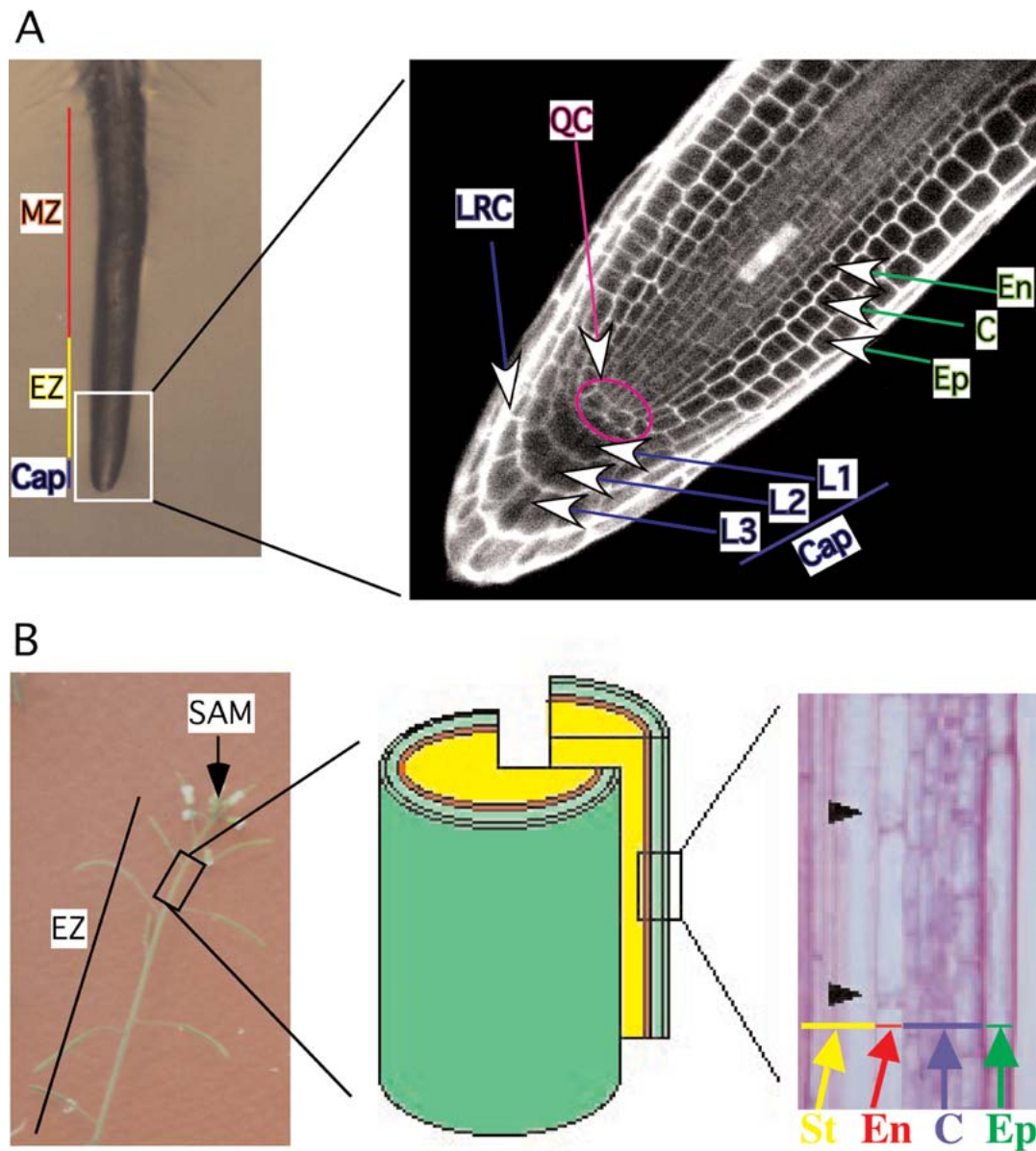
Figure 1. Plant organs grow using a combination of cell division in apical meristems and cell expansion in elongation zones. **A.** Root from a 3 day-old light-grown Arabidopsis seedling (left), showing the cap, elongation zones (EZ) and mature zone (MZ). The insert (right) represents a confocal image of a propidium iodide –stained Arabidopsis root tip showing the root cap (with its L1, L2 and L3 layers of columella cells, and lateral cap (LRC) cells), the promeristem (with its quiescent center cells (QC) surrounded by initials), the distal elongation zone (DEZ) and a small region of the central elongation zone (CEZ). The root proper is composed of several cell layers, including the epidermis (Ep), the cortex (C), the endodermis (En) and the stele (St). **B.** Top of an inflorescence stem showing the stem ended by a shoot apical meristem (SAM), flowers and siliques. The entire stem region located below the SAM in this picture is part of the elongation zone (EZ). The middle drawing corresponds to a region of the shoot stem. Microscopical image of a longitudinal section of this region (indicated by the rectangle in the drawing) is represented on the right, showing the epidermis (Ep), three layers of cortical cells (C), one layer of endodermis (En), and the stele (St). Sedimenting amyloplasts are represented by black arrowheads.

izontally, the inflorescence stem of Arabidopsis bends upward about 90 degrees within 90 min at 23°C in the dark (Figure 3B). Decapitated stem segments without leaves, flowers, flower buds, and apices show similar kinetics of
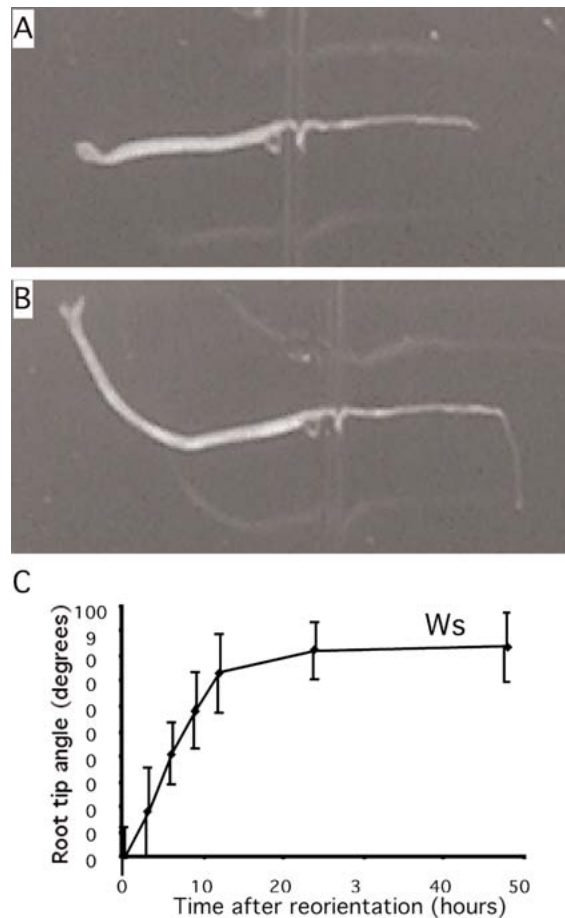

Figure 2. Root gravitropism in *Arabidopsis thaliana* seedlings. **(A)** shows a 4 day-old *Arabidopsis thaliana* seedling (WS ecotype) transferred onto the surface of a 0.8% agar-based 1/2 MS medium (Sedbrook et al., 1999), immediately after reorientation of the plate to position the seedling horizontally (gravistimulation). **(B)** 33 hours later, both root and hypocotyl have reoriented their growth such that the root is growing vertically downward, and the hypocotyl upward. In both A and B, the gravity vector was directed downward on the picture. **(C)** Kinetics of root gravitropism after a 90° reorientation similar to the one described in A. The mean root tip angle from the horizontal is shown on the Y axis (in degrees), while the time elapsed since gravistimulation is represented on the X axis (in hours). n = 150 – 200. Error bars represent standard deviations.

gravitropic response as intact stems. As shown in Figure 3C, gravistimulated stem segments began to bend upward within 30 min. The curvature reached 90 degrees after approximately 90 min, and overshot before curving in the opposite direction. Eventually, the stem segment stabilized after reaching the vertical (Fukaki et al., 1996). Hence, the stem segment contains all of the essential components for the gravitropic response.

Experiments in which the stem curvature was traced over time indicated that stems do not appear to contain a 'master zone' that would organize gravitropic responses. Each part of the elongation zone appeared able to respond to gravity. However, different regions of a gravistimulated stem segment appeared to possess different elongation abilities, explaining the overshooting phenomenon described above. In these experiments, the apical region was shown to initially curve faster than the basal region (Figure 3C). When the apical region reached a vertical position, the basal region was still bending. Since the basal region pushes up the apical region, the apical one overshot the vertical line while the basal region pursued its bending toward a vertical stature, generating a U-shaped stem segment. At this time, the apical region was again gravistimulated, and started bending in the opposite direction, while the basal region continued to bend in the original direction (Figure 3C; Fukaki et al., 1996). Hence, each region of the stem appeared capable of recognizing its own position within the gravity field independently of the other regions, and the response of the whole stem appeared to be the sum of all local responses (Figure 3B, C; Tasaka et al., 1999).

Arabidopsis inflorescence stems share common gravitropic-response characteristics with other plant shoots. For instance, the gravitropic curvature is inhibited at 4°C, but at least the gravity perception step can be executed at that temperature (Fukaki et al., 1996).

It should be cautioned here that gravitropism does not always dictate vertical upward growth in shoots and vertical downward growth in roots. In fact, each plant organ is characterized by a preferential direction of growth relative to the gravity vector named gravitational set point angle (GSPA). The GSPA varies from organ to organ, species to species, and depends upon other environmental parameters. Typically, a gravitropic response characterized by the development of a curvature is initiated when the plant organ deviates significantly its growth vector from the GSPA (Firn and Digby, 1997).
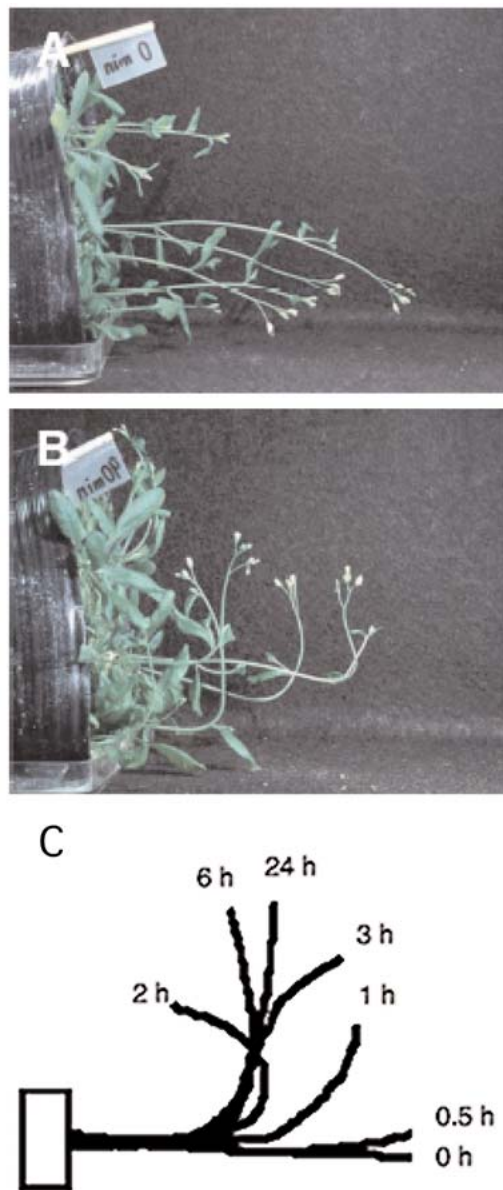
GRAVITY SUSCEPTION IN ROOTS AND SHOOTS

Root-cap columella and stem endodermal cells are important for gravity perception in roots and shoots, respectively

Gravity is best defined by the force it exerts on objects of specific mass. This force assigns specific weights to these objects, and displaces or deforms them. Therefore, in order for a living system to perceive gravity, a specific object within that system must be affected by the corresponding force. Then, the effect has to be transduced into a physiological signal that regulates the corresponding response.

Experiments performed over a period of more than a century allowed identification of the root tip as a primary site for gravity sensing in roots. Surgical experiments by Ciesielski (Ciesielski, 1872) and the Darwins (Darwin, 1880) demonstrated that the root cap is essential for root gravitropism. Centrifugation experiments in which seedlings were rotated such that different regions of the root were positioned along the centrifuge axis indicated that the primary site for gravity sensing in roots is located within the apical region. In these experiments, the sensory site mapped to a region of the root tip that included the cap, the meristem and parts of the DEZ. In shoots, similar experiments demonstrated that the primary site for gravity sensing was more diffuse, and covered the entire elongation zone (reviewed in Poff and Martin, 1989).

An important contribution to the field of gravitropism was made by Haberlandt and Nemec, who independently observed that specialized cells within the gravity-sensing regions of both roots and shoots contain sedimentable amyloplasts (Haberlandt, 1900; Nemec, 1900). Based on these observations, these authors proposed the starch-statolith hypothesis, which postulates that gravity susception involves the sedimentation of amyloplasts (named statoliths) within specialized cells, named statocytes. Statocytes occupy the central columella of the cap in roots, and the endodermis in hypocotyls and stems (Figure 1).

The starch-statolith hypothesis was essentially based on correlative data at its inception. Experimental support for this model first came from more careful surgical experiments which demonstrated that ablation of the root cap almost completely eliminated root gravitropism while still allowing normal root growth (Juniper et al., 1966).

Figure 3. Shoot Gravitropism of *Arabidopsis thaliana.* **(A)** and **(B)** Gravitropic response of inflorescence stems after 0 min (A) and 90-min (B) of horizontal gravistimulation at 23°C in the dark. 5-week-old wild-type plants (Columbia) were used. **(C)** Gravitropic response of a decapitated inflorescence stem segment of *Arabidopsis thaliana*. The stem segment was placed horizontally at time 0 h at 23°C in the dark. The shape of the stem was traced at the indicated times. A white rectangle indicates gel block as a holder. The basal side of the stem segment was embedded in the gel block. The apical side was free to move.

Subsequently, the genetic ablation of Arabidopsis root-cap cells by root-cap specific expression of a diphteria toxin A gene also resulted in transgenic seedlings with agravitropic roots, even though the rates of root growth were dramatically altered in this case (Tsugeki and Fedoroff, 1999).

The advent of laser ablation technology, coupled with the utilization of *Arabidopsis thaliana* as an experimental system, allowed a more refined analysis of the relative contribution to gravity sensing of different root-cap columella cells (Blancaflor et al., 1998). In one elegant study, laser ablation was used to kill specific cells in the cap and analyze its effects on gravitropism. The gravitropic response of ablated roots was analyzed using three parameters: time course of curvature, gravistimulation time needed to commit to bending (presentation time, a measure of gravitropic sensitivity), and deviation from vertical growth. Ablation of the peripheral cap cells and tip cells did not alter root curvature. Ablation of the innermost columella cells caused the strongest inhibitory effect on root curvature without affecting growth rates, and significantly increased the presentation time. The central cells of columella story 2, which exhibited the largest amyloplast sedimentation velocities, contributed the most to root gravitropism (Blancaflor et al., 1998).

In inflorescence stems of *Arabidopsis*, genetic studies of endodermis development have provided evidence in support of the starch-statolith hypothesis. Some genes involved in the development or differentiation of the gravity-sensing endodermal cells or of amyloplasts have been identified and cloned. The *shoot gravitropism1* (*sgr1*) and *sgr7* mutants exhibited no gravitropism in the inflorescence stem or in the hypocotyl, whereas they showed normal phototropism in both organs (Fukaki et al., 1996). Histological analysis revealed that the inflorescence stems of both mutants lacked a properly differentiated endodermal cell layer, and no tissues containing sedimented amyloplasts were found in the stems of these mutants (Figure 4) (Fukaki et al., 1998). In *sgr1,* hypocotyls contained two irregular cortical layers and no apparent endodermal cell layer. The abnormal layer of cortical cells occasionally contained small amyloplasts which hardly sedimented. Hypocotyls of *sgr7* also had no endodermal cell layer. Interestingly, both mutants contained normally sedimented amyloplasts in their columella cells and displayed normal gravitropism in roots (Fukaki et al., 1998). Genetic analysis indicated that *sgr1* and *sgr7* are allelic to *scarecrow (scr)* and *short-root (shr)*, respectively (Fukaki et al., 1998; Scheres et al., 1995).

Both *SGR1/SCR* and *SGR7/SHR* encode putative transcription factors of the GRAS family (Di Laurenzio et al., 1996; Helariutta et al., 2000; Pysh et al., 1999). *SGR1/SCR* is expressed specifically in the endodermal cell layer and in its presumptive initial cells in the shoot as well as in the root (Wysocka-Diller et al., 2000). *SGR7/SHR*, on the other

hand, is expressed in the stele and appears to function upstream of *SCR* in the regulation of radial patterning and endodermal cell specification in both shoots and roots, suggesting a cell-nonautonomous mode of action for *SHR* (Helariutta et al., 2000).

Hence, *SGR1/SCR* and *SGR7/SHR* are essential for radial patterning and endodermal cell specification in both roots and shoots. Furthermore, *sgr1/scr* and *sgr7/shr* display defects in shoot gravitropism. Therefore, it can be concluded that the endodermal cell layer is essential for shoot gravitropism. Because the endodermis is the only tissue containing sedimented amyloplasts in shoots, these studies strongly suggested that the endodermis is the gravity sensing tissue in shoots (Fukaki et al., 1998; Tasaka et al., 2001).

More experimental evidence for the starch-statolith hypothesis came from studies of gravitropic sensitivity of starch-deficient and starchless *Arabidopsis thaliana* mutants, such as *phosphoglucomutase deficient (pgm)* (Caspar et al., 1985). Indeed, the gravitropic sensitivity (presentation times) of starch-deficient mutants correlated with starch content within the columella cells of the root cap (Kiss et al., 1996). Similar results were obtained when the gravitropic response of hypocotyls was analyzed in wild type and starch-deficient mutants (Kiss et al., 1999), as well as when inflorescence stems of mature plants were analyzed (Weise and Kiss, 1999) (see below).

A recent report on the *endodermal-amyloplast less1 (eal1)* mutant provided further support for the starch-statolith hypothesis, and for an involvement of endodermal cells in stem gravitropism. *eal1* exhibited reduced and no gravitropism in hypocotyls and in inflorescence stems,

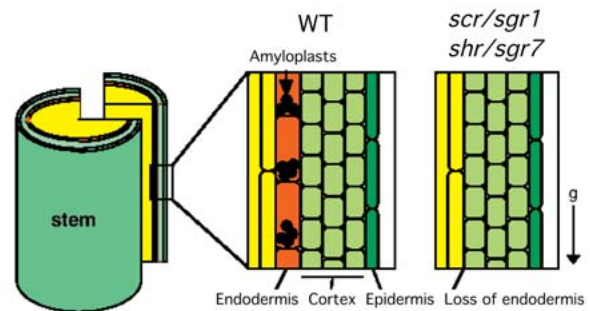**Figure 4.** *sgr1/scr* and *sgr7/shr* inflorescence stems lack an endodermal layer. Left and middle: Schematic model of longitudinal section of inflorescence stem, as defined in figure 1B legend. Generally, wild-type inflorescence stem has one layer of epidermis, three layers of cortex, and one layer of endodermis (middle, and figure 1B). In contrast, inflorescence stems of *sgr1/scr* and *sgr7/shr* lack the endodermis (right).

respectively, whereas its roots displayed normal gravitropism. The mutant lacked amyloplasts in the hypocotyl endodermal cells, whereas it contained normal amyloplasts in the root cap. The hypocotyls of *eal1* clearly showed normal radial patterning, including an endodermal layer. Furthermore, starch synthesis was not affected in hypocotyl tissues other than the endodermis (Fujihira et al., 2000). Taken together, these data suggested that *EAL1* might be involved in the differentiation or development of amyloplasts within the endodermis. The molecular cloning of this gene is awaited.

Magnetophoresis as a test for the starch-statolith hypothesis

Taken together, the studies described above supported a role in gravity perception for the sedimentation of amyloplasts within the columella cells of the root cap and in endodermal cells of shoots. However, an important test for this model had yet to be performed: demonstrating that a curvature would develop at the tip of a vertical organ if one were to artificially displace amyloplasts within the statocytes. This test was very elegantly carried out by investigators at the University of Louisiana, who set up to use high gradient magnetic fields (HGMF) to displace the diamagnetic starch grains within the cells of live plant organs (magnetophoresis) (Kuznetsov and Hasenstein, 1996; Kuznetsov and Hasenstein, 1997). They demonstrated that the lateral displacement of amyloplasts within the columella cells of vertical roots triggered a curvature whose direction was predicted by the direction of amyloplast movement (Kuznetsov and Hasenstein, 1996). No curvature response to magnetophoresis was observed in starch-deficient mutants of *Arabidopsis*. Furthermore, the curvature response to magnetophoresis occurred in a direction opposite to the direction of amyloplast displacement when hypocotyls and inflorescence stems were analyzed (Kuznetsov and Hasenstein, 1996; Kuznetsov and Hasenstein, 1997; Kuznetsov et al., 1999; Weise et al., 2000). Because hypocotyls and inflorescence stems develop a negative gravitropic response, while roots display positive gravitropism, the results of these magnetophoretic experiments supported the starch-statolith theory of gravitropic sensing and suggested that HGMF - induced ponderomotive forces can substitute for gravity.

Vacuoles appear to be involved in the early steps of

gravity perception in shoots

The experiments discussed above did not address whether other organelles within the statocytes might also be involved in gravity perception. Some answers to this question were obtained through an analysis of two other shoot-gravitropic mutations in *Arabidopsis thaliana*.

Both *sgr2* and *zig/sgr4* mutants showed abnormal gravitropic response in hypocotyls and inflorescence stems, whereas their roots displayed normal gravitropism. Besides, a normal phototropic response was observed in all three organs of these mutants (Fukaki et al., 1996). An amyloplast-containing endodermis clearly existed in both mutants. However, not all amyloplasts sedimented; some remained at the top or middle of the endodermal cells (Figure 5). The abnormal gravitropic response appeared to be caused by an abnormal distribution of amyloplasts in both mutants.

The *SGR2* and the *ZIG/SGR4* genes have recently been cloned (Kato et al., 2002). *SGR2* encodes a novel protein homologous to the bovine phosphatidic acid-preferring phospholipase A1 enzyme, which contains a putative transmembrane domain. This gene family has been reported only in eukaryotes, and *sgr2* is the first mutant found in this family. Expression studies using SGR2-GFP fusions revealed that GFP fluorescence localized to the vacuole and in small organelles (Morita et al., 2002). Although the enzymatic activity associated with the SGR2 protein has not been analyzed yet, SGR2 may affect vacuole function.

The *ZIG/SGR4* gene has also been cloned and characterized. It encodes a v-SNARE-like protein (*AtVTI11*) homologous to the yeast v-SNARE *VTI1* which is involved in the transport of vesicles to the vacuoles (Kato et al., 2002). ZIG/AtVTI1a is partially functional in yeast cells,
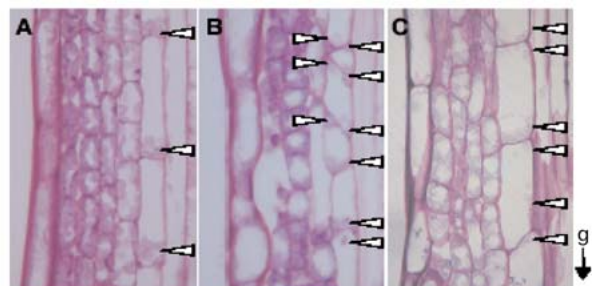

Figure 5. Endodermal cells of *sgr2* and *sgr4/zig* mutants contain abnormally positioned amyloplasts. Longitudinal sections through inflorescence stems of wild-type (A), *sgr2-1* (B), and *zig-1* (C). Growth orientation of stems was maintained during fixation. Arrowheads indicate amyloplasts. Vertical arrows represent the gravity vector.

and is localized in the prevacuolar compartment and trans-Golgi network in *Arabidopsis* root cells (Zheng et al., 1999).

Interestingly, the structure of the vacuoles was altered in the endodermis of *sgr2* and *zig/sgr4* inflorescence stems (Morita et al., 2002). *zig/sgr4* also displayed remarkable abnormalities in vacuole structure in other tissues. *SCARECROW* promoter-driven expression of the wild-type *SGR2* and *ZIG/SGR4* genes within mutant endodermal cells was sufficient to simultaneously complement amyloplast sedimentation and shoot gravitropism in their respective mutants. This important result indicated that these phenotypes were not the consequence of global abnormalities in other tissues, and that both genes are crucial for shoot gravitropism. Taken together, the data strongly suggest that vacuolar function is involved in the early step of gravitropism, probably in amyloplast sedimentation, through the function of *SGR2* and *ZIG/SGR4*.

The hydrostatic-pressure model of gravity sensing in plants: an alternative to the starch-statolith model

Even though amyloplasts appear to be good candidates as gravity susceptors, other systems may contribute to gravity perception in higher plants, either as alternatives or as complementary mechanisms. Indeed, starchless *Arabidopsis* mutants still developed significant root, hypocotyl and inflorescence-stem gravitropic curvatures, even though their amyloplasts did not sediment (Caspar and Pickard, 1989; Weise and Kiss, 1999). Furthermore, laser ablation of the entire *Arabidopsis* root cap did not completely obliterate root gravitropism (Blancaflor et al., 1998).

One alternate system of gravity perception in plants may implicate the whole protoplast as a gravity susceptor. The gravitational-pressure model postulates that plant cells perceive gravity by sensing their relative buoyancy to that of the surrounding medium (Staves, 1997). Accordingly, the weight of the cell protoplast is translated into membrane tension at sites of focal attachment to the cell wall by cytoskeletal elements. A change in cell orientation within the gravity field would result in a different distribution of plasma membrane tensions at these focal attachment points, resulting in the activation of stretch-activated ion channels. Resultant increases in cytosolic Ca^{2+} levels would then trigger a gravity signal transduction pathway, responsible for at least part of the gravitropic curvature (Staves et al., 1995). This model appears to adequately explain gravity sensing and its involvement in regulating the polarity of cytoplasmic streaming in the large *Chara*

internodal cells, and it may also function in gravitropic sensing in rice roots (Staves et al., 1997).

GRAVITY RECEPTORS AND SIGNAL TRANSDUCTION IN ROOTS AND SHOOTS

In order to function in gravity perception, susceptors have to activate specific receptors upon gravistimulation. Unfortunately, little is known about the nature of this (these) receptor(s). Sievers and collaborators postulated that amyloplast sedimentation might act upon the distal ER or upon plasma membranes through the displacement of cytoskeleton tethers, to generate membrane tensions which would trigger the opening of mechano-sensitive ion channels. The resulting increase in cytosolic Ca^{2+} levels would then trigger a gravity signal transduction pathway (Hensel and Sievers, 1981; Sievers et al., 1991).

A variation of this model was more recently formulated by Dr. Staehelin and collaborators (Yoder et al., 2001), who observed that the cytoplasm of statocytes is pervaded by an actin-based cytoskeletal network which is denser at the middle of the cell than in the ER-enriched region. Amyloplasts often sedimented along the interface between the ER-enriched cortex and the microfilament-enriched central region. These authors postulated that amyloplast sedimentation might produce a directional signal by locally disrupting the actin network, which may be connected to mechanosensitive receptors at the plasma membrane. Hence, microfilament-network disruption would alter the balance of forces acting on the mechanosensitive receptors in the plasma membrane, activating them (Yoder et al., 2001).

Interestingly, several observations suggest that amyloplasts might be connected, directly or indirectly, to microfilaments in statocytes. For instance, root-cap amyloplasts appear surrounded by a dense network of microfilaments, and the center of the columella cells is occupied by an actin-based cytoskeletal network (Baluska et al., 1995; Collings et al., 2001; Driss-Ecole et al., 2000; Hensel, 1989). Furthermore, root-cap amyloplasts saltate, and their position within the root-cap statocytes becomes more proximal when the seedlings are exposed to microgravity for short periods of time during parabolic rocket flights (Volkmann et al., 1991). Interestingly, treatments of roots with cytochalasins B and D (drugs that disrupt actin microfilaments) altered the distribution of plastids and nuclei (Hensel, 1985; Lorenzy and Perbal, 1990), and increased the sedimentation of statoliths in statocytes (Sievers et al., 1989; Yoder et al., 2001).

Even though the nature of the plant gravity receptors

remains elusive, several potential gravity signal transducers have recently been identified. These include the second messengers Ca^{2+}, pH, and $InsP_3$, and proteins belonging to the *ARG1/RHG* group. Here again, Arabidopsis has contributed enormously to the identification of some of these signal transducers, as discussed below.

Ca^{2+} has long been hypothesized to be a second messenger in gravity signaling in plants. Indeed, pharmacological inhibitors of ion channels, of calmodulin and calmodulin-related proteins, and of Ca^{2+}-ATPases were shown to negatively effect gravitropism in plants (reviewed in Sinclair et al., 1996; Sinclair and Trewavas, 1997). Furthermore, the columella cells of the root cap contain unusually high concentrations of calmodulin or calmodulin activity compared to other cells in the plant, such that relatively small changes in cytosolic Ca^{2+} levels may have a signaling effect on the transduction pathway (also reviewed in Sinclair and Trewavas, 1997). Unfortunately, attempts at identifying gravity-induced changes in cytosolic Ca^{2+} levels in Arabidopsis root-cap statocytes have failed (Legue et al., 1997). On the other hand, $InsP_3$ has also been implicated in the gravity signal transduction pathway in cereal leaf pulvini (Perera et al., 1999; Perera et al., 2001). However, the potential effect of this important signaling molecule in root or shoot gravitropism of dicots has not been documented yet.

Changes in cytosolic and apoplastic pH levels within the columella region of *Arabidopsis* root caps constitute some of the fastest physiological responses to gravistimulation observed to date (Fasano et al., 2001; Scott and Allen, 1999). The apoplast of Arabidopsis root caps was found to acidify from pH 5.5 to 4.5 within 2 min of gravistimulation, while cytoplasmic pH increased in columella cells from 7.2 to 7.6. These pH changes preceded initiation of the graviresponse by 10 min, and alterations of the cytosolic pH changes by injection of caged protons in statocytes delayed the graviresponse (Fasano et al., 2001). Therefore, it appears likely that alterations in root cap pH contribute to the initial phases of gravity perception or signal tranduction (Fasano et al., 2001). While one group reported that similar pH changes affected the statocytes on upper and lower sides of gravistimulated root caps (Fasano et al., 2001), the other group showed that statocytes on the top and bottom sides responded differently to gravistimulation (Scott and Allen, 1999). Solving this difference in experimental results will be essential, because asymmetry in the response could suggest a role for pH changes in establishment of response polarity. Conversely, a symmetrical response across the root cap would suggest that the pH changes facilitate or contribute to the gravity signal transduction pathway, but do not contribute to the establishment of a final response polarity (Fasano et al., 2001).

In addition to providing an outstanding system with which to study the physiological changes associated with gravity signal transduction in the root cap, *Arabidopsis* has also proven very powerful in the identification of genes involved in this phase of the process. In addition to the starch-deficient mutants described above, a number of root gravitropic mutants have been identified in *Arabidopsis thaliana*, using the procedure described in Figure 2 legend. However, only few of these mutations were shown to affect early phases of gravity signal transduction. Most mutations affected the polar transport of auxin, or the organs' response to auxin.

The *altered response to gravity 1 / root and hypocotyl gravitropism (arg1/rhg)* mutants displayed altered root and hypocotyl gravitropism. Mutant seedlings accumulated starch in their statocytes, displayed wild type rates of root and hypocotyl growth, and developed wild-type root growth responses to exogenously supplied phytohormones and inhibitors of polar auxin transport. They also displayed wild type kinetics of hypocotyl curvature in response to lateral light (phototropism), suggesting that the *arg1/rhg* mutants are not generally defective in their ability to undergo differential growth across organs. This combination of phenotypes suggested that *ARG1/RHG* encodes a protein involved in early phases of gravity signal transduction, or that it functions in the auxin-gradient-independent phase of gravitropism (Fukaki et al., 1997; Sedbrook et al., 1999).

The *ARG1* gene encodes a dnaJ-like protein which carries a J domain at its amino terminus, a potential transmembrane domain and a coiled-coil domain which shares homologies with similar coiled-coils found in a number of proteins that interact with the actin microfilaments or with microtubules (Sedbrook et al., 1999). DnaJ-like proteins often act as molecular chaperones, alone or complexed with hsp70. In fact, the J domain has been implicated in interactions with the latter protein, while other regions have been implicated in substrate recognition. As such, dnaJ-like proteins have been implicated in protein folding, protein trafficking within cells, protein degradation, formation of macromolecular complexes and facilitation of signal transduction pathways (discussed in Sedbrook et al., 1999). Based on the phenotypes of *arg1* null mutants, it was hypothesized that *ARG1* functions in gravity signal transduction, either through potential interactions with the microfilaments in the statocytes, or by bringing together a complex of gravity signal-transducing proteins in the vicinity of the cytoskeleton within these cells. Interestingly, even though *ARG1* is expressed in all plant tissues examined, its expression in the root cap is sufficient to rescue the root gravitropic defect associated with *arg1-2* (Boonsirichai and Masson, unpublished). This result supports the potential involvement of ARG1 in early phases of gravity signal transduction.

ARG1 belongs to a small gene family in *Arabidopsis thaliana* (Arabidopsis Genome Initiative, 2000). Two other

genes in this species encode proteins that are highly similar to ARG1. Amongst these two proteins, ARG1-LIKE2 (ARL2) also appears essential for wild-type gravitropism. The phenotype associated with *arl2* mutations is similar to that of *arg1-2*. Interestingly, *arg1-2 arl2-1* double mutants developed an intermediate gravitropic phenotype that was similar to that of the *arg1-2* single mutant. These results suggest that *ARG1* and *ARL2* may interact to facilitate an important step of the gravity signal transduction pathway (Rosen, Boonsirichai, Guan, Poff and Masson, in preparation).

Given the potential role of *ARG1* and *ARL2* in facilitating the gravity signal transduction pathway, it is reasonable to hypothesize that the *arg1-2* mutation may serve as a useful genetic tool with which to identify other components of this pathway. Two genetic modifiers of *arg1-2* have recently been isolated and initially characterized. These modifiers enhance the gravitropic defect associated with *arg1-2*, but have little effect in a wild-type *ARG1* background. In addition to increasing the randomization of root-growth vector relative to gravity, these enhancers also convert a typically positive root gravitropic response into a negative one for the few seedlings that still respond to gravistimulation (Boonsirichai, Sedbrook and Masson, unpublished results). Hence, both *ARG1* and the modifiers play an important role in the interpretation of gravity signals by plant organs. Molecular cloning of these modifiers should contribute to a better understanding of the gravity signal transduction pathway in roots and shoots (Boonsirichai, Sedbrook and Masson, unpublished data).

Other mutants with abnormal shoot gravitropism also appear to be involved in the signal transduction phase of gravitropism. The *sgr3*, *sgr5*, and *sgr6* mutants exhibited reduced gravitropic response only in inflorescence stems. The existence of an endodermis containing normal amyloplasts, and normal phototropic response in these mutants suggested that the corresponding genes might be involved in the signal transduction phase of gravitropism (Fukaki et al., 1996; Yamauchi et al., 1997). Molecular cloning and functional analysis of these genes will provide important insights into the molecular mechanisms involved in gravity signal transduction in shoots.

A ROLE FOR AUXIN IN SIGNAL TRANSMISSION FROM THE SITE OF GRAVITY SENSING TO THE SITE OF CURVATURE RESPONSE

The direction of gravity-signal transmission differs between shoots and roots

The previous discussion points to a fundamental difference between shoots and roots in the spatial distribution of the gravity-sensing and –response sites. In roots, there is a physical separation between both sites (root cap and elongation zones, respectively), whereas they overlap in shoots. This implies that the physiological signal generated by activation of the gravity signal transduction pathway has to be transmitted longitudinally in roots, whereas it can be transmitted laterally in shoots (figure 6). We will now discuss the probable nature of this signal.
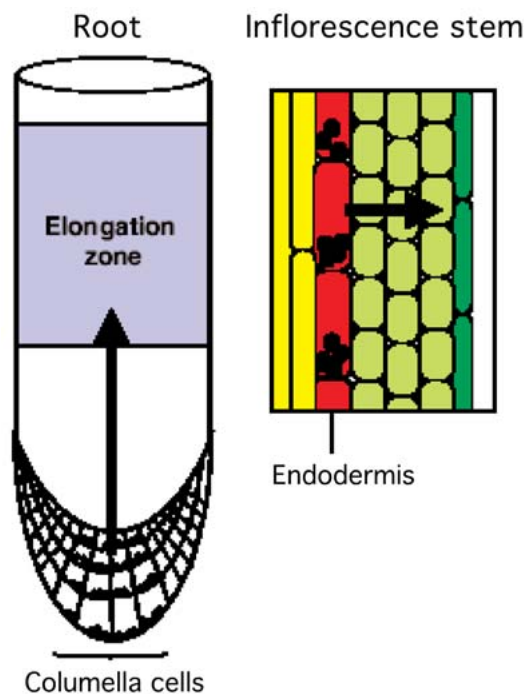
Figure 6. Spatial relationship between gravity sensing tissue and elongation zone in roots and shoots. In a root (left), the gravity vector (g) is perceived in the columella cells in the root cap. A signal (arrow) is transmitted from the columella cells to the elongation zone in an apical-to-basal fashion. In an inflorescence stem (right), gravity sensing is carried out in the endodermal cells and a signal (arrow) is transmitted in an inner-to-outer fashion.

Auxin is redistributed laterally across gravistimulated organs

In 1937, Cholodny and Went independently proposed that gravistimulation promotes the lateral transport of auxin across plant organs, resulting in its accumulation at the bottom side. This lateral auxin gradient would then promote a differential cellular elongation on opposite flanks of the stimulated organ, responsible for organ curvature. The differential sensitivity of root and shoot cells to auxin would be responsible for the differential curvature in shoots and roots: upward and downward, respectively (reviewed in Trewavas, 1992).

Even though the specific properties of shoot and root gravitropism suggest that the process is more complex than originally hypothesized, it appears that a lateral transport of auxin does indeed take place in both shoots and roots upon gravistimulation. Firstly, lateral redistribution of radiolabeled IAA occurred in both shoots and roots upon gravistimulation. It preceded the gravitropic curvature response, and appeared to depend upon active metabolism and Ca^{2+} (Parker and Briggs, 1990; Young et al., 1990). Secondly, application of auxin transport inhibitors to gravistimulated plants completely inhibited root gravitropism, even when the compounds were used at concentrations that were not sufficient to inhibit regular growth (Muday and Haworth, 1994; Rashotte et al., 2000). Similarly, mutations in genes that encode components of the auxin transport machinery altered gravitropism, as discussed below. Thirdly, gravistimulation of shoots and roots promoted a differential activation of auxin-response gene expression at the bottom side of each organ (Li et al., 1991; Luschnig et al., 1998; Rashotte et al., 2001; Sabatini et al., 1999).

Hence, activation of the gravity signal transduction pathway in shoots or roots promotes the development of a lateral auxin gradient. Even though the precise molecular mechanisms underlying this process have not been elucidated yet, recent progress in our understanding of auxin transport allows us to postulate the potential involvement of specific mechanisms in the regulation of this process, as discussed below. These include protein phosphorylation and membrane targeting of transporter proteins.

Polar auxin transport involves auxin influx and efflux carriers

The most abundant auxin in plants, indole-3-acetic acid (IAA) is synthesized in young tissues of shoots, and transported basipetally into more mature regions of the plant where it regulates cell division, expansion and differentiation. IAA is also transported through the vasculature into the root tip, where it is redistributed laterally to more peripheral tissues. It is then transported back to the elongation zones, where it also regulates cell division, expansion and differentiation (reviewed in Lomax et al., 1998).

Early biochemical studies had shown that auxin transport occurs within cell files (Figure 7). Auxin enters cells by passive diffusion of its protonated form through the plasma membrane or by import though a transmembrane influx carrier. It exits the cell through a plasma-membrane
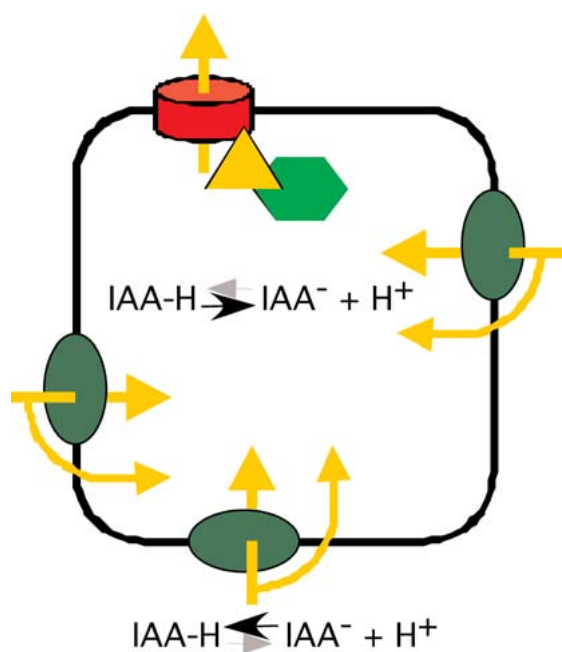
Figure 7. The chemiosmotic model of polar auxin transport in plants. IAA is in equilibrium between its protonated and ionic forms. In the apoplast, a reasonably large amount of IAA is in the protonated form, due to the low pH. Protonated IAA can enter the cell by passive diffusion through the plasma membrane, as well as through a transmembrane auxin influx carrier (green oval). Once within the cell, most of this IAA is converted into the ionized form. It can exit the cell by active transport through an auxin efflux carrier, made of at least one transmembrane protein (red cylinder), a regulatory NPA-binding protein (green octogon), and a potential linker protein (yellow triangle). The direction of auxin transport is dictated by the polar distribution of the auxin efflux carrier within the transporting cells (Müller et al., 1998). In this model auxin transport is represented by yellow arrows.

efflux carrier complex whose location is restricted within the transporting cells. Hence, the basipetal or acropetal polarity of auxin transport is governed by specific distribution of the auxin efflux carrier complex to the basal or apical side of the transporting cell, respectively (Figure 7). Biochemical studies suggested that the auxin efflux carrier complex is composed of at least three proteins, including a transmembrane carrier, an actin-associated peripheral regulatory protein that binds an inhibitor of polar auxin transport (naphthylphthalamic acid, or NPA), and a putative linker protein (reviewed in Lomax et al., 1998).

The AUX1 and AGR1/EIR1/PIN2/WAV6 genes of Arabidopsis thaliana, essential for root gravitropism, respectively encode components of the auxin influx and efflux carriers involved in basipetal auxin transport

The *AUX1* gene of *Arabidopsis thaliana* appears to encode a component of the auxin influx carrier. It was isolated through a screen for mutations that affect root-growth response to auxin, and root gravitropism (Maher and Martindale, 1980). *aux1* mutant seedlings displayed increased root-growth resistance to auxin and ethylene, altered root gravitropism, defects in lateral root formation, and altered auxin transport (Bennett et al., 1996; Marchant et al., 1999). Interestingly, the gravitropic phenotype of *aux1* mutant roots was rescued by adding to the medium low concentrations of naphthaleneacetic acid (NAA, an auxin analog that efficiently diffuses through the plasma membrane), but not by adding 2,4-dichlorophenoxyacetic acid (2,4-D) or IAA (two hormones known to require the auxin influx carrier to penetrate the cells) (Marchant et al., 1999; Yamamoto and Yamamoto, 1998). The *AUX1* gene was cloned and shown to encode a transmembrane protein that shares homologies with bacterial amino acid permeases (Bennett et al., 1996). Taken together, the data suggested that the AUX1 protein is a component of the auxin influx carrier involved in polar auxin transport in roots (Marchant et al., 1999; Yamamoto and Yamamoto, 1998). Interestingly, shoot gravitropism was not affected by mutations in the *AUX1* gene. This difference may simply reflect functional redundancy with other *AUX1* – like genes expressed in the shoot (Arabidopsis Genome Initiative, 2001).

A probable transmembrane component of the auxin efflux carrier has also been identified by genetic approaches in *Arabidopsis*. Mutations in *AGR1/EIR1/PIN2/WAV6* resulted in altered root gravitropism, increased root-growth sensitivity to high concentrations of NAA, and increased root-growth resistance to ethylene and polar auxin trans-

port inhibitors (Bell and Maher, 1990; Chen et al., 1998; Luschnig et al., 1998; Müller et al., 1998; Utsuno et al., 1998). Furthermore, explants from *agr1* mutant roots retained more pre-loaded radiolabeled IAA than wild type after 2 hours of washing (Chen et al., 1998), and basipetal auxin transport was affected in roots (Rashotte et al., 2000). Taken together, these data suggested that the mutations affected auxin efflux. *AGR1/EIR1/PIN2/WAV6* was shown to encode a transmembrane protein that shares some homology with bacterial transporters. Its expression was restricted to the epidermis and cortical cells of the root elongation zones, as well as in hypocotyls of etiolated seedlings during the first few days of growth (Chen et al., 1998; Luschnig et al., 1998; Müller et al., 1998; Utsuno et al., 1998).

Immunolocalization experiments indicated that the PIN2 protein localizes on the basal side of epidermal and cortical cells in the distal and central elongation zones of Arabidopsis roots, as well as in the outermost lateral membrane of cortical cells in this region (Müller et al., 1998). Interestingly, when expressed in yeast, this protein conferred an increased ability to export pre-loaded radiolabeled IAA, and an increased resistance to toxic IAA derivatives (Chen et al., 1998; Luschnig et al., 1998). Taken together, these data strongly suggest that *AGR1/EIR1/PIN2/WAV6* encodes a component of the auxin efflux carrier complex in roots. Because its expression was localized in the elongation zones of roots, but not in the root cap, it was postulated that AGR1/EIR1/PIN2/WAV6 might mediate the transport of auxin from the root cap to the responding elongation zones. Accordingly, AGR1/EIR1/PIN2/WAV6 would be required for gravitropism because it mediates the transport of a preformed auxin gradient from the root cap to the responding elongation zone cells (see below). Alternatively, it is also possible that AGR1/EIR1/PIN2/WAV6 plays a more active role in root gravitropism by contributing to lateral gradient formation. The latter model would imply that another signal, possibly electrical (Björkman and Cleland, 1991; Lee et al., 1983) or chemical (Stevenson et al., 2000), might be transmitted from the root cap to the distal elongation zone (Chen et al., 1998; Luschnig et al., 1998; Müller et al., 1998; Utsuno et al., 1998). Alternatively, it is also possible that distal elongation zone cells can also perceive gravity (reviewed in Chen et al., 1999).

Some members of the AGR/PIN gene family may regulate the lateral redistribution of auxin across

gravistimulated root tips

It is interesting to note that *AGR1/EIR1/PIN2/WAV6* is not expressed in the root cap. Yet, lateral redistribution of auxin in response to gravistimulation is believed to occur at, or in the vicinity of the root cap (Sabatini et al., 1999; Young et al., 1990). Hence, one would have to conclude that another auxin efflux carrier controls the lateral transport of auxin across the root cap in response to gravistimulation. The *AGR1/EIR1/PIN2/WAV6* gene belongs to a family that contains 8 genes, and other members of this family may modulate the lateral transport of auxin across the cap.

Interestingly, at least one member of the *AGR1/EIR1/PIN2/WAV6* gene family is expressed in the root quiescent center and columella cells of the root cap (Chen, Woody and Masson, unpublished data). Preliminary in situ immunolocalization data suggest that the corresponding protein might be distributed symmetrically at the periphery of the columella cells in vertical roots. However, more protein appeared to accumulate at the new physical bottom of the columella cells upon gravistimulation. Furthermore, the domain of expression of this gene appeared to expand to additional peripheral cap cells at the bottom side of gravistimulated roots (Chen and Masson, in preparation). Because the efflux carriers might be turned over rapidly (Delbarre et al., 1998; Morris and Robinson, 1998; Sieberer et al., 2000), these interesting results suggested a mechanism allowing lateral auxin redistribution across gravistimulated root caps. The gravity signal transduction pathway could affect the trafficking of AGR/EIR/PIN/WAV-like proteins within the statocytes, establishing a polar distribution of auxin carriers that would allow lateral auxin redistribution upon gravistimulation. Furthermore, differential regulation of efflux carrier activity by reversible protein phosphorylation (Delbarre et al., 1998; Rashotte et al., 2001) might also contribute to lateral polarity establishment or maintenance upon gravistimulation. Experiments are in progress in several laboratories to test these interesting models.

Lateral auxin redistribution may directly affect the curvature response in shoots, without a need for longitudinal signal transmission

As discussed earlier in this review, statocytes constitute endodermal cells in shoots, and sedimentable amyloplasts are found along the entire length of the elongation zone, which constitutes the site of curvature response to gravis-

timulation (Tasaka et al., 1999). Hence, gravity-induced lateral auxin transport can directly promote a curvature response in shoots, without a need for signal transmission (Figure 6) (Tasaka et al., 1999; Tasaka et al., 2001). It is likely that other members of the AGR/PIN gene family are the target of the gravity signal transduction pathway in shoot statocytes, and regulate lateral auxin redistribution in a way that resembles the processes discussed for roots. Systematic expression and functional analysis of members of this gene family will likely allow identification of the proteins involved.

Mutations affecting genes that control expression, localization, stability or activity of efflux and influx carriers also affect gravitropism

Other *Arabidopsis* mutations affecting gravitropism appeared to alter the regulation of auxin transport. Ruegger et al. (1997) reported that mutations in the *TRANSPORT INHIBITOR RESISTANT 3* (*TIR3)* locus resulted in altered responses to auxin transport inhibitors, a decrease in polar auxin transport, a reduction in apical dominance, a decrease in root and shoot growth rates, and defects in lateral root formation. Interestingly, *tir3* also appeared to affect the number of NPA binding sites within Arabidopsis seedlings. Taken together, these data strongly suggest that *TIR3* is required for expression, localization or stabilization of the NPA-binding protein, believed to regulate the activity of auxin efflux carriers (Ruegger et al., 1997).

The *roots curling in NPA 1 (rcn1)* mutation affects root growth behavior in the presence of NPA: mutant roots curled in the presence of NPA, while wild type roots did not. *RCN1* was cloned and shown to encode the regulatory subunit A of protein phosphatase 2A (Garbers et al., 1996). Interestingly, *rcn1* mutant roots displayed altered kinetics of gravitropism in roots. Furthermore, the basipetal transport of auxin at the root tip was enhanced by mutations in *rcn1* and by protein phosphatase inhibitors (Rashotte et al., 2001). Using a *GUS* reporter construct that fuses the *GUS* gene to a minimal promoter carrying multiple copies of the auxin-response element at its 5'end (DR5-GUS), Rashotte and collaborators demonstrated a delay in the asymmetrical activation of *GUS* gene expression in the tip of gravistimulated *rcn1* roots when compared to wild type. Interestingly, wild type kinetics of gravitropism were restored when low concentrations of NPA were added to the medium, suggesting that the gravitropism defect derived from an increase in the flow of auxin from the tip to the elongation zones. This effect was not

dependent upon the existence of AGR1/EIR1/PIN2/WAV6 in the background, suggesting that RCN1 might regulate auxin transport by affecting other carriers (Rashotte et al., 2001). Amazingly, *rcn1* did not appear to affect the acropetal transport of auxin in roots in the absence of NPA. However, it affected its NPA sensitivity. Taken together, these results suggested that reversible phosphorylation is crucial for the regulation of auxin transport and gravitropism, and that the acropetal and basipetal auxin transport systems in roots are differentially regulated.

The *polar auxin transport inhibitor sensitive 1* (*pis1*) mutation also affected root curling in the presence of NPA. However, in this case mutant seedlings displayed increased sensitivity to two polar auxin transport inhibitors (NPA and triiodobenzoic acid (TIBA)) for a variety of phenotypes, including root gravitropism and phototropism, root curling, root elongation, and seedlings growth (Fujita and Syono, 1997). It was suggested that the *PIS1* gene might be specifically involved in the response pathway to NPA/TIBA leading to auxin efflux inhibition (Fujita and Syono, 1997).

reduced gravitropism 1 (rgr1) / auxin resistance 4 (axr4) affects root gravitropism and confers specific root-growth resistance to auxin and polar auxin transport inhibitors (Hobbie and Estelle, 1995; Simmons et al., 1995). Interestingly, analysis of root gravitropism in the presence of auxin indicated that NAA added at low concentrations, but not IAA or 2,4-D, could rescue the gravitropic phenotype of *axr4*. This specific rescue of *axr4* gravitropism by NAA is quite reminiscent of a similar rescue of *aux1* gravitropism by the same compound, as discussed above. Because NAA is capable of efficiently diffusing through the membranes while 2,4-D and IAA require an influx carrier to penetrate the cells, Yamamoto and Yamamoto proposed that *axr4* might also affect auxin influx within the cells (Yamamoto and Yamamoto, 1999). Molecular cloning of this gene should provide clues on its biochemical role in auxin transport and/or response.

The gravitropic curvature involves auxin-response processes

Auxin is essential for gravitropism in roots and shoots

The gravity-induced auxin gradient generated across organs appears to drive at least some of the curvature response to gravistimulation in both shoots and roots. Indeed, mutations that affect auxin transport and/or auxin response interfere with gravitropism.

Auxin controls cell expansion by regulating the activity of plasma membrane proton pumps (Ephritikhine et al., 1987), resulting in cell wall acidification and increased extensibility. It also activates intracellular processes involving protein degradation, resulting in the differential activation of auxin-responsive gene expression (reviewed in del Pozo and Estelle, 1999).

Auxin promotes cell elongation in shoots and inhibits it in roots. This reflects a dramatic difference in sensitivity of these two organs to auxin, as discussed in Kaufman et al. (1995). Hence, the top to bottom lateral auxin transport occurring across gravistimulated organs results in the development of a downward curvature in roots (positive gravitropism), and an upward curvature in shoots (negative gravitropism) (Kaufman et al., 1995).

As expected, a variety of auxin-response mutations were shown to affect shoot and/or root gravitropism. These mutations can be classified into two major groups, depending on the type of gene they affect. A first group of mutations appears to affect genes involved in the regulation of protein degradation, while the second group includes mutations that affect genes involved directly or indirectly in the regulation of expression of auxin-response genes.

Several auxin-response mutations affect genes involved in the regulation of protein stability

The *auxin-resistance 1* (*axr1*) mutant displays a variety of phenotypes, including defects in root gravitropism, apical dominance, lateral branching, vasculature development, hypocotyl elongation, fertility and accumulation of auxin-response genes. *AXR1* encodes a nuclear protein that shares similarity with the N-terminus of E1 ubiquitin-activating enzymes (del Pozo and Estelle, 1999; Leyser et al., 1993), suggesting a role in the regulation of protein stability. Interestingly, AXR1 was shown to interact with ECR1, a protein that shares homology with the C-terminal end of E1s. The AXR1-ECR1 complex was shown to activate RUB1, a ubiquitin-like protein (del Pozo et al., 1998). Upon activation RUB1 conjugates with another protein, AtCUL1, which is part of an E3 ubiquitin-ligase SCF complex containing ASK1, a homolog of the yeast Skp1p protein, RBX1 and the F-box containing protein TIR1 (del Pozo and Estelle, 1999; Gray et al., 1999). This SCF^{TIR1} complex is believed to ubiquitinate specific proteins acting as repressors of auxin action, possibly including the short-lived Aux/IAA proteins, targeting them for degradation (del Pozo and Estelle, 1999).

Interestingly, mutations in several of the genes involved

in this protein degradation pathway affect root gravitropism and auxin response, along with other auxin-related processes. Such mutations include *tir1* and *ask1*. Furthermore, overexpression of *TIR1* promotes auxin response, suggesting that SCF^{TIR1} is limiting in the process (Gray et al., 1999). Taken together, the data strongly suggest that the SCF^{TIR1} complex functions by modulating the stability of a group of proteins involved in the regulation of auxin response and gravitropism (Gray et al., 1999).

Some mutations altering gravitropism and auxin sensitivity affect genes that are involved in the regulation of expression of other auxin-response genes

The *AUX/IAA* genes encode short-lived nuclear proteins that may serve as transcriptional regulators. Their transcription is rapidly upregulated in the presence of auxin (Abel et al., 1995; Ulmasov et al., 1997). The Aux/IAA proteins share four conserved domains, one of which (domain II) being involved in their destabilization (Rouse et al., 1998; Worley et al., 2000). Interestingly, the rapid degradation of Aux/IAA proteins is necessary for a normal auxin response (Rouse et al., 1998; Worley et al., 2000).

Some Aux/IAA proteins were found to repress expression of auxin-responsive genes (Ulmasov et al., 1997). It has been suggested that the SCF^{TIR1} complex described above might target Aux/IAA proteins for degradation by the proteasome (Gray et al., 1999).

Considering the observations discussed above, it is not surprising that some mutants with defects in gravitropism and auxin response turned out to carry mutations in some of the *AUX/IAA* genes. The dominant mutant *axr2-1* shows abnormal gravitropism in all organs (Timpte et al., 1992; Wilson et al., 1990). The *AXR2* gene encodes a member of the Aux/IAA family, IAA7 (Nagpal et al., 2000). The *SUPRESSOR OF HY2 (SHY2)*, *AXR3*, and *SOLITARY ROOT (SLR)* genes, whose mutations cause abnormal gravitropism both in hypocotyls and roots, also encode Aux/IAA family members: IAA3, IAA17, and IAA14, respectively (Kim et al., 1998; Leyser et al., 1996; Reed et al., 1998; Tian and Reed, 1999; Tasaka et al., 2001). The auxin-insensitive *massugu 2* (*msg2*) mutant was also shown to carry a mutation in the *IAA19* gene and exhibited decreased gravitropism (Tatematsu et al., 1999). In all cases, the mutations responsible for these phenotypes were semi-dominant and affected conserved domain II, involved in protein destabilization. It should however be emphasized that loss of function mutations in *SHY2/IAA3*

also affected root gravitropism, root-growth response to auxin, and lateral root formation, strongly supporting a role for this gene, at least, in the regulation of these processes in wild type plants (Tian and Reed, 1999).

A group of transcription factors, named "auxin-response factors" (ARFs), was recently found to bind to the auxin-response elements found in the promoters of many auxin-regulated genes (Guilfoyle et al., 1998; Jungmook et al., 1997). These transcription factors share two of the conserved domains (III and IV) found in Aux/IAA proteins. Interestingly, some mutations affecting gravitropism and auxin response were in one of the *ARF* genes. The *massugu1 (msg1)* mutant, which displays abnormal gravitropism in hypocotyls only, was isolated as a mutant whose hypocotyl was less sensitive to exogenous auxin compared to wild type (Watahiki and Yamamoto, 1997). Genetic studies revealed that *msg1* is allelic to *non phototropic hypocotyl 4 (nph4)*, a mutation which results in reduced phototropism in hypocotyls (Liscum and Briggs, 1996; Stowe-Evans et al., 1998). Therefore, it was suggested that *MSG1/NPH4* is necessary for the auxin-regulated differential cell elongation underlying the gravitropic and phototropic responses of hypocotyls. The *MSG1/NPH4* gene encodes a putative transcription factor which belongs to the *ARF* gene family (*ARF7*) (Harper et al., 2000), and the *msg1/nph4* mutations caused a decrease in expression of auxin-regulated genes, as expected (Stowe-Evans et al., 1998).

ARF and Aux/IAA proteins can interact with each other through their conserved domains (III and IV), forming homo- or hetero-dimers, probably in various combinations (Kim et al., 1997; Ulmasov et al., 1997). In Arabidopsis, there are at least 23 *ARF* genes and 29 *AUX/IAA* genes (Arabidopsis Genome Initiative, 2000). It is expected that ARF and Aux/IAA proteins can generate a large number of homo- and hetero-dimers, forming a complex genetic network to control gravitropism and to regulate many other genetic and developmental mechanisms important for plant life. The altered sensitivity to auxin found in the auxin-related mutants described above causes abnormal changes in growth rates on both sides of gravistimulated organs, resulting in a defect in the differential growth. Therefore, elucidation of auxin signaling networks is required for a better understanding of the molecular mechanisms underlying gravitropism.

Posttranscriptional regulation of gene expression may also be important for the gravitropic response of roots. A mutation in *HYPONASTIC LEAVES 1* (*HYL1*) resulted in altered kinetics of root gravitropism, coupled with shorter plant stature, increased branching, delayed flowering, leaf hyponasty and reduced fertility. This variety of phenotypes was associated with increased root-growth resistance to auxin and cytokinin, and hypersensitivity of seed germination and root growth to ABA. The *HYL1* gene was shown

to encode a double-stranded RNA-binding, nuclear protein that contains two double-stranded RNA binding motifs, a nuclear localization motif, and a C-terminal repeat that may be involved in protein-protein interaction. It was hypothesized that this protein might regulate multiple aspects of plant growth and development, including gravitropism, through a differential effect on the expression of genes that modulate the transmission of, or the response to phytohormones (Lu and Fedoroff, 2000).

Other mutations affecting root gravitropism and auxin response are in genes whose functions remain uncharacterized

The *AXR6* gene of *Arabidopsis thaliana* also appears important for root gravitropism and other plant responses to auxin. Mutations in this locus are semi-dominant. While homozygous *axr6* embryos usually arrested growth after germination, displaying cotyledons with abnormal vasculature and missing hypocotyl and roots, heterozygous *AXR6/axr6* plants grew to maturity. Their roots displayed altered kinetics of gravitropism and increased root-growth resistance to IAA, 2,4-D and NAA, but not to a precursor of ethylene biosynthesis (ACC), to abscisic acid or to kinetin. Heterozygous plants also displayed a variety of phenotypes typically associated with defects in auxin physiology or response (Hobbie et al., 2000). Molecular characterization of this gene is needed to clarify its function in auxin response and embryogenesis.

clg1 is another root-gravitropism mutation that affects auxin response, though to a lesser extent than the other auxin-response mutations described above. *clg1* seedlings exhibited only a modest increase in root-growth resistance to 2,4-D, NAA and auxin transport inhibitors, but displayed wild-type root-growth resistance to IAA (Ferrari et al., 2000). Interestingly, mutant roots also displayed a notable resistance to ethylene, suggesting a role for this phytohormone in root gravitropism (Ferrari et al., 2000).

Compounds other than IAA also function as auxins in plants. Amongst them, indole-3-butyric acid (IBA) also appears to play an important role in root gravitropism (Poupart and Waddell, 2000). The semi-dominant *resistant to IBA 1* (*rib1*) mutant of *Arabidopsis thaliana* displayed increased root-growth resistance to IBA, but showed a wild-type response to IAA. This mutation also conferred increased root-growth resistance to 2,4D and to polar auxin transport inhibitors. Interestingly, mutant roots displayed a variety of phenotypes, including altered gravitropism. The authors proposed that the *RIB1* gene functions

in either IBA transport or response (Poupart and Waddell, 2000). Molecular cloning of *RIB1* will also be essential to the understanding of the role of this gene in auxin transport or response.

The Root curvature response to gravistimulation also involves a phase that appears to be independent of an auxin gradient

Careful analyses of early phases of the root curvature response to gravistimulation revealed a response complexity that was not consistent with the classical Cholodny-Went theory. As discussed earlier in this review, cells at both the top and bottom sides of the CEZ and at the bottom side of the DEZ displayed a decrease in their rate of elongation early after gravistimulation. Then, cells at the topside of the DEZ exhibited an increased rate of elongation, resulting in initiation of curvature. Subsequently, the curvature developed as it migrated through the CEZ, eventually reaching the maturation zone (Ishikawa and Evans, 1993). Interestingly, early phases of gravicurvature were maintained and developed vigorously in the DEZ even when roots were exposed to high concentrations of auxin, otherwise sufficient to completely eliminate root growth (Muday and Haworth, 1994). This observation was interpreted as indicating that the DEZ-dependent early-response phase of gravitropism might be independent of an auxin gradient.

This interpretation is, in fact, consistent with the results of several physiological studies of cell elongation at the DEZ, which show that the wall of DEZ cells rapidly acidifies upon gravistimulation (Fasano et al., 2001). Cell wall acidification of DEZ cells on the topside occurred within 10 min of gravistimulation, much faster than one would expect if it followed the transfer of an auxin signal from the root cap to the DEZ (Fasano et al., 2001). Hence, DEZ cells may sense gravity by themselves, or they may receive another signal from the root cap, possibly electrical (Fasano et al., 2001). More work is needed to characterize this interesting phase of gravitropism.

Similarity and difference in mechanism of gravitropism between root and shoot – Conclusions and perspectives

In this review, we have attempted to summarize the current

state of our knowledge on the genetic basis of gravitropism in shoots and roots of *Arabidopsis thaliana* (Table 1). The mutants described here can be divided into at least six classes: some are deficient in gravitropism in only one organ and some display altered gravitropism in several organs simultaneously (Table 1; Tasaka et al., 2001). This indicates that the mechanism of gravitropism is, at least in part, genetically different in each organ (roots, hypocotyls, and inflorescence stems), and that some genetic components of the mechanism are shared among organs. The difference between shoots and roots is not surprising since these organs exhibit opposite gravitropism. Maybe more surprising is the observation of only a partial overlap in the genetic mechanisms that underlie hypocotyl and inflorescence stem gravitropism, in spite of the fact that both organs can be classified as shoots, and that both exhibit negative gravitropism. Two possible mechanisms can explain this observation. Firstly, many of the genes uncovered in these studies belong to multigene families whose members exhibit different expression patterns, despite encoding very similar gene products. Hence, functional redundancy may explain the differential gravitropic phenotype observed between organs for some of these mutants. Secondly, hypocotyls and inflorescence stems differ in their developmental origin: the hypocotyl originates from the middle part of the embryo, whereas the inflorescence stem arises from the shoot apical meristem after germination.

Roots and shoots appear to utilize the same gravity-susceptible organelles, the amyloplasts, as statoliths. Yet, they exhibit opposite gravitropism. Interestingly, many features of the gravity sensing and response machinery differ between roots and shoots, and may be partly responsible for some of the genetic differences in gravitropism between these two organs, as discussed in this review, and summarized in Table 1. Developmental origin of the endodermal cells in inflorescence stems is still obscure, even though cells expressing the *SCR* gene appear at the basal side of the peripheral zone of the shoot apical meristem (Wysocka-Diller et al., 2000). Shoot endodermal cells are filled with a large central vacuole which appears to surround their amyloplasts (Kiss et al., 1997; Morita et al., in preparation). Such a large vacuole may affect sedimentation of amyloplasts and/or the signaling that follows. On the other hand, columella cells originate from columella initials located at the apical side of the quiescent center at the root apical meristem. They are polarized, with their amyloplasts sedimenting on top of distally localized peripheral ER. Their vacuoles are very small, and do not interfere with amyloplast sedimentation (Zheng and Staehelin, 2001). Hence, the difference in subcellular organization of statocytes between shoots and roots is compatible with a differential contribution of vacuolar function to gravitropism between these two organs. This is in

agreement with the fact that *sgr2* and *sgr4* display normal root gravitropism (Fukaki et al., 1996; Yamauchi et al., 1997).

The general arrangement of sensing cells within gravity-sensing tissues also differs between roots and shoots. As discussed earlier, columella cells form a conical tissue at the root tip, whereas the endodermal cells form a one-layer cylindrical tissue (Figure 6). The radially symmetrical arrangement of endodermal cells in shoots may enable stems to recognize a change of gravity vector in any direction (Figure 8). The differential sensing between upper and the lower sides of the endodermis in a thin stem unit may occur as follows. Each endodermal cell may be able to sense its own orientation relative to the gravity vector, possibly via amyloplast sedimentation. The orientation of a specific cell relative to the gravity vector may be determined by comparing the vertical orientation pointed by gravity with some cellular asymmetry that defines a radial vector within the shoot (toward the center of the stem). Although the molecular structure that gives radial information to each endodermal cell is not known yet, it may be generated by an intracellular polarity, as observed in the columella cells of roots (see above).

The spatial relationship between gravity-sensing and –responding tissues also differs between shoots and roots. The gravity-sensing columella tissue of roots is physically separated from the gravity-responding elongation zones, whereas the gravity-sensing endodermal cell layer of
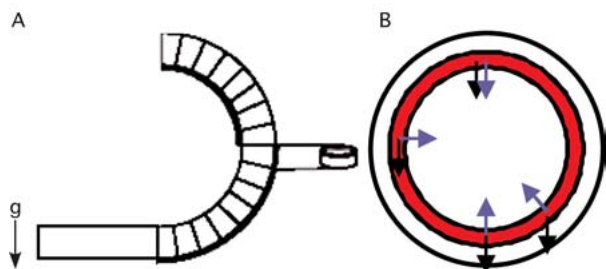

Figure 8. Model for gravity perception in inflorescence stems in Arabidopsis. **(A)** Inflorescence stem segment after gravitropic overshooting, displaying a 'U' shape. This stem segment was analyzed 2 hr after gravistimulation, as shown in Figure 3C. Thick lines indicate the lower side of the stem. Thin stem units located at the apical half of the stem segment should perceive a gravistimulus that is opposite to the initial stimulus. **(B)** Cross-section of a horizontally-oriented stem. Black arrows indicate the orientation of gravity. Blue arrows indicate the radial vector. A comparison between these two directions may provide each endodermal cell with a unique directional information. g, gravity orientation

Table 1. Gravitropic mutants of Arabidopsis thaliana*

Mutant	Gravitropism**	Other phenotypes***	Gene product
Gravity perception and signal transduction			
arg1/rhg	Reduced (r & hyp)	None	DnaJ-like protein
arl2	Reduced (r & hyp)	None	DnaJ-like protein
eal1	Reduced (hy) No (inf)	Loss of amyloplast	Unknown
pgm	Reduced (r, hy & inf)	No starch synthesis	Plastidic phosphoglucomutase
sgr1/scr	None (hy & inf)	Loss of endodermis	Transcription factor (GRAS family)
sgr2	Reduced (hy) No (inf)	Abnomal embryogenesis	Phospholipase A1 protein
sgr3	Reduced (inf)	None	Unknown
sgr4/zig	Reduced (hy) No (inf)	Zigzag-shaped stem	v-SNARE (AtVTI1a)
sgr5	Reduced (inf)	None	Unknown
sgr6	Reduced (inf)	None	Unknown
sgr7/shr	None (hy & inf)	Loss of endodermis	Transcription factor (GRAS family)
Auxin transport			
agr1/eir1 /pin2/wav6	Reduced (r; young etiolated hyp)	NAA-hypersensitive Ethylene-, NPA- and TIBA- resistant	Transmembrane component of auxin efflux carrier complex
aux1	Reduced (r)	Auxin and ethylene resistant Lateral root formation	Auxin influx carrier
pis1	Reduced (r) in the presence of NPA	Hypersensitive to NPA	Unknown
rcn1	Reduced (r)	Roots curling in presence of NPA	Regulatory subunit A of protein phosphatase A1
rib1[#]	Reduced (r)	IBA-resistant Resistant to polar auxin Transport inhibitors	Unknown
tir3	reduced (r)	auxin and NPA resistant Lateral root formation	Unknown
Auxin response			
axr1	Reduced (r)	Increased resistance to auxin	Similar to N-terminus of El-ubiquitin ligase
axr2	Reduced (r, hy & inf)	Insensitive to auxin	IAA7
axr3	Reduced (r & hy)	Auxin resistant	IAA17
rgr1/axr4	Reduced (r)	Resistant to auxin and NPA	Unknown
axr6	Reduced (r)	Auxin resistant Embryo patterning defect in homozygous individuals	Unknown

Table 1 continued

Mutant	Gravitropism**	Other phenotypes***	Gene product
clg1	Reduced (r)	Slightly resistant to 2,4-D, NAA and polar auxin transport inhibitors Ethylene resistant	Unknown
dwf	Reduced (hy)	Auxin resistant	Unknown
msg1/nph4	Reduced (hy)	Auxin resistant	ARF7
msg2	Abnormal (hy)	Insensitive to auxin	IAA19
rib1[#]	Reduced (r)	IBA-resistant Resistant to polar auxin Transport inhibitors	Unknown
shy2	Reduced (r & hy)	Auxin resistant	IAA3
slr	Reduced (r & hy)	Auxin resistant Reduced lateral root formation	IAA14
tir1	Reduced (r)	Increased resistance to auxin and polar auxin transport inhibitors	F-box containing protein

- *Discussion on the phenotypes associated with each mutation, on the function of the corresponding gene, and the corresponding references, is provided in the text
- **r = root; hyp = hypocotyl; Inf = inflorescence stem
- ***Most of the auxin-resistant mutants also display a variety of morphological phenotypes which were not indicated here
- [#]*rib1* could affect either the transport of, or the response to IBA (Pouppart and Waddell, 2000)

shoots overlaps with the gravity-responding elongation zone in these organs (Figure 6). In fact, stem segments dissected from any part of the elongation zone are gravitropic (Fukaki et al., 1996). Consequently, one expects that the pattern of gravity signal transmission should differ between roots and shoots. In roots, the gravity signal perceived in the columella cells is transmitted to the distant elongation zones, and auxin transport is involved in this process. In shoots, the gravity signal perceived in the endodermal cell layer is probably transmitted in an inner-to-outer fashion to the cortex or epidermal cell layers, which may execute the differential cell elongation responsible for the gravitropic curvature (Figure 6). Although the identity of the corresponding signal(s) in shoots is not known yet, existence of auxin-related shoot gravitropism mutants suggests that auxin also plays an important role in gravitropic signaling and response.

The utilization of *Arabidopsis thaliana* as a model system for the study of shoot and root gravitropism has been instrumental in enhancing our understanding of this important and complex plant response to its environment, as discussed in this review. It is anticipated that a genetic approach similar to the one summarized here, along with novel genomic and proteomic procedures (based on the enormous amount of information derived from the recent completion of the genome sequencing project), and refined cell biological, biochemical, and physiological strategies, will allow us to elucidate the molecular mechanisms underlying gravitropism in plants.

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