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The Role of the Distal Elongation Zone in the Response of Maize Roots to Auxin and Gravity[1]

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We used a video digitizer system to (a) measure changes in the pattern of longitudinal surface extension in primary roots of maize (*Zea mays* L.) upon application and withdrawal of auxin and (b) compare these patterns during gravitropism in control roots and roots pretreated with auxin. Special attention was paid to the distal elongation zone (DEZ), arbitrarily defined as the region between the meristem and the point within the elongation zone at which the rate of elongation reaches 0.3 of the peak rate. For roots in aqueous solution, the basal limit of the DEZ is about 2.5 mm behind the tip of the root cap. Auxin suppressed elongation throughout the elongation zone, but, after 1 to 3 h, elongation resumed, primarily as a result of induction of rapid elongation in the DEZ. Withdrawal of auxin during the period of strong inhibition resulted in exceptionally rapid elongation attributable to the initiation of rapid elongation in the DEZ plus recovery in the main elongation zone. Gravistimulation of auxin-inhibited roots induced rapid elongation in the DEZ along the top of the root. This resulted in rapid gravitropism even though the elongation rate of the root was zero before gravistimulation. The results indicate that cells of the DEZ differ from cells in the bulk of the elongation zone with respect to auxin sensitivity and that DEZ cells play an important role in gravitropism.

In a study of surface extension patterns in maize roots (Ishikawa et al., 1991), we reported that the pattern of differential elongation responsible for gravitropic curvature is complex. During rapid downward curvature, acceleration of elongation along the top results largely from initiation of rapid elongation in a group of cells located between the meristem and central portion of the elongation zone. This amounts to a gravity-induced encroachment of the elongation zone closer to the tip along the upper surface. During the phase of transient backward curvature (which often occurs before reaching the vertical) and during the phase of backward curvature following overshoot past the vertical, the corresponding group of cells along the lower side begins to elongate rapidly, i.e. the elongation zone extends closer to the root tip along the lower side of the root. Zieschang and Sievers (1991) reported similar results from a study of the gravitropic response of *Phleum* roots. In this case, the cells showing the initial response to gravity were cells within the apical region of the elongation zone.

Baluška et al. (1990) characterized the cells in maize roots between the meristem and the point at which rapid elongation is initiated as a special zone in which growth is nearly isodiametric. They referred to this region as the postmitotic "isodiametric" growth zone. The cells that show rapid gravi-induced enhancement of elongation rate (Ishikawa et al., 1991) include cells in the postmitotic isodiametric growth zone (compare Baluška et al., 1990, and Ishikawa et al., 1991).

There is evidence that cells between the meristem and the CEZ also play a special role in the ability of roots to adapt to inhibitory levels of auxin. Burström (1957) reported that the elongation of roots of wheat seedlings was initially inhibited by IAA but soon recovered even in the presence of the hormone. The resumption of elongation resulted from initiation of rapid elongation in cells near the meristem including the apical region of the elongation zone. Cells that were in the main elongation zone at the time of auxin treatment remained inhibited. Similar data were obtained by Hejnowicz (1961) using wheat roots and by Goodwin (1972) using roots of *Phleum*.

In this study, we examined the auxin response of cells throughout the apical region of the root. Because IAA redistribution is thought to mediate gravitropic responses, we compared the elongation rate distribution pattern in auxin-treated roots with that of gravistimulated roots. In view of the suggestion that adaptation to localized changes in auxin level contributes to the kinetics of gravitropism (Evans, 1991), we also examined the gravitropic response of roots exposed to IAA before stimulation. We discuss the surprising finding that roots whose elongation rate is reduced to zero by auxin treatment still exhibit a rapid and strong gravitropic response upon gravistimulation. Preliminary results of these studies were reported by Evans et al. (1990) and Ishikawa and Evans (1992).

MATERIALS AND METHODS

Plant Material

Caryopses of maize (*Zea mays* L. cv Merit; Asgrow Seeds, Vineland, NJ) were soaked in tap water overnight and planted between wet paper towels in opaque plastic trays. Several trays were stacked together and held vertically under fluorescent lights (Sylvania cool-white, approximately 5 μmol

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Abbreviations: AEZ, apical elongation zone; BEZ, basal elongation zone; CEZ, central elongation zone; DEZ, distal elongation zone; PEZ, proximal elongation zone.

m^{-2} s^{-1}). Seedlings were used when the primary roots were 2 to 3 cm long (about 3 d after soaking).

Measurement of Elongation and Curvature

Elongation and curvature were measured using the video digitizer system described by Ishikawa et al. (1991). For the gravitropism experiments, seedlings were mounted in a clear plastic Petri dish under near-saturating humidity. Elongation and curvature were recorded and displayed automatically by a custom software program called SECANT (surface extension and curvature analysis technique). To measure localized extension rates, glass beads (approximately 0.25 mm in diameter) stained with India ink were placed on the root surface. Data concerning the velocity of movement of each bead relative to the root tip was used to generate relative elongation rate versus position curves (Ishikawa et al., 1991). To measure localized relative elongation rates before and after auxin treatment, seedlings were mounted in a plastic chamber with the roots immersed in an aerated solution (1 mM KCl, 1 mM $CaCl_2$, Mes/Tris [pH 6.5]). Before immersion, the root surface was marked with small dots of black Speedball oil-base block printer's ink. The dots served as reference points for surface extension analysis by the SECANT software. A small volume of a concentrated solution of IAA was added to the chamber to obtain the desired final concentration.

In some experiments, roots were marked with ink and immersed in a solution of auxin long enough to obtain complete inhibition of elongation (measured as net tip displacement using the ADAPT software described by Ishikawa and Evans, 1990). The seedling was then removed from the auxin solution and mounted horizontally in a plastic chamber at high humidity. The video digitizer system (SECANT software) was used to determine surface extension patterns during the ensuing gravitropic response.

Terminology for Various Regions of the Root Elongation Zone Based on Elongation Rate Profiles

Baluška et al. (1990) defined the postmitotic isodiametric growth zone as that region between the meristem and the onset of rapid cell elongation. They noted that, within this zone, cells grow in width as well as in length, and they retain an approximate isodiametric shape. There is no difficulty in defining the apical limit of this zone because it is represented by the point at which cell division ceases. In the absence of the kind of morphometric measurements made by Baluška et al. (1990), it is difficult to establish the basal limit of this zone because the point at which "rapid" cell elongation begins is not well defined.

For the purposes of this report, we have arbitrarily divided the elongation zone into five subzones based on rates of elongation relative to the peak rate in the CEZ (Fig. 1). The zones are defined as follows: DEZ, that region between the meristem and the point at which the rate of elongation has increased to 0.3 the peak elongation rate; AEZ, that region from the basal limit of the DEZ to the point at which the rate of elongation has increased to 0.7 the peak elongation rate; CEZ, that region from the basal limit of the AEZ to the point beyond the locus of the peak elongation rate where the rate has declined to 0.7 the peak elongation rate; BEZ, that region from the basal limit of the CEZ to the point at which the rate has declined to 0.3 the peak elongation rate; and PEZ; that region from the basal limit of the BEZ to the point at which the growth rate has decreased to zero.

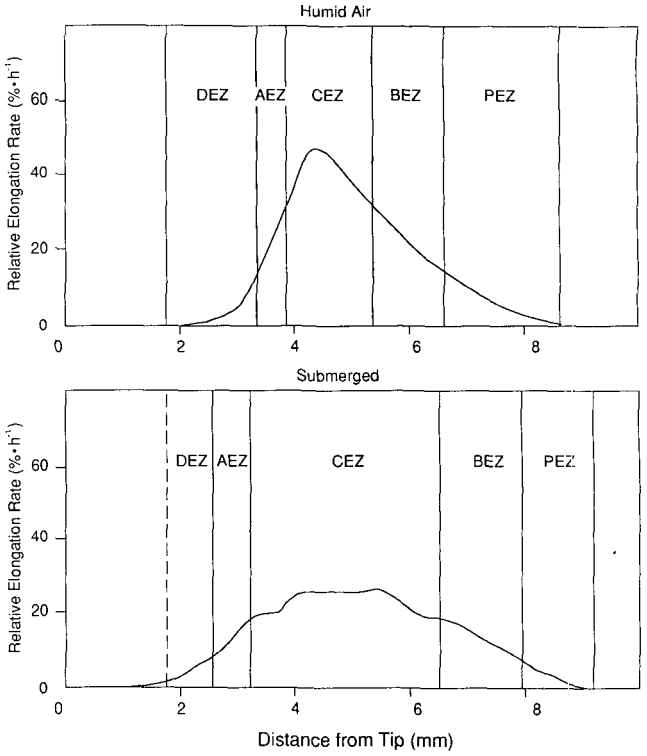

Figure 1. Relative elongation rate (% h^{-1}) as a function of position from the root tip in primary roots of maize growing in humid air (top) or immersed in solution (bottom). In each curve, the five arbitrarily defined subregions (DEZ, AEZ, CEZ, BEZ, and PEZ) of the elongation zone are indicated. In the case of submerged roots, the line indicating the basal limit of the meristem (apical limit of the DEZ) is shown as a dashed line because we are using the value reported by Baluška et al. (1990) for this position, and we cannot be certain that the area of the meristem is unaffected by immersion. There were small variations in the precise shape of the curves from experiment to experiment, but the general shape and position of the curves were as shown in this representative example. Because of this, we have used the experiment with submerged roots as the control curve for Figures 3 and 5. Each experiment was repeated more than 100 times.

The data of Baluška et al. (1990) are plotted as distances from the root apex with the basal limit of the meristem located at about 1.2 mm. The figures in this paper are plotted as distances from the tip of the root cap, which places the basal limit of the meristem at about 1.7 mm. For the experiments in which the roots were immersed in aqueous solution, we have used the value of Baluška et al. (1990) (corrected for the length of the root cap) for the basal limit of the meristem (apical limit of the DEZ) even though we cannot be certain that immersion has no effect on the area of the meristem. Because of this uncertainty, lines indicating the apical limit

of the DEZ in submerged roots are shown as dashed lines, and lines delimiting the other regions of the root elongation zone are shown as solid lines.

For maize roots growing in humid air, the DEZ corresponds to the region of the root from 1.7 to 3.4 mm from the root tip (Fig. 1). This corresponds closely with the postmitotic isodiametric growth zone of Baluška et al. (1990) as measured in the rhizodermis of maize roots.

RESULTS

Pattern of Localized Surface Extension Rates in Vertical Roots before and after Treatment with Auxin

Figure 1 shows the distribution of relative elongation rates along the surface of a primary root of maize growing in humid air or submerged in aerated solution. In humid air, the elongation zone extended from 2 to 8.6 mm behind the root tip, and the peak rate (about 46% h^{-1}) occurred about 4.4 mm behind the tip. It is likely that there was also slow expansion closer to the root tip than 2 mm and farther back than 8.6 mm but that the rate was below the resolution of the digitizer system during these short-term experiments. The relative positions of the five subdivisions of the elongation zone are as indicated in the figure.

In submerged roots, the elongation zone extended from about 1.5 to 9.2 mm behind the tip, and there was a broad peak of maximum relative elongation rate (about 26% h^{-1}) extending from 3.2 to 6.5 mm behind the tip. The apparent broadening of the elongation zone in submerged roots was accounted for, at least in part, by extension of detectable elongation closer to the root tip, possibly including part of the region occupied by the proximal meristem in nonsubmerged roots. Because enhancement of elongation near the tip on the upper side of gravistimulated roots makes a major contribution to curvature (see below and Ishikawa et al., 1991; Zieschang and Sievers, 1991), the activation of elongation near the tip in immersed roots may contribute to the weak gravitropic response of immersed roots (Haberkorn and Sievers, 1977; Moore and Fondren, 1986; Lee et al., 1992).

Figure 2 shows an example of auxin-induced inhibition of elongation followed by partial recovery in the presence of the hormone. The figure also illustrates the rapid elongation following removal of auxin from adapted roots. Each line represents the changing position of a separate marker bead with the intercept of the line with the *y* axis indicating the initial position of the bead. Position is expressed relative to the apical-most bead (generally in the meristem region) as determined by summing the straight-line distances between intervening beads.

Figure 3 displays data concerning elongation rate distribution before, during, and after auxin application. The data are plotted as relative elongation rates versus positions along the root with the curves corresponding to the times covered by the lettered slopes in Figure 2. In the absence of applied auxin, there was a broad elongation peak (also see Fig. 1). When auxin was applied, elongation was inhibited throughout the elongation zone (curve b). As the root adapted, resumption of elongation was accounted for largely by enhanced elongation in the DEZ and AEZ (curve c). Although

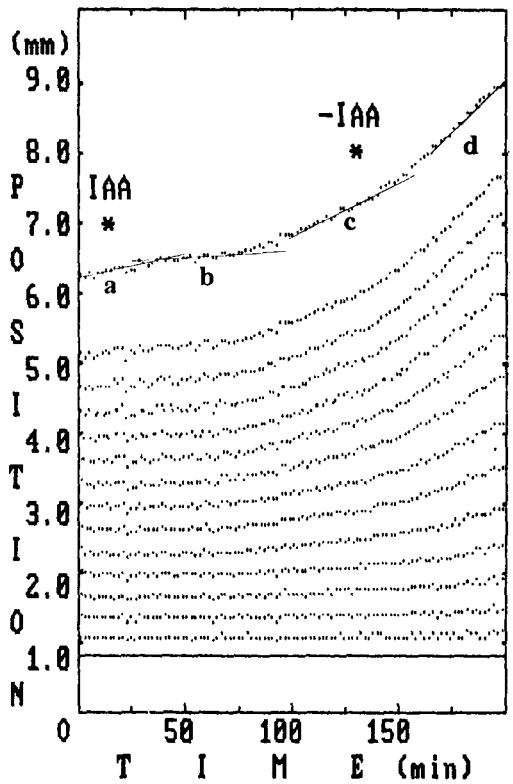

Figure 2. Raw data from digitizer tracking of beads along vertically oriented maize roots immersed in solution. IAA (1 nM) was added at the first asterisk and was withdrawn at the second asterisk. Each line is for a separate marker bead with the intercept of the line with the y axis indicating the initial position of the bead. The position of the bead is expressed relative to the position of the apical-most bead (generally in the meristem region) as determined by summing the straight line distances between intervening beads. The solid black line near the bottom indicates the position of the apical-most marker bead used as a reference for displacement of the other beads. The solid lines in the uppermost curve indicate the slopes for overall root elongation before addition of auxin (a), during maximal inhibition by auxin (b), during recovery in the presence of auxin (c), and during the rapid growth following auxin removal (d). Analysis of growth rates during these periods for all bead positions along the root was used to generate the corresponding growth rate distribution curves in Figure 3. This experiment was repeated 17 times with similar results. A representative example is shown.

cells in the AEZ contributed to the recovery of growth in this experiment and those described below, it should be noted that, during the course of the experiment, cells in the DEZ were displaced basipetally; therefore, some of the cells in the AEZ at the conclusion of the experiment were in the DEZ at the time of treatment.

The exceptionally rapid elongation upon auxin withdrawal resulted from acceleration of elongation in the DEZ, the AEZ, and most of the CEZ (curve d). Cells in the basal portion of the CEZ and cells in the BEZ and PEZ remained inhibited. The finding that auxin inhibits elongation in the PEZ, the BEZ, and part of the CEZ and that these cells do not recover in the presence of the hormone cannot be extended to auxin effects on overall cell expansion. In a preliminary investiga-

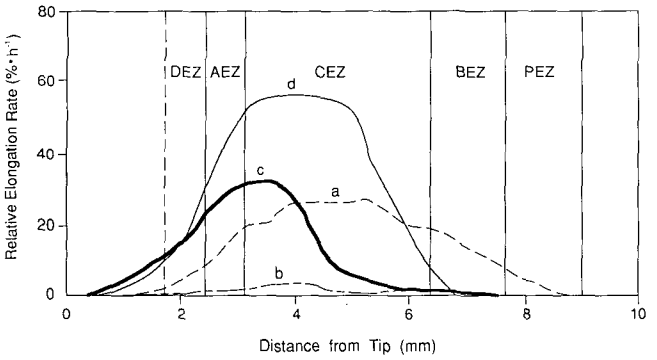

Figure 3. Relative elongation rate distribution pattern of an immersed, vertically oriented maize root before and after application or application and withdrawal of auxin. a, Control. Remaining curves show the elongation rate distribution pattern during the period of inhibition following application of auxin (b), following recovery in the presence of auxin (c), and following recovery upon withdrawal of auxin (d). Curves a, b, c, and d correspond with the periods indicated by lines a, b, c, and d in Figure 2. The vertical lines delineate the five subregions of the elongation zone. In the case of submerged roots, the line indicating the basal limit of the meristem (apical limit of the DEZ) is shown as a dashed line because we are using the value reported by Baluška et al. (1990) for this position and we cannot be certain that the area of the meristem is unaffected by immersion. Auxin concentration was 1 nM in b and c and 20 nM in d. This experiment was repeated 17 times with similar results. A representative example is shown.

tion of the effects of auxin on radial expansion in maize roots, we found (data not shown) that auxin inhibition of elongation is accompanied by enhanced radial expansion. The conclusions reached in the present study apply to auxin effects on elongation only.

Changes in the Elongation Rate Distribution Pattern upon Application and Withdrawal of Mannitol

Although we hypothesize that the exaggerated elongation upon removal of auxin is related to prior adaptation to elevated auxin, it is also plausible that it represents stored growth. It is well known, for example, that a burst of elongation occurs upon removal of inhibitors from coleoptile segments (Ray, 1961). This is referred to as stored growth in recognition of the possibility that growth potential accumulated during inhibition is expressed upon removal of the inhibitor.

To test this possibility, we examined the response of maize roots to application and withdrawal of mannitol. Figure 4 shows data concerning surface marker displacement before, during, and after application of 400 mM mannitol. Figure 5 shows the change in distribution of relative elongation rates determined from the data of Figure 4. Mannitol inhibited elongation throughout the elongation zone with the exception of the apical portion of the DEZ, where the rate remained unchanged (Fig. 5). When mannitol was replaced with control solution, there was a burst of elongation (Fig. 4). Osmotic swelling contributed to the initial phase of rapid elongation. However, rapid elongation occurred beyond the time of the osmotic transient, indicating a true burst of elongation. The rapid elongation was accounted for by exceptionally rapid elongation throughout most of the elongation zone. Although there was some enhancement of elongation in the DEZ (Fig. 5), the relative enhancement was small compared with that seen upon withdrawal of auxin (Fig. 3, curve d).

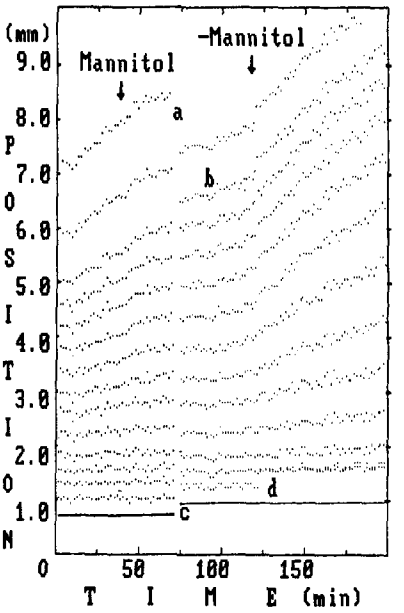

Figure 4. The response of maize roots to application and withdrawal of 400 mM mannitol. Raw data were from digitizer tracking of beads along vertically oriented maize roots immersed in solution. Mannitol was added at the first arrow and was withdrawn at the second arrow. Each line is for a separate marker bead with the intercept of the line with the y axis indicating the initial position of the bead. The position of the bead is expressed relative to the position of the apical-most bead (generally in the meristem region) as determined by summing the straight-line distances between intervening beads. The solid black line near the bottom indicates the position of the apical-most marker bead used as a reference for displacement of the other beads. There are some discontinuities (indicated as a, b, c, and d) in the data that are accounted for as follows: a, Data for this marker bead ends abruptly at about 68 min. We anticipated a strong stimulation of elongation upon withdrawal of mannitol (at 118 min). At 68 min, the apical-most bead was approaching the lower range of view of the camera. Consequently, we shifted the entire root upward slightly causing the uppermost bead to move out of view. b, Data for this marker bead begins at about 73 min. When the root was shifted upward, losing the most distal bead, the digitizer was assigned to track a bead at point b. This bead was on the root throughout the experiment but was not tracked until the 73-min point. c, There is a shift in the solid black line (indicating reference position of apical-most bead) at about 73 min. This occurred because the initial reference bead became unsteady because of excess mucilage production. Consequently, the next most apical bead was selected as the reference position for the remainder of the experiment. d, Data for this marker bead ends abruptly at 123 min. This corresponds to the time of mannitol removal when the rate of overall root extension was very rapid, causing the digitizer to lose track of this rapidly moving bead. This experiment was repeated seven times with similar results. A representative example is shown.

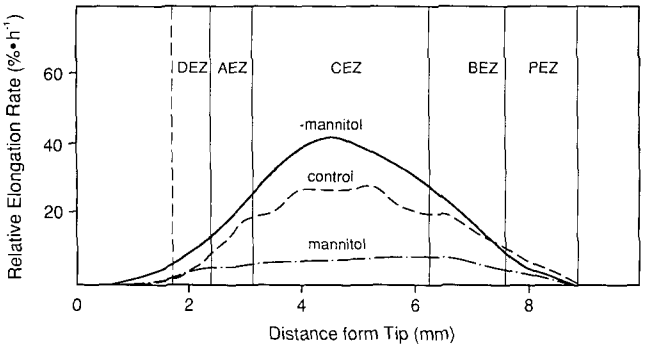

Figure 5. Elongation rate distribution profiles before, during, and after treatment with mannitol. The data of Figure 4 are plotted as relative elongation rate versus position along the root. Control (- - -), 20 min after application of mannitol (· — · — ·), 20 min after withdrawal of mannitol (———). Rates for curves in the presence of mannitol and following mannitol removal were calculated from consecutive 20-min data points at the indicated times. The vertical lines delineate the five subregions of the elongation zone. In the case of submerged roots, the line indicating the basal limit of the meristem (apical limit of the DEZ) is shown as a dashed line because we are using the value reported by Baluška et al. (1990) for this position and we cannot be certain that the area of the meristem is unaffected by immersion. Root elongation rates were 0.7, 0.3, and 1.2 mm h^{-1} before, during, and after mannitol treatment, respectively. This experiment was repeated seven times with similar results. A representative example is shown.

Gravitropic Curvature of Auxin-Inhibited Roots

According to the Cholodny-Went theory (Trewavas, 1992) gravity-induced redistribution of auxin is the key factor in the development of the differential elongation pattern causing downward curvature of gravistimulated roots. If so, we expected that roots pretreated with auxin at a concentration high enough to stop elongation would show no response to gravistimulation, both because they are not growing and because auxin pretreatment should mask subtle gradients generated by redistribution of endogenous auxin. We were surprised to find that roots pretreated with auxin at a concentration that completely suppresses elongation still exhibited strong gravitropism (Fig. 6). In fact, the rate of curvature in pretreated roots was sometimes greater than that of controls (data not shown). The final angle of curvature was as large or larger than that of controls (Fig. 6), but the oscillation of the root tip around the final angle of curvature, which is typical in control roots (Fig. 6; Ishikawa et al., 1991), did not occur in auxin-pretreated roots.

The pattern of elongation rate distribution during the gravitropic response of auxin-pretreated roots is compared with that of controls in Figure 7. Data for controls are taken from the study by Ishikawa et al. (1991) and are reproduced here for purposes of comparison. In control roots, curvature resulted from an acceleration of the elongation rate along the top and a reduction along the bottom. Along the upper side, the zone of elongation became bimodal with a large peak of elongation in the DEZ and AEZ (Fig. 7a). The rate of elongation was reduced in the region that had shown peak elongation in the vertical control (CEZ). A small peak of elongation was retained in the BEZ. Along the lower side, elongation was reduced throughout the elongation zone, and there appeared to be some shortening (negative relative elongation rate) in the region 2 to 5 mm from the tip. A small peak of elongation was retained in the BEZ/PEZ region (Fig. 7b).

In auxin-pretreated roots, the elongation rate was zero throughout the root before gravistimulation (Fig. 7c, dashed line). Following stimulation, a strong peak of rapid elongation developed in the DEZ and extended into the AEZ and CEZ. This peak was similar to the major peak along the upper side of controls (Fig. 7a) but shifted somewhat toward the root tip. Elongation remained inhibited along the lower surface of the root during the gravitropic response (Fig. 7d).

DISCUSSION

The results of this study confirm that maize roots adapt to inhibitory levels of auxin (Gougler and Evans, 1981) and show that, in adapted roots, elongation is accounted for primarily by rapid elongation in the DEZ and AEZ (Fig. 3). Cells of the DEZ (especially those at the apical extremity of the DEZ) exhibit minimal elongation before auxin application. In contrast to cells of the DEZ, the elongation of cells in the PEZ, the BEZ, and most of the CEZ remains suppressed in the presence of auxin. These results are consistent with the findings from earlier studies with roots of wheat (Burström, 1957; Hejnowicz, 1961) and *Phleum* (Goodwin, 1972). Our findings extend these earlier results with the observation that,

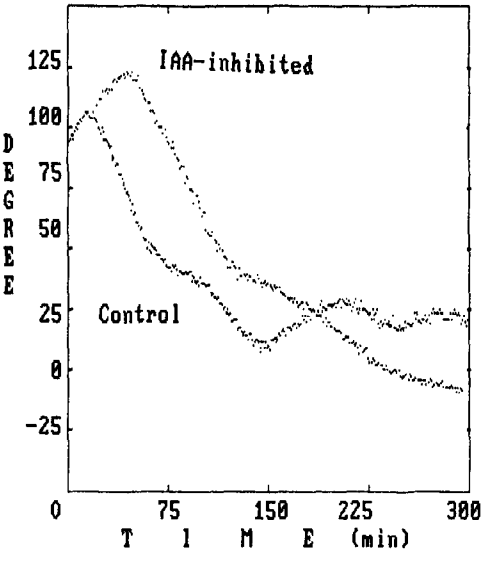

Figure 6. Comparison of the kinetics of gravitropic curvature in control roots with that of auxin-inhibited roots. In the case of auxin-inhibited roots, the root was immersed in 1 μM IAA long enough to obtain maximal inhibition of elongation. The root was then removed from the auxin solution (no rinsing) and oriented horizontally in humid air. Gravistimulation was begun at −5 min. The initial upward curvature following gravistimulation may be caused by completion of a phase of tip nutation that is commonly observed in vertical roots. This experiment was repeated three times with similar results. A representative example is shown.

at least in roots of maize, cells close to the apical meristem make a major contribution to recovery in the presence of auxin.

Although there is agreement that auxin is a powerful inhibitor of root-cell elongation, we found that the effect depends on the cells in question and time elapsed since exposure to the hormone. The initial effect of auxin is to inhibit cell elongation throughout the elongation zone (Fig. 3), with the possible exception of cells immediately adjacent to the meristem that are elongating only very slowly even in the absence of applied auxin. Beginning about 1 h after application of auxin, cell elongation is stimulated in the DEZ, the AEZ, and apical portion of the CEZ.

The promotive effect of auxin in the DEZ is shown diagrammatically in Figure 8, which depicts the time-dependent response of DEZ zone cells in intact maize roots to applied auxin. As indicated in the diagram, auxin causes long-term enhancement of cell elongation within the DEZ (solid line). However, with the passage of time, cells that were in the DEZ at the time of exposure to auxin become displaced toward the base of the elongation zone. As the cells move into the basal regions of the elongation zone, they become sensitive to long-term auxin inhibition similar to the cells that were in the basal region of the elongation zone at the time of auxin application (Fig. 8, dashed line).

The response of roots to a brief exposure to auxin is particularly interesting. The elongation of cells throughout the main elongation zone is initially suppressed by auxin (Fig. 3, curve b). These cells resume elongation when auxin is removed (Fig. 3, curve d), and cells in the DEZ, AEZ, and CEZ actually grow more rapidly than they did before auxin application. As discussed above, the (minimal) extension of DEZ cells is promoted by auxin following a lag of about 90 min. Surprisingly, if the auxin is withdrawn during the 90-min latent period, there is a sudden large stimulation of extension in the DEZ (Fig. 3, curve d). Collectively, the data indicate that the elongation of DEZ cells is enhanced by long-term exposure to auxin and even more so by brief exposure to auxin that is withdrawn before the expression of growth acceleration.

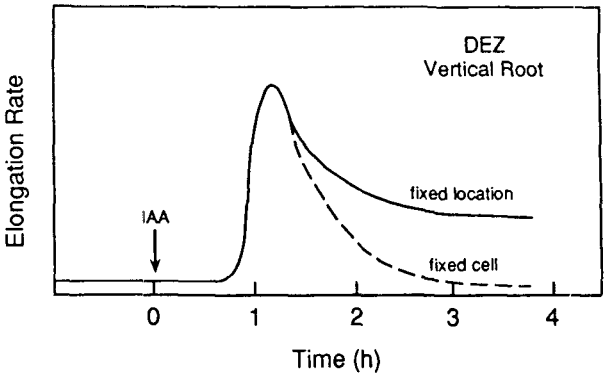

Figure 8. Diagrammatic representation of the time course of the response of DEZ cells to low levels of applied auxin. The elongation of cells within the DEZ is promoted after a lag of about 1 h. Solid line, Elongation rate of cells at a fixed position within the DEZ reaches a peak and then decreases to a lower steady rate that is greater than the pretreatment rate. Dashed line, A cell in the DEZ at the time of auxin treatment is followed as it moves from the DEZ into the main elongation zone. Auxin stimulates the elongation of the cell while it is in the DEZ and AEZ, but its elongation becomes severely inhibited (relative to control cells in the elongation zone) as it moves farther back into the elongation zone with the passage of time.

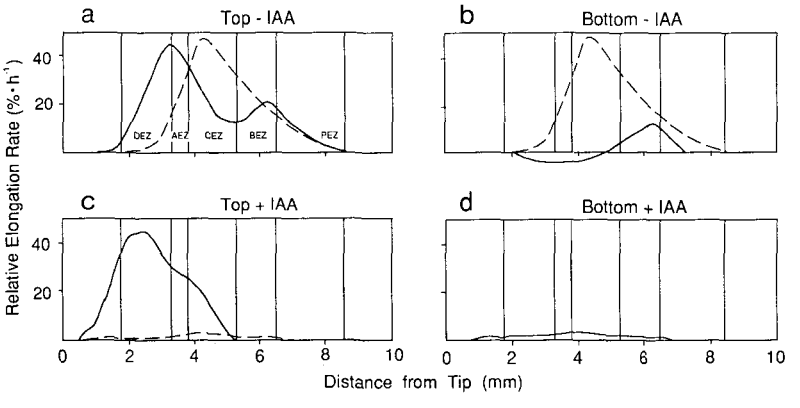

Figure 7. Pattern of elongation rate distribution along the top and bottom of gravistimulated control and auxin-pretreated (as described in legend to Fig. 6) roots. In each panel, the dashed line indicates the elongation rate distribution pattern in the vertical control, and the solid line indicates the pattern in the corresponding gravistimulated root. a, Elongation rate distribution along the upper side compared with vertical control; b, elongation rate distribution along the lower side compared with vertical control; c, elongation rate distribution along the upper side of a gravistimulated auxin-inhibited root compared with an auxin-inhibited vertical control; d, elongation rate distribution along the lower side of a gravistimulated auxin-pretreated root. The distribution pattern along auxin-treated vertical roots (during the period of strong auxin inhibition) was essentially identical with this. In all cases, the elongation rate distribution patterns are for the initial period of rapid downward curvature as shown in Figure 6. The vertical lines delineate the five subregions of the elongation zone. Experiments of parts a and b were repeated more than 40 times. Experiments of parts c and d were repeated three times.

It is surprising to find enhancement of elongation in the DEZ both upon long-term exposure to IAA and upon withdrawal of IAA during the latent period before promotion of elongation. Although we hypothesize that these responses are related to auxin-induced changes in auxin sensitivity in the DEZ, testing this idea will require more detailed experiments.

The pattern of elongation upon withdrawal of auxin differed from that upon withdrawal of mannitol. Upon withdrawal of mannitol, elongation throughout the elongation zone (except the basal region) increased to a value exceeding that of the control (Fig. 5). Upon withdrawal of auxin, elongation in the PEZ, the BEZ, and basal portion of the CEZ showed little or no recovery, whereas elongation in the DEZ, the AEZ, and most of the CEZ was very rapid. The stimulation of elongation in the DEZ upon withdrawal of auxin was larger than the stimulation upon withdrawal of mannitol. Although the effects of mannitol on cell elongation may include factors other than its osmotic action (Zhu and Boyer, 1992), the differing response patterns upon withdrawal of auxin and mannitol indicate that the burst of elongation upon withdrawal of auxin is not a stored growth phenomenon.

The observation that mannitol inhibited elongation throughout the elongation zone, with the exception of the apical portion of the DEZ, is consistent with the data of Sharp et al. (1988), who found that maintenance of root elongation at low water potential resulted from sustained elongation near the apex of the elongation zone.

The observation of rapid gravitropism in maize roots pretreated with a sufficient concentration of auxin to cause complete inhibition of elongation was surprising. Katekar and Geissler (1992) also observed this phenomenon in roots of maize, and G.K. Muday (personal communication) found a similar phenomenon in tomato roots. The data of Barlow et al. (1991, compare their figs. 1 and 4) indicate that the DEZ covers the same relative position in tomato roots as in roots of maize.

Our data (Fig. 7, c and d) indicate that gravitropic curvature in auxin-pretreated roots results mainly from enhancement of elongation in the DEZ on the top, coupled with a general suppression of elongation along the lower side. Although the overall rate of elongation is much less than that of controls, the gradient of elongation across the root near the tip is as large or larger. This is a consequence of the complete inhibition (Fig. 7d) of elongation along the lower side (compared with partial inhibition along the lower side of controls, Fig. 7b) coupled with strong stimulation of elongation in the DEZ on the upper side.

The results reported here are relevant to the continuing controversy (Trewavas, 1992) regarding the validity of the Cholodny-Went theory of gravitropism. The pattern of localized elongation rate following the adaptation of vertically oriented roots to applied auxin (Fig. 3, curve c) is nearly identical with that along the lower side of gravistimulated roots late in the gravitropic response (fig. 19 of Ishikawa et al., 1991). This is consistent with the Cholodny-Went theory, which holds that the auxin level increases along the lower side. On the other hand, the fact that roots exhibit gravitropism while immersed in high levels of auxin is contrary to the Cholodny-Went hypothesis, especially in view of the fact that the pattern of elongation along the top was similar in the presence and absence of applied auxin. In general, our results are consistent with the Cholodny-Went theory as it applies to the inhibition of elongation along the lower side but inconsistent with a role for auxin in the acceleration of elongation along the upper side. The possibility of direct gravity detection and/or auxin-independent acceleration of elongation in the DEZ and AEZ deserves serious consideration.

The most significant aspects of the findings reported here are as follows: (a) There are at least two classes of cells in maize roots in terms of auxin effects on cell elongation. Auxin stimulates elongation in cells of the DEZ and AEZ but inhibits elongation of cells in the remainder of the elongation zone. (b) Auxin-induced enhancement of elongation in the DEZ and AEZ accounts for the ability of roots to adapt to inhibitory levels of the hormone. (c) The pattern of elongation rate along the lower side of gravistimulated roots late in the gravitropic response is similar to that of auxin-treated vertical roots. This is consistent with the Cholodny-Went theory. (d) Gravitropism in auxin-inhibited roots results primarily from gravity-induced stimulation of elongation in the DEZ on the upper side. This indicates that redistribution of endogenous auxin does not mediate the localized enhancement of elongation during gravitropism and that the Cholodny-Went theory cannot account for this aspect of the generation of elongation rate asymmetry.

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