

Directional Gravity Sensing in Gravitropism

Miyo Terao Morita

Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan 630-0192; email: mimorita@bs.naist.jp

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Key Words

statocyte, amyloplast, plastid, vacuole, actin, environmental response

Abstract

Plants can reorient their growth direction by sensing organ tilt relative to the direction of gravity. With respect to gravity sensing in gravitropism, the classic starch statolith hypothesis, i.e., that starch-accumulating amyloplast movement along the gravity vector within gravity-sensing cells (statocytes) is the probable trigger of subsequent intracellular signaling, is widely accepted. Several lines of experimental evidence have demonstrated that starch is important but not essential for gravity sensing and have suggested that it is reasonable to regard plastids (containers of starch) as statoliths. Although the word *statolith* means sedimented stone, actual amyloplasts are not static but instead possess dynamic movement. Recent studies combining genetic and cell biological approaches, using *Arabidopsis thaliana*, have demonstrated that amyloplast movement is an intricate process involving vacuolar membrane structures and the actin cytoskeleton. This review covers current knowledge regarding gravity sensing, particularly gravity susception, and the factors modulating the function of amyloplasts for sensing the directional change of gravity. Specific emphasis is made on the remarkable differences in the cytological properties, developmental origins, tissue locations, and response of statocytes between root and shoot systems. Such an approach reveals a common theme in directional gravity-sensing mechanisms in these two disparate organs.

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INTRODUCTION

Plant organs are capable of sensing various vectorial stimuli, e.g., light, gravity, touch, and humidity. Plants then reorient their growth direction so as to be in a suitable position for absorption of water or nutrients, photosynthesis, and reproduction. The response of a plant organ to a directional stimulus is called tropism. Gravity is one of the most important directional environmental cues that control growth direction. In general, plant shoots grow upward (negative gravitropism), whereas roots grow downward (positive gravitropism) (41). Because the direction and the magnitude of gravity are almost constant on the surface of the earth, gravitropism can be regarded as a posture control, triggered by sensing the tilt of organs relative to the direction of gravity.

In higher, multicellular plants, it is thought that the relative directional change of gravity is detected by specialized cells called statocytes, and this is followed by signal conversion from physical information to physiological information within the statocytes. Subsequently, the signal is transmitted to the neighboring cells and other tissues, which leads to differential cell growth between the lower and upper flanks of the responsive organ.

Readers may be interested in the signal conversion, or transmission, process after susception of the directional change of gravity. Numerous historical studies involving various experimental systems using various species have led to several appealing and plausible models (57, 92, 102). The endoplasmic reticulum (ER) is thought to be involved in the signal conversion process within the statocyte. Studies on mechanotransduction in animal cells have inspired a model that incorporates mechanosensitive ion channels, actin microfilaments, and integrin-like molecules (1, 34, 61, 89). Unfortunately, however, the direct or substantial evidence is probably insufficient. With respect to signaling molecules, changes in pH, intracellular Ca^{2+} , or inositol trisphosphate (IP₃) levels have been experimentally detected in response to gravistimulation (13, 31, 45, 58–60, 62, 88). In addition to temporal linkage, the spacial relationship between statocytes and the site where such further signaling is perceived is important to understand the initial signal conversion process of gravitropism. Which tissue or cells generate such signal molecules, however, remains undetermined, except for the change in pH that occurs in the gravity-sensing cells (13). Recent review articles have discussed in detail the signal conversion and transmission in gravitropism (e.g., 24). It is also important to clarify whether the signal reflects a tropic response or some other gravitational response. Plants have another response to gravity, gravity resistance, which is a reinforcement of the cell wall in response to hypergravity (28, 76). In gravitropism, the directional change of gravity is perceived, whereas in gravity resistance the magnitude of gravitational force or mechanical

Sensing: includes both susception and signal conversion by a sensor/receptor

Statocytes: cells and tissues that are specialized for gravity sensing and contain sinking cellular components

Susception: here defined as amyloplast sedimentation or movement that contributes to gravity sensing

ER: endoplasmic reticulum

pressure is sensed. Notably, the gravity perception mechanism of gravity resistance is likely different from that of gravitropism (77, 78).

In this review, I focus on the process of gravity sensing related to gravitropism. It is essential to decipher the exact mechanism of action at the site of gravity sensing (within the statocytes) to understand the susception mechanism of directional change in response to gravity. Recent studies, consisting primarily of genetic investigation using *Arabidopsis thaliana*, have demonstrated the molecular mechanism of gravitropism, at least in part (5, 15, 35, 70, 80, 82). This review covers current views on gravity perception in gravitropism in higher plants with specific emphasis on *Arabidopsis*.

IDENTIFICATION OF STATOCYTES

Sensing Site in Roots

In the 1900s, experimental evidence demonstrated that the orientation of the root apex in particular is important for gravitropism. The tip of the root consists of the root cap and the root apical meristem. In some plant species,

the root cap can be removed from the root tip without damaging root growth. Such decapped roots lose the gravitropic response, but the responsiveness is recovered following the regeneration of a new root cap (2). In addition, genetic manipulation to remove the *Arabidopsis* root cap—via root cap-specific expression of diphtheria toxin A, which kills only root cap cells—abolishes the gravitropic response in roots (90).

In *Arabidopsis*, the root cap comprises four tiers of columella cells that originate from columella initial cells, and lateral root cap cells that originate from the same source as root epidermal cells (11). Ablation of specific cells in the root cap with a laser has shown that the inner cells of the second tier of columella cells contribute maximally to root gravitropism (4). In contrast, ablation of the lateral root cap cells exhibits little effect on root gravitropism (4). The fact that columella cells in the root cap contain sedimenting amyloplasts is highly correlated to the functional importance of columella cells in root gravitropism. Thus, central columella cells in the root cap are the most important sites for gravity sensing in root gravitropism (**Figure 1**).

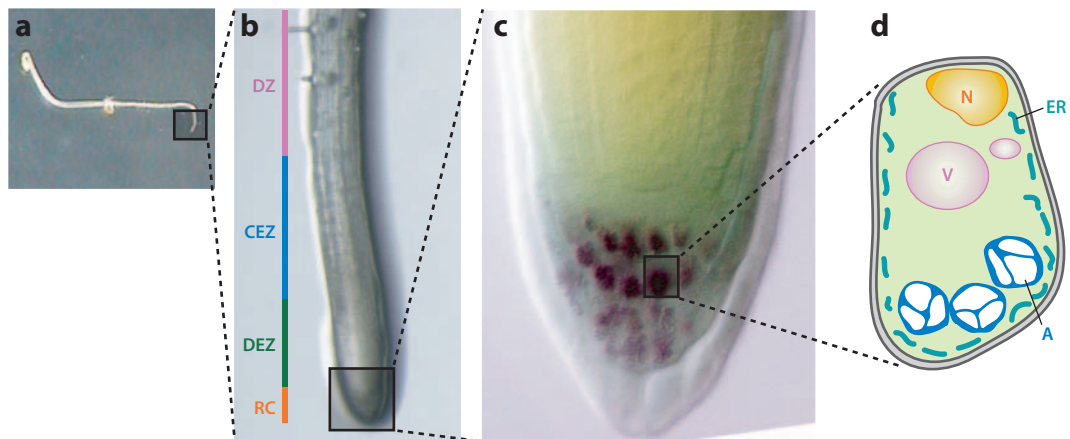


Figure 1

Root structure and root statocyte in *Arabidopsis*. (a) A three-day-old, dark-grown seedling was gravistimulated by placing it in a horizontal position, and leaving it in the dark for one day. Root and hypocotyls show a gravitropic response. (b) Root of the three-day-old seedling dissected from the tip, with the root cap (RC), distal elongation zone (DEZ), central elongation zone (CEZ), and differentiation zone (DZ) visible. (c) Root cap structure. A three-day-old seedling was stained with IKI (iodine–potassium iodide) solution. Amyloplasts are visible in columella cells in the root cap. (d) Schematic structure of the columella cell, showing the nucleus (N), vacuole (V), amyloplast (A), and endoplasmic reticulum (ER). Panel (c) is adapted from Reference 49, with the permission of Elsevier.

GRAS: gene family name based on the locus designations of three genes: GIBBERELLIN-INSENSITIVE (GAI), REPRESSOR of GA1 (RGA), and SCARECROW (SCR)

The existence of a site for gravity perception outside the cap in maize roots has been suggested via the use of a specific device for imaging analysis linked to a rotating stage (50, 98). The device enables the researcher to maintain the root cap in a vertical position while retaining the selected region within the elongation zone at a gravistimulated angle. The maize root continues to show differential growth even under these conditions. Although the gravitropic response elicited by a site outside the cap is estimated to contribute up to 20% of the total response, this study (98) suggested the existence of a second type of gravity perception site in maize roots.

Sensing Site in Shoots

Several graviresponsive organs have been well studied in shoots, such as coleoptiles and pulvini of monocotyledonous plants and hypocotyls of dicotyledonous plants. Tissues containing sedimentable amyloplasts have been commonly observed in these graviresponsive organs, suggesting that such tissues are the sites for gravity sensing (64). However, unlike the root cap, the

surgical removal of the putative statocytes or the treatment of a specific site of an organ is difficult in shoots because in many cases the tissues exist deep within the organs. Thus, researchers were unclear as to which cells act as statocytes in shoots until the genetic approach using *Arabidopsis* was developed.

In addition to the fact that the endodermis contains sedimented amyloplasts in the shoots of a number of plant species, genetic studies have established the role of endodermal cells as statocytes in shoots (**Figure 2**). *Arabidopsis shoot gravitropism* mutants *sgr1* and *sgr7* exhibit no gravitropic response in their hypocotyls and inflorescence stems (18). These mutants are allelic to *scarecrow* (*scr*) and *short-root* (*shr*), respectively (20), which were isolated as mutants defective in the formation of the endodermis both in roots and in hypocotyls (3, 10). *SGR1/SCR* and *SGR7/SHR*, as members of the GRAS gene family, encode putative transcription factors (10, 26). *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 (99). In roots, *SHR* is non-cell-autonomously involved in the expression

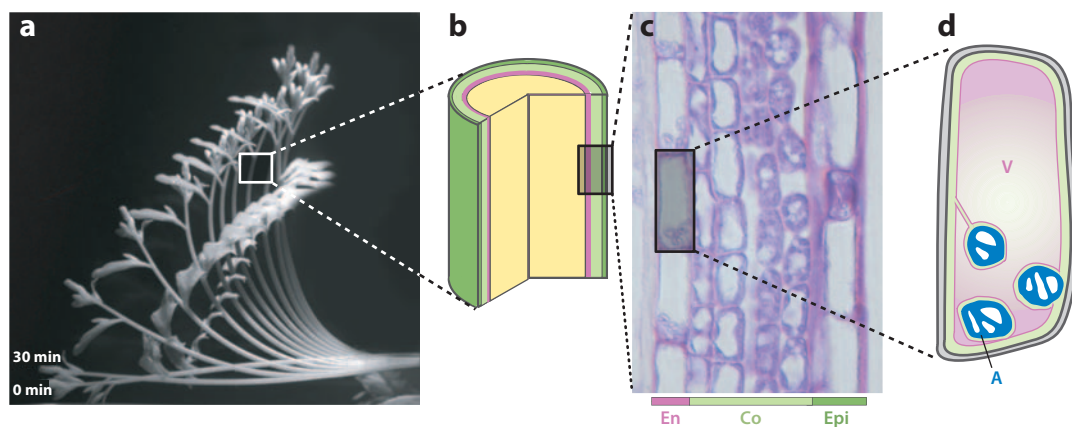


Figure 2

Shoot structure and shoot statocyte in *Arabidopsis*. (a) A five-week-old inflorescence stem (Columbia accession) was gravistimulated by orienting it in a horizontal position. Following 30 min of gravistimulation, photographs were taken every 10 min for 100 min, and a composite was made. (b) Schematic structure of stem tissue. The position of a single layer of endodermis is indicated (pink). (c) Longitudinal section of stem. Sample was stained with toluidine blue and observed by microscopy. The epidermis (epi), cortex (co), and endodermis (en) are visible. In endodermal cells, amyloplasts are sedimented in the direction of gravity. (d) Schematic structure of the endodermal cell, showing the vacuole (V) and amyloplast (A). Panel (c) is adapted from Reference 49, with the permission of Elsevier.

of *SCR* because the expression domains of *SCR* and *SHR* transcripts do not overlap (26, 52). These mutants also lack the endodermis in the inflorescence stems. In *sgr1* hypocotyls, one layer of cells is lost and one of the remaining cell layers is abnormal (i.e., contains a few amyloplasts without sedimentation) (20). Therefore, *SGR1/SCR* and *SGR7/SHR* genes are essential for the formation of the endodermis in both shoots and roots. The fact that *sgr1/scr* and *sgr7/sbr* exhibit almost normal root gravitropism is consistent with the difference in developmental origin between the columella cells, which are the statocytes in roots, and the endodermal cells in shoots. Recent research on shoot gravitropic mutants in Japanese morning glory (*Pharbitis nil*) expands the role of the endodermis for gravitropism beyond *Arabidopsis*. The agravitropic phenotypes of *weeping* (*we*) and *weeping2* (*we2*) are due to mutations in the putative morning glory orthologs of *SCR* and *SHR1* genes, respectively (39, 40). Indeed, both mutants form abnormal endodermal cells (25, 40).

PLASTIDS AS STATOLITHS

Amyloplasts Are Commonly Found in Almost All Gravity-Responding Organs

Because gravity acts on a mass, heavier bodies that sink inside the cell are ideal susceptors (e.g., statoliths) of information about directional change. Starch grain sedimentation in the direction of gravity within specific cells was first observed by Harberlandt and Nemeč independently (22), and similar observations have been reported in almost all graviresponsive organs in various higher plants (43, 64). In *Arabidopsis*, starch is highly accumulated in plastids within statocytes—both columella cells and endodermal cells. Such plastids, known as amyloplasts, are considered to act as statoliths in gravity suspension.

Starch Accumulation Is Not an Essential Feature of a Statolith

The most remarkable characteristic of amyloplasts is the dense accumulation of starch;

however, this characteristic is unlikely to be the sole vital feature of a statolith. An *Arabidopsis* starchless *phosphoglucosyltransferase* (*pgm*) mutant exhibits a reduced but significant gravitropic response in both roots and shoots (6, 36, 37, 95). Amyloplasts are unlikely to sediment to the bottom of cells in the mutant statocytes. The extent of reduction in gravitropism is positively correlated with the reduction in starch content, suggesting that the mass of the amyloplast (i.e., of the starch inside) indeed affects the magnitude of the gravitropic response (38, 64). A series of studies on starchless mutants has indicated that starch is not absolutely essential but does play a role in sensing gravity and is necessary for a full gravitropic response. As discussed in detail by Sack (64, 65), amyloplasts without starch, i.e., plastids, can act as susceptors and trigger a residual gravity response in starchless mutants. Thus, it is not the starch but the plastid itself that is likely to function as a statolith.

Plastids in Statocytes

The definition of an amyloplast is rather ambiguous, but the term generally indicates a plastid that accumulates high quantities of starch. Although amyloplasts are commonly found both in shoots and in roots, amyloplasts in shoot endodermis do not appear to be identical to those in root columella cells. Amyloplasts in root columella cells are derived from proplastids in columella initial cells, which contain large starch granules but do not have an organized thylakoid membrane structure or photosynthetic pigments, thereby representing the orthotypical definition of amyloplasts (64). Similar amyloplasts are also found in dark-grown hypocotyls, although prolamellar bodies are occasionally observed in such hypocotyls (63). Amyloplasts in hypocotyls probably develop their thylakoid membrane under light conditions (6) and produce photosynthetic pigments.

In contrast, amyloplasts in the shoot endodermal cells are likely chloroplasts that are specifically accumulating starch. In fact,

Susceptor: a cellular component whose mass is acted upon directly by gravity, such as amyloplasts

Statolith: A small, movable dense granule found in statocytes that can sediment according to the gravity vector

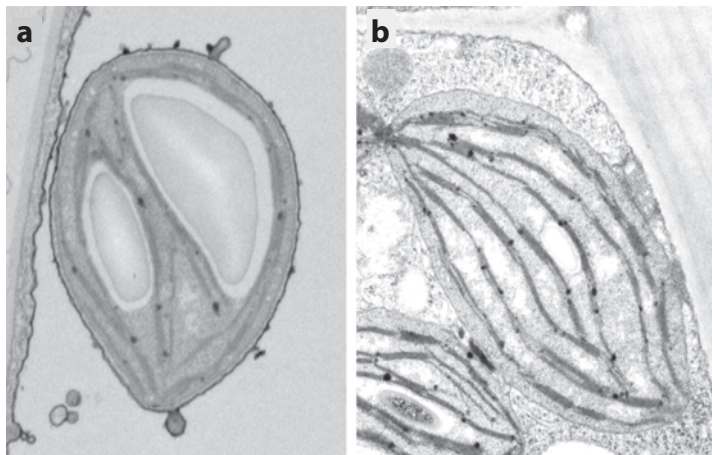


Figure 3
Electron microscopy of plastids in shoot. (a) Wild-type endodermal cell. (b) Wild-type cortical cell. Panels (a) and (b) are reprinted from Reference 101, with the permission of the publisher.

amyloplasts in *Arabidopsis* stems or in cereal grass pulvini contain developed thylakoid membranes (47, 79). Chlorophyll autofluorescence is observed in amyloplasts in endodermal cells as well as in chloroplasts in the neighboring cortical cells in *Arabidopsis* inflorescence stems (48). The chloroplasts in cortical cells occasionally contain small starch granules in their stromal space (Figure 3). The photosynthetic competence of shoot amyloplasts may lead to a functional difference from root amyloplasts, such as production of ATP, generation of reactive oxygen species (ROS), and ionic homeostasis. Such a difference could affect gravity sensing in shoot statocytes. Other prominent differences between plastids of endodermal cells and cortical cells in the *Arabidopsis* stem involve their shape and mobility. Chloroplasts in cortical cells are spindlelike in shape, are almost equally distributed around the cell periphery, and appear to be anchored somewhere, possibly to the plasma membrane, because they hardly move. In contrast, amyloplasts in endodermal cells are round and located mainly at the bottom of the cell, although they sometimes exhibit saltatory movement toward the upper part of the cell.

Presentation time:
the minimum amount of time necessary to evoke a tropic response

AMYLOPLAST MOVEMENTS

Amyloplast Movement Toward the Direction of Gravity

The motility of plastids (specifically, of amyloplasts), which are not anchored to the plasma membrane, seems significant to their function as statoliths. Several studies (e.g., 67, 68) have described amyloplast movement in living statocytes in maize roots and shoots. Further, amyloplast sedimentation occurs toward the new bottom of the cell upon gravitational stimulation within the presentation time (67, 68). More correlative evidence has been provided by unique experiments using high-gradient magnetic field (HGMF), which were adopted to mimic a gravitational field and thereby exploit the differences in diamagnetic susceptibilities between starch and cytoplasm. Amyloplast displacement was induced under the HGMF, which in turn results in organ curvature similar to that of the gravitropic response (42, 44, 96). The fact that HGMF does not affect root gravitropism of starchless mutants indicates that HGMF is unlikely to act on substances other than starch. These studies suggest that amyloplast movement toward the direction of gravity is important and is probably the key event triggering gravity sensing.

“Noisy” Saltatory Movement

The word *statolith*, derived from Greek, means a “stationary stone.” However, the behavior of amyloplasts differs considerably from that of the ideal statolith, particularly in shoot statocytes. Some amyloplasts exhibit saltatory movement; these saltations are directed (though not necessarily toward gravity), non-Brownian movements constituting a simple type of cytoplasmic streaming (66). In *Arabidopsis* endodermal cells, amyloplasts perform F-actin-dependent saltatory movements (70). Saltation-like movements of amyloplasts were also observed in maize root columella cells, but these occur much more infrequently, more slowly, and over shorter distances (69). These

observations suggest that intracellular environments surrounding amyloplasts differ considerably between shoot statocytes and root statocytes.

In endodermal cells, a stationary sedimented state of plastids is unlikely to be necessary for gravity sensing, but directional movement of plastids toward gravity is important for triggering a gravitropic response (70). In contrast, saltatory movement, which is often observed and especially in shoot statocytes, appears as a noisy movement for gravity sensing (66, 70). How can endodermal cells discriminate between gravitational movement and noisy movement? One possible explanation is that sedimenting plastids and saltating plastids may have somewhat distinct molecular features and that only the former plastids are distinguishable by a hypothetical sensor/receptor. To clarify the sensing mechanism of signal-to-noise ratio in plastid movement, further extensive studies will be required. Genetic screening under conditions free from noisy amyloplast movements would be an effective strategy to identify completely unknown sensors/receptors.

CYTOLOGICAL FEATURES OF STATOCYTES

Endoplasmic Reticulum

Columella cells have a specific polarity: The nucleus and endoplasmic reticulum (ER) are localized at the proximal side to the root meristem and the periphery of the cell, respectively. In addition to the peripheral arrangement of ER, its function as an intracellular Ca^{2+} reservoir has been the preferred hypothesis for a possible gravity-sensing mechanism in columella cells; that is, amyloplasts coming into contact with peripheral ER can trigger the release of Ca^{2+} stored in the ER (57). In tobacco root columella cells, a specialized form of ER termed nodal ER, localized at the cell periphery, has been reported (104). Recent research using electron micrographs, with high-pressure freezing and freeze-substitution methods, shows a close contact between amyloplasts and the cortical ER.

Tomographic imaging techniques reveal sheet-like ER membrane deformation at the contact site (46). Endodermal cells in shoots generally have a large central vacuole surrounded by a peripheral layer of cytoplasm (47). Thus, any ER in the cytoplasm would be localized to the cell periphery and possibly in contact with amyloplasts located in the peripheral region. Unfortunately, there is no direct or molecular evidence that supports this model of attraction, e.g., Ca^{2+} release within statocytes in response to gravistimulation, or ER involvement in gravity sensing.

Actin Microfilaments

F-actin has been thought to play a major role in gravity perception. In several models of gravity perception, sedimenting amyloplasts were hypothesized to distort the F-actin network prompting a signal conversion; e.g., tension produced by F-actin could activate mechanosensitive channels on a certain membrane (57, 74). Consistent with this hypothesis, a network of fine, randomly oriented actin filaments surrounds the amyloplasts in root columella cells (9). As such, the disruption of F-actin should inhibit gravity perception and lead to a reduction in the gravitropic response. However, a number of recent studies using drugs to disrupt actin filaments have provided rather controversial results (81, 91). In fact, organ curvature, sensitivity to gravity, and amyloplast sedimentation in maize and *Arabidopsis* roots were not inhibited, but instead enhanced, by treatment with the F-actin disrupting drug Latrunculin B (Lat-B) (29, 30). However, it should be noted that drug treatment affects the entire plant, and we cannot attribute the effect of the drug to statocytes alone. Statocyte-specific manipulation of the actin cytoskeleton will be required for precise understanding of the role of F-actin in gravity perception.

Extensive analyses of an *Arabidopsis* mutant, *altered response to gravity 1* (*arg1*), with a reduced gravitropic response in hypocotyls and roots imply that interaction between plastids and actin cytoskeleton affects early steps of

Sensor/receptor: a cellular component that receives information of susceptor movement and converts the information into a physiological intracellular signal

Lat-B: Latrunculin B, a drug used to inhibit actin microfilament formation

gravity sensing (5, 19, 21, 23, 71, 80). ARG1, a J-domain protein localized to endomembrane organelles, together with its paralog ARL2, acts in the root statocytes and the endodermis of hypocotyls to facilitate gravitropism (5, 21, 71). In the *arg1* mutant, early reactions such as the changes in cytosolic pH and PIN3 relocalization (see below) that occur in response to gravistimulation in columella cells are rarely observed. Notably, ARG1 interacts with the cytoskeleton, most probably with actin (5). Recently, two enhancers of *arg1*, *mar* (*modifier of arg*) 1 and *mar2* have been reported (80). Both *MAR1* and *MAR2* encode different components of the Translocon of Outer Membrane of Chloroplasts (TOC) complex (80). Although no obvious gravitropic phenotype is observed in *mar2* single mutants, *mar2 agr1* shows little root gravitropism. A recent biochemical attempt to identify chloroplast envelope proteins involved in actin binding in pea revealed that one TOC component, a paralog of MAR2, can bind to actin (32), though its biological significance is not known. This finding supports the functional interaction between plastids and actin cytoskeleton, possibly via functions of TOC and ARG1. Although ARG1 is thought to play a role in the gravity-signaling process rather than in the gravity-sensing process, it seems intriguing to investigate the functional significance of the interaction between ARG1 and the actin cytoskeleton in root gravitropism.

In contrast to the fine mesh of F-actin in root collumela cells, thick actin bundles have been observed in endodermal cells of *Arabidopsis* (70, 100). Lat-B treatment also promotes shoot gravitropism of *Arabidopsis* despite the disruption of actin filaments in the endodermal cells (56, 100). Interestingly, amyloplast sedimentation in *Arabidopsis* endodermal cells appears to be enhanced by Lat-B treatment, because the saltatory movement of amyloplasts is greatly reduced and all amyloplasts settle to the bottom of the cell. These amyloplasts are still able to respond and relocate upon gravistimulation in the presence of Lat B (70).

A new genetic study (M. Nakamura, M. Tasaka, M. T. Morita, unpublished data)

indicates that amyloplasts' saltatory movement and sedimentation can be uncoupled in shoots. A novel mutant with reduced gravitropism in inflorescence stems was recently isolated. The endodermis of the mutant inflorescence stem possesses amyloplasts, which despite undergoing dynamic organelle movement, fail to sediment in response to the vector of gravity. However, the gravitropic response of the mutant is almost completely recovered to the level of the wild type with Lat-B treatment. Simultaneously, amyloplast sedimentation in the endodermal cells of the mutant is also recovered by Lat-B treatment. These results suggest that F-actin may act negatively on amyloplast sedimentation toward gravity, at least in shoot statocytes. The gene responsible for the novel mutant could be involved in releasing the amyloplasts from the negative effect of F-actin on sedimentation in the direction of gravity. The corresponding gene product may be the key regulator of amyloplast dynamics.

F-actin also plays a role in nonamyloplast plastid movement. Movement of chloroplasts in response to a directional light signal (photorelocation movement) is dependent on F-actin (33, 54, 93). However, the manner in which F-actin acts appears to be completely different in plastid movements in the gravity response as opposed to those in photorelocation, despite the similar context of the control of plastid movements based on environmental cues. In shoot statocytes, the release of amyloplasts from the effect of F-actin could be important to gravity susception.

Vacuole Dynamics

Are amyloplasts the only organelle important for gravity perception? Genetic screening for *Arabidopsis* mutants with little or reduced shoot gravitropism (i.e., *sgr* mutants) brought the vacuole to the forefront as an additional organelle important for gravity perception. Abnormal amyloplast localization within endodermal cells is a common cellular defect observed among *sgr* (*shoot gravitropism*) 2, *sgr3*, *zig* (*zigzag*)/*sgr4*, and *grv* (*gravitropism*

defective) *2/sgr8/kam* (*katamari*) 2 mutants (35, 47, 75, 101). Molecular cloning of *SGR2*, *SGR3*, *ZIG/SGR4*, and *GRV2/SGR8/KAM2* indicates that vacuolar functions supported by membrane trafficking in the endodermal cell are implicated in amyloplast behavior (47, 75, 101). Qb-SNARE VTI11 is encoded by *ZIG/SGR4* localized to the trans-Golgi network (TGN), prevacuolar compartment (PVC), and vacuole; Qa-SNARE syntaxin VAM3/SYP22 is encoded by *SGR3* localized to the PVC and vacuole. These two proteins form a SNARE complex and probably play roles in TGN-PVC/vacuole vesicular trafficking. *SGR2* encodes a phosphatidic acid–preferring phospholipase A1-like protein that localizes to the vacuolar membrane and to small, unidentified compartments. Although the role of *SGR2* in membrane trafficking is not known, it is suggested that *SGR2* orthologs in animal cells are involved in membrane trafficking (53, 73, 84). *GRV2/SGR8/KAM2* is a homolog of RME-8 suggested to be involved in endocytosis in *Caenorhabditis elegans*, *Drosophila*, and human cells (7, 17, 103). Recently, *grv2/sgr8/kam2* was shown to have a defect in protein sorting to the protein storage vacuole (PSV) that results in secretion of storage proteins to the intercellular space in mutant seeds (16). Endodermis-specific expression of *SGR2*, *ZIG*, or *SGR3* using the *SGR1/SCR* promoter can restore shoot gravitropism in the corresponding mutants. These results indicate that the functions of the proteins encoded by these genes in the endodermis are sufficient to trigger gravitropism, although the genes are expressed universally and are probably required for general membrane trafficking.

How does vacuolar function or membrane trafficking affect amyloplast sedimentation? Shoot statocytes are mostly filled by a large central vacuole (8, 66). Amyloplasts in *Arabidopsis* endodermal cells are almost completely enclosed by vacuolar membrane, with only a thin layer of cytoplasm (70). Most amyloplasts are sedimented in the direction of gravity with slight movement, and some amyloplasts with saltatory movements can move through

transvacuolar strands or narrow cytoplasmic spaces (70). In contrast, in *sgr2*, *sgr3*, *zig/sgr4*, and *grv2/sgr8/kam2* mutants, amyloplasts do not sediment in the direction of gravity and appear to stay at the cell periphery with the cytoplasm (47, 75, 101). In addition, amyloplasts in *zig/sgr4* mutants hardly move around the cell at all and never demonstrate saltatory movements. Although a large central vacuole was observed to form in the mutants, the membrane structure lost its ruggedness and transvacuolar strands were rarely observed (70; M. T. Morita and M. Tasaka, unpublished data). Because loss of amyloplast dynamics and loss of flexible vacuolar membrane structure were simultaneously observed in these four independent *sgr* mutants, the flexible structure of the space-filling vacuolar membrane likely affects amyloplast dynamics indirectly. The loss of transvacuolar strands, the formation of which is largely dependent on F-actin (27, 72), in these mutants could result from a deficiency in the interaction between the vacuolar membrane and F-actin. This deficiency could lead to a phenotype of immobile amyloplasts in the mutants. The possibility that vacuolar functions, such as ion homeostasis, may influence gravity sensing or signaling along with affecting amyloplast dynamics cannot be excluded. In any case, maintaining dynamic vacuolar structure or vacuolar function would be an important requirement for transforming an endodermal cell into a statocyte.

In contrast to shoot endodermal cells, root columella cells that contribute to gravity sensing have small vacuoles and abundant cytoplasm. Consistently, *sgr2*, *zig/sgr4*, *sgr3*, and *sgr8/grv2/kam2* mutants have normal root gravitropism. This result further reinforces the idea that the intracellular environment surrounding amyloplasts is different between shoot and root statocytes.

ARRANGEMENT OF STATOCYTES IN ORGANS AND SIGNAL TRANSMISSION

Once a directional cue is perceived by statocytes, the directional signal is converted into a

SNARE: soluble N-ethylmaleimide fusion protein attachment protein (SNAP) receptor

biochemical signal within the statocytes. Subsequently, the signal is transmitted intercellularly, and asymmetric auxin distribution follows (49, 85).

Roots and shoots exhibit a remarkable difference in their spatial relationship between statocytes and the elongation zone, where the actual differential growth occurs in response to gravity signal transmission. Columella cells form conical tissue at the root tip, whereas the endodermal cells form a single layer of cylindrical tissue (**Figures 1** and **2**). In roots, the elongation zones are located at the basal sides of the root meristem, detached from the root cap columella cells. In shoots, the endodermal cell layer is distributed over the whole of the elongation zone (**Figure 2**). As a consequence of this arrangement, the direction of signal transmission in the organ would also be different between roots and shoots. The directional signal sensed in the columella cells is transmitted to the distant elongation zone in roots. Auxin transport is involved in the apical-to-basal signal transmission in roots, probably via the lateral root cap and the epidermis (14, 55, 82, 83). In shoots, the gravity signal perceived in the endodermal cell layer is possibly transmitted laterally in an inner-to-outer fashion to the cortex or epidermal cell layers, which may undergo differential cell elongation (85). Although no substantial signal(s) is yet known that is transmitted in an inner-to-outer fashion, a number of studies suggest that auxin may also play a role in shoot gravitropism (12, 51, 86, 87, 94).

Based on the fountain model of auxin flow at the root tip, auxin transported from the shoot to the root tip is redistributed laterally and then transported back to the elongation zone (97). When we focus on the relative directional relationship of the statocyte and auxin flow, auxin is commonly transmitted in a lateral direction in the statocyte of roots and shoots. Thus, a directional signal sensed in the statocyte may be translated as a directional regulation of auxin flow upon gravistimulation. A member of the auxin transport facilitator PIN family, PIN3, is an ideal candidate as a regula-

tor of lateral auxin flow upon gravistimulation (15). In addition to expression of PIN3 in columella cells and in endodermis, PIN3 changes its intracellular distribution laterally within columella cells in response to gravistimulation (15). There are still several ambiguous points to resolve, e.g., the minor genetic contribution of PIN3 and the experimental reproducibility of PIN3 relocalization (23). PIN3's contribution to shoot gravitropism and changes in protein distribution in shoots is as yet unknown. However, determining the regulatory mechanism of PIN distribution within statocytes may tell us more about the role of signal transduction in gravitropism.

FUTURE DIRECTIONS

There are numerous issues to be addressed in order to understand the molecular mechanisms behind gravity sensing. These can be divided into two categories: issues common to all gravitropic organs, and issues specific to each.

Two important open questions have been answered for those issues common to all organs: (1) how statocytes sense the directional movement of plastids toward gravity (i.e., identification of the suceptor/receptor), and (2) how statocytes transmit gravity signals laterally to neighboring cells. To address these questions, real-time imaging provides a powerful strategy to disclose the actual spatiotemporal processes *in situ*. More important, substantial evidence at the molecular level will be required to explain the phenomena that take place in statocytes. Evaluation of whether changes detected in statocytes are actually linked to gravitropic response is also significant. To overcome these challenges, molecular markers to monitor the early signaling process within statocytes will be required for further analysis using single-cell imaging. For example, pH can serve as a marker for root gravitropism if the functional linkage to gravitropism at the molecular level is clarified. To find new molecular markers, a molecular genetics approach may be suitable because it can link function and structure at the molecular level with causality.

At present it is unclear whether factors involved in processes commonly accepted as important to gravitropism are identical in all organs or not. Such factors might differ between roots and shoots yet belong to the same gene family. They might instead be totally different proteins that share a similar molecular function. To elucidate the molecular mechanism of gravity sensing, experimental approaches must be adapted to capitalize on the advantages of each organ as a study material. For instance, root is the most suitable material for noninvasive, live-cell imaging. On the other hand, environmental signals other than gravity, e.g., touch or humidity, can be easily excluded in shoots. As another example, since columella formation and differentiation are regulated by auxin, which is an important signaling molecule of gravitropism, and auxin flow is in turn affected by columella integrity, the inseparable relationship might make it difficult to interpret experimental results. Such disadvantages may be reduced by using shoots. In any case, combined experimental results from studies using roots

and shoots may provide clues to address issues common to both.

With respect to rather specific characteristics in each organ, such as cytological features, an intriguing question about the biological significance of such differences arise. F-actin appears to act negatively against gravitropism both in roots and in shoots. However, active saltatory movement, which is dependent on F-actin triggering inhibition of amyloplast sedimentation, is prominently observed in shoot statocytes. This negative effect of F-actin could be a kind of damper that sets a threshold for gravity sensing. In contrast to roots in the soil, shoots (especially inflorescence stems) are easily exposed to acute environmental agitation by wind, rain, animals, etc. F-actin could prevent amyloplasts from agitation that could cause unnecessary gravitropic response. By enclosing amyloplasts, vacuoles in the epidermis might also act as a damper. Possibly such organ-specific cytological features may have evolved as modulators that adapt the characteristics of each organ for gravity sensing.

SUMMARY POINTS

1. Although the starch-statolith hypothesis was proposed more than 100 years ago, genetic studies demonstrate that starch is not essential for gravity sensing.
2. Plastids with motility—but not necessarily amyloplasts that accumulate starch—have an important role as statoliths.
3. Although there are several differences in cytological features between shoot and root statocytes, plastid movement toward the direction of gravity is the common trigger of gravitropic response.
4. Shoot statocytes may discriminate between plastid movement toward the direction of gravity and noisy, random plastid movement that is probably dependent on F-actin, via an unknown mechanism.
5. Once statocytes perceive a directional cue, they transmit signal molecules (i.e., auxin) laterally from cell to cell.

FUTURE ISSUES

1. To understand gravity perception, it is crucial to investigate the regulatory and/or modulation mechanisms of amyloplast dynamics.

2. Clarification of the cytological biochemical events that commonly take place within the statocytes of roots and shoots is critical to our understanding of the gravity sensing mechanism in plants.
3. Live-cell imaging combined with molecular genetic strategies can link cytological events to underlying molecular mechanisms and provide valuable information about gravitropism in future.

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Errata

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