Polarity of Indoleacetic Acid in Young Coleus Stems

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ABSTRACT
Young internodes of Coleus blumei Benth. have long been known for their sizable amount of acropetal indoleacetic acid movement. However, plants of the same clone, under improved growing conditions, now show almost absolute basipetal polarity of 14C-indoleacetic acid, as measured by liquid scintillation counting of 14C in the receiver cylinders of agar. The ratio of basipetal to acropetal movement is now as much as 85:1, instead of the 3:1 ratio found years ago under conditions providing slower growth.

Although auxins such as IAA were first known for their unvarying and strongly polar basipetal movement through the organs of etiolated seedlings such as Avena coleoptiles, later work showed that this movement was subject to regulation (e.g. 3, 4, 8). Furthermore, in green shoots of plants past the seedling stage, even cases of acropetal movement were discovered. The most thoroughly investigated of these are the 3:1 ratio of basipetal to acropetal IAA movement through sections cut from young internodes of Coleus (first found with bioassay measurements by Jacobs [5, 6], then confirmed with radioisotope counting of 14C-IAA by Naqvi and Gordon [23] and Leopold and de la Fuente [19]) and the increasing acropetal movement of IAA that occurs through sections of bean petioles of increasing age (20, 21). The auxin-type herbicide, 2,4-D, gave similar results in bean petioles (9, 14, 21).

Is this acropetal movement through excised sections merely an artifact of excision? For bean petioles the only evidence on this point comes from elongation measurements after such sections were provided with IAA from the base only or from the apex only: basally applied IAA produced relatively more growth as the petioles from which the sections were cut were progressively older (10, 21). For young Coleus internodes the evidence that the 3:1 ratio of IAA movement is meaningful has been related somewhat more closely to the intact plant: if vascular strands in matching internodes were cut, the regeneration of tracheary cells was mostly basipetal, but with some strands regenerating acropetally from the cut strand below the wound (5)—thus qualitatively paralleling the 3:1 ratio of IAA movement. Quantitative data in support of the physiological significance of acropetal IAA movement came from experiments in which all leaves below the regenerating internode were excised in one set of plants and that set compared with a set which had all leaves excised above the regenerating internode. Leaves had already been shown to be the main source of endogenous auxin in the Coleus shoot (5), hence those wounds presumably had auxin available to them only from above or only from below, respectively. The number of strands of tracheary cells that regenerated in a week under these two treatments averaged 16 versus 5.6—that is, the numbers were proportional to the ratio of the amounts of IAA moved in the two directions through transport sections from matching internodes (6, 7). Various lines of evidence support the view that the endogenous auxin of Coleus is IAA and solely IAA (2, 25). Because other quantitative data support the hypothesis that the amount of IAA available determines how many tracheary cells differentiate in Coleus (5, 7, 15, 27), the conclusion was drawn that the acropetal movement of IAA, observed in transport sections cut from internode #2, also occurred in the more nearly intact plant, where it caused the regeneration of the 5.6 strands of tracheary cells found 1 week after the leaves above the wound were excised (6).

To understand more about the polarity of hormone movement in plants we needed to investigate the characteristics of acropetal IAA movement in those few cases where it was known to occur in amounts sufficient to measure. A start was made with the time course of IAA movement in aged bean petioles, and statistical analysis of the linear regressions showed that IAA moved acropetally at the same velocity with which it moved basipetally through matching sections—only the slope of the linear regression lines was less for acropetal movement (10). Leopold and de la Fuente (19) arrived independently at the same conclusion about the velocities in the two directions, based on visual estimates of the intercepts of curved lines fitted “by eye” to their data points. Naturally, it was thought important to extend this investigation of acropetal IAA movement to Coleus #2 internodes, the one system for which the most quantitative data had been collected.

Accordingly, this paper investigates further the polarity of IAA movement in the young internode #2 of Coleus. A separate paper (1) reports on associated phenomena of xylem regeneration. (A preliminary report of some of these results has been published [11].)

MATERIALS AND METHODS

Plants of the Princeton clone of Coleus blumei Benth. were grown in the greenhouse under natural day lengths that were supplemented by continuous light from incandescent bulbs. All plants in a given experiment were of identical age (as measured by the days since the cuttings were started) and were furthermore selected for uniformity of length of the leaf-blade on the #2 leaves (defined as the leaves whose blades were between 60 and 100 mm long and which were the second pair of unfolded leaves below the apical bud). Plants were closely matched by leaf length (usually within 3 mm), and treatments were assigned within each matched set by a mathematically random method, so that no subconscious bias could influence which plant was used for a given treatment.

The polarity of movement of IAA was determined with IAA labeled in the carboxyl group with 14C, using the methods that have been standard in this laboratory for more than 10 years (10, 16, 22, 28). Transport sections 5 mm long were cut with a double-bladed cutter from the middle of #2 internode (the

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internode below leaf pair #2). Each section was placed in upright (normal) orientation on a cylinder of 1.5% agar, which was made up to contain 14C-IAA at 5 µg/ml if acropetal movement was being tested. An agar cylinder was then placed on the top cut surface of the transport section (the cylinder containing 14C-IAA if basipetal movement was being tested, otherwise being a plain agar receiver for the acropetally moving IAA). The concentration of 14C-IAA was selected so that basipetal movement would be saturated, as shown to occur for internode #2 for this clone at donor concentrations of 2 to 5 µg/ml (8, 22, 24). The exact concentration of 14C-IAA in the donor cylinders was routinely determined by counting five or more separate donors in the liquid scintillation counter, calibrating the counter for efficiency with 14C standard, then using the stated specific radioactivity of the 14C-IAA to calculate the concentration of IAA in the average “original donor.” The purity of the stock solution of 14C-IAA was regularly checked with paper chromatography and counting of the dried zones. The counter was about 90% efficient, but the efficiency was determined with each batch of counted vials. The average background (based on counts from plain agar blocks placed directly in the counting vials, and determined separately for each experiment) was subtracted from the gross count: it was about 40 cpm.

Statistical methods followed Snedecor (26), with the t test being used routinely where two samples were compared. The usual conventions were followed in calling the 5 to 2% level of probability “statistically significant” and the 1% level or beyond “highly significant.” Sample size was five, unless stated differently.

The 14C in the basal receivers was counted directly because it has been the general finding that the label is still all with IAA in cases of polar transport lasting 8 hr or less.

RESULTS

Movement of 14C-IAA Through Transport Sections Was Very Strongly Polar in a Basipetal Direction. Four full scale experiments were run, two in January 1974, and two in February 1975. One was run in a dark incubator at 26 C (our standard conditions for previous isotope transport studies). After 4 hr the average cpm in the basal receivers was 762 as contrasted to only 9 cpm in the apical receivers, for a polar ratio of 85:1, instead of the 3:1 expected. Because the 3:1 bioassay experiments of the 1950s had been run under laboratory lighting and temperature, I thought that illuminating the sections during transport might change the polarity of transport. (Significant changes in the amount of basipetal transport resulting from illumination had, in fact, recently been found [17].) Hence, the rest of the experiments were run under laboratory lighting. The first such experiment, run for 3 hr during the 1974 oil crisis at an ambient temperature of 16 C, showed 384 cpm in the basal receivers compared to 18 cpm in the apical, for a ratio of 21:1. The third experiment (run for 3.5 hr at an ambient temperature of 20 C and with laboratory lights providing 49 ft-c at table level) showed 211 cpm in basal compared to 8 cpm in apical receivers, for a ratio of 26:1. The fourth transport experiment was part of a vascular regeneration experiment (1) in which regeneration was checked at 0, 3, 5, and 7 days and transport polarity was checked at 4 days on matching, randomly assigned plants. After 3.5 hr at ambient temperature the basal receivers averaged 1,030 cpm, the apical ones 14 cpm, for a ratio of 74:1 (Table I).

Searching for other possible explanations as to why #2 internode failed showed that even 25 years ago should now seem to be so strongly polar, we checked the effect of excising all axillary branches. My recollection was that in the early experiments axillary branches were removed at potting time and kept off thereafter. This induced compensatory growth of the remaining shoot (12) and significantly speeded flowering (13). Such striking developmental effects might easily change hormone movement; too, so in one experiment the axillaries of one group of plants were excised 4 days before the transport (so that compensatory growth would have time to occur), while the axillaries of the other group were not excised until the afternoon of the day before the transport. The average cpm in the basal receivers of the two groups were identical at 211 cpm: the apical receivers, at only 6 and 8 cpm, respectively, above the background of 36 cpm, were of course not significantly different by statistical test.

The effect of early floral development was also checked by selecting from a group of the same age those plants that showed the first macroscopically visible sign of an inflorescence primordium. This group was compared with plants that looked clearly vegetative. All axillary branches were excised on day zero. At day 4, sections were cut from each of the two groups for transport tests. Sections from the plants with the very small inflorescence primordia showed no statistically significant difference in IAA transport from the sections from vegetative plants: basal receivers averaged 1,109 and 1,030 cpm, respectively; apical receivers, 11 and 14 cpm, respectively (Table I).

DISCUSSION

The polarity of movement of 14C added as carboxyl-labeled IAA was very strongly basipetally polar through excised sections of young internode #2, in contrast to my earlier results with

Table I. Polarity of movement of 14C from 14C-IAA through 5-mm sections cut from young internode #2 of Coleus, as measured by liquid scintillation counting of receiver and donor cylinders.

<table>
<thead>
<tr>
<th></th>
<th>Basipetal Movement</th>
<th>Acropetal Movement</th>
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<tbody>
<tr>
<td></td>
<td>ADcpm</td>
<td>Rcpm</td>
</tr>
<tr>
<td>Vegetative</td>
<td>47,429</td>
<td>1030 ± 90b</td>
</tr>
<tr>
<td>Inflorescence primordium</td>
<td>53,066</td>
<td>1109 ± 95</td>
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</tbody>
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*a = loss from donors in the 3.5 hr of the experiment; R = net gain in receivers during the 3.5 hr; donors at zero time = 261,874 ± 2903 cpm, which, in terms of the stated specific activity, the cylinder volume, and the LSC efficiency, represents a concentration of 5.3 ppm. Plain receivers gave 36 ± 1.1 cpm, the value subtracted as background from all other counts. Values of cpm are given as Mean ± S. Error. Sample size was 5. The length of blades #2 averaged 94.4 mm one day before the experiment started.

b The cpm in the vegetative receivers were not significantly different by the 't' test from those in the corresponding receivers from plants that had an inflorescence primordium macroscopically visible at the start of the experiment.
Coleus, where one-third as much IAA moved acropetally as moved basipetally (5-7), as well as to Naqvi's results with a different variety of C. blumei (22, 23), and to Leopold and de la Fuente's (19).

What caused the change in IAA polarity from 3:1 25 years ago to as much as 85:1 now, since I used sections cut from internodes of a specific developmental stage, all from the Princeton clone of C. blumei, in each case? One might guess at first that the bioassays used then, compared to 14C counting now, might explain the difference (e.g. perhaps IAA added to the basal end in the old experiments was transformed during acropetal passage into another compound, which showed auxin activity in the bioassay but had lost its carboxyl group—and hence would not be counted in the 14C assay). But this is very unlikely because Naqvi and Gordon (23) found the same 3:1 ratio of 14C-IAA movement through sections from the "Golden Bedder" variety of C. blumei by counting only the 14C that ran to the R, of IAA on chromatograms. Also, Figure 6 of Leopold and de la Fuente (19) shows similar sizable acropetal movement through sections cut from the second internode of C. blumei of 14C that had been added as 14C-IAA. Furthermore, the very strong polarity of 14C-IAA movement currently found in sections is paralleled by a strong polarity of tracheary regeneration (1) —just as such "xylem bioassays" confirmed the 3:1 polarity reported in the past.

The change in polarity is probably due to changing growing conditions. Plants of the Princeton clone of 25 years ago were grown in an old greenhouse with minimal supplementary illumination. Since the early 1960s the plants have been grown in a new greenhouse with a different orientation to the sun and with round-the-clock supplementary illumination provided. The extra light was needed to maintain a fast and uniform growth rate. Specific evidence of the faster growth produced under our current growing conditions comes from comparing the elongation of 24 mm/week recorded for leaf blades initially 87 mm long (5) with the 45 mm/week under current conditions (the 85 mm leaf of Phaseolus vulgaris, Am J Bot 41: 725-730). Also, Naqvi (22), who was the first to report on 14C-IAA movement in plants from this new facility, noted that sections from winter-grown plants of the Princeton clone of Coleus did not show the sizable acropetal movement that he observed in his more detailed studies of the "Golden Bedder" variety grown in the summer near Chicago —although differences in section length might have accounted for it, also. Veen and Jacobs (28) called attention to the fact that petiolar transport sections from plants of the Princeton clone grown in the new facility showed essentially absolute polarity of 14C-IAA movement, in contrast to the sizable acropetal movement reported by Leopold (18). The current experiments found no sign of increased acropetal movement from any of the following: (a) the presence or absence of the axillary shoots; (b) the presence or absence of macroscopic primordia of the inflorescence at the tip of the main shoot; (c) illumination of the sections during the transport as contrasted to putting them in a dark incubator (as was done in the first experiment reported above).

The relation of this changed IAA polarity to xylem regeneration is discussed in Aloni and Jacobs (1).

LITERATURE CITED


17. NAQVI SM 1963 Transport studies with C4-2,4-dichlorophenoxyacetic acid in Coleus stems. PhD thesis. Princeton University (No. 64-9144 from Univ Microfilms).


