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Comparison of Developmental Gradients for Growth, ATPase, and Fusicoccin-Binding Activity in Mung Bean Hypocotyls[1]

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ABSTRACT

A comparison has been made of the developmental gradients along a mung bean (*Vigna radiata* L.) hypocotyl of the growth rate, plasma membrane ATPase, and fusicoccin-binding protein (FCBP) activity to determine whether they are interrelated. The hook and four sequential 7.5 millimeter segments of the hypocotyl below the hook were cut. A plasma membrane-enriched fraction was isolated from each section by aqueous two-phase partitioning and assayed for vanadate-sensitive ATPase and FCBP activity. Each gradient had a distinctive and different pattern. Endogenous growth rate was maximal in the second section and much lower in the others. Vanadate-sensitive ATPase activity was maximal in the third section, but remained high in the older sections. Amounts of ATPase protein, shown by specific antibody binding, did not correlate with the amount of vanadate-sensitive ATPase activity in the three youngest sections. FCBP activity was almost absent in the first section, then increased to a maximum in the oldest sections. These data show that the growth rate is not determined by the ATPase activity, and that there are no fixed ratios between the ATPase and FCBP.

As cells develop, they undergo changes in physiological potential and in their spectrum of proteins. The challenge is to relate changes in proteins to changes in physiological potential. Cells in a hypocotyl go through a period of elongation prior to maturation (7). This elongation is due, at least in part, to auxin-induced wall acidification, mediated by the PM[2] ATPase (11). The first question addressed here is whether the peak in elongation in mung bean hypocotyls is paralleled by a peak of PM ATPase activity. Because hypocotyl development progresses from apex to base, with the youngest, meristematic cells contained in the hook, the growth rate and PM ATPase activity were compared in successive sections along the hypocotyl, starting with the hook region.

Proton excretion via the PM ATPase is greatly enhanced in most higher plant cells by the fungal toxin FC (19, 24). FC does not bind to the catalytic peptide of the ATPase, but to a separate FCBP (1, 5, 6, 20). It has been suggested that the FCBP is a constitutive regulatory subunit of the ATPase (5, 10); if so, one would expect a constant ratio between ATPase and FCBP activities. The second question addressed here is the quantitative relationship between these two activities along the mung bean hypocotyl. Mung beans (*Vigna radiata* L.) were selected because of the large body of information about other developmental gradients along its hypocotyl (7), and the relative ease of obtaining PM using aqueous two-phase partitioning (21, 31).

MATERIALS AND METHODS

Plant Material

Mung bean (*Vigna radiata* L. cv Berken) seeds were obtained from Burpee Seed Co. Seeds were soaked with aeration overnight, then dark-grown at 25°C on soaked and drained coarse vermiculite. Three hundred milliliters of a 1 mM $CaSO_4$ solution were added to the vermiculite to prevent damping-off symptoms. Hypocotyls were harvested after 5 d, when their lengths were between 6 and 7 cm.

Harvest and Sectioning

All harvest and sectioning procedures were carried out under dim green light. Mung bean hypocotyls were harvested at ground level, the cotyledons were removed, and then the hypocotyls were placed into ice-cold water. Free hand sections were cut in a similar manner to Goldberg and Prat (7). The upper cut was the cut that removed the cotyledons, then sequential sections were cut. Briefly, section a includes the whole hook region; section b is the region 0.0 to 7.5 mm below the hook; section c is the region cut 7.5 to 15.0 mm from the hook; section d is the region 15.0 to 22.5 mm from the hook; and section e is the region cut 22.5 to 30.0 mm from the hook. The five sets of sections were wrapped in known weights of aluminum foil, and the foil packets containing the sets of sections were weighed. The fresh weight of each set of sections was defined as the difference between the two weights.

Elongation Measurements

All manipulations were undertaken in dim green light. Glass beads dyed with India ink were smeared with lanolin,

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[2] Abbreviations: PM, plasma membrane; FC, fusicoccin; FCBP, fusicoccin-binding protein; [^{3}H]FC, tritiated dihydrofusicoccin; LPC, lysophosphatidylcholine; U_3, the third upper phase of a phase partitioned extraction.

then the sticky beads were placed along the hypocotyls of intact mung bean seedlings in the same places where cuts would have been made during sectioning. Photographs of these hypocotyls were taken at 0 h and 5 h later. The lengths between the beads were measured from each photographic negative, and the change in the distance between beads was obtained by subtraction.

In Vitro IAA and FC-Induced Growth Assays

Growth assays were performed under dim green light. Hypocotyl sections ($n = 40$, section a; $n = 20$, sections b, c, d, and e) were preincubated for 1 h in 10 mM K-phosphate buffer, pH 6.1, with 0.1 mM $CaCl_2$. Preincubated sections were placed in 1 mM K-phosphate buffer (pH 6.1) with either 10 μM IAA or 1 μM FC, then reweighed after 4 h (IAA) or after 1 h (FC). Growth was expressed by a percentage change in fresh weight over initial fresh weight.

Plasma Membrane Preparation

Extraction steps were done at 4°C, as described by Sandstrom *et al.* (25), with the exception that the Dextran/PEG concentration for phase partitioning was 6.2% (w/w). Sections from each of the five regions were ground twice in 20 mL freshly made and filtered extraction buffer composed of 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 250 mM sucrose. The five resulting homogenates were each filtered through four layers of cheesecloth into centrifuge tubes. The filtrate obtained was first centrifuged at 3,000*g* for 30 min, then the supernatant was centrifuged at 100,000*g* for 30 min to form the five microsomal pellets. These pellets were then resuspended in 250 μL buffer II, composed of 5 mM K-phosphate buffer (pH 7.8), 4 mM KCl, and 250 mM sucrose. PM vesicles (U_3 extractions) were obtained by three successive aqueous two-phase partitioning steps (14) at 6.2% (w/w) Dextran/PEG concentration. The resulting U_3 fractions were diluted in buffer III, which was composed of 10 mM Tris-HCl, 1 mM EDTA, and 20% (v/v) glycerol, then were centrifuged at 100,000*g* for 30 min. The pellets were each resuspended in 50 μL buffer III, then stored in a −70°C freezer.

Microsomal Membrane Preparation

Microsomal pellets obtained from the five sections were each resuspended in a buffer containing 25 mM Tris-base (pH 7.0, adjusted with Mes), 1 mM EDTA, 1 mM DTT, and 20% (v/v) glycerol. Samples were stored as 100 μL aliquots in a −70°C freezer.

Electrophoresis and Immunodetection

SDS-PAGE of the five U_3 fractions was run according to method of Laemmli (13) using a 12% acrylamide concentration. Samples were prepared in a sample buffer containing 5% (w/v) SDS, 30% (w/v) sucrose, 0.01% (w/v) bromphenol blue, and 100 mM DTT, then were boiled for 2 min. Electroblotting to nitrocellulose paper was accomplished with a Bio-Rad Transblot apparatus using a transfer buffer containing 25 mM Tris-glycine (pH 8.3), 20% (v/v) methanol, and 0.067% (w/v) SDS. Immunodetection of the blotted protein was accomplished using a Bio-Rad Immuno-Blot Assay Kit. Ammonium sulfate-precipitated rabbit polyclonal primary antisera 758 and 759, obtained from Dr. Ramon Serrano, were utilized at 1/10,000 dilution. Antiserum 758 was made against the central domain of the H^+ ATPase, corresponding to amino acids 340 to 650 (2); antiserum 759 was made against the carboxyl terminus of the H^+ ATPase, corresponding to amino acids 851 to 949 (23). Goat anti-rabbit second antibody coupled to alkaline phosphatase (Bio-Rad, 1 mg/mL concentration) was utilized at 1/5000 dilution. Blots were developed using 5-bromo-4-chloro-3-indoyl phosphate and nitroblue tetrazolium.

Quantitation of PM H^+ ATPase

Dot blots were made in a similar fashion to Altabella *et al.* (2). Briefly, triplicate U_3 samples (0.5 μg in 2 mL) from each of the five sections were spotted onto nitrocellulose paper previously soaked with transfer buffer as described above. The immunodetection and development of the spotted U_3 fractions were each performed according to the protocols listed earlier, then the dot blots were photographed. Peak heights of the spots on the negatives were measured by densitometry. A dilution series of a separate U_3 extraction, spotted beside the samples, was used as an internal standard. Peak heights *versus* protein amounts were linear between 0.1 to 0.8 μg U_3 protein using either antisera 758 or 759. Arbitrary units are peak heights normalized to the 0.5 μg internal standard.

Enzyme Assays

Vanadate-sensitive ATPase activity was measured according to Sandstrom *et al.* (25). The ATPase assay included 0.02% (w/v) LPC to eliminate latency, so that complete ATPase activity was measured. FC binding was assayed according to the filter disc assay of de Boer *et al.* (6). Cyt *c* oxidase was estimated according to Tolbert (29); antimycin A-resistant NADH reductase was measured according to Lord (16); latent IDPase and latent UDPase were assayed according to Nagahashi and Nagahashi (22). Nitrate-sensitive ATPase was measured according to Mandala and Taiz (17). Protein concentrations were determined by the method of Markwell *et al.* (18).

Fluorescence Quench Assay

Proton transport into microsomal vesicles was measured as the initial rate of the fluorescence quench of quinacrine. Microsomal vesicles (150 μg protein) were added to a cuvette containing 5 mM Na-ATP (adjusted to pH 6.5 with Tris base), 30 mM Mes/Tris (pH 6.7), 50 mM KNO_3, 0.5 mM NaN_3, 250 mM sorbitol, 160 μM Na-orthovanadate (where applicable), and 3 μM quinacrine. The final volume in the cuvette was 2 mL. Changes in fluorescence were measured at room temperature with a Perkin-Elmer LS-5B luminescence spectrometer set at 425 nm excitation, 500 nm emission. Proton influx was initiated by the addition of 5 mM $MgSO_4$.

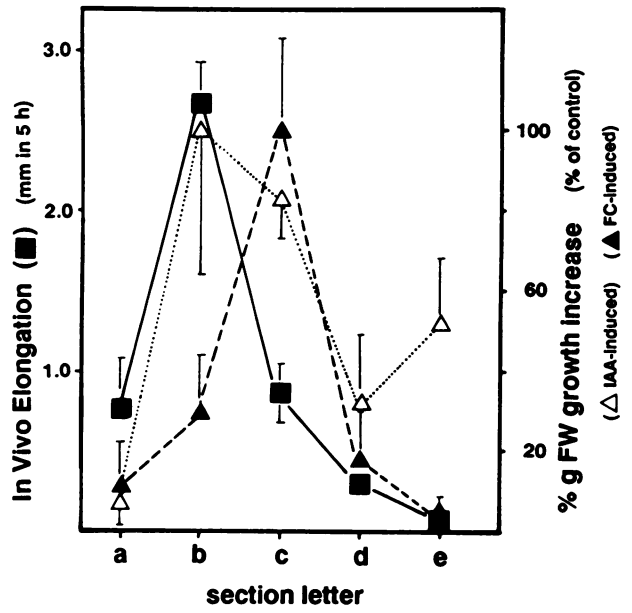

Figure 1. Comparison of *in vivo* elongation, IAA-induced growth after 4 h, and FC-induced growth after 1 h. Five-day-old mung beans (heights between 6 and 6.5 cm) were marked or sectioned as described in "Materials and Methods." Section letters are defined in "Materials and Methods." Control increases in percent gram fresh weight were 5.2% for FC, 11.9% for IAA. Values for *in vivo* elongation represent the mean lengths ± SE, $n = 4$; values for induced growth represent the mean increase in gram fresh weight ± SE, $n = 7$.

Lipid Phosphorus Assay

Lipids were extracted from U_3 preparations by the method of Bligh and Dyer (4). Total lipid phosphorus was assessed by the method of Ames (3) after digestion in hot nitric acid.

Chemicals

[^{3}H]FC (130 TBq/mmol) was obtained from New England Nuclear. The biological effect and binding activity of dihydrofusicoccin is the same as FC (1). Ammonium sulfate-precipitated polyclonal antisera 758 and 759 were produced by Dr. J.M. Pardo, and were generous gifts from Dr. Ramon Serrano. Dextran (mol wt 500,000), polyethylene glycol (mol wt 4000), sodium-ATP, LPC, and quinacrine were obtained from Sigma. Solvents were redistilled in glass before use. All other chemicals were reagent grade.

Replication

Each measurement was triplicated, using three different sets of extractions, unless otherwise specified.

RESULTS

Growth Gradients along the Mung Bean Hypocotyl

Mung bean hypocotyls were characterized *in vivo* for their ability to elongate. Section b, 0 to 7.5 mm from the top of the hypocotyl hook, was clearly the elongation zone (Fig. 1, closed squares). Average maximal elongation rate was about 0.6 mm/h. At that rate, an individual plant cell below the base of the hook can be expected to go through the bulk of the elongation zone (section b) in about 13 h. Section a, the region between cotyledon and hook, and section c, the region 7.5 to 15.0 mm from the hook, each elongated at less than one-third the rate of section b. Sections d and e, 15.0 mm below the hook, elongated very little, and were considered fully mature. These results are slightly different from the published results of Goldberg and Prat (7), where the elongation zone was indicated as extending 10.0 mm below the hook. Because the sections were 7.5 mm long in this work, section b encompassed more of the elongation zone, whereas section c encompassed more of the maturation zone.

Cut sections were also characterized for their ability to respond to IAA and FC. IAA-induced growth results (Fig. 1, open triangles) are similar to the ones obtained by Goldberg and Prat (7), in which section a showed no increase in g fresh weight, sections b and c showed the highest increases, and sections d and e showed only modest increases. FC-induced growth in these sections after 1 h (Fig. 1, closed triangles) has a different pattern. Section c showed the largest FC-induced increase in g fresh weight, five times greater than the growth responses from other sections. Neither IAA nor FC-induced growth correlated well with the *in vivo* elongation data.

Characterization of PM Vesicles by Aqueous Two-Phase Partitioning

Using unsectioned mung bean hypocotyls, the purity of the U_3 extraction obtained from aqueous two phase partitioning was determined by measuring the activities of contaminating enzymes (Table I). The characteristics of the ATPase activity were obtained from these vesicles (Table II); similar results were obtained with U_3 fractions from the individual sections (data not shown). The data from Table I indicate that enzyme markers for membrane contaminants declined upon preparation of the U_3 fraction. The concentration of the contami-

Table I. *Specific Activities of Selected Membrane Markers*

Assays were conducted on microsomal and U_3 PM vesicles isolated by phase partitioning on 5-d-old mung bean hypocotyls. The Mg^{2+},K^+-ATPase assay buffer contained 3 μg protein, 5 mM $MgSO_4$, 50 mM KCl, 125 mM sucrose, 5 mM NaATP (pH 6.5), 0.1 mM $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 30 mM Mes/Tris (pH 6.5), and 0.02% (w/v) LPC. Assay temperature was 37°C. The NO_3^--sensitive ATPase buffer contained the same reagents as the Mg^{2+},K^+-ATPase plus 0.5 mM $NaNO_3$ and assayed at pH 7.0. Values represent the means ± SE; $n = 3$.

Marker Enzyme	Organelle	Specific Activity	
		Microsomal	U_3
		μmol·mg^{-1} protein·min^{-1}	
Cyt *c* oxidase	Mitochondria	0.20 ± 0.01	0.05 ± 0.03
NADH Cyt *c* reductase	ER	0.01 ± 0.00	0.00 ± 0.00
Latent IDPase	Golgi	0.39 ± 0.08	0.07 ± 0.01
Latent UDPase	Golgi	0.25 ± 0.03	0.14 ± 0.06
Mg^{2+},K^+-ATPase	PM	0.32 ± 0.09	0.87 ± 0.08
NO_3^--sensitive ATPase	Tonoplast	0.28 ± 0.01	0.11 ± 0.01

Table II. *Effect of Ions and Inhibitors on Mung Bean U_3 PM ATPase Activity*

ATPase activity was assayed as described in Table I. Where appropriate, the following salts were included in the assay; 5 mM $MgSO_4$, 50 mM KCl, 0.1 mM $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 0.5 mM NaN_3, 50 mM $NaNO_3$, and 0.1 mM Na_3VO_4. Control activity obtained was 1.1 μmol Pi mg^{-1} min^{-1} during the ion studies, and 0.9 μmol Pi mg^{-1} min^{-1} during the inhibitor studies. Values represent the mean of three readings.

Ion/Inhibitor	% Control
Mg^{2+} + K^+ (control)	100
Mg^{2+} ($-K^+$)	88
K^+ ($-Mg^{2+}$)	12
Molybdate	94
Azide	93
Nitrate	91
Vanadate	16

nants left in the U_3 corresponds well with other published characterizations of mung bean hypocotyl PM (21, 31). In the U_3 extractions, the activities of NADH Cyt *c* reductase, an ER marker, and Cyt *c* oxidase, a mitochondrial marker, were each reduced to one-quarter of the activity seen in the microsomal fraction. The Golgi markers latent IDPase and latent UDPase activity were both significantly reduced; the results correspond favorably with other published characterizations (31).

On the other hand, the specific activity of a Mg^{2+},K^+,ATPase, a PM marker, increased threefold in the U_3 as compared with the microsomal fraction. The ATP-dependent hydrolysis in the U_3 vesicles (Table II) is vanadate-sensitive, insensitive to nitrate, azide, and molybdate, has a pH optimum of 6.5 (data not shown), and is dependent upon Mg^{2+} for full activity. All of these characteristics correspond well with characteristics obtained from published PM H^+ ATPase results (8, 21, 25, 27, 31). This PM H^+ ATPase activity was only slightly stimulated by potassium; however, this effect was also seen in mung bean hypocotyl by Yoshida *et al.* (31), who showed that strong potassium stimulation occurs only when this ATPase is measured under nonoptimal pHs (31). When taken together, Table I and Table II indicate that PM vesicles were enriched in the U_3 fraction, and that little ATP-dependent hydrolysis was seen other than the PM H^+ ATPase.

Gradient of PM Protein in Sections

The amount of U_3 protein extracted from mung bean sections was variable (Fig. 2, open triangles). The highest PM protein recovery was obtained in sections a and b, whereas it was considerably lower in sections c, d, and e. This variability in protein recovery may be due, at least in part, to differences in the efficiency of phase partitioning technique in the different regions. The protein to phospholipid phosphorus ratio (Fig. 2, closed triangles) was similar in the U_3 fractions from each section, which suggests that there is no major change in the amount of protein per unit area in the PM during cell development.

Gradient of PM H^+ ATPase Activity

Enriched PM vesicles extracted from individual sections were tested for their PM H^+ ATPase activity, assessed by the rate of vanadate-sensitive, ATP-dependent hydrolysis. Vanadate-sensitive H^+ ATPase specific activity was highest in section c, the young mature region, past the elongation zone (Fig. 3, open circles). A small decrease is seen in the later mature regions of sections d and e. In section a, the meristematic region, PM H^+ ATPase specific was only one-third of that of the peak. The specific activity gradient of ATP-dependent proton pumping measured from these sections parallels the specific activity gradient obtained from vanadate-sensitive ATP hydrolysis (Fig. 3, closed circles). Thus, it appears that the ability to pump protons also changes between the sections and is also highest in the youngest mature region, section c. Proton pumping was measured using microsomal vesicles, rather than phase partitioned material, because U_3 vesicles are mostly right side out, rather than inside out (data not shown). Neither the ATP hydrolysis nor the proton pumping activity seen (Fig. 3) correlates well with the amount of IAA-induced growth seen (Fig. 1, open triangles).

Next, we compared PM H^+ ATPase enzyme kinetics from each of the five section extractions to assess whether the PM H^+ ATPase activity varies kinetically during any stage of plant development. Three representative Hanes-Woolf plots (Fig. 4) show that the K_m values of the PM H^+ ATPase do not differ in any of the sections. The K_m values (0.22–0.25 mM) for the mung bean hypocotyl sections are lower than published K_m values obtained from other plant materials (8, 15, 25). The V_{max} values obtained from the Hanes-Woolf plots (data not shown) correlate well with the PM H^+ ATPase

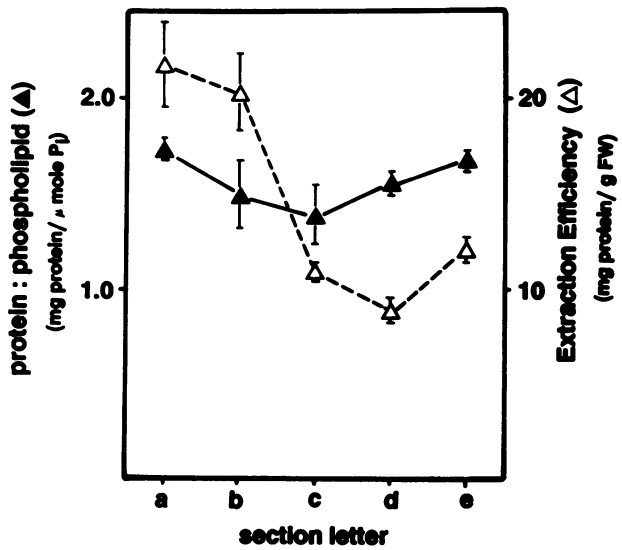

Figure 2. Comparison of protein to phospholipid ratios and milligram PM protein extracted divided by gram fresh weight of harvested sections. Total lipid phosphorus was assayed as described in "Materials and Methods," protein was assayed according to Markwell *et al.* (18). Section letters are defined in "Materials and Methods." Values represent the means ± SE of five different U_3 extractions obtained from each section.

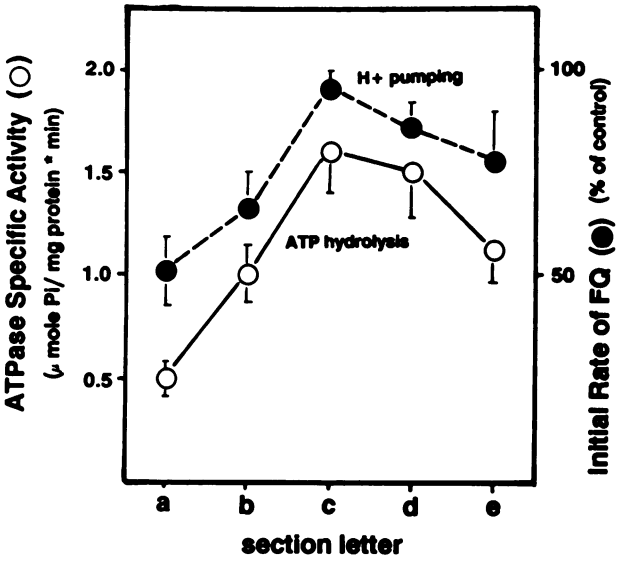

Figure 3. Plot of vanadate-sensitive ATP hydrolysis and the initial rate of vanadate-sensitive fluorescence quench. Vanadate-sensitive ATP hydrolysis was assayed as described in Table II; values represent the means ± SE from five different U_3 extractions. Fluorescence quench (FQ) measurements were made in 25 mM Tris/Mes (pH 6.7), 250 mM sorbitol, 100 mM KNO_3, 0.5 mM NaN_3, 5 mM NaATP (pH 6.7), 3 μM quinacrine, 150 μg membrane protein, ± 160 μM vanadate. The reaction was started by the addition of $MgSO_4$ to a final concentration of 5 mM. Values obtained from FQ measurements represent the normalized means ± SE from three different sets of microsomal extractions.

activity shown in Figure 3. These data imply that the change in PM H^+ ATPase activity seen in these mung bean hypocotyls is due to variable proportions of active enzyme on the PM, rather than to changes in affinity for MgATP.

Gradient of H^+ ATPase Protein

Differences in ATPase activity do not necessarily mean that differences exist in the amount of ATPase protein (2). Therefore, we assessed the amount of 100 kD PM H^+ ATPase protein in the U_3 fractions from the five sections, using two different polyclonal antisera made against specific sites of the *Arabidopsis* PM H^+ ATPase (2, 23) and generously donated by R. Serrano. Both antibodies were found to bind to the 100 kD band in mung bean U_3 (Fig. 5). Antisera 759, made against the carboxyl terminus, corresponding to amino acids 851 to 949 of the *Arabidopsis* PM H^+ ATPase (23), was specific for the 100 kD band (Fig. 5, right blot). Antisera 758, made against the central domain, corresponding to amino acids 340 to 650 of the *Arabidopsis* PM H^+ ATPase (2), also cross-reacted to a 70 kD and a 50 kD band (Fig. 5, left blot), even at 1/10,000 dilution. These bands may be proteolysis products of the 100 kD protein. Alternatively, they might be the 67 and 55 kD peptides reported by Mito *et al.* (21) as an alternative PM ATPase in mung bean hypocotyls, although antibodies to a maize root 90 kD ATPase failed to bind to either peptide (12). Smudges above 100 kD, seen with both antibodies, were due to aggregation during boiling.

Immunological quantification of PM H^+ ATPase protein shows that the amount of PM H^+ ATPase antibody binding (Fig. 6, open circles) is not parallel to vanadate-sensitive ATPase activity (Fig. 6, closed circles) in the two youngest sections, a and b. The results were the same whether antisera 758 (Fig. 6A) or 759 (Fig. 6B) was used. The vanadate-sensitive specific activities obtained were lower in these experiments than in previous ones due to several unavoidable freeze-thaw cycles.

Normalizing the ratios between antibody binding and vanadate-sensitive activity in section c, it appears that three to four times more antibody binds to the U_3 of section a, and twice the antibody binds to the U_3 of section b than would be expected from the vanadate-sensitive activity seen. Compared with section c, mature sections d and e show less than a 20% discrepancy between antibody binding and vanadate-sensitive activity.

Fusicoccin-Binding Gradient

As a comparison to the PM H^+ ATPase, FC-binding activity was measured in the U_3 fractions from the five sections. As Figure 7 shows, FC binding was extremely low in the youngest section, a, low in the elongation zone, and became progressively greater in each mature section. The specific activity of FC binding seen in the mature sections d and e was approximately six to seven times greater than in section b.

Comparison of Developmental Gradients

The developmental gradients for *in vivo* elongation and the specific activities for PM H^+ ATPase and FCBP activities are compared in Figure 8. None of these gradients coincide. This indicates that the rate of elongation is not controlled by the amount of PM H^+ ATPase activity. Likewise, it is unlikely

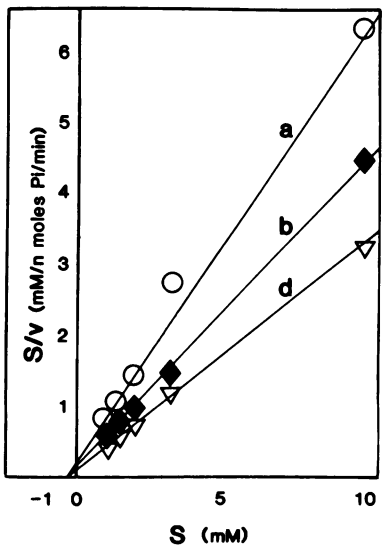

Figure 4. Similarity of ATPase kinetics in U_3 extractions obtained from three representative hypocotyl sections. Kinetics were plotted using the Hanes-Woolf equations. Each assay was run in the presence of ATPase assay buffer described in Table I.

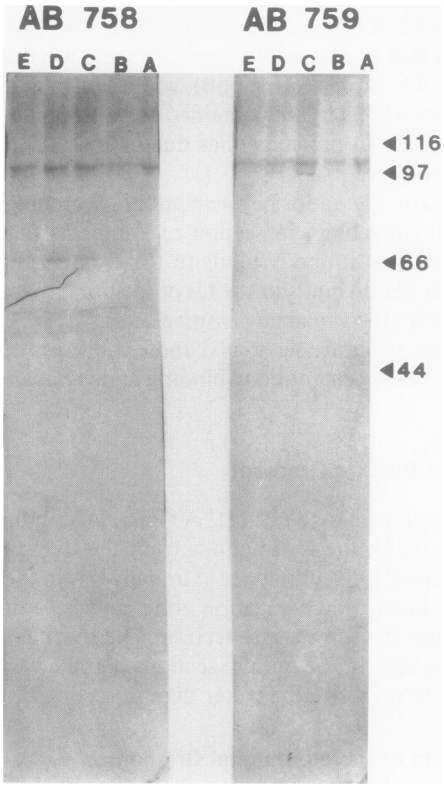

Figure 5. Western blot analysis of the U_3 extractions. U_3 extractions of 10 μg were separated by SDS-PAGE, transferred to nitrocellulose, then immunodecorated with either antisera 758 or 759 at 1/10,000 dilution. Lanes are labeled by section letter.

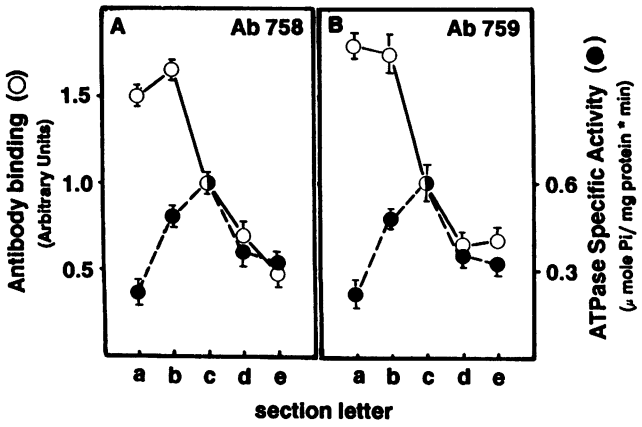

Figure 6. Comparison of the amount and activity of the PM H^+ ATPase obtained from the five sequential U_3 extractions. Vanadate-sensitive ATP hydrolysis was assayed as described in Table II; values represent the means ± SE from three different experiments. Antibody binding was assessed as described in "Materials and Methods"; values represent the means ± SE, $n = 12$.

that the FCBP is a subunit of an ATPase complex, because its activity differs greatly from that of either the activity (Fig. 8) or the amount (Fig. 6) of PM H^+ ATPase. The FCBP activity does correlate well with the amount of growth induced by FC in 1 h (Fig. 1) in sections a, b, and c, but not in d or e.

DISCUSSION

The developmental gradients for *in vivo* elongation and for IAA- and FC-induced section growth for mung bean hypocotyls agree well with those obtained by Goldberg and Prat (7). Although *in vivo* elongation is believed to be controlled by auxin, the difference in gradients of *in vivo* elongation and IAA section growth are not unexpected, because gradients exist *in vivo* in auxin concentration (26) and in availability of solutes for osmotic adjustment. Thus, the *in vivo* elongation gradients indicate that the cells in sections b and c have the highest capacity for enlargement if optimal auxin is present.

There have been a few attempts to relate the PM H^+ ATPase activity with the developmental status of cells. In soybean roots (30), K^+ stimulated ATPase activity was greatest in the meristematic region; it was minimal in the elongation zone and then increased as the cells matured. In tobacco calli, the PM ATPase activity changed with time, but was not correlated with the growth rate (2). In bean leaf, the PM ATPase decreased by 50% in light-grown *versus* dark-grown leaves (15). In the present study, the ATPase activity was not correlated with either the *in vivo* elongation rate or with the capacity of the isolated sections to grow. It is concluded that the growth rate is not controlled by the maximum activity of the ATPase. Because the rate of proton excretion *in vivo* is certainly

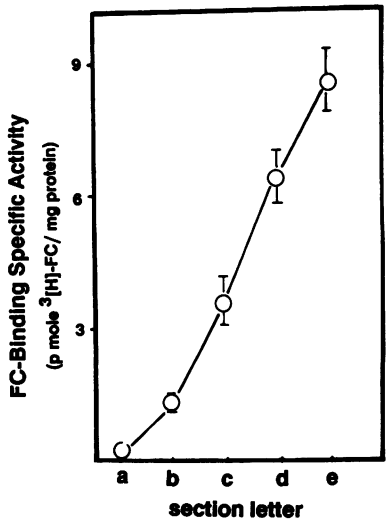

Figure 7. Specific activity of FC binding in the U_3 extractions obtained from the five different hypocotyl sections. The FC-binding assay was conducted by adding [^{3}H]FC to a final concentration of $2 \cdot 10^{-9}$ M in the presence of 20 μg PM protein, in a buffer composed of 25 mM Mes/Tris (pH 7.0), 1 mM MgCl, 0.5 mM EDTA, 25 mM potassium fluoride, 10% (v/v) glycerol, and 0.02% (v/w) NaN_3. During the assay, membranes were incubated at 30°C for 2 h. Values represent the mean ± SE, $n = 5$.

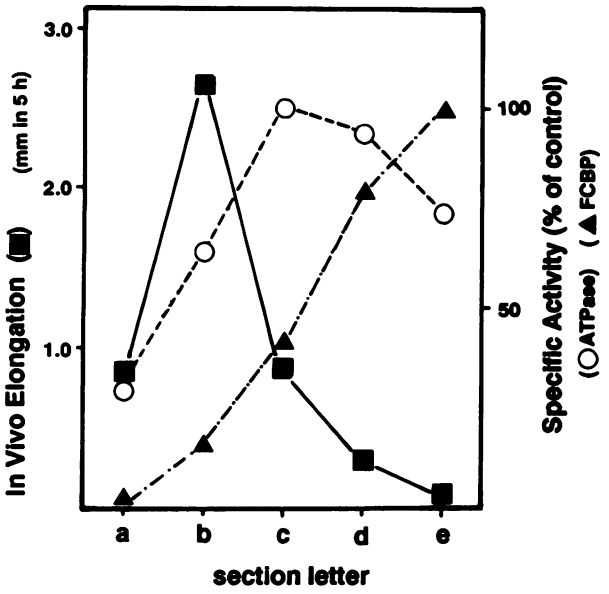

Figure 8. Comparison of *in vivo* elongation rates with normalized specific activities of vanadate-sensitive ATPase, and FC binding. Measurements were made as described in Figures 1, 3, and 7.

suboptimal (8), the control of the rate of proton excretion will be by other factors such as the cytoplasmic pH (9).

The developmental gradient in PM H^+ ATPase activity would suggest that this enzyme is developmentally regulated. However, the pattern of change in the amount of 100 kD PM ATPase protein gives a different picture. The ATPase protein is most abundant in the youngest sections (a and b) and then decreases with cell maturation. This might indicate that the ATPase is synthesized preferentially in the younger cells and is diluted out with other PM proteins as the cells enlarge and mature. Alternatively, it may be that the conversion of inactive to active ATPase is under developmental control. Altabella *et al.* (2) have reported on a possible developmental control of ATPase activation in tobacco calli cells. Finally, an apparent conversion of inactive to active ATPase might be a representation of different mixes of PM H^+ ATPase isoforms, each differentially regulated.

The FCBP is a 31 to 33 kD protein, different from the 105 kD PM ATPase (5, 6, 20, 24). However, it has been suggested that the FCBP is a regulatory subunit of the PM ATPase complex (5, 10), perhaps involved with maintaining ATPase activity during wound recovery (10). The idea has been strengthened by the finding of Cocucci and Marrè (5), that both the solubilized FCBP and PM H^+ ATPase comigrate on sucrose gradients. If this idea is correct, there should be a constant stoichiometry between the PM H^+ ATPase and the FCBP. Although it is only the FCBP activity, not the amount of protein, that has been measured, the difference in gradients of PM H^+ ATPase and FCBP activities (Fig. 8) speaks against the subunit idea.

The fact that virtually all higher plants excrete protons in response to FC suggests that the FCBP must be a highly conserved protein whose presence is required in all cells. It has been suggested that the role of the FCBP, *in vivo,* is not to bind FC but some other essential function (6, 10, 19). It was a surprise, then, to find that FCBP activity was nearly absent from cells in the hook region. The very low growth response of section a to FC is consistent with the lack of FCBP activity in this region. The development of strong FCBP activity as cells mature suggests that this protein plays some role in mature, differentiated cells that is not needed in meristematic cells. FCBP activity has been detected, however, in callus cells from *Arabidopsis thaliana* (28). It would be interesting to know if the FCBP activity was present in all cells or restricted to the more mature, nondividing cells.

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