Short Communication

Polar Movement of Gibberellic Acid through Young Coleus Petioles

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For several decades the only type of growth regulator believed to move with strong polarity through plants was the endogenous auxin indole-3-acetic acid (9, 15, 25). After it had been demonstrated that both naphthalene-ace tic acid (8, 16) and the weed killer 2,4-D also moved with strong polarity when tested under the same conditions as IAA (17, 18), investigations were started on other types of growth regulators.

Several cytokinins have been tested for polar movement through excised sections. The first reports of polarity in the movement of benzyladenine (1, 20) could not be confirmed in more detailed investigations by Fox and Weis (7). If kinetin moved at all through excised sections, it usually moved without polarity (14, 24). Adenine, a weak cytokinin, also moved without polarity through Coleus petiole sections (24).

The two authors who have studied gibberellin movement through excised sections concluded that gibberellin moved through pea stems, but without polarity. Kato (13) used primarily ultraviolet absorption to estimate the gibberellinic acid extracted from receiver blocks of agar. Presumably forced to do so because of the insensitivity of this method, he used extremely high concentrations of GA$_3$ in the donor blocks (1.4 grams per liter, in contrast to the milligram per liter concentration of GA$_3$ typically used in physiological experiments). Clor (3), purporting to confirm Kato, added tritium-labeled gibberellin of unstated purity, concentration, or even structure and then counted the label extractable from tissue slices. No evidence was presented that any of the label thus counted was still with the "gibberellinic acid."

Basipetally polar movement has been reported for both synthetic and natural abscisic acid isolated from tomato fruits in transport experiments with explants, petiole, and internode segments of Coleus blumei (6). Milborrow (19) has stated that abscisic acid labeled with radioisotope moves with 3:1 basipetal polarity through petiolar sections.

By analogy with the active transport systems studied in cell physiology (22), we could expect more than just two plant hormones to show polar movement. The two negative reports with GA$_3$ did not convince us that this expectation was unfounded—Clor's because of its cited inadequacies, and Kato's because the levels of GA$_3$ in the donor were so high. (The polarity of IAA movement, so striking at physiological levels, decreases as the donor concentration is increased [26].) In addition, although Kato reported extra absorption in extracts of both apical and basal receiver blocks at a wave length where gibberellinic acid absorbed, he could find no gibberellinic activity in extracts of similar receivers from 3-hr transports when he tested them in the dwarf corn bioassay.

Our plan was to add GA$_3$ at a low, physiological concentration to young Coleus petioles, as a tissue whose strongly polar auxin transport properties we had thoroughly investigated already (23, 27), and whose younger petioles we knew responded to physiological levels of GA$_3$ (10). We selected barley endosperm as the bioassay for GA$_3$ because of its requisite sensitivity and the physiologicalsimplicity of the reacting tissue.

MATERIALS AND METHODS

The barley endosperm bioassay for gibberellins, basically as described by Coombe et al. (4, 5), was used with a few modifications. The barley cultivar Himalaya was used in the experiments. Following a suggestion of Nicholls (personal communication), dry seeds were initially heated for 30 min at 50 C to improve uniformity of response. The seeds were then cut into halves. The endosperm halves, surface-sterilized for 1 hr in 1% (w/v) calcium hypochlorite, were spread as a single layer over sterile filter paper covered with a thin layer of autoclaved distilled water in a Petri dish and imbibed for 3 days in the refrigerator at 1.5 C. The response of the preimbibed endosperm halves to GA$_3$ was calibrated in each experiment with five or six known concentrations of GA$_3$ in 1.5% (w/v) agar, in addition to a blank agar control. The agar cylinders to be tested for GA$_3$ content, as well as the calibration cylinders containing known amounts of GA$_3$, were added directly, one to each bioassay vial, along with 1 ml of distilled water, then autoclaved for 18 min at 120 C. Two preincubated endosperm halves were then added to each vial. Three replications, each with two endosperm halves, were used for each determination. A 24-hr incubation at 30 C was found to be sufficient for sizable production of reducing sugars by the endosperm halves, as measured by optical density in the Nelson-Somogyi sugar test, read at 560 nm in a Cary spectrophotometer. Efficiency and reproducibility were improved by reading the unknowns from a graph of absorbance against GA$_3$ calibrations, thus omitting the extra calibration of absorbance against reducing sugars that Coombe et al. suggested.

The movement of GA$_3$ through transport sections was checked by the same general methods used earlier in this laboratory for studies of auxin and cytokinin transport. Five-millimeter sections were cut from the middle of young number 3 petioles (23) of plants of the Princeton clone of Coleus blumei Benth. and were assigned treatments on a mathematically random basis. Small

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3 All the curves in Kato's Figure 1 are labeled incorrectly, judging by the description in his text.

4 Abbreviation: GA$_3$: gibberellic acid.
cylinders of 1.5% (w/v) agar (2.85 mm long and 5.0 mm diameter) were placed on each cut end to act as donors (if they contained 0.1 μg of GA₃ or receivers (if they contained no GA₃). Sections were oriented so that movement of the added GA₃ was always against gravity (i.e., the sections used for basipetal movement were inverted). Also inverted were the control sections, which had plain agar cylinders on both cut ends to correct for both whatever endogenous gibberellins might move out of the sections and gibberellin activity in the agar itself. Three transport sections were used for each treatment, with movement tested for 3 hr in moist Petri dishes in a dark incubator at 26 C.

Preliminary experiments run on young internode sections used methanol extracts of the agar cylinders with or without subsequent partitioning in ethyl acetate as described by Jones and Phillips (11). The averages indicated basipetally polar movement as we expected by analogy with the 3:1 ratio of basipetal to acropetal movement found in our earlier IAA studies. However, the results were too variable to reach satisfactory levels of probability in statistical tests (abstract by Greenblatt and Jacobs, 1966, Plant Physiol. 41: xxxiii). Because ethyl acetate residues have since been found to increase the level of reducing sugars (2, 4), we tried to avoid such artifacts. In addition, we decided to switch to young petioles, which we had found to be more polar in IAA movement than were the internodes.

For the thin layer chromatograms we used silica gel that had not been activated by heating, chloroform-ethyl acetate-acetic acid solvent (60 + 40 + 5), a spray of concentrated sulphuric acid and ethanol (5 + 95), and heating to 120 C. The expected separations and colors are shown in Tables 92 and 93 of Kaldewey (12).

The t test was used for estimating statistical significance.

RESULTS

The pooled averages of the 11 experiments are shown in Table I. Vanishingly small amounts of GA₃ activity were found in either the apical or basal control receivers; the values were typically not significantly different from those obtained with the plain agar blank in the corresponding GA₃ calibrations.

A striking polarity of movement of the added GA₃ was manifest in the transport receivers. At least 10 times as much GA₃ moved basipetally as acropetally. The difference was statistically significant at the 2% level of probability. The net increase in 3 hr in the amount of GA₃ activity in the basal receivers represented 0.7% of the 100 ng added in the apical donors. In the apical receivers the amount of GA₃ after 3 hr was so small that in individual experiments it was usually not significantly different statistically from the plain agar calibrations. After subtraction of the GA₃ activity found in the apical control receiver, only 0.07 ng of GA₃ remained in the apical "transport" receiver.

Further evidence that the "gibberellin activity" found in the basal receivers by bioassay was indeed due to GA₃ came from thin layer chromatography. Tentatively assuming that all the gibberellin activity in the basal receivers was from GA₃ and taking 0.7 ng as the average amount of GA₃ to be expected in each receiver (Table I), we calculated that 260 basipetal transporters would need to be set up to increase the total GA₃ to a level detectable by faint fluorescence in ultraviolet light. When methanolic extracts of 260 frozen receivers (11) were run on a thin layer chromatogram, fluorescence was found of the expected color, intensity, and Rₜ for GA₃. The fast development of fluorescence after spraying and heating (12) confirmed that the extracts contained GA₃ rather than GA₁ (which is not well separated from GA₃ by Rₜ in the solvent used). Evidence that these physical and chemical properties typical of GA₃ were still associated on the chromatogram with the biological activity of gibberellin was found by checking various zones of another chromatogram with the barley endosperm assay. The ethyl acetate fraction of an extract of 20 basal receivers showed gibberellin activity only at the Rₜ of GA₃. (Roughly half as much gibberellin activity was found in the aqueous phase of the extract, all remaining at the origin.)

The minute amounts of GA₃ moved into the apical receivers were not due to lack of uptake from the basal donor cylinders. This is indicated by the rough values for GA₃ activity in the halves of the sections (Table I). These values are not quantitatively comparable with those from the agar cylinders, because only 75 to 85% of the GA₃ added to such sections was recovered. However, they show that substantially more GA₃ was in both types of treated sections than in the control sections; the amounts found in the section halves near the donor being highly significantly larger than in the corresponding control halves.

An unexpected result was the values for the donors after 3 hr of "transport." Instead of decreasing in gibberellin content, as we would expect from both the earlier results with other growth regulators and the increased GA₃ in the treated sections and receivers after 3 hr, the apical donors contained as much or more gibberellin activity at the end as at the beginning of the experiment. This was particularly striking with the basal donors, which after 3 hr showed gibberellin activity equivalent to 134 ng of GA₃ in the barley endosperm assay. The most obvious interpretation is that some substance moves from the sections into the donor agar cylinders and interacts with the GA₃ there to give increased activity in the barley endosperm assay. Preliminary tests support this view, and further experiments on this topic are in progress.

In conclusion, when GA₃ was added at physiological levels to transport sections cut from Coleus petiolar tissue of a developmental stage known to respond to GA₃ and to show strong polarity of IAA movement, a strongly basipetally polar movement of GA₃ occurred.

**Note Added in Proof.** The following paper has recently reported that gibberellic acid, did not show polarity in movement through the

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### Table 1: Amount of Gibberellin Activity (Calculated as ng GA₃ Found in Each Agar Cylinder and Petiolar Section Half 3 hr after 100 ng GA₃ Added in Each Donor Cylinder (1.8 × 10⁻⁶ g/ml).

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>MOVEMENT OF ADDED GA₃</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Basipetal</td>
<td>Acropetal</td>
<td></td>
</tr>
<tr>
<td>Basal receiver</td>
<td>0.08 ± 0.02</td>
<td>0.70 ± 0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Section's basal half</td>
<td>0.35</td>
<td>±0.23</td>
<td>0.73</td>
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<tr>
<td>Section's apical half</td>
<td>0.40</td>
<td>±0.18</td>
<td>±0.32</td>
</tr>
<tr>
<td>Apical receiver</td>
<td>0.17</td>
<td>±0.43</td>
<td>±0.36</td>
</tr>
<tr>
<td></td>
<td>100.65</td>
<td>134.47</td>
<td>0.05</td>
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<tr>
<td></td>
<td>±16.05</td>
<td>±25.87</td>
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1 Control values subtracted.

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LITERATURE CITED