THE OHIO STATE UNIVERSITY
Research Foundation
1960 Kenny Road
Columbus, OH 43210-1063

The Role of Calcium and Calmodulin in the Response of Roots to Gravity

HQ. GRANT
IN-51-CR
64302
P-7

Michael L. Evans
Department of Plant Biology

National Aeronautics and Space Administration
Washington, D.C. 20546

Grant No. NAGW-297
Final Report
RF Project No. 763005/714376

January 1992

(NASA-CR-189800) THE ROLE OF CALCIUM AND CALMODULIN IN THE RESPONSE OF ROOTS TO GRAVITY Final Report, Mar. 1989 - Feb. 1991 (Ohio State Univ.) 7 p CSCL 06C
N92-16545
Unclas
G3/51 0064302

Final Report
NASA GRANT NAGW-297
March 1989 - February 1991

The role of calcium and calmodulin in the response of roots to gravity.

Michael L. Evans, Principal Investigator
(Ohio State University Research Foundation Project 714376)

Background: root gravitropism. There is general agreement that, in roots, the primary detection of the gravitropic signal occurs in the columella cells of the cap and that this results in the generation of a signal that moves into the elongation zone causing the asymmetric growth that leads to downward curvature. Recent work in this laboratory has focused on the nature of the signal moving basipetally in gravistimulated roots and how this signal might be generated. Considerable evidence indicates that auxin is the ultimate mediator of differential growth during root (and shoot) gravitropism. The theory that auxin gradients mediate curvature (Cholodny-Went theory) received support in a recent study by Li et al. who generated transgenic tobacco seedlings containing a chimeric gene consisting of an auxin up-regulated RNA promoter fused to an E. coli ß-glucuronidase (GUS) open reading frame. GUS expression was enhanced by physiological doses of auxin and, in gravistimulated plants, GUS expression was greatest on the lower side of the stem. The asymmetry of GUS expression was blocked by inhibitors of polar auxin transport.

While these studies add to the evidence that auxin mediates gravitropic curvature, they do not address the question of how the auxin asymmetry is generated or the question of the nature of the signal moving from the cap to the elongation zone. If we assume that an auxin gradient across the elongation zone is a key element in gravitropic curvature, there seem to be two general means of generating such asymmetry: 1) The auxin asymmetry may be generated in the tip and maintained as hormone is transported toward the elongation zone or 2) A signal may move in some other form (e.g. electrical) from the cap to the elongation zone where it initiates local asymmetric distribution of auxin. Because calcium appears to be necessary for root gravitropism and because tip-applied calcium gradients can induce curvature in maize roots we have also investigated the potential involvement of this cation either as a factor in the regulation of auxin transport or as a factor in generating a signal moving from the cap to the elongation zone.

Our recent work has addressed the following questions relevant to this issue: 1) does gravistimulation induce asymmetric auxin movement within the cap?; 2) do treatments that enhance gravitropism alter cytoplasmic calcium levels in root cap protoplasts?; 3) are the electrical perturbations that occur in the caps of gravistimulated roots restricted to the cap tissues or do they also occur in the elongation zone?; and 4) to what extent do changes in responsiveness to auxin (as opposed to changes in auxin concentration) contribute to the motor response of root gravitropism?

Asymmetric auxin movement across the cap. If gravi-detection by the cap leads to local auxin asymmetry, one would predict asymmetric movement across the root cap of label from applied ^{3}H-IAA. We tested auxin movement across the caps of stimulated roots and found that: 1) stimulation induces movement of label toward the bottom of the cap; 2) the kinetics of curvature and the kinetics of auxin movement are correlated; 3) roots preadapted to gravity (repeated brief stimulation without curvature) show parallel alterations in the kinetics of auxin movement and curvature upon prolonged stimulation; 4) neither curvature nor asymmetric movement of auxin occurs in roots depleted of Ca; and 5) gravistimulated asymmetric movement of auxin occurs in caps isolated from the root. These findings indicate that both Ca and auxin are critical in the communication between the cap and the elongation zone.

Cytoplasmic calcium levels in root protoplasts. Incentive for measuring intracellular Ca derives from the observations that: 1) Ca is necessary for root gravitropism; 2) stimulation induces Ca movement toward the lower side of root caps; 3) calmodulin antagonists inhibit root gravitropism; and 4) in roots of dark-grown seedlings that require light for orthogravitropism, treatments (red light, ABA, water stress) that allow orthogravitropism may increase cytoplasmic Ca levels in cap cells. Sievers and coworkers have shown that ER vesicles accumulate Ca, and they postulate that sedimentation of amyloplasts triggers release of Ca from the ER. This is consistent with the model we proposed linking elevation of Ca to asymmetric movement of auxin across the cap. However, we have found that asymmetric movement of Ca across the cap is not essential to gravitropism. When caps of intact roots were plasmolyzed and then rehydrated, the roots showed gravitropism with little or no asymmetric Ca movement. Also, Takahashi et al. recently reported that curvature induction by tip-applied gradients of calcium is away from the high side of the calcium gradient rather than toward the high side of the calcium gradient as reported in our initial papers. We have also observed curvature away from the high side of the calcium gradient in some of our more recent experiments. This indicates that, although calcium can induce directional growth responses in roots, the nature of calcium involvement in root gravitropism requires more study.

In order to assess the calcium status of root cells, we loaded protoplasts from the cap and elongation zone of primary roots of maize with fluorescent Ca indicators including the potassium salts and acetoxymethyl esters of indo-1 and fura-2. We found the free calcium level to be higher in protoplasts from the elongation zone (257 ± 27 nM) than in protoplasts from the cap (160 ± 40 nM). It may be significant that cytoplasmic free calcium levels are low in the cap. The cap contains the putative gravity-detecting cells and the cells across which asymmetric calcium redistribution occurs. Cells low in free calcium should be especially sensitive to sudden elevation of calcium.

As with similar studies in related plant systems the interpretation of these data is complicated by the possibility of alteration of the protoplasts during isolation. Also, we are using a heterogeneous population of "root cap cells" instead of purified columella cells.

Gravity-induced changes in intracellular potential. Dr. Hideo Ishikawa of Kyorin University was a visiting researcher in this laboratory from May of 1989 through August of 1990, during which time he was supported by Project NAGW-297. While at Kyorin University, Dr. Ishikawa studied gravity-induced changes in intracellular potentials in primary roots of 2-day-old mung bean seedlings. The electrodes were inserted into outer cortical cells within the elongation zone. In vertical roots the potentials of the cortical cells (2 mm behind the apex) were about -115 mV. When the roots were placed horizontally the cells on the upper side hyperpolarized to -154 mV within 30 s while cells on the lower side depolarized to about -62 mV. This did not occur in cells of the maturation zone.

These results may be very significant to understanding the gravitropic response mechanism. Earlier studies of changes in potential in stimulated roots reported responses in the cap but not in the elongation zone. The timing (<30 s) of gravity-induced changes in intracellular potentials in the elongation zone is too rapid to be accounted for by hormonal transmission of a signal moving back from the cap. It seems possible that the changes in intracellular potential in the elongation zone result from an early effect related to the transduction event itself or even to direct gravity detection by cells in the elongation zone.

Kinetics of localized growth rate changes. We used a computer-based video digitizer system to analyze localized relative growth rates along the top and bottom sides of graviresponding maize roots. We found that curvature results both from enhanced growth along the top and reduced growth along the bottom relative to vertically oriented controls. There is also a period of backward curvature (partial reversal of curvature) during the response. During this phase, the growth pattern is reversed, i.e., growth is enhanced along the bottom and reduced along the top relative to vertically oriented controls.

Analysis shows that, during gravitropism, the normally unimodal growth rate distribution along the elongation zone becomes bimodal with two peaks of rapid elongation separated by a region of reduced elongation rate. This occurs at different times on the convex and concave sides of the responding root. Also, during downward curvature the elongation zone along the convex side extends farther toward the tip. During the period of reduced rate of curvature or partial reversal of curvature, the zone of elongation extends farther toward the tip along the concave side. We believe that this shift in the boundaries of the elongation zone is an important factor in root gravitropism.

Time-dependent changes in responsiveness to auxin

Because of the evidence for changes in auxin sensitivity along the top and bottom sides of gravi-stimulated soybean hypocotyls, we became interested in the possibility that time-dependent changes in auxin sensitivity contribute to the kinetics of root gravitropism. We tested for "adaptation" to elevated auxin levels by continuously recording the response of vertically oriented roots to a sudden step-up in auxin concentration. The roots were severely inhibited (as expected) by addition of 0.2 uM IAA. However, after about 60 min some of the roots began to recover and by 100 min they had resumed rapid growth, in some cases as rapid as the rate prior to exposure to auxin. The rapid growth continued even though the roots remained immersed in auxin. A comparison of the growth rate distribution pattern before addition of auxin and after recovery from auxin revealed that, whereas growth was accounted for by cells throughout the elongation zone in the controls, growth following recovery from auxin inhibition was accounted for almost entirely by a group of cells near the apical extremity of the elongation zone. We were particularly struck by the fact that the pattern of growth rate distribution following "adaptation" to elevated auxin was essentially identical to that along the lower side of gravistimulated roots during the phase of partial reversal of curvature. From these and related observations we conclude that: 1) Maize roots can adjust their sensitivity to auxin in a time-dependent manner following a step-up or step-down in ambient auxin levels, and 2) such time-dependent adjustments in auxin sensitivity are initiated by localized shifts in auxin concentration and they play an important role in determining the kinetics of the gravitropic response in roots.

Summary. Our studies of the time course of curvature, auxin redistribution/adaptation, and electrical potential changes in maize roots have led to the following generalizations. 1) Downward curvature begins 18-32 min following gravistimulation. 2) Asymmetric auxin redistribution across the root cap begins at about the same time as curvature or perhaps slightly earlier. 3) There is a lag of approximately 15 min in the response of roots to applied auxin. 4) Gravi-induced changes in intracellular potentials of cortical cells within the elongation zone (data obtained from mung bean, not maize) occur within 30 s following stimulation.

Collectively, items 1-3 above appear to invalidate the Cholodny-Went hypothesis as applied in its simplest form to root gravitropism. This is especially true when one takes into account that estimated rates of auxin transport are approximately 0.5 to 1 cm h^{-1}. According to the Cholodny-Went hypothesis, gravistimulation leads to auxin asymmetry in the cap and this asymmetry is transmitted basipetally to the elongation zone where it induces the asymmetric growth that leads to curvature. But how can this be reconciled with the observation that auxin asymmetry in the cap develops at the same time as or only slightly earlier than the initiation of curvature in the elongation zone? This leaves little time for 1) basipetal migration of auxin into the elongation zone and 2) events associated with the latent period of auxin action on cell elongation.

Apparent conflicts in the timing of events during root gravitropism may be resolved if one considers the possibility that the changes in intracellular potential in the elongation zone are in response to a rapidly moving non-hormonal (electrical?) signal generated by transduction events in the cap. Hejnowicz and Sievers recently reported rapid waves of electrical activity moving basipetally along the apical portion of vertically oriented roots of Lepidium. Alternatively, the changes in intracellular potential in the elongation zone may occur by a mechanism involving direct gravity detection by cells in the elongation zone.

The kinetics of auxin action on root growth may also be more consistent with gravitropism kinetics than previously thought. We have preliminary data indicating that the inhibitory action of auxin on root growth occurs within a few minutes in small roots such as those of Arabidopsis or tomato. This indicates that the latent period for auxin action is much shorter than previously thought and that much of the latent period observed when auxin is applied to larger roots relates to time required for uptake or time to establish a particular local concentration profile.

In our future research we propose to 1) re-investigate the kinetics of auxin action in small roots in order to determine the true lag for auxin action on growth and assess the effect of root geometry on the apparent lag; 2) investigate the potential role of gated ion channels in the generation of electrical signals in roots; 3) determine the relationship between intracellular potential and growth rate; and 4) examine the biomechanical basis for growth rate changes in the elongation zone and in the group of cells just proximal to the meristem.

Publications from most recent funding period (NAGW-297)

Hasenstein KH, Evans ML, Stinemetz CL, Moore R, Fondren WM, Koon EC, Higby MA, Smucker, AJM. 1988 Comparative effectiveness of metal ions in inducing curvature of primary roots of Zea mays. Plant Physiol 86: 885-889

Hasenstein KH, Evans, ML 1988 Effects of cations on hormone transport in primary roots of Zea mays. Plant Physiol 86: 890-894

Evans ML 1989 Second messengers in stimulus-response coupling in plant cells. Proc Plant Growth Regul Soc Amer 15: 114-127

Evans ML, Stinemetz CL, Young LM, Fondren WM. 1990 The role of calcium in the response of roots to auxin and gravity. Plant Growth Substances 1988, R. Pharis, ed. Springer-Verlag, pp 209-215

Moore R, Evans ML, Fondren WM 1990 Inducing gravitropic curvature of primary roots of Zea mays cv Ageotropic. Plant Physiol 92: 310-315

Young LM, Evans ML, Hertel R 1990 Correlations between gravitropic curvature and auxin movement across gravistimulated roots of *Zea mays*. Plant Physiol 92: 792-796

Ishikawa H, Evans ML 1990 Gravity-induced changes in intracellular potentials in elongating cortical cells of mung bean roots. Plant Cell Physiol 31: 457-462

Yang RL, Evans ML, Moore R 1990 Surgical removal of epidermal and cortical cells: evidence that the gravitropic signal moves through the outer cell layers in primary roots of maize. Planta 180: 530-536

Lee JS, Hasenstein KH, Mulkey TJ, Yang RL, Evans ML 1990 Effects of abscisic acid and xanthoxin on elongation and gravitropism in primary roots of *Zea mays*. Plant Sci 68: 17-26

Evans ML, Kiss HG, Ishikawa H 1990 Interaction of calcium and auxin in the regulation of root elongation. *In* Calcium in Plant Growth and Development, 13th Annual Riverside Symposium in Plant Physiology, RT Leonard and PK Hepler, eds., Amer Soc Plant Physiologists, pp 168-175

Ishikawa H, Evans ML 1990 Electrotropism of maize roots. Role of the root cap and relationship to gravitropism. Plant Physiol 94: 913-918

Lee JS, Chang WK, Evans ML 1990 Effects of ethylene on the kinetics of curvature and auxin redistribution in gravistimulated roots of *Zea mays*. Plant Physiol 94:1770-1775

Ishikawa H, Hasenstein K, Evans ML 1990 Computer-based video digitizer analysis of surface extension in maize roots: Kinetics of growth rate changes during gravitropism. Planta 183: 381-390

Evans ML 1990 Gravitropism: Interaction of sensitivity modulation and effector redistribution (mini-review). Plant Physiol 95: 1-5

Kiss HG, Evans ML, Johnson JD Cytoplasmic calcium levels in protoplasts from the cap and elongation zone of maize roots. Protoplasma 163: 181-188

Evans ML, Young LM, Hasenstein KH. 1990 The role of calcium in the regulation of hormone transport in gravistimulated roots. Advances in Space Research (in press)