Plant Physiol. (1990) 94, 1763–1769
0032-0889/90/94/1763/07/$01.00/0

Received for publication June 13, 1990
Accepted September 1, 1990

Transport of Indole-3-Acetic Acid during Gravitropism in Intact Maize Coleoptiles[1]

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ABSTRACT

We have investigated the transport of tritiated indole-3-acetic acid (IAA) in intact, red light-grown maize (*Zea mays*) coleoptiles during gravitropic induction and the subsequent development of curvature. This auxin is transported down the length of gravistimulated coleoptiles at a rate comparable to that in normal, upright plants. Transport is initially symmetrical across the coleoptile, but between 30 and 40 minutes after plants are turned horizontal a lateral redistribution of the IAA already present in the transport stream occurs. By 60 minutes after the beginning of the gravitropic stimulus, the ratio of tritiated tracer auxin in the lower half with respect to the upper half is approximately 2:1. The redistribution of growth that causes gravitropic curvature follows the IAA redistribution by 5 or 10 minutes at the minimum in most regions of the coleoptile. Immobilization of tracer auxin from the transport stream during gravitropism was not detectable in the most apical 10 millimeters. Previous reports have shown that in intact, red light-grown maize coleoptiles, endogenous auxin is limiting for growth, the tissue is linearly responsive to linearly increasing concentrations of small amounts of added auxin, and the lag time for the stimulation of straight growth by added IAA is approximately 8 or 9 minutes (TI Baskin, M Iino, PB Green, WR Briggs [1985] Plant Cell Environ 8: 595–603; TI Baskin, WR Briggs, M Iino [1986] Plant Physiol 81: 306–309). We conclude that redistribution of IAA in the transport stream occurs in maize coleoptiles during gravitropism, and is sufficient in degree and timing to be the immediate cause of gravitropic curvature.

There are many circumstances under which plants abruptly change their pattern or rate of growth in response to external stimuli such as light or gravity or in response to internal signals, as happens during normal maturation. For many years experimenters have studied the transport of IAA in seedlings and in more mature plants, to determine whether or not plants redistribute or regulate the concentration of this endogenous compound to effect growth changes. It has been established that shoots generally produce IAA in their tips and that the IAA produced there flows basipetally from the apex. Very fast, nonpolar components of transport may be present in mature tissues (15, 19), but in seedlings IAA is basipetally transported at a rate that has been estimated to be between 10 and 20 mm/h, depending on the species, developmental stage, and method of assay of the transport rate (12).

Most investigators have found that the distribution or transport of IAA in maize coleoptile tissue changes during gravitropism. The amount of growth-promoting activity (as determined with the *Avena* coleoptile curvature test) flowing from the cut end of a horizontal coleoptile is greater from the lower half than from the upper half (5). The amount of radioactively labeled IAA flowing from the cut end of a horizontal coleoptile that has been symmetrically supplied with the tracer at the apical end is greater from the lower half than from the upper half (8, 10). Although asymmetrically supplied ^{14}C-IAA is normally transported almost exclusively down the side to which it is applied to vertical coleoptiles, when it is applied to the upper side of a horizontal coleoptile section, a large proportion of the ^{14}C-IAA is recoverable from the lower half (11). Finally, when endogenous free IAA is assayed in coleoptiles showing a gravitropic response, it is found that the lower half of the coleoptile contains significantly more IAA than the upper half (2). It is clear that IAA becomes redistributed in response to a gravitropic stimulus.

What is not perfectly clear, and what has been debated for a number of years, is whether the observed IAA redistributions cause gravitropic curvature or are correlates of the response. It has been argued that the amount of IAA redistributed is too small to cause the large changes in growth rate necessary to account for gravitropic curvature, given the sensitivity of coleoptile sections to exogenously added IAA (21). It has also been argued that redistributions of endogenous IAA might not occur early enough, given the lag time between IAA application and the resulting change in straight growth response, to be the immediate cause of gravitropic curvature (9). Alternative scenarios have been proposed, in which the immediate cause of tropic curvature is an asymmetric change in a seedling's sensitivity to the IAA already in the tissue (20, 21), perhaps by modification of the availability of its endogenous IAA to receptors, or through conjugation or compartmentation of the auxin. We have addressed these questions in this study of the timing and extent of IAA transport changes with respect to the development of gravitropic curvature.

The maize coleoptile shows a number of interacting, regulated growth changes in response to developmental stage, red light, blue light, and gravity. Studies on the transport of IAA during regulated growth changes have therefore often been difficult to compare because experimenters used different cultivars of maize at different developmental stages and exposed to different light conditions for their work. Differences such as these profoundly affect the growth pattern of a seed-

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[1] CIW-DPB publication number 1079. This work was supported in part by National Science Foundation grant DCB-88 19137 to W. R. B.

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ling, as well as its sensitivity and speed of response to tropic stimuli and to the application of growth regulators such as IAA. One notable example is that etiolated maize coleoptiles show their greatest rate of growth near the apex, with growth declining toward the basal regions near the mesocotyl, whereas red light-grown maize coleoptiles show exactly the reverse pattern, with highest rates of growth near the mesocotyl node and growth rates declining toward the apex (2, 13). By working with a well-characterized, standardized material—4d-old maize coleoptiles of a previously studied cultivar, grown under dim red light—we have been able to avoid some of these problems. We have already characterized the transport of IAA in these intact, upright maize seedlings (18), and now are in a position to compare it with IAA transport in coleoptiles responding to gravity.

MATERIAL AND METHODS

Plant Material

Maize seeds (*Zea mays,* cv PX9540, Northrup King) were soaked in deionized water on a shaker at about 60 cycles per min for 5 to 8 h and were planted in 30 mL glass beakers filled with damp vermiculite, 2 or 3 seeds per beaker. Imbibition and growth were under constant red light, at a temperature of 24°C. The light source was as described before (18). Plants with coleoptiles 14 to 18 mm long (unless otherwise noted) were used for experiments on the morning or early afternoon of the fourth day. The average coleoptile was 15 mm long, with a primary leaf 12 mm long (mesocotyls varied in length).

Growth Rate Experiments

Plants were chosen to be straight and upright, with coleoptiles 15 mm long. In control experiments, plants were marked with dots of carbon black in immersion oil at 2 mm intervals, starting 1 mm from the apical tip. They were marked down the midline of the broad side of the seedling, between the vascular bundles, to delineate seven 2-mm zones. Preliminary experiments indicated that this handling caused transient perturbations in growth rate, so the plants were allowed to remain undisturbed for 1 h before beginning the experiment. After this time, in control experiments, the plants were turned horizontal for 1 s, returned to the vertical position, and immediately photographed at 5 min intervals for 85 min using Kodak technical pan film (TP 135-36). In gravitropism experiments, the plants were left horizontal. All plants were irradiated with about 15 s of bright red light (light source described in ref. 3) to allow the camera to be focused. The camera was a Minolta Maxxum 7000i with a Minolta Maxxum AF 50 mm F/2.8 macro lens and a Minolta Maxxum PB-7 Program Back.

The film was developed with Kodak D-19 developer. The negatives were cut, inserted into slide holders, and projected with a slide projector at about 50-fold magnification. The projected lengths of individual zones were marked onto paper and measured on the digitizer pad. Time courses for individual plants were normalized to 100% length at time 0, averaged, and standard errors were calculated.

In gravitropism experiments, the coleoptile was marked and photographed as in the control experiments. Dots of carbon black were, however, placed down the sides of the coleoptile in these experiments, rather than down the midline, so that when the coleoptile was turned horizontal, with the direction of gravity parallel to the plane through the vascular bundles, the dots marked the upper and lower edges. Growth rates were calculated separately for the two sides of the coleoptile.

Transport Experiments

[^{3}H]IAA[3] was obtained at a specific activity of 21.7 Ci/mmol, and a concentration of 46 μM in ethanol from Amersham. Label was used at a concentration of 0.92 μM [^{3}H]IAA, a 1:50 dilution of primary label into 95% ethanol (v/v), 1% Triton X-100 (v/v). A volume of 0.5 μL of label was applied to the tip of each coleoptile as a drop with a Hamilton syringe. The applied drop clung to the tip of the coleoptile on the broad side, approximately centered between the two vascular bundles, and covered an area between 2 and 3 mm in diameter. We did not control which side of the coleoptile received the drop. The solution evaporated rapidly—after about 5 min the drop was no longer visible. Fifteen min after the application of the drop of label the plants were turned horizontal and held there until the end of the experiment.

At the time the plants were turned horizontal, and at 10 min intervals thereafter, seedlings were harvested. Their tips were wiped upward several times, gently, with a Kimwipe soaked in 95% ethanol, 1% Triton X-100, and they were sectioned down their length between the vascular bundles into upper and lower halves. Each half was sectioned into 1.1 mm long pieces. For most time points, individual tissue pieces were pooled and placed in 4 mL of Aqueous Counting Scintillant (Amersham) or BudgetSolv (Research Products International Co., Mount Prospect, IL), extracted overnight in the dark at room temperature, and counted the next day for 5 min each. In any one experiment, only one type of scintillation fluid was used. For one time point, 40 min after the plants were turned horizontal, tissue pieces were counted individually. Profiles were averaged and standard errors were determined. Transport experiments were performed on 5 separate days, with five plants each day harvested for each time point.

Redistribution Experiments

Plants were given label, then allowed to remain vertical for a period of 15 min to let the solvent evaporate. Then half of the seedlings were turned horizontal and half were left vertical. Sixty min later, the section 5 to 10 mm from the tip was taken from each plant, split lengthwise with a razor blade, placed into 4 mL of ACS or BudgetSolv, extracted overnight in the dark, and counted the next day for 5 min. The halves of control coleoptiles were alternately assigned to be 'upper' or 'lower' at the time they were split lengthwise. The amounts of radioactivity in the upper and lower sides of horizontal plants were compared with each other and with the radioactivity in the 'upper' and 'lower' halves of the vertical coleoptiles.

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[3] Abbreviations: [^{3}H]IAA, 5-[^{3}H]indole-3-yl acetic acid;

Efflux Determinations

Coleoptiles were cut 10 mm below the tip. Microfuge tubes (0.5 mL) were cut so that they held about 200 μL. Each coleoptile was placed upright in one of these small tubes with just the basal 2 or 3 mm in 200 μL of glass-distilled water. About 30 min later, each coleoptile tip received a 0.5 μL droplet of the tritiated IAA solution. Fifteen min later, half the plants were turned horizontal with the direction of gravity parallel with the plane through the vascular bundles, their bases still in water. When plants were turned horizontal, the surface tension of the small volume of water kept it from flowing, and the sides of the containers kept the coleoptiles oriented and horizontal. Half the plants were left vertical. At that time, and at 10 min intervals thereafter, batches of plants were removed from their containers. The water from each individual plant was put into 4 mL ACS or BudgetSolv, left in the dark overnight at room temperature, and counted for 5 min the next day. Average effluxes and standard errors were determined.

RESULTS

Growth Changes Occurring during Gravitropism

The straight growth rates of 4 d-old, red-grown maize coleoptiles, by 2 mm zones, are reported in Figures 1 and 2 for upright and horizontal plants. Under both conditions, the basal regions of the coleoptiles grow faster than the apical regions. While this pattern has been reported before for red light-grown coleoptiles (14), it contrasts with that reported for totally etiolated maize coleoptiles, which show a greater rate of growth in apical than in basal regions (2). Our coleoptiles normally grow about 2% over 90 min in the zone 1 to 3 mm from the tip, and approximately 3, 3.5, 4, 4, 4, and 5% over 90 min for the zones 3 to 5 mm, 5 to 7 mm, 7 to 9 mm, 9 to 11 mm, 11 to 13 mm, and 13 to 15 mm from the tip, respectively (Figs. 1 and 2). These control coleoptiles show a short inhibition of growth, lasting about 15 min, resulting from handling shock. The pronounced dip in the growth curve shown in Figure 2, for the zone 9 to 11 mm from the tip between 0 and 5 min, probably results from an inaccurate determination of the length of the average section at 0 min.

The growth rate response during gravitropism in these coleoptiles is complex. After the initial period of growth inhibition, the upper and lower sides of the coleoptile begin to recover equally, at least in the 5 to 15 mm subapical region of the coleoptile. This period during which the upper and lower sides of the coleoptile are growing at similar rates lasts 10 to 30 min, longer for the basal regions than the apical regions. The growth rates of the upper and lower sides of the other regions then diverge from each other. The times during which the growth rates diverge are represented in Figure 4, described below, and are earlier for the more apical zones than for the more basal zones. This growth rate divergence represents the onset of gravitropic curvature. The time course of gravitropic curvature in 4 d-old, red-grown corn coleoptiles has been described elsewhere (18).

The degree of error involved in these measurements does not allow us to determine if small differences in total growth occur during gravitropism, but in all but the two most basal zones (Fig. 2) there are no clear differences in the first 90 min between the average total growth of comparable regions of horizontal and of vertical coleoptiles. The upper half of a horizontal coleoptile between 1 and 11 mm below the apex essentially stops growing during gravitropism, but the growth rate of the lower half increases to compensate.

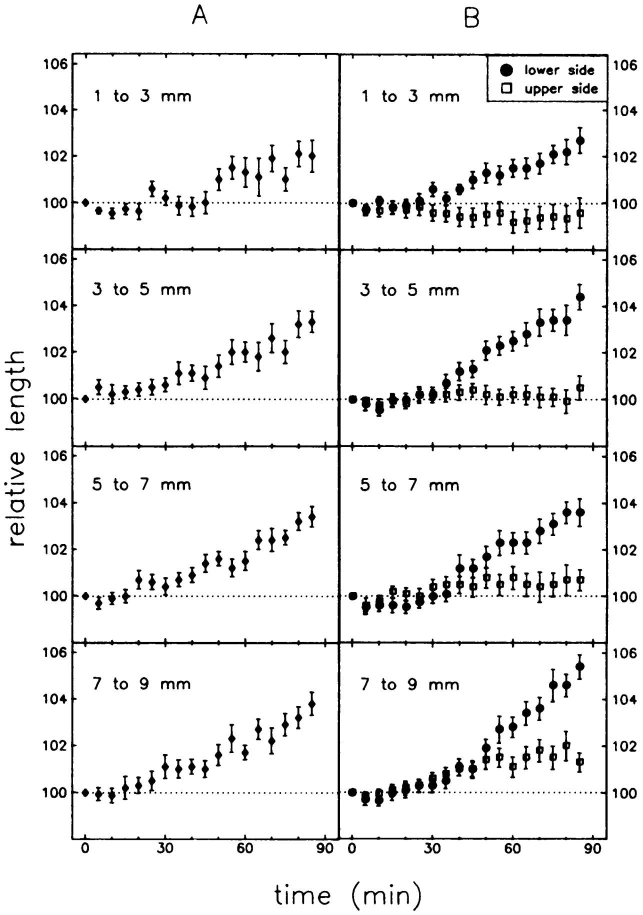

Figure 1. Comparison of growth rates in upright and horizontal plants over 90 min, for the apical 9 mm of the coleoptile. Column A: upright plants; error bars represent the SEM, $n = 12$. Column B: horizontal plants; open symbols represent the growth rate of the upper side of the coleoptile, and closed symbols represent the growth rate of the lower side of the coleoptile. Plants were turned horizontal at 0 min. Error bars represent the SEM, $n = 18$.

In the two most basal zones of the coleoptile, the differential in growth rate between the upper and lower sides seems to result primarily from the partial inhibition of growth of the upper half, while the growth rate of the lower half of the coleoptile is unchanged. So, although the differences in growth between the upper and lower sides of gravistimulated coleoptiles are approximately compensatory for most zones, the basal 4 mm of a coleoptile may experience a decrease in total growth rate during gravitropism.

Redistribution of IAA during Gravitropism

Figure 3 shows the progress of tritiated IAA down gravistimulated coleoptiles. Thirty minutes after the plants are turned

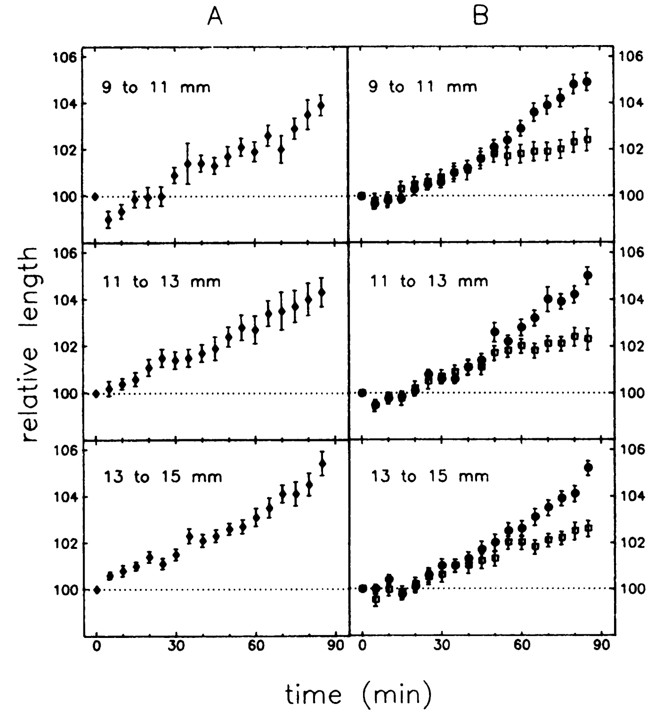

Figure 2. Comparison of growth rates in upright and horizontal plants over 90 min, for the basal 6 mm of the coleoptile. Column A: upright plants; error bars and *n* as in Figure 1. Column B: horizontal plants; symbols, error bars and *n* as in Figure 1.

horizontal, they are still transporting IAA symmetrically and at a normal rate (*cf.* the 50 min poststimulus time point in Fig. 3—representing 65 min of transport of tracer—with the 60 min time point for tracer IAA transport in Fig. 1 of ref. 18). However, between 30 and 40 min after the beginning of the stimulus, the amounts of tritiated IAA in the upper and lower halves of coleoptiles begin to differ. The region 6.6 to 7.7 mm from the tip has a 2:1 ratio of tracer IAA in the lower *versus* the upper sides at 40 min, and this difference is statistically significant ($P < 0.05$, one-tailed Student's *t* test). Although this is the only statistically significant difference at this time, the whole region 4 to 10 mm below the tip probably shows an elevated level of tracer IAA in the lower half of the coleoptiles. Between 40 and 50 min after the beginning of the stimulus, the apical 10 mm shows an approximately 2:1 ratio of ^{3}H-IAA, lower:upper side. By 60 min the lower half contains significantly more tracer than the upper half along the whole length of the 15 mm coleoptile, although the basal 3 or 4 mm of the coleoptile do not achieve as great a redistribution of label as do more apical regions. The approximately 2:1 pattern of tracer IAA distribution is maintained until at least 80 min after the beginning of the stimulus, the longest time interval we tested (data for that time point not shown).

Figure 4 shows the time intervals during which significant IAA redistribution occurred, compared to the growth rate redistributions. In all zones but one the IAA redistribution precedes the growth rate distribution by between 5 and 15 min. There is, however, one zone that appears to show a simultaneous redistribution of IAA and growth rates. The region approximately 3 to 5 mm from the apex first shows both redistributions at approximately 50 min after the beginning of the stimulus. We must emphasize, however, that we sampled the IAA distribution at 10 min intervals, while we sampled the growth rate distribution at 5 min intervals. Significant IAA redistribution in the 3 to 5 mm zone occurred between 40 and 50 min, and significant growth rate redistribution between 45 and 50 min, after the plants were turned horizontal. The IAA redistribution could, therefore, have preceded the growth rate redistribution by as much as 6 or 8 min, but the resolution of these experiments is not fine enough to tell whether the two events occurred simultaneously or at different times.

We could not compare tracer IAA distribution with growth redistribution in the apical 3 or 3.5 mm of the coleoptile because we could not accurately determine the distribution of tracer IAA in that region. We applied the ^{3}H-IAA as a drop to the tip of the coleoptile. The drop rested on the side of the coleoptile, dried onto it and partitioned into the cuticle. We did not control the side of the coleoptile, with respect to the seed, that received the label, and so did not control whether label seeped into the coleoptile pore. The variability of the

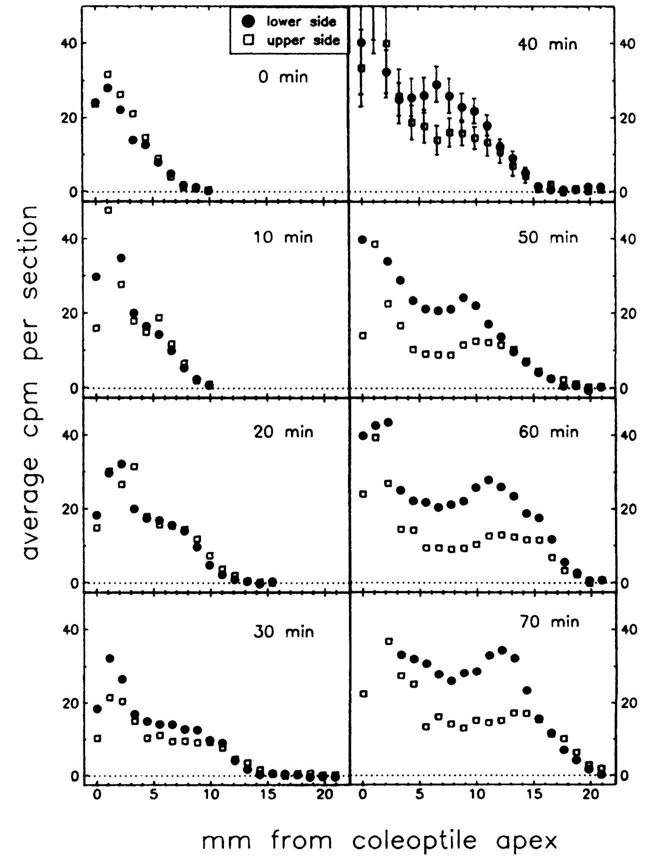

Figure 3. The transport of tracer IAA during gravitropism. Label was applied to coleoptile tips at −15 min. At 0 min, plants were turned horizontal. Open symbols represent the amount of tracer present in the upper half of an average coleoptile section. Closed symbols represent the amount of tracer present in the lower half of an average coleoptile section. Error bars (shown only for the 40 min time point) represent the SEM, $n = 25$.

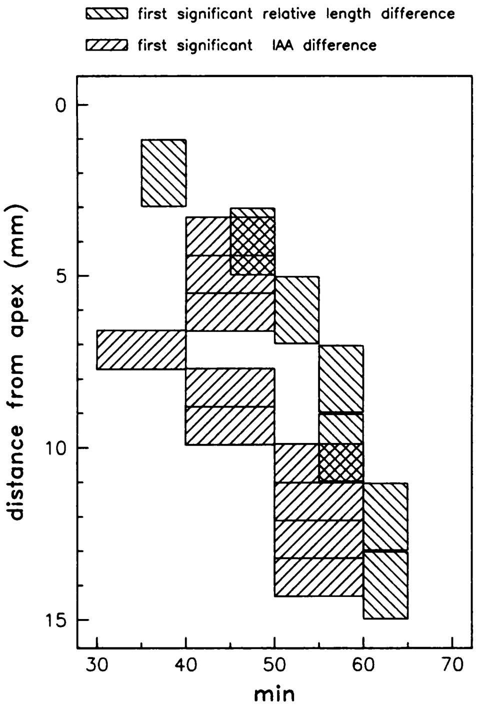

Figure 4. Comparison of the times and locations of the divergences of relative lengths and of tracer IAA concentrations. The longer bars represent the time periods during which the tracer IAA concentrations of the upper and lower sides of the coleoptile become statistically significantly different ($P < 0.05$, one-tailed Student's *t* test), and the regions of the coleoptile for which those determinations were made. For example, 30 min after the beginning of the gravitropic stimulus the tracer concentrations in the upper and lower sides are indistinguishable in the zone 6.6 to 7.7 mm from the tip of the apex, whereas at 40 min after the stimulus starts they are significantly different. The shorter bars represent the time periods during which redistributions of growth first become significant ($P < 0.05$, one-tailed Student's *t* test), and the regions of the coleoptile for which those determinations were made. For example, in the region 5 to 7 mm below the coleoptile apex, the relative lengths of the upper and lower sides become significantly different between 50 and 55 min after the start of the gravitropic stimulus, and in the 7 to 9 mm zone between 55 and 60 min. These data are consistent with a lag period of between 10 and 30 min between the IAA transport change and the subsequent growth redistribution.

radioactivity recovered from sections just under the site of label application was much larger than that recovered from further down the coleoptile, leading us to suspect that radioactivity counted in the apical three sections represented a substantial amount of ^{3}H-IAA outside of the transport stream, possibly in the pore, or dried onto the surface of the cuticle. Consequently, we cannot say how IAA in the transport stream in the apical 3 or 3.5 mm of the coleoptile is being transported either in control or gravistimulated seedlings.

Figure 4 contains one more interesting piece of information. The IAA transport redistribution generally precedes the growth rate redistribution. However, if the IAA redistribution is causing the growth rate redistribution, then the response times for different regions of the coleoptile may vary, with the zone 6.5 to 10 mm from the apex responding more slowly than more apical or more basal zones. However, because of the sampling intervals chosen for the growth experiments and the IAA redistribution experiments, the data shown in Figure 4 are also consistent with a lag time (between IAA concentration change and a resulting relative length change) of about 10 min.

Site of IAA Redistribution

The results of an experiment to test whether IAA is being redistributed within coleoptiles side to side, or whether IAA transport is being asymmetrically enhanced or inhibited at the coleoptile tip, are shown in Figure 5. The kinetics of the divergences in growth rates of the lower and upper sides (Figs. 1 and 2) argue against the latter. The first apical zone shows a clear divergence in growth rate between the lower and upper sides by 40 min (Fig. 1), and the most basal zone shows the divergence by 65 min (Fig. 2) after the beginning of the stimulus, an apparent basipetal rate of movement of the response of about 30 mm/h, considerably faster than the rate of IAA transport observed in these coleoptiles. As seen in Figure 5, it appears instead that IAA is redistributed laterally within the coleoptile, for the total amount of radioactivity in the coleoptile in the 5 to 10 mm region is the same in upright and in horizontal plants. In horizontal coleoptiles, however, the lower half of the 5 to 10 mm region contains almost twice the tritiated IAA of the upper half.

Test for IAA Immobilization

Finally, we were interested in knowing whether changes in growth pattern could result not only from the lateral transport of IAA, but also from its immobilization. This point is especially important in light of the apparent cessation of growth of the upper half of the apical 9 mm of the horizontal coleoptile (Fig. 1) while there is still measurable tracer auxin in that half (Fig. 3). The efflux of tritiated IAA from horizontal coleoptiles into water compared with that from upright co-

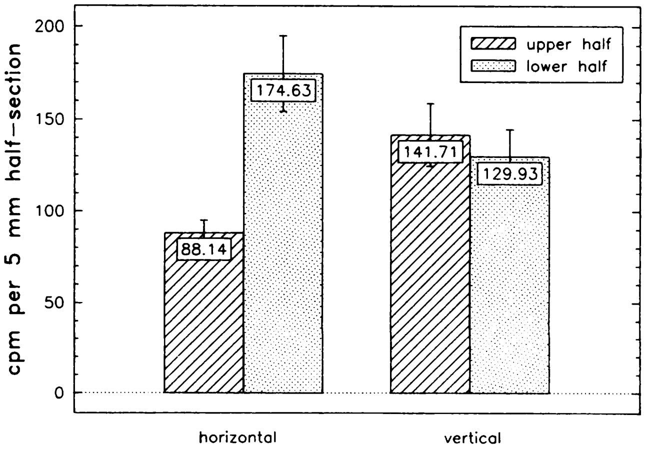

Figure 5. The distribution of tracer IAA in the 5 to 10 mm subapical zone 60 min after plants are turned horizontal. Plants were given label at −15 min. At 0 min, half the plants were turned horizontal. Bars represent the label in an average half-section. Error bars represent the SEM, $n = 63$.

leoptiles is shown in Figure 6. The two efflux curves are indistinguishable from each other. So during the first 80 min after maize coleoptiles are turned horizontal, at least in the apical 10 mm, they transport radiolabeled IAA down their lengths and out into water at the same flux as vertical controls. If there is any difference between the fluxes of tracer IAA in upright and horizontal coleoptiles after 80 min, it is not in the direction of immobilization of IAA away from the transport stream. Therefore, the apparent cessation of growth on the upper side of the apical 9 mm of a coleoptile responding to gravity may reflect the lowering of the concentration of endogenous IAA below the level needed to maintain detectable growth.

DISCUSSION

A maize coleoptile responds to gravity first by altering its transport of IAA, then by altering its growth pattern. The change in IAA transport is first statistically significant 40 min after plants are turned horizontal, in a zone 6.6 to 7.7 mm from the apex (although real redistributions of IAA are probably occurring in the region 5-9 mm from the apex by then). The transport differential spreads outward, apically and basipetally, until by 60 min after the plant is turned horizontal the whole 15 mm coleoptile shows an approximately 2:1 ratio of ^{3}H-IAA in the lower versus the upper half. This 2:1 ratio is stable until at least 80 min after the beginning of the stimulus.

A gravistimulated coleoptile next shows a redistribution of growth, with the upper side of the coleoptile growing slowly, if at all, relative to the lower side. In the apical 11 mm of the coleoptile, the growth rate redistribution is compensatory. In all zones between 5 and 15 mm below the apex, the redistribution of growth follows the redistribution of IAA transport by 5 min at the minimum. For example, in the zone 11 to 13 mm from the apex, the IAA transport change first becomes statistically significant between 50 and 60 min, while the growth differential between the upper and lower sides becomes significant between 60 and 65 min after the plants are turned horizontal, giving a response time of between 5 and 15 min (Fig. 4). This is comparable to the lag times reported before for the growth rate response of etiolated maize coleoptile sections to IAA added in aqueous solution—13 and 12 min (6, 7)—and to the 8 or 9 min lag time reported for the growth rate response of intact, red light-grown coleoptiles to IAA supplied to the epidermis in lanolin (3).

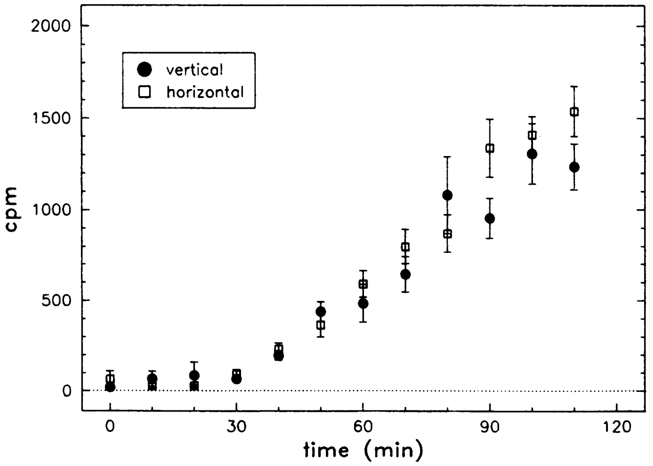

Figure 6. The flow of tracer IAA into water from the ends of cut coleoptiles in either upright or horizontal position. Coleoptiles were cut at 10 mm below the apex. At −15 min, plants were given label. Closed symbols represent label flowing from upright coleoptiles; open symbols represent the label flowing from coleoptiles turned horizontal at 0 min. Error bars represent the SEM, n = 17 to 19.

We were able to compare the first appearance of statistically significant growth differences with the first appearance of statistically significant tracer IAA redistributions because the standard errors on the determinations of the two functions were similar in magnitude. If anything, the errors on the determinations of tracer IAA content are larger than for the growth rate determinations, potentially leading us to underestimate the time between IAA redistribution and subsequent growth rate changes. We were unable to calculate accurate mean lag times because we sampled the IAA distributions too infrequently to obtain accurate estimates of when the IAA redistribution along the length of the plant began, relative to the start of the growth rate redistributions.

When ^{3}H-IAA is applied to intact coleoptiles, it enters the transport stream in the same total amounts in upright plants and in horizontal plants (Fig. 5). IAA is transported at the same flux in the two conditions—at least up to 80 min after the beginning of the stimulus, well after the differential growth that leads to gravitropic curvature is established—and is not significantly immobilized in either condition (see Fig. 6, and ref. 18). We conclude that conjugation or compartmentation of IAA away from the transport stream is not a significant cause of gravitropic curvature, at least for the apical 10 mm of the coleoptile. We have no information on whether endogenous conjugates of IAA are cleaved to liberate free IAA during gravitropism in these seedlings, but the lack of an overall stimulation of growth during gravitropism argues against that possibility.

If any significant changes in coleoptile sensitivity to IAA, or in the conjugation or compartmentation of IAA away from the transport stream are occurring during gravitropism in these seedlings, it is in the basal 4 mm of the coleoptile. In this region, there does not appear to be an increase in the growth of the lower side to balance the decrease in growth of the upper side. The total growth of this region during the first 90 min of gravitropic stimulus seems to be decreased by about a third, relative to the controls. So if IAA sensitivity is regulated during gravitropism, it may be decreased in this zone in response to a change in gravitropic orientation. Unfortunately, we did not test for IAA immobilization in this region.

Two major questions that have been asked are, first, whether changes in distributions of tracer IAA reflect changes in endogenous IAA content, and second, whether observed redistributions of IAA during gravitropism are sufficient in degree and timing to account for the correlated redistributions of growth. In response to the first question, the endogenous IAA content of red-grown maize coleoptiles has been measured, as has their efflux of endogenous IAA into water (M Iino, unpublished data). If one assumes that all the endogenous IAA in these coleoptiles is in the transport stream, the transport rate that one calculates from Iino's data for the concentration and flux of the endogenous IAA is about 12 mm per h—very close to the average rate of tracer IAA transport we measure. Although this kind of calculation is too coarse to use to demonstrate or rule out the presence of small pools of untransported IAA in the coleoptile, it indicates to us that at least the majority of the endogenous, free IAA in a coleoptile is in the transport stream. Because we can observe the transport and metabolism of tracer IAA in the transport stream, we conclude that we can therefore observe the transport and metabolism of the majority of the endogenous IAA in the coleoptile. The distribution of free, endogenous IAA in red light-grown maize coleoptile tissue during gravitropism has also been measured, and was found to be approximately 2:1, lower:upper side (M Iino, unpublished data), just as we report here for the distribution of tracer IAA during gravitropism.

In response to the second question, there are two kinds of evidence that observed redistributions are sufficient to account for observed growth rates during the development of tropic curvature. The first is that when IAA is supplied to intact, red light-grown maize coleoptiles, the coleoptiles respond with a linear increase in growth rate to linearly increasing concentrations of auxin over the range of growth rates normally found during gravitropism or phototropism (4). Beyond the range of normal growth rates, the incremental response to increasing exogenous IAA drops off, becoming more nearly exponential, or log linear, as if the tissue's capacity to transport or to respond to additional IAA is becoming saturated. The other, less direct, line of evidence is from experiments performed in light-grown sunflower hypocotyls. These seedlings can be decapitated, given different IAA concentrations on their right and left sides, and observed for development of curvature. They respond with significant curvature to gradients of applied IAA as small as 1:1.3. IAA applied in a gradient of 1:1.3 might be expected to produce more shallow internal IAA gradients, because decapitated sunflower seedlings still contain measurable, symmetrically distributed IAA. Internal IAA gradients smaller than 1:1.3 might, therefore, be expected to produce detectable curvature in sunflower seedlings (17).

It is clear that sensitivity to IAA may be an important, regulated component of a coleoptile's response to external stimuli. When etiolated maize coleoptiles are irradiated with very small amounts of red light, for example, their overall growth rate increases even as their free and diffusible IAA content decreases (2, 13). In this case, a change in tissue sensitivity to IAA may well be the proximate cause of the growth response. In etiolated oat seedlings, similar increases in coleoptile growth rates have been reported to result from exposing the seedlings to the dim green "safe-lights" commonly used in studies of gravitropism in etiolated seedlings (16). By both growing and experimenting with maize seedlings under standard red light conditions, we hoped to be able to separate any effect of light on the sensitivity of coleoptiles to IAA, from any effects of gravitational orientation.

Many of the issues we have discussed here are also relevant to the debate on how plants convert the sensing of a phototropic stimulus to a change in growth rates. To address this, we need to investigate IAA transport during phototropism in these same red light-grown maize coleoptiles.

ACKNOWLEDGMENTS

We thank Tim Short and Marta Laskowski for their good suggestions during the course of the experiments, and for their careful and critical readings of the manuscript.

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