Plant Physiol. (1984) 76, 347–352
0032-0889/84/76/0347/06/$01.00/0

Polar Calcium Flux in Sunflower Hypocotyl Segments[1]

I. THE EFFECT OF AUXIN

Received for publication February 1, 1984 and in revised form May 28, 1984

C. C. DE GUZMAN AND R. K. DELA FUENTE*
Department of Biological Sciences, Kent State University, Kent, Ohio 44242

ABSTRACT

The flux of Ca^{2+} at the apical or basal ends of short sunflower (*Helianthus annuus* L.) hypocotyl segments was monitored using a Ca^{2+}-specific electrode. A higher Ca^{2+} efflux was observed at the apical end relative to the basal end, indicating a net polar flux of Ca^{2+}. The extreme low mobility of Ca^{2+} in the isolated segment makes it likely that the observed Ca^{2+} fluxes are of localized origin, that is, from the parenchyma cells close to the exposed cut ends and may represent acropetal transport of Ca^{2+} at the cellular level. The rate of Ca^{2+} efflux depended on the concentration of Ca in the seedling medium. Incubation of hypocotyl segments in 10 mM $CaCl_2$ for 24 h did not eliminate the net acropetal flux of Ca^{2+} at the apical end.

IAA, as well as the synthetic auxin α-naphthaleneacetic acid, significantly enhanced Ca^{2+} efflux; the non-auxin analog, β-naphthaleneacetic acid, was ineffective. The transport of auxin, not merely its presence in the medium, was found to be a requisite for the enhancement of Ca^{2+} efflux since the presence of the auxin transport inhibitor 2,3,5-triiodobenzoic acid eliminated the auxin-promoted Ca^{2+} efflux. A model for how auxin promotion of Ca^{2+} efflux could play a role in promoting subsequent auxin secretion is proposed. Calcium probably serves as a 'second messenger', as it does in the secretion of various substances by animal cells.

One of the early indications of a polarity of Ca movement in plants was the finding that an apex-to-apex graft of the lateral stems of two tomato plants presented a barrier for the movement of ^{45}Ca, but not for ^{32}P (2). Indications of an interrelationship between the movement of Ca and the polar basipetal movement of IAA were shown by the findings that the auxin transport inhibitor, TIBA,[2] sprayed on the shoot, prevented the acropetal movement of Ca to young apple fruits (27) as well as to young stems and leaves in peas and beans (30). Subsequently, it was shown that the basipetal transport of IAA was significantly reduced in sunflower hypocotyl segments washed with the Ca chelator, EDTA (6). Furthermore, in gravitropically stimulated coleoptile and hypocotyl segments, ^{45}Ca moved to the slow-growing upper side (25), while ^{42}K and ^{32}P moved to the fast-growing lower side, as does IAA (11). These findings suggest that Ca and IAA move in opposite directions in gravistimulated shoot organs.

Work in our laboratory has set out to probe further the relation between Ca^{2+} and IAA transport. The divalent cation requirement in IAA transport is much more specific for Ca^{2+} with segments from seedlings grown in Ca-deficient medium (4) than for EDTA-washed segments (6). Data on the relationship between IAA transport and other elements, such as boron, an element related to Ca nutrition, will be forthcoming. In this paper, we present evidence for a polar flux of Ca^{2+} in short hypocotyl segments of sunflower that is enhanced by IAA but inhibited by the auxin transport inhibitor, TIBA.

MATERIALS AND METHODS

Plant Material and Growing Conditions. Sunflower (*Helianthus annuus* L. cv 'Russian Mammoth'; L. L. Olds Seed Co., Madison, WI) seeds were germinated on wet paper towels overnight. Approximately 60 seeds were selected for good radicle break and then placed on fiberglass netting stretched over plastic dishes with about 450 ml one-fifth strength Hoagland solution (1 mM Ca), unless indicated otherwise. The dishes with seeds were placed in a transparent tray lined with wet paper towels at the bottom. A transparent plastic cover placed ajar over the tray provided for medium humidity and aeration.

The seedlings were grown under a bank of fluorescent and incandescent lamps with a net intensity of 13.5 $J \cdot m^{-2} \cdot s^{-1}$ at the level of the cotyledons; the light regime was 16 h light/8 h dark. The temperature in the growth chamber was about 30°C when the lights were on and about 25°C when off.

Preparation of Stock Solutions. Measured amounts of IAA, TIBA, and α- and β-NAA were dissolved in 0.30 ml of 1 N KOH and made up to 250 ml using deionized distilled H_2O to yield stock solutions of 1 mM. The pH of the solutions was adjusted to 5.7 to 5.9 with 1 N HCl. In the course of the experiments, it was found that potassium could stimulate Ca^{2+} flux; since then, care was taken to equalize the K^+ concentration in the hypocotyl segment media.

Determination of Endogenous Ca^{2+} Flux. A 2-cm hypocotyl segment was taken from each seedling, beginning about 1 cm below the cotyledonary node, washed in deionized distilled H_2O for 1 h and gently blotted dry before it was transferred to the treatment solution. Each replication consisted of 20 segments held in a vertical position by embedding 1 to 2 mm of either the apical or basal end of the segment in lanolin in planchets attached to glass slides. The segments were then inverted into small plastic beakers containing 3 to 10 ml solution, henceforth called 'segment medium'. Only about 1 to 2 mm of the segments came in contact with the segment medium which always contained an initial concentration of 10 μM $CaCl_2$. The segments were placed inside a moist chamber and kept in the dark at 26°C.

At periodic intervals, or at the end of the experiment, the segment medium was monitored for Ca^{2+} using a Ca^{2+}-specific electrode (Orion model 93-20) attached to an Orion Research Microprocessor Ionalyzer model 901. The response of the electrode was linear between 4 and 1000 μM $CaCl_2$. The Ca^{2+} level of most of the samples fell between 10 and 100 μM. Standard solutions were prepared from Orion Research Calcium Standardizing Solution 92-20-06.

[1] Supported by National Science Foundation Grant PCM 78-04920.

[2] Abbreviations: TIBA, 2,3,5-triiodobenzoic acid; NAA, naphthaleneacetic acid.

The experiments were run in triplicate unless noted otherwise. The values given in the figures are means of the replicates; vertical lines represent the SE.

RESULTS

Rationale for the Methods. Evidence indicates that the Ca^{2+} in the extracellular space is several orders of magnitude higher than that present in the cytosol (3, 31). This soluble extracellular Ca^{2+} in the water-free space is in equilibrium with Ca^{2+} present on the exchange sites of the cell wall and the external surface of the plasmalemma. The nonexchangeable Ca bound to the cell wall or deposited as insoluble salts (1) would be of little significance to the putative role of Ca^{2+} as a second messenger and is not considered here.

The main objective is to determine whether there is a polar flux of Ca^{2+} in the isolated hypocotyl segment. We hypothesized that the promotion of basipetal auxin transport by Ca^{2+} (4, 6) could mean that Ca^{2+} may be moving in an acropetal direction. If such were the case, then there should be more Ca^{2+} close to the apical end relative to the basal end. Furthermore, because of the extracellular nature of most of the Ca^{2+}, it would tend to diffuse and equilibrate with an aqueous solution in contact with the exposed ends of the segment. With the use of a Ca^{2+}-specific electrode, a direct reading of the Ca^{2+} in the segment medium can be made and should more or less indicate the status of Ca^{2+} in the free space, at least of the tissues close to the exposed ends. These relationships are shown in Figure 1.

Polarity of Endogenous Ca^{2+} Flux. An example of the changes in Ca^{2+} concentration in the segment medium as a result of Ca^{2+} flux at the apical or basal ends of the segments is shown in Figure 2. During the first 2 h, both apical and basal ends of the segments absorbed Ca^{2+} from the segment medium. However, at about the 3rd h, a steady increase in Ca^{2+} concentration was observed in the apical segment medium. By 12 h Ca^{2+} efflux at the apical end had increased the concentration in the medium by about 90 nmol Ca^{2+}/20 segments. The basal ends, on the other hand, did not show Ca^{2+} efflux until 12 h for a net of about 15 nmol Ca^{2+}/20 segments.

The actual kinetics of apical and basal Ca^{2+} flux were found to be different in different experiments depending on the initial Ca content of the segments and the treatments imposed during the experiment. The averages from many experiments, with several treatments, comparing apical and basal Ca^{2+} fluxes (Tables I and II) show that Ca^{2+} efflux at the apical end is significantly greater than at the basal end. This indicates the possibility that the movement of Ca^{2+} in the hypocotyl segment is acropetal.

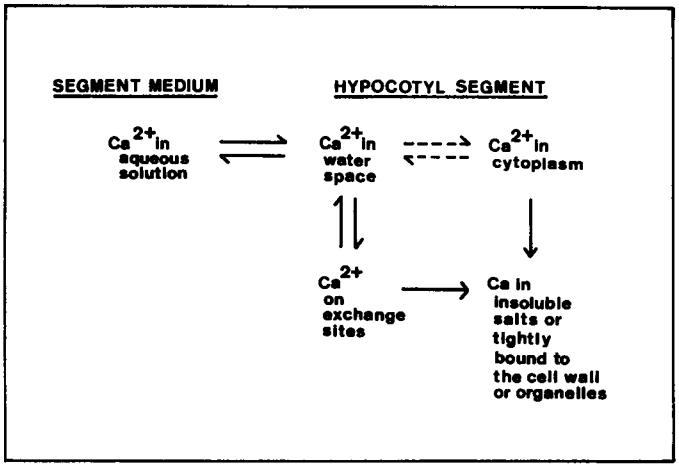

FIG. 1. Calcium compartmentation in the hypocotyl tissue and relationship to segment medium.

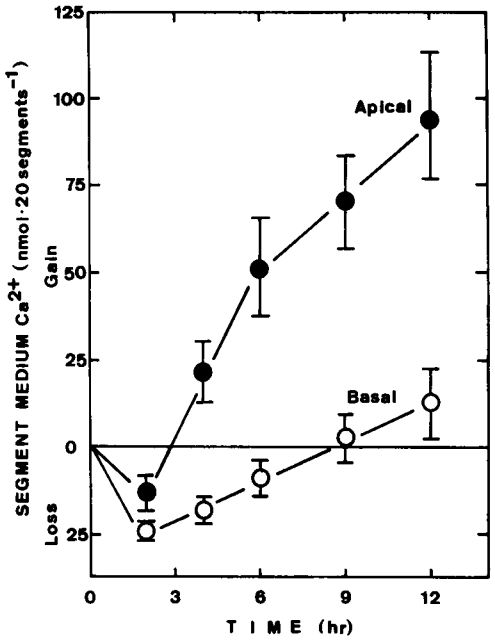

FIG. 2. Apical and basal flux of Ca^{2+} in 2-cm hypocotyl segments of 7-d-old sunflower grown in vermiculite. The seedlings were watered once with full-strength Hoagland solution and tap water for the rest of the growing period. The segment medium (5 ml) contained 10 μM $CaCl_2$. At the indicated intervals, the segments were lifted from the solution and the Ca^{2+} content of the segment medium determined with the Ca^{2+} electrode before returning the segments back to the same solution.

Table I. *Effect of Exogenous K^+ on IAA-Promoted Ca^{2+} Efflux*

Segments were taken from 4-d-old seedlings grown either in one-fifth strength Hoagland or 1 mM $CaCl_2$ solution. Values given are means of 11 separate experiments followed by SE. The K^+, when added, ranged from 0.4 to 1.4 mM.

Segment Medium	Ca^{2+} Efflux			
	Control		+ IAA	
	$-K^+$	$+K^+$	$-K^+$	$+K^+$
	nmol (20 segments·h)$^{-1}$			
Apical	13.4 ± 1.4	17.2 ± 1.7	21.1 ± 2.4	28.6 ± 2.7
Basal	7.2 ± 0.9	11.9 ± 1.4	13.2 ± 1.7	18.4 ± 1.9

Table II. *Effect of TIBA on the IAA-Promoted Ca^{2+} Efflux*

Segments were obtained from 4-d-old seedlings grown in one-fifth strength Hoagland solution. All segment media contained K^+ at a concentration range of 0.4 to 1 mM. Concentration of TIBA was 100 μM while IAA was 10 μM. Values given are means of several separate experiments followed by SE.

Treatment	Ca^{2+} Efflux	
	Apical (10)[a]	Basal (6)[a]
	nmol (20 segments·h)$^{-1}$	
Control	22.4 ± 0.7	15.4 ± 1.7
IAA	37.1 ± 1.4	25.6 ± 3.2
TIBA	25.4 ± 0.9	17.2 ± 1.7
IAA + TIBA	25.4 ± 2.4	19.2 ± 1.9

[a] Number in parentheses refers to number of separate experiments.

In the above experiments, the segments were in the normal orientation (apical end up, basal end down) only in the case where basal Ca^{2+} flux was being monitored. We have modified the above experiments to test whether the orientation of the

segments had an effect on the observed polarity of Ca^{2+} flux. In the modified experiments only one set of segments, oriented in the normal position, was used to measure both apical and basal Ca^{2+} flux. This modified procedure has the advantage of using only half as many hypocotyl segments and paired data comparison is possible; however, it is much more difficult to manipulate and is unwieldy for routine experiments. Nevertheless, results from such experiments showed the same higher rate of Ca^{2+} efflux at the apical end as compared to the basal end (manuscript in preparation).

Calcium Concentration in the Seedling. To determine the effect of Ca^{2+} concentration in the seedling on the rate of hypocotyl Ca^{2+} flux, the seedlings were grown in distilled H_2O with different concentrations of $CaCl_2$. In this experiment the hypocotyl segment medium containing 10 μM $CaCl_2$ was changed every 2 h to minimize the effect of changes in Ca^{2+} concentration on the rate of hypocotyl Ca^{2+} flux.

In general, when higher concentrations of Ca were provided in the root medium, the influx or absorption of Ca^{2+} at the basal end of the hypocotyl segment was lessened, while Ca^{2+} efflux at the apical end was increased (Fig. 3). At 10 μM $CaCl_2$ in the root medium, both apical and basal ends of the hypocotyl segment absorbed Ca^{2+} from the segment medium. Still, the lesser absorption at the apical end is consistent with the interpretation of a flux of Ca^{2+} in the hypocotyl from the basal to the apical end. At 1.0 or 0.1 mM $CaCl_2$ in the root medium, a net apical Ca^{2+} efflux in the hypocotyl segment medium was observed.

Calcium Gradient in the Hypocotyl Segment. A possible explanation for the higher efflux of Ca^{2+} at the apical end of the hypocotyl segments is that the Ca^{2+} concentration may be higher near the apical end due to its proximity to the Ca reserve in the cotyledons. An attempt was made to nullify the effect of such a gradient, if it exists, by incubating the uppermost 3-cm segments for 24 h in 10 mM $CaCl_2$. After incubation, the middle 2 cm portion of the segment was excised and washed in deionized distilled H_2O for 1 h. The results (Fig. 4) show a greater Ca^{2+} release from the pretreated segments compared to segments not incubated in Ca^{2+} (Figs. 2 or 3). The data also show that incubation in a high concentration of Ca^{2+} may have lessened the apical/basal ratio of Ca^{2+} efflux but did not eliminate the higher rate of Ca^{2+} efflux at the apical end.

Effect of Auxin on Ca^{2+} Efflux. It has been shown that the concentration of Ca^{2+} in the sunflower hypocotyl segments affects the rate of basipetal auxin transport (4, 6). The reciprocal effect of IAA on Ca^{2+} efflux is shown in Figure 5. The control segments show the usual difference between apical and basal Ca^{2+} efflux; the addition of 10 μM IAA to the segment medium significantly enhanced both apical and basal Ca^{2+} efflux. Table I shows Ca^{2+} efflux into segment media with or without IAA taken from 11 separate experiments that involve other treatments; the presence of IAA significantly increased Ca^{2+} efflux in the presence or absence of exogenous K^+.

The promotive effect of IAA on Ca^{2+} efflux can also be obtained with the synthetic auxin α-NAA (Fig. 6), the growth-promoting activity of which, as well as its polar transport, is similar to that of IAA (13). In contrast, its structural analog β-NAA, which lacks auxin activity, has virtually no effect on apical Ca^{2+} efflux.

The Effect of K^+ on Ca^{2+} Efflux. A further enhancement of Ca^{2+} efflux was observed when K^+ was added to the segment media with or without IAA (Table I). The effect of K^+ appears to be a displacement of Ca^{2+} from exchange sites (Fig. 1) since the divalent cations Mg^{2+} and Sr^{2+} were later found to be more effective than an equivalent molar concentration of K^+ (data not shown).

Effect of TIBA on Ca^{2+} Efflux. As seen earlier the presence of auxin in the segment medium promotes the net Ca^{2+} efflux. To test whether simple contact of the cells with IAA is sufficient to

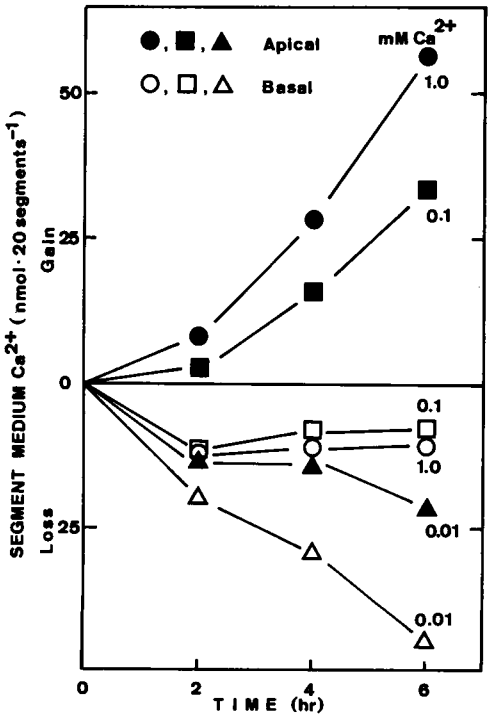

FIG. 3. Effect of Ca^{2+} concentration in which the seedling grew on Ca^{2+} flux. The segments were taken from 4-d-old seedlings grown in deionized distilled H_2O with the indicated levels of $CaCl_2$. The segment medium (10 ml), containing 10 μM $CaCl_2$, was changed with fresh solution at 2 and 4 h.

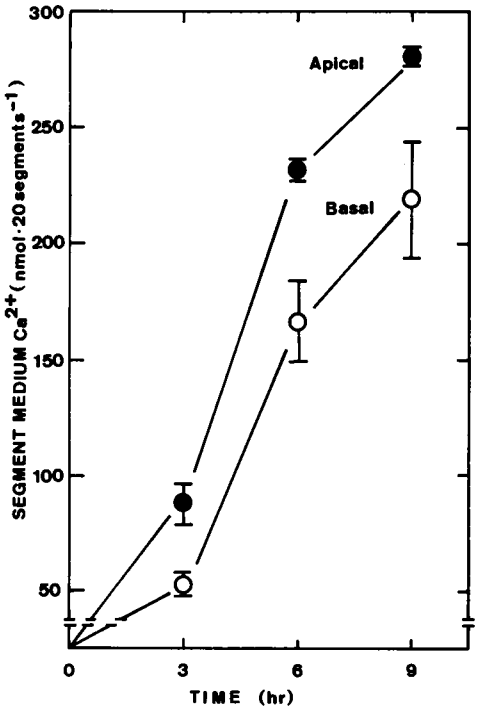

FIG. 4. Effect of pretreating segments in 10 mM $CaCl_2$ solution for 24 h on Ca^{2+} efflux. The seedlings were grown in 1 mM $CaCl_2$. Segments (3 cm) were taken from 4-d-old seedlings and incubated in 10 mM $CaCl_2$. After 24 h, the middle 2-cm segment was obtained, washed in deionized distilled H_2O for 1 h, and Ca^{2+} loss to the segment medium was determined as usual. The segment medium (5 ml) with an initial concentration 10 μM $CaCl_2$ was not changed during the course of the experiment.

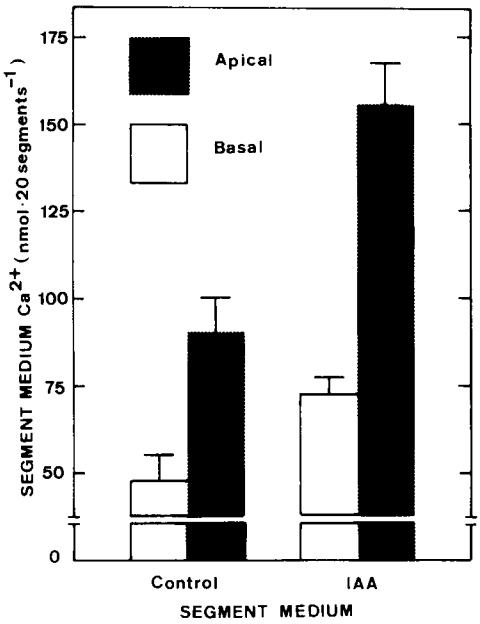

FIG. 5. Effect of IAA on apical and basal Ca^{2+} efflux. The segments were taken from 4-d-old seedlings grown in 1 mM $CaCl_2$. The segment medium (5 ml) contained 10 μM $CaCl_2$ and 10 μM IAA when added. The medium was monitored for its Ca^{2+} content after 9 h.

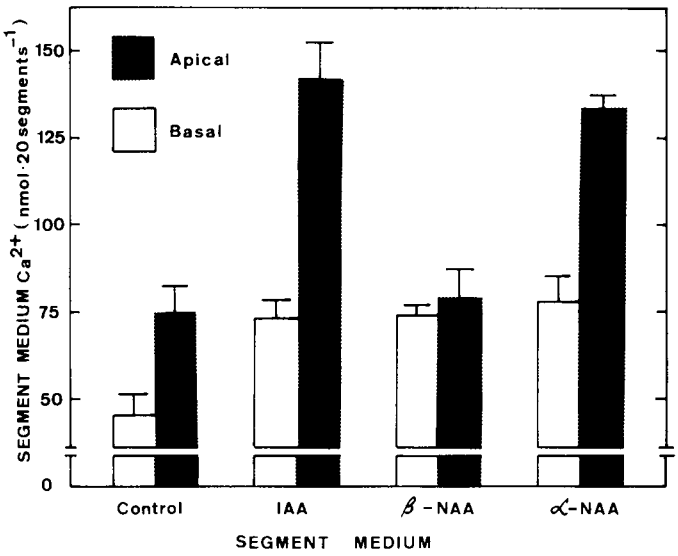

FIG. 6. Comparison of the effects of IAA and the α and β isomers of NAA on apical and basal efflux of Ca^{2+}. Segments were taken from 4-d-old seedlings grown in one-fifth strength Hoagland solution. Segments were preincubated with 1 mM KCl for 2 h. All segment media (3 ml) contained 1 mM KCl and 10 μM $CaCl_2$. IAA, α-, and β-NAA concentrations were 10 μM. Ca^{2+} was determined in 8 h.

stimulate Ca^{2+} efflux or whether the actual transport of IAA is required, the auxin transport inhibitor, TIBA, was used. Results from one of the experiments are shown in Figure 7.

The addition of TIBA alone to the segment medium has a slight but consistent promotive effect on Ca^{2+} efflux by about 10% (Fig. 7; Table II). As usual the presence of IAA promoted Ca^{2+} efflux. When both IAA and TIBA were added to the segment medium, Ca^{2+} efflux was reduced to the same level as that of TIBA alone.

DISCUSSION

Ca^{2+} in sunflower hypocotyl segments, believed to be mostly extracellular, tends to equilibrate with an aqueous medium

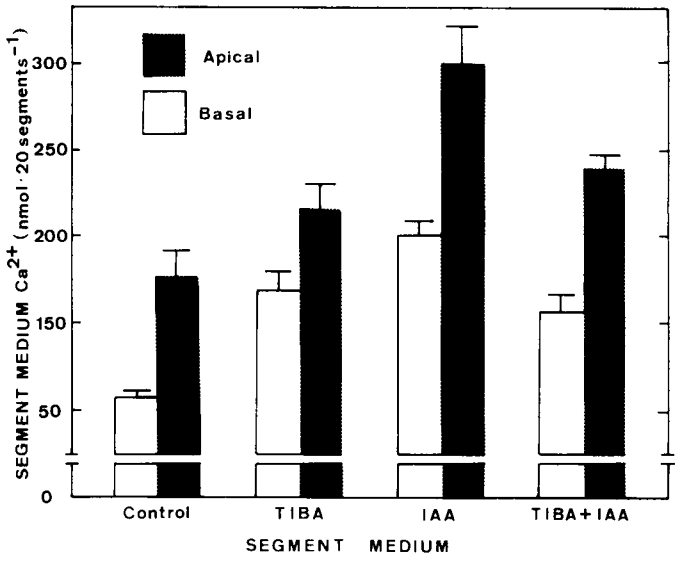

FIG. 7. Effect of 2,3,5-TIBA on the IAA-induced Ca^{2+} efflux. Ca^{2+} was determined in 10.5 h. Segments were taken from 4-d-old seedlings grown in one-fifth strength Hoagland solution. All segment media (7 ml) contained 0.44 mM KCl and 10 μM $CaCl_2$. The concentration of IAA was 10 μM while TIBA was 100 μM. Each column represents an average of three replicates for apical efflux and two for basal efflux.

through the cut ends. Hypocotyl segments from seedlings cultured in low concentrations of Ca (10 μM) absorb Ca^{2+} when incubated in 10 μM Ca, while segments from seedlings cultured in higher Ca concentrations (0.1 or 1.0 mM) release Ca^{2+} into the segment medium containing 10 μM (Fig. 3). The results indicate the rate of Ca^{2+} efflux was higher at the apical end relative to the basal end, indicating the existence of a net acropetal Ca^{2+} flux. The higher rate of Ca^{2+} efflux at the apical end cannot be accounted for by assuming an initially higher concentration of Ca^{2+} at the apical end because of its proximity to the reserve Ca in the cotyledons. The incubation of the segments in 10 mM Ca, in order to nullify any Ca^{2+} gradient, did not cancel the net acropetal Ca^{2+} flux (Fig. 4). Furthermore, an acropetal Ca^{2+} flux was also observed in corn coleoptile and pea epicotyl segments (data not shown). In these organs, the basal end is closer to the reserve Ca. These observations, together with the absence of any transpiring surface or mobilizing centers attached to the 2-cm segments, indicate that the observed acropetal Ca^{2+} flux, presumably driven by cytoplasmic activity, arises from polar, acropetal Ca^{2+} transport. The net acropetal Ca^{2+} flux, as well as the efflux of Ca^{2+} into the medium with 10 μM Ca, was reduced or eliminated when the segments were treated with cyanide or subjected to low temperatures (5–15°C); (manuscript in preparation).

We have not conclusively determined how far along the 2-cm segment the effluxing Ca^{2+} is coming from, although we have found that the rate of apical Ca^{2+} efflux was higher with the 2 cm segment compared to segments half as long. However, attempts to use ^{45}Ca applied at one end of the 2-cm segment failed to indicate the appearance of ^{45}Ca in significant amounts in the segment medium at the other end for up to 24 h. It is thus likely that the Ca^{2+} found in the segment medium is of localized origin, probably from the parenchyma cells close to the cut ends of the hypocotyl segment. Adsorption of Ca^{2+} to exchange sites in the cell wall (26) is most likely the principal reason for the very slow movement of this element in the plant. On this basis, we believe that the acropetal flux of Ca^{2+} in the isolated segment may be insignificant for long distance transport of Ca^{2+} but is essential to the stimulus-response coupling mechanisms at the cellular

level. This acropetal polarity at the cellular level may be significant in the long distance movement of Ca^{2+} in the intact plant for reasons of time, root pressure, and the transpiration stream (19, 29).

Our view is that the net acropetal polarity may be attained through the differential entry or exit of Ca^{2+} in the cell (see later discussion on mechanisms involved). It is of course possible that the net acropetal Ca^{2+} flux observed is due to some other attribute quantitatively different between the apical and basal cells in the 2-cm segment. One aspect we have recently looked into is the Ca exchange capacity of the cell wall (26); data from several experiments showed the same values for apical as well as basal cell walls (data not shown).

Acropetal Ca^{2+} Efflux and Basipetal Auxin Transport Relationship. The basipetal transport of auxin was first shown to require Ca in EDTA-treated sunflower hypocotyl segments (6). In seedlings nutritionally deficient in Ca, the requirement for this element in auxin transport was quite specific (4). The transport of auxin in hypocotyl segments deficient in Ca exhibited a slower velocity as well as a lower flux.

In the work reported here, IAA was likewise found to promote the acropetal flux of Ca^{2+} (Fig. 5). This action of IAA is duplicated by the synthetic auxin α-NAA but not by the non-auxin analog β-NAA (Fig. 6). The evidence also indicates that the basipetal transport of auxin is a factor in the Ca^{2+} flux since the presence of the auxin transport inhibitor, TIBA, eliminated the IAA-promoted Ca^{2+} efflux (Fig. 7; Table II). The overall evidence points to a mutually interdependent transport of Ca^{2+} and IAA in stem tissue.

Interestingly, the available evidence in roots also indicates an opposite direction of movement for Ca and IAA. However, in roots the direction of movement appears to be the reverse of that found in stems. Ca moves basipetally in roots (8, 17, 24) while IAA moves in the acropetal direction (10, 23, 28).

The apical/basal Ca^{2+} flux ratio in the 2-cm stem segment is rarely more than two while the basal/apical ratio of IAA flux in a 1-cm segment could reach infinitely high values (5). The difference in relative polarity between Ca^{2+} and IAA flux may be a reflection of where each substance is located in the tissue. Most of the IAA in the plant is in the ionic form (IAA^-) in the cytoplasm and is secreted almost exclusively at the basal end (5, 14) into the acidic free space. The thermodynamic gradient for the nonionic IAA (IAAH) is to enter the cell (10). On the other hand, most of the tissue Ca^{2+} is extracellular (31), and thus, at any given time, only a small fraction is under cytoplasmic control. This, together with the high Ca exchange capacity of the cell wall (26), could account for the relatively weak polarity and sluggish movement of Ca^{2+} in young nontranspiring tissues.

As with apical Ca^{2+} efflux, basal Ca^{2+} efflux was promoted by exogenous IAA. The IAA effect on basal Ca^{2+} efflux was also cancelled in the presence of TIBA. We believe the IAA-promoted basal Ca^{2+} efflux may actually be due to basipetal IAA transport. Significant amounts of IAA from basal donors are trasported acropetally in 2-mm plant segments (5). These IAA molecules may be expected to enter the cells, just as IAA coming from apical donors, and then be transported basipetally (9).

We do not have a good explanation why TIBA inhibits only the Ca^{2+} flux promoted by exogenous IAA. TIBA does not inhibit the Ca^{2+} flux observed in segments without exogenous auxin; in fact, a slight but consistent promotion is the rule (Table II). TIBA, however, is a well known inhibitor of Ca movement in intact plants, especially in young shoots and fruits (27, 30).

Probably the most compelling evidence for a Ca^{2+}-IAA transport relationship is provided by the experiments of Bukovac (2). He found that ^{32}P crossed an apex-to-apex graft union in tomatoes while ^{45}Ca did not. This observation can be explained if we assume that the opposite direction of transport of Ca^{2+} and IAA is coupled to each other. In this case ^{45}Ca does not cross the graft union because IAA can not be transported from one apical end to another.

Possible Mechanisms Involved. In the accompanying paper (4), evidence was presented showing that Ca^{2+} is required in auxin transport and the suggestion made is that this might be a manifestation of the mechanism known as 'stimulus-secretion coupling'. Stimulus-secretion coupling refers to the coupling of passive entry of Ca^{2+} to the secretion of many kinds of substances in animal cells (22). Passive Ca^{2+} entry may be triggered by a variety of stimuli, and the increased internal level of Ca^{2+} is proposed to act as a 'second messenger' that conveys the information of the stimulus from the environment (20). The increase in Ca^{2+} in the cytoplasm causes the activation of the Ca^{2+}-binding protein calmodulin, which in turn activates the Ca^{2+}-dependent processes. In plant cells the secretion of IAA may be controlled by Ca^{2+} via a stimulus-secretion mechanism. Since the cells normally maintain a low concentration of Ca^{2+} in the cytosol, the increase in Ca^{2+} by passive influx may also activate Ca^{2+} efflux pumps. The active uptake of Ca^{2+} by inside-out plasmalemma-enriched microsomal vesicles (12) is enhanced by calmodulin (7). This is believed to result from Ca^{2+}-calmodulin activation of Ca^{2+} efflux pumps in the membranes (18). Interestingly, this ATP-dependent uptake of Ca^{2+} in microsomal vesicles was also found to be stimulated indirectly by IAA (15).

We believe the characteristic slow movement of Ca in plant tissues and the relatively weak polarity observed here, can be accounted for by either of two models. The first model assumes that there is simply a greater entry of Ca^{2+} at the basal plasmalemma relative to all other sides of the cell. The second model postulates a greater exit of Ca^{2+} at the apical plasmalemma than at other sides.

The first model seems to be more compatible with the concept that Ca^{2+} acts as a second messenger and that the entry at the basal end activates the basipetal secretion of IAA. Since IAA also increases the rate of Ca^{2+} flux, the model can be represented as a mechanism whereby the cell constantly monitors its environment. With the initial entry of Ca^{2+} into the cytoplasm, the internal concentration of Ca^{2+} may rise above a threshold level leading to Ca^{2+}-enhanced secretion of IAA. The increased secretion of IAA may in turn cause more Ca^{2+} to enter the cell. The cycle probably continues until either substance reaches a low threshold level unable to stimulate the secretion or uptake of the other. This model would also account for the opposite lateral movement of Ca and IAA in gravitropically stimulated plant organs (11, 16, 25). The second model assumes a high Ca^{2+} efflux at the apical plasmalemma. This model is based on one by Raven (21) who contends that, as a result of membrane electrophoresis, the IAA^- carriers with a net negative charge would concentrate at the positive basal end of the cell. Carriers with a net positive charge would concentrate at the negative apical end. Perhaps the Ca^{2+} efflux pump is an example of a carrier with a net positive charge.

Acknowledgments—We would like to thank Drs. M. L. Evans, M. H. M. Goldsmith, J. B. Hanson, A. C. Leopold, B. G. Pickard, and S. J. Roux for their critical reading of this manuscript.

LITERATURE CITED

1. ARNOTT HJ, FGE PAUTARD 1970 Calcification in plants. *In* H Scharaer, ed, Biological Calcification: Cellular and Molecular Aspects. Appleton-Century-Crofts, New York, pp 375–446
2. BUKOVAC MJ 1957 Effect of stock-scion interrelationships and graft unions upon nutrient absorption and transport in higher plants as indicated by radioactive isotopes. PhD dissertation. Michigan State University
3. CLARKSON DT, JB HANSON 1980 The mineral nutrition of higher plants. Annu Rev Plant Physiol 31: 239–298
4. DELA FUENTE RK 1984 Role of calcium in the polar secretion of indoleacetic acid. Plant Physiol 76: 343–347
5. DELA FUENTE RK, AC LEOPOLD 1966 Kinetics of polar auxin transport. Plant

Physiol 41: 1481–1484
6. DELA FUENTE RK, AC LEOPOLD 1973 A role for Ca in auxin transport. Plant Physiol 51: 845–847
7. DIETER P, D MARME 1980 Calmodulin activation of plant microsomal Ca uptake. Proc Natl Acad Sci USA 77: 7311–7314
8. EVANS EC III 1964 Polar transport of calcium in the primary roots of *Zea mays*. Science 144: 174–177
9. GOLDSMITH MHM 1966 Movement of indoleacetic acid in coleoptiles of *Avena sativa* L. II. Suspension of polarity by total inhibition of the basipetal transport. Plant Physiol 41: 15–27
10. GOLDSMITH MHM 1977 The polar transport of auxin. Annu Rev Plant Physiol 28: 439–478
11. GOSWAMI KKA, LJ AUDUS 1976 Distribution of calcium, potassium and phosphorous in *Helianthus annuus* hypocotyls and *Zea mays* coleoptiles in relation to tropic stimuli and curvatures. Ann Bot 40: 49–64
12. GROSS J, D MARME 1978 ATP-dependent Ca^{2+} uptake into plant membrane vesicles. Proc Natl Acad Sci USA 75: 1232–1236
13. HERTEL R, ML EVANS, AC LEOPOLD, HM SELL 1969 The specificity of the auxin transport system. Planta 85; 238–249
14. JACOBS M, SF GILBERT 1983 Basal localization of the presumptive auxin transport carrier in pea stem cells. Science 220: 1297–1300
15. KUBOWICZ BD, LN VANDERHOEF, JB HANSON 1982 ATP-dependent calcium transport in plasmalemma preparations from soybean hypocotyls. Effect of hormone treatments. Plant Physiol 69: 187–191
16. LEE JS, TJ MULKEY, ML EVANS 1983 Reversible loss of gravitropic sensitivity in maize roots after tip application of calcium chelators. Science 220: 1375–1376
17. MACKLON AES 1975 Cortical cell fluxes and transport to the stele in excised root segments of *Allium cepa* L. II. Calcium. Planta 122: 131–141
18. MARME D 1982 The role of Ca and calmodulin in plants. What's New Plant Physiol 13: 37–40
19. PALZKILL DA, TW TIBBITTS 1977 Evidence that root pressure flow is required for calcium transport to head leaves of cabbage. Plant Physiol 60: 854–856
20. RASMUSSEN H 1981 Calcium and cAMP as synarchic messengers. John Wiley and Sons, New York
21. RAVEN JA 1979 The possible role of membrane electrophoresis in the polar transport of IAA and other solutes in plant tissues. New Phytol 82: 285–291
22. RUBIN RP 1982 Calcium and Cellular Secretion. Plenum Press, New York
23. SCOTT TM 1972 Auxins and roots. Annu Rev Plant Physiol 23: 235–258
24. SINGH C, L JACOBSON 1979 The accumulation and transport of calcium in barley roots. Physiol Plant 45: 443–447
25. SLOCUM RD, SJ ROUX 1983 Cellular and subcellular localization of calcium in gravistimulated oat coleoptiles and its possible significance in the establishment of tropic curvature. Planta 157: 481–492
26. SOMERS GF 1973 The affinity of onion cell walls for calcium ions. Am J Bot 60: 987–990
27. STAHLY EA, NR BENSON 1970 Calcium levels of "Golden Delicious" apples sprayed with 2,3,5-triiodobenzoic acid. J Am Hortic Sci 95: 726–727
28. TANAKA Y, I URITANI 1977 Polarity of production of polyphenols and development of various enzyme activities in cut-injured sweet potato root tissue. Plant Physiol 60: 563–566
29. VAN DE GEIJN SC, F SMEULDERS 1981 Diurnal changes in the flux of calcium toward meristems and transpiring leaves in tomato and maize plants. Planta 151: 265–271
30. WIENEKE I, O BIDDULPH, CG WOODBRIDGE 1971 Influence of growth regulating substance on absorption and translocation of calcium in pea and bean. J Am Hortic Sci 96: 721–724
31. WILLIAMSON RE, CC ASHLEY 1982 Free Ca^{2+} and cytoplasmic streaming in the alga *Chara*. Nature 296: 647–651