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The Kinetics of Root Gravitropism: Dual Motors and Sensors

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ABSTRACT

The Cholodny-Went theory of tropisms has served as a framework for investigation of root gravitropism for nearly three quarters of a century. Recent investigations using modern techniques have generated findings consistent with the classical theory, including confirmation of asymmetrical distribution of polar auxin transport carriers, molecular evidence for auxin asymmetry following gravistimulation, and generation of auxin response mutants with predictable lesions in gravitropism. Other results indicate that the classical model is inadequate to account for key features of root gravitropism. Initiation of curvature, for example, occurs outside the region of most rapid elongation and is driven by differential acceleration rather than differential inhibition of elongation. The evidence indicates that there are two motors driving root gravitropism, one of which appears not to be auxin regulated. We have recently developed technology that is capable of maintaining a constant angle of gravistimulation at any selected target region of a root while continuously monitoring growth and curvature kinetics. This review elaborates on the advantages of this new technology for analyzing gravitropism and describes applications of the technology that reveal (1) the existence of at least two phases to gravitropic motor output, even under conditions of constant stimulus input and (2) the existence of gravity sensing outside of the root cap. We propose a revised model of root gravitropism including dual sensors and dual motors interacting to accomplish root gravitropism, with only one of the systems linked to the classical Cholodny-Went theory.

Key words: Auxin; Central elongation zone; Distal elongation zone; Electrotropism; Gravitropism; Gravity; Gravity sensing; Roots

INTRODUCTION

For the past 75 years the Cholodny-Went theory has been the predominant paradigm guiding investigations into the nature of gravitropism in plants (Cholodny 1926; Went and Thimann 1937). As applied to roots, the theory holds that downward curvature following gravistimulation is caused by accumulation of the growth hormone, auxin, to inhibitory levels along the lower side of the elongation zone. This results in suppression of elongation along the lower side of the root and leads to downward curvature. The Cholodny-Went theory was formulated at a time prior to the discovery of other classes of plant hormones, prior to the development of sophisticated methods for probing gravitropism at the molecular level, and prior to the capability of computer assisted measurement of precise differential growth patterns and kinetics during gravitropic curvature. As such, it is not surprising that the Cholodny-Went theory is now

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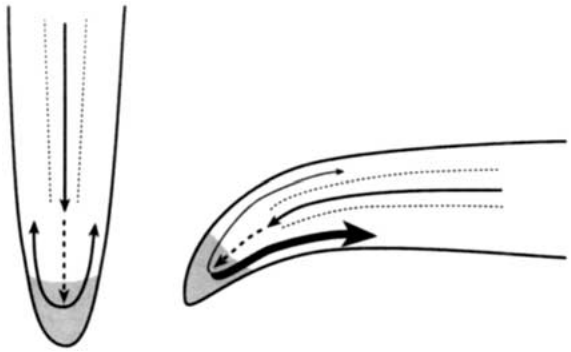

Figure 1. The fountain model of auxin-regulated root gravitropism. Left: In vertical roots, auxin moves toward the root tip through the stele eventually entering the root cap (shaded). Auxin accumulating in the central portion of the root cap is loaded symmetrically into basipetally moving transport streams that direct the hormone back into the elongation zone through the cortex. Right: In gravistimulated roots the flow of auxin from the cap toward the elongation zone becomes asymmetrical with more auxin moving into the elongation zone along the lower side.

considered inadequate to explain the many complexities of gravitropism (see Firn and others 2000; Trewavas 1992). The surprising thing, perhaps, is that, even after 75 years of research, the theory is still highly visible, an indication that, even though the theory may be inadequate to account for the many complex features of gravitropism, it may account for a core portion of the response.

The ''fountain model'' of auxin-mediated root gravitropism (Figure 1) (Evans and others 1986; Trewavas 1981) provides a more specific application of the Cholodny-Went theory to root gravitropism. The model proposes that auxin moves toward the tip of the root within the stele and that the root cap functions to redirect auxin arriving at the tip into symmetrical polar transport streams moving back toward the elongation zone through the cortex. Gravistimulation is thought to induce an asymmetry in the basipetally moving auxin transport stream so that more auxin moves into the elongation zone along the lower side. Much of the recent research on gravitropism is compatible with the basic tenets of the Cholodny-Went theory and with the more specific aspects of the fountain model. This includes the finding of asymmetric distribution of auxin transport carriers (Estelle 1998; Gälweiler and others 1998; Müller and others 1998) and the molecular evidence for auxin redistribution following gravistimulation (Friml and others, 2002; Li and others 1999).

As we have continued to examine root gravitropism in greater detail, however, it has become increasingly clear that the response kinetics are too complex to be accounted for by a simple transient suppression of elongation along the lower side of the region of maximum elongation (Ishikawa and others 1991). This review focuses on recent advances in methodology that have led to a more thorough understanding of the kinetics of root gravitropic curvature and how classical ideas on the nature of gravitropic responses can be integrated into newly developing models. Advances in the technology of stimulus application and growth measurement along with advances in understanding the molecular biology of tropisms are ushering in a new era of gravitropism research, one that is revealing the complexity of the response and promising to provide answers to long-standing questions regarding mechanisms.

The Dual Motors of Root Gravitropism

The Cholodny-Went theory of root gravitropism predicts that curvature should be driven primarily by suppression of elongation along the lower side. It is logical that the greatest growth asymmetry would therefore be obtained by affecting growth in the region of most rapid elongation, within the central elongation zone. However, curvature initiates in a region of the root apical to the zone of maximum elongation. This was shown by Iversen (1973) for cress, by Barlow and Rathfelder (1985) for maize, and by Zieschang and Sievers (1991) for Phleum. In all of these studies, curvature initiation near the root apex was accompanied by stimulation of elongation along the upper side of the root in addition to suppression along the lower side.

In a computer-based automated analysis of maize root gravitropism, Ishikawa and colleagues (Ishikawa and others 1991; Ishikawa and Evans 1993, 1997) showed that gravitropic curvature is initiated in a group of cells between the meristern and the region of most rapid elongation and that a key feature of the response is acceleration of elongation along the upper side of the root. They referred to this region of the root as the distal elongation zone (DEZ) and arbitrarily defined its basal limit as the point at which the relative elongation rate reaches 0.3 that of the peak rate in the central elongation zone (CEZ). In the case of 3-day-old primary roots of maize growing in humid air, the DEZ extends back about 3.4 mm from the tip of the root cap while the peak elongation rate within the CEZ is located at about 5 mm. In the case of roots of *Arabidopsis*, the basal limit of the DEZ is

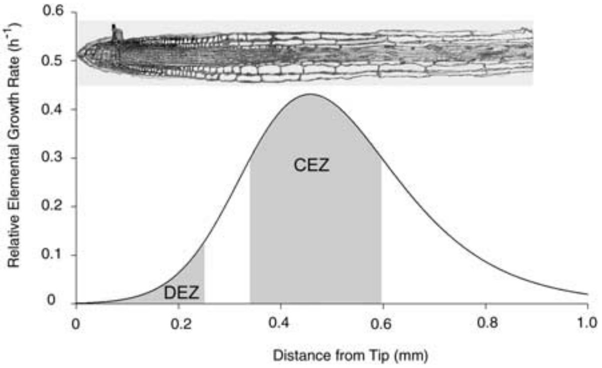

Figure 2. Relative elemental growth rate (REGR) profile for a vertically growing *Arabidopsis* root. The basal limit of the DEZ is defined as that point in the apical region of the elongation zone where the REGR is 30% of the maximum REGR. The inset is a longitudinal section of the root and is scaled to the graph (from Mullen and others 1998).

about 250 μm from the extreme root tip (Figure 2). The cells in the DEZ appear to differ strikingly from cells in the CEZ with regard to their physiological properties (Baluŝka and others 1994; Ishikawa and Evans 1995). For example, while applied auxin inhibits the elongation of cells in the CEZ, it promotes the elongation of cells within the DEZ (Ishikawa and Evans 1993). Although it cannot be ruled out that enhancement of cell elongation in the DEZ by applied auxin is a secondary response triggered by suppression of elongation in the CEZ, it is clear that auxin does not inhibit elongation in the DEZ. We must therefore conclude that the Cholodny-Went theory of gravitropic curvature does not apply to the DEZ component of gravitropic curvature. In fact there are reports that exposure of roots (or even shoots, Rice and Lomax 2000) to high levels of auxin does not prevent gravitropism (Ishikawa and Evans 1993; Katekar and Geissler 1992; Muday and Haworth 1994), a result not unexpected if at least one component of the response is auxin-independent. On the other hand, numerous studies show that there is curvature generation within the CEZ during root gravitropism and that a key feature of the response in the CEZ is suppression of elongation along the lower side (Ishikawa and Evans 1993), Therefore, the CEZ component of gravitropic curvature has properties quite compatible with the fountain model of gravitropism.

Based on these observations, we propose that there are dual motors for root gravitropism, a motor in the DEZ that is not controlled by auxin asymmetry and is responsible for the initiation of curvature, and an auxin-regulated motor in the CEZ that contributes to overall curvature once the response is under way. Consistent with this model, Zieschang and Sievers (1991) identified two zones of curvature during the response of Phleum roots to gravity, one near the root apex and one farther from the root tip. Although the molecular nature of the two motors and their control systems remains unknown, it appears that sensory input to the two systems can be differentiated. We have shown, for example, that the relative contribution of the DEZ motor to the gravity response increases as the angle of gravistimulation increases (Ishikawa and Evans 1997; Mullen and others 2000). The evidence that the DEZ motor may not be auxin controlled raises the question of the nature of its control. As described below, there is evidence that electrical signaling may play a key role.

A ROLE FOR ELECTRICAL SIGNALING IN CONTROL OF THE DEZ MOTOR

There are electrical currents along the root surface resulting from the activities of ion transporters (Shrank 1959; Weisenseel and others 1979, 1992). These currents form loops in vertically growing roots (Collings and others 1992; Iwabuchi and others 1989; Weisenseel and others 1979), leaving the CEZ and root cap and entering the meristem and DEZ. Upon gravistimulation, the current patterns change. For example, the current flow along the upper flank of the DEZ reverses, resulting in current efflux from this region (Collings and others 1992; Iwabuchi and others 1989). This is accompanied by rapid (less than one min) hyperpolarization of cells in the cortex along the upper side of the DEZ with depolarization along the lower side (Ishikawa and Evans 1990a). The correlation between changes in electrical patterns and alterations in growth patterns during gravitropism raises the possibility that electrical signaling may play a role in establishing the growth asymmetry that drives curvature in the DEZ.

In support of this idea, it is known that applied electrical fields can cause directional growth responses in roots (electrotropism) (Ishikawa and Evans 1990b; Schrank 1959; Stenz and Weisenseel 1991). In a detailed study of electrotropism in roots of *Vigna mungo,* Wolverton and others (2000) found that applied DC electric fields (5 V cm^{-1}) induced curvature toward the cathode (−) in the region of the DEZ with curvature resulting from stimulation of elongation along the region of the DEZ opposite the cathode. The observed growth pattern in the DEZ during electrotropism was qualitatively similar to that of gravistimulated roots, that is, enhanced

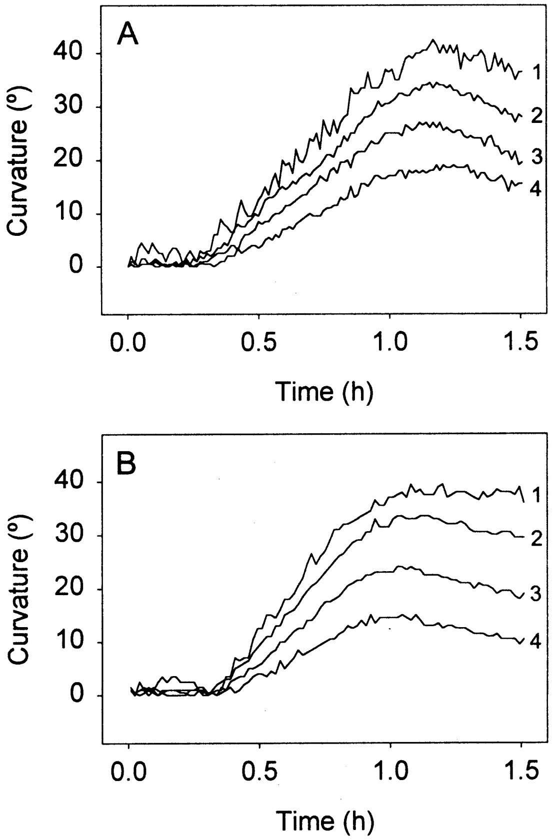

Figure 3. Comparative curvature pattern within the DEZ of roots responding to (A) gravity or (B) electrotropic stimulation. Each curve represents time-dependent change in angle of one of a series of segments of the root. Each segment remains a fixed distance from the root tip (that is, is defined by distance from the root tip). (A) Kinetics of curvature of a root of *Zea mays* in response to gravistimulation. Root reoriented from vertical to 90° at zero time. Root segments (distance from tip of cap): 1 = 0–1 mm, 2 = 1.5–2.5 mm, 3 = 2.5–3.5 mm, 4 = 3.5–4.5 mm. (B) Kinetics of curvature of a root of *Vigna mungo* in response to a localized electric field (5 V cm^{-1}) applied to the DEZ. The field was applied at 0 h and removed at 0.5 h. Root segments (distance from tip of cap): 1 = 0–1 mm, 2 = 0.5–1.5 mm, 3 = 1–2 mm, 4 = 2.5–3.5 mm. The sets of segments represent anatomically comparable regions of the apical portion of the elongation zone in these two species.

elongation on the convex side of the responding root (Figure 3).

Additional evidence that electrical signaling may regulate the gravitropic response of the DEZ is provided by the recent finding that the anion channel blocker 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) inhibits gravitropic curvature in a dose-dependent manner without affecting elongation rate (Wolverton and others, unpublished data). Although gravitropic curvature in NPPB-treated roots was initiated in the DEZ just as in control roots, the rate of curvature was greatly retarded in the treated roots.

A New Tool for Investigating Gravitropism

The kinetics of gravitropic curvature are complex, with the rate of curvature slowing or even reversing in the later stages of the response (Ishikawa and others 1991; Selker and Sievers 1987). During the measurement of curvature kinetics in standard experiments, it is difficult to separate the effects of diminishing stimulus strength from effects related to the inherent properties of the response system. To overcome this difficulty, Mullen and others (2000) devised a feedback system linking video digitizer analysis of curvature with a mechanical stage capable of rotating seedlings in tiny steps. This device (dubbed "ROTATO") provides a number of new capabilities for gravitropism research (Table 1). For example, the controlled smooth rotation provided by ROTATO can be used for gentle, repeatable application of a gravitropic stimulus. Mullen and others (2000) reported that the latent period of the gravitropic response of *Arabidopsis* roots when stimulated gently using ROTATO was approximately 10 min as compared with 20 min when the roots were stimulated by the standard method (abrupt rotation by hand). They speculated that the slower onset of curvature following stimulation by the standard method might be due to mechanical perturbation associated with the standard method of stimulation. If so, use of the standard stimulation method may confound investigations of early gravitropic events.

A second important capability introduced by ROTATO is the ability to hold a targeted region of a root or other organ at a constant angle with respect to gravity throughout a gravitropic response (Table 1, Figure 4). In standard gravitropism experiments the strength of the gravity stimulus declines as curvature occurs. Consequently, during investigations of curvature kinetics, it is difficult to separate effects of declining stimulus strength from true changes in motor output. Attempts to circumvent this difficulty have included rotating specimens on two-dimensional or three-dimensional clinostats following an initial gravistimulus so that the gravitropic vector is randomized during the ensuing gravitropic response (Hoson and others 1997; Kiss and others 1989; Salisbury 1993). However, there is evidence of undesirable side effects from such treatment, including an increase in ethylene pro-

Table 1. Comparison of Alternative Methods of Cavistimulation.

	Method		
Variable	Traditional	Clinostat	ROTATO
g vector	Varies	Randomized	Constant
Nature of stimulation	Abrupt	Hyperstimulation	Smooth, gentle
Kinetics of stimulation	Immediate	NA	Slow (e.g., 60° min^{-1})
Interaction of multiple stimuli	Competing	ambiguous	Controlled

The traditional method involves abrupt rotation of the organ from vertical to a particular stimulation angle (for example, 90°). The clinostat method involves continuous rotation of the specimen on single (2-dimensional clinostat) or dual axes (3-dimensional clinostat). The ROTATO method uses a motorized feedback system to gently rotate the organ from vertical to a particular, stimulation angle (for example, 90°) and subsequently maintains a selected target region (for instance, root tip) at the prescribed angle.

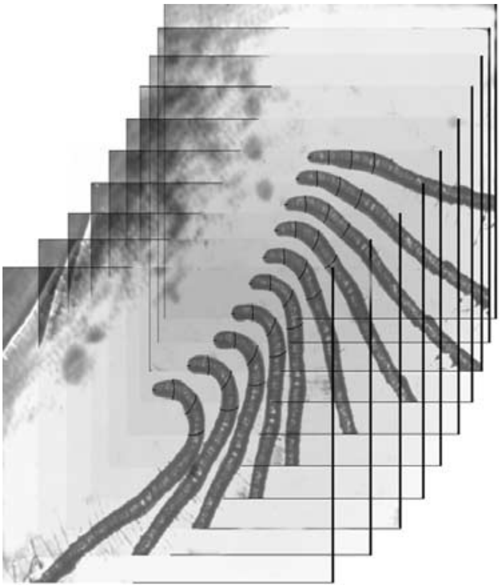

Figure 4. Gravitropic response of an *Arabidopsis* root on ROTATO. The images represent a time-lapse sequence of an *Arabidopsis* root undergoing gravitropism while the tip-most region of the root is maintained at 90° by the ROTATO system. As the root curves downward, the stage automatically rotates clockwise to maintain the tip at 90°. Total time elapsed from the beginning of gravistimulation is approximately 4 h.

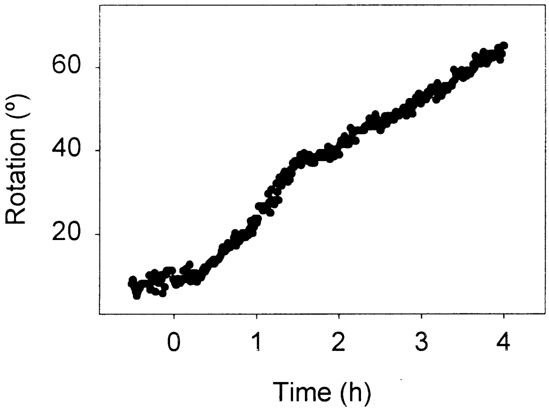

Figure 5. Kinetics of the gravitropic response of an *Arabidopsis* root. The root tip was constrained at 20° (relative to vertical) at 0 h. The measured rotation was the rotation of the vertical stage necessary to keep the root tip at 20° (see Figure 3).

duction and/or a destruction of cellular polarity (Hensel and Iversen 1980; Hensel and Sievers 1980; Hoson and others 1997). Also, the gravity stimulus is not maintained at a constant vectorial value in clinostat experiments, it is simply randomized (Table 1). ROTATO has the advantage that it can maintain a constant gravity stimulus to a targeted region over a prolonged period while measuring the detailed kinetics of curvature.

A third advantage of ROTATO is that it is capable of controlling the gravity stimulus vector while measuring the response of seedlings to other environmental stimuli such as light (Table 1). During phototropism experiments, there is interaction between responses to light and to gravity. In standard phototropism experiments with roots (Okada and Shimura 1992; Ruppel and others 2001; Vitha and others 2000), for example, displacement of the root tip from vertical during the phototropic response induces a counteracting gravitropic response. This makes it difficult to separate effects of phototropic and gravitropic stimulation. ROTATO resolves this problem by maintaining the root tip in a constant vertical orientation throughout a phototropic response, thus eliminating gravitropic stimulation and maintaining constant phototropic stimulation. The phototropic response is then quantified as the rotational activity required to maintain the root tip vertical in the face of phototropic curvature. This capability of ROTATO should prove particularly useful in designing ground-based controls for space flight experiments where it is desirable to eliminate lateral gravistimulation.

ROOT GRAVITROPISM RESPONSE KINETICS USING ROTATO

We have used the ROTATO technology to investigate the kinetics of gravitropic curvature under conditions of constant gravistimulation of the root tip of *Arabidopsis* (Figures 4 and 5). With constant stimulus input, the gravitropic curvature response continues long-term, that is, the sensing/response mechanism appears not to adapt strongly. However, there is evidence for some complexity in the output. It appears that there are distinct phases of curvature even under conditions of constant stimulation (Figure 5), with an initial rapid phase of curvature followed by a slower phase. For roots of *Arabidopsis*, the two phases are more evident during responses to smaller angles of gravistimulation. In the case of maize roots, the two phases are distinct for both small and large angles of stimulation (Wolverton and others, unpublished data). The occurrence of two phases of curvature in roots of these two species, one a monocotyledonous species and one a dicotyledonous species, suggests the possibility that the biphasic response kinetics may be a general feature of root gravitropism. An early phase of rapid curvature followed by a prolonged phase of slower curvature was shown for roots of cress that were rotated on a clinostat following gravistimulation (Iversen 1973) and for roots of lentil that were maintained under microgravity following gravistimulation at 1g (Perbal and Driss-Ecole 1994). In these cases, of course, the stimulus input was not maintained at a constant value. Instead, the stimulus was either canceled through randomization (Iversen 1973) or removed by ceasing centrifugation while in microgravity (Perbal and Driss-Ecole 1994).

Prior to the development of ROTATO, it was not possible to identify the phases of the gravitropic motor response under conditions of constant stimulation. By exploiting the capabilities of this new technology, we should be able to go beyond simply identifying the phases of the motor response and begin to address their specific properties. Do mutations leading to reduced root gravitropism specifically affect one of the phases of the motor response? If so, what can we learn about the nature of that phase from the nature of the mutants that affect it? Are the phases of the motor response interconnected or are they independent? Does adaptation to the gravity stimulus or to an effector such as auxin (Evans 1991) contribute to the appearance of the two phases? Are the phases of the motor response controlled by a single sensor or by separate sensors? Do the two phases show the same or different dose-response properties with respect to angle of gravistimulation? These kinds of questions will be addressed in future experiments using ROTATO.

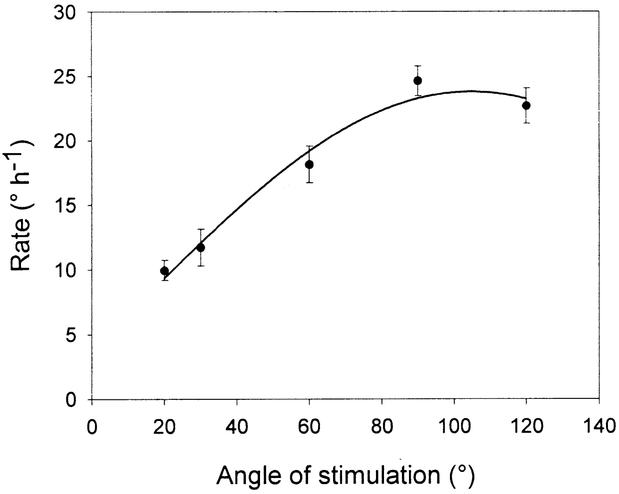

Figure 6. Dependence of the rate of root curvature on stimulation angle. ROTATO was used to hold the root tip at the indicated angle and the rate of curvature was measured from the rate of rotation required to maintain the selected angle. The data represent the average rate of curvature as determined by linear regression during the early (Phase 1) steady rate of curvature. Error bars indicate SE (n = 10–12). The curve is a sinusoidal function fitted to the data by nonlinear regression. (From Mullen and others 2000).

RE-NVESTIGATING DOSE RESPONSE RELATIONSHIPS

The ability of ROTATO to maintain the root cap at a constant angle with respect to gravity throughout a gravitropic response has been exploited to reexamine the dose response relationship of root gravitropism. The advantage to investigating dose response relationships using ROTATO, of course, is that stimulus strength remains unchanged, allowing measurement of response output without the complication of changing stimulus input as the response proceeds. We used ROTATO to examine the dose-response relationship of the early phase of root gravitropism in *Arabidopsis* and found a sinusoidal curve with a phase shift of 15° (Mullen and others 2000) (Figure 6). This finding contrasts with earlier reports (Audus 1964; Larsen 1969; Perbal 1974) that indicate a "modified sine" relationship with maximal curvature at angles of 120°–135°. It is not clear why the data obtained using ROTATO indicate a more pure sine relationship for the dose response than the earlier studies. We considered the possibility that the large curvature rates observed at large angles of stimulation in studies lacking control of stimulus input could be due in part to the root tip spending more time at large angles rather than to a larger magnitude of response at these high angles. However, in the earlier studies, care was taken to determine the initial rate of curvature prior to significant change in stimulus strength, so this explanation may be untenable. The earlier work was done with roots such as those of *Vicia*, *Lepidium*, or *Lupinus* (see Audus 1964) while our ROTATO experiments were done using roots of *Arabidopsis*. Thus, it is possible that the dose response relationship for root gravitropism may be species dependent. We have found, for example, that the dose-response curve for the early phase of maize root gravitropism differs markedly from that of *Arabidopsis* roots (Wolverton and others unpublished data). As discussed above, there appear to be two motors driving root gravitropism and the relative activation of these motors shifts with changing angle of stimulation. Furthermore, recent findings (see below) indicate that there is gravitropic sensing not only in the root cap, but also within the elongation zone, at least in the case of maize roots. In view of the complex nature of the gravitropic response system, apparently involving dual motors and dual sensors with differing dependencies on angle of stimulation, it is not surprising that gravitropic dose-response relationships might vary from species to species or even within a species, depending upon the extent to which stimulus input is controlled during experimentation.

DUAL SENSORS

It is widely accepted that the root cap is the site of gravity sensing. This conclusion is based on a variety of experiments showing that removal or disruption of the cap causes nearly complete loss of gravitropic responsiveness with little or no effect on the rate of elongation (Blancaflor and others 1998; Ciesielski 1872; Juniper and others 1966; Konings 1968). The conclusion that the cap is the site of gravity sensing is also supported by the correlation of amyloplast sedimentation in the columella of the root cap with the induction of gravitropic curvature (Kiss 2000; Sack 1991) and the observation that mutants with amyloplasts devoid of starch show impaired gravitropism (Kiss and others 1989).

The impairment of gravitropism upon removal of the cap, of course, does not in itself justify the conclusion that the cap is the site of gravity sensing. In addition to a role in gravity sensing, the cap appears to function in the regulation of the motor response, either as a source of auxin or in the establishment of auxin asymmetry following gravistimulation (Young and others 1990). In fact, there is evidence for limited gravity sensing even in the absence of the root cap. When Keeble and others (1929) gravistimulated decapped maize roots, returned them to vertical, and replaced the missing cap with a cap from an unstimulated root, the roots curved in the direction predicted by the stimulation of the decapped root. Others have shown that decapped roots placed horizontally exhibit positive gravitropism provided that the tip of the decapped root is treated with a source of auxin (Geiger-Huber and Huber 1945; Pilet 1953). Evidently, exposure of decapped roots to a lateral gravitational stimulus can induce an internal change in the extending region that results in a gravitropic response once auxin is applied at the root tip.

The existence of gravity sensing outside the cap has also been demonstrated using centrifugation experiments (Haberlandt 1908; Piccard 1904; reviewed by Poff and Martin 1989). Haberlandt (1908), for example, oriented roots of several species at 45° with respect to the vertical axis of a centrifuge, with the effective axis located at various distances from the root tip. When the point of rotation was located 1.5-2 mm from the root tip of, for example, *Vicia faba*, the roots curved in the direction predicted by tip stimulation. However, when the point of rotation was closer to the root tip (1 mm), the roots curved in the direction corresponding to the stimulation of the main body of the root (see discussion by Haberlandt 1908). It was concluded that there is gravity sensing in regions of the root basal to the root cap.

We have examined the possibility of gravity sensing outside of the cap using ROTATO to control the orientation of selected regions of the root. Using maize roots, we held the region of the elongation zone 4–5 mm from the tip of the root cap at 60° while allowing the root tip (cap) to reorient to vertical (Wolverton and others, 2002). The software for these experiments is designed such that the target region of the root is defined by its position relative to the root tip, that is, in this case, the target segment remains at 4–5 mm from the root tip as the root extends and cells flow into and out of the target region as growth continues. Using ROTATO to maintain the 4–5 mm target region at 60°, we found that gravitropic curvature continued long after the cap reached vertical (Figure 7). This observation indicates that a signal from outside the cap can

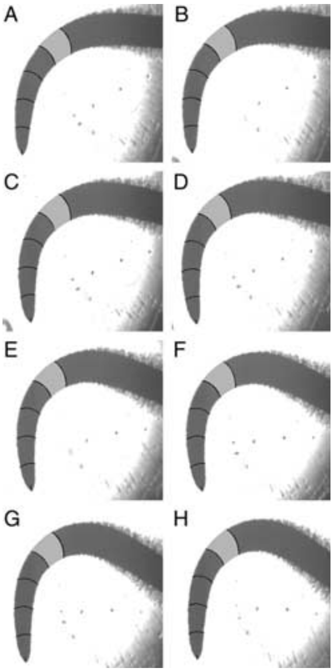

Figure 7. Signals arising from outside the root cap can contribute to gravitropic curvature. Images of a root responding to gravity while the root cap remains vertical. The root was initially stimulated at 60° with the highlighted segment (4-5 mm from the root tip) maintained at 60° throughout the response by continuous clockwise rotation of the ROTATO stage. The images, captured every 15 min, show changes in root shape during the 2 h time period after the root tip reached vertical. The root generated 30° of curvature during this time as indicated by the rotation of the root base. The ROTATO software is designed to focus on a target region that remains a fixed distance from the root tip. Therefore, the highlighted region remains at 4–5 mm from the root tip and cells flow into and out of this region as root growth continues. The fact that the apical portion of the root remains straight and in a vertical orientation in spite of the continued clockwise rotation imposed by ROTATO indicates that there is continued curvature generation within the root apex. In the absence of such curvature generation, the root tip would be displaced upward in a clockwise direction as the stage rotates. (From Wolverton and others, 2002).

contribute to the curvature response. When the target region of constraint was farther back in the root (6–7 mm from the tip of the root cap), curvature ceased soon after the cap reached vertical, suggesting that the sensing associated with the response originating outside of the root cap may be restricted to a more apical region of the elongation zone. The results are in agreement with the earlier work cited above indicating that a signal from outside of the cap can contribute to the curvature response. Because the capacity of the root for localized curvature generation is finite, ROTATO cannot hold a specific target region of the root at a predetermined angle without the surrounding regions approximating the same angle. Consequently, based upon experiments done to date, we can only conclude that the region of second sensing is somewhere within the root apex, most likely the DEZ or the apical portion of the CEZ. Based upon the specialized properties of the DEZ (altered auxin responsiveness, rapid electrophysiological changes upon gravistimulation) we speculate that the area of second sensing is within the DEZ.

Although we do not understand the nature of the signal originating outside the root cap, measurements of the rate of curvature with and without ROTATO indicate that non-cap sensing accounts for only about 20% of the total rate of curvature (Wolverton and others, 2002). Nevertheless, the apparent existence of curvature induction arising from events occurring outside the cap may help explain certain aspects of root gravitropism that have been difficult to reconcile with the single sensor model. For example, freely responding roots are known to curve well past vertical in many instances (Ishikawa and others 1991). This behavior would be consistent with continued signal generation in the curved elongation zone after the cap has reached vertical. Also, roots of starchless mutants are known to retain some gravitropic competence (Caspar and Pickard 1989; Kiss and others 1989; Kiss and others 1996; Vitha and others 2000). The remaining responsiveness in these mutants has been attributed to either persistent plastid-based sensing or the activity of some alternative mechanism of perception (Barlow 1995; MacCleery and Kiss 1999). If the persistence of gravitropic curvature in starchless roots can be attributed to sensing from outside the cap, such sensing must occur by some mechanism other than displacement or sedimentation of starch-filled amyloplasts.

The apparent existence of dual sensors in root gravitropism should be taken into account in efforts to understand the existence of two phases of gravitropic motor activity revealed by ROTATO experiments. Our initial assumption was that these phases are expressed during constant signal input as the root tip is held at a fixed angle during the gravitropic response. However, if there is a second (weaker) sensor in the elongation zone, then it should be recognized that signal input to this region of the root is not constant during ROTATO experiments in which the root tip is held at a constant angle. Instead, the angle of orientation in the region of the elongation zone continuously increases as gravitropism proceeds and ROTATO actively main-

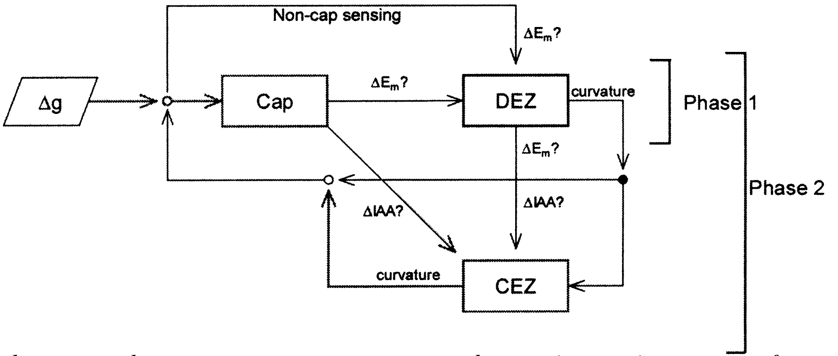

Figure 8. Proposed model for root gravitropism. This working model depicts two sites of stimulus input, one at the root cap and one in the DEZ. Stimulation at the root cap leads to auxin-mediated growth asymmetry in the CEZ according to the "fountain model" of root gravitropism. Direct gravistimulation of the DEZ is thought to induce growth asymmetry in the DEZ mediated by stimulus-induced changes in membrane potential (ΔE_m). The DEZ and CEZ response zones represent the two interacting motors of root gravitropism. The DEZ motor may account for the Phase 1 gravitropic response (the early phase of gravitropism), while the CEZ motor may contribute to Phase 2 gravitropism along with the DEZ motor.

tains the tip at a fixed angle (Figure 4). Although it seems unlikely that the increased stimulation of the second sensing region could account for the decrease in rate of curvature during the later phase of curvature, the changing stimulation of the second sensing region during experiments such as that shown in Figure 4 could contribute to the precise kinetics observed.

How do we reconcile the classical model of root gravitropism with these recent findings? It is tempting to ascribe the dual sensors, dual motors, and dual phases of the gravitropic response to two somewhat independently behaving gravitropic response systems (Figure 8). According to this view, one system might consist of the classical model entailing amyloplast-based sensing in the columella linked to auxin control of differential growth in the CEZ and accounting for curvature during the later phase of gravitropism. The second system would then consist of a still uncharacterized sensor in the DEZ linked to electrical control of differential growth in the DEZ and accounting for the early phase of curvature. This view is certainly oversimplified. We know, for example, that the DEZ contributes to curvature throughout the gravitropic response (Wolverton and others unpublished data). However, the model of Figure 8 can serve as a guide for further investigations into the nature of root gravitropism and as a framework for assigning functions to the wide variety of root gravitropism response mutants that have been identified (Firn and others 2000).

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