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Short Communication

Organ Distribution of Auxin-Binding Protein 1 in the Etiolated Maize Seedling

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Abstract: The auxin-binding protein designated ABP1 has been proposed to mediate auxin-induced cellular changes such as cell expansion. Its exact mode of action is unknown, but currently several approaches to elucidate its function are being pursued. One of these approaches, described here, is to determine the organ distribution of this putative auxin receptor in order to correlate spatially the abundance of the protein with some auxin-regulated activity such as cell elongation. The absolute and relative amounts of ABP1 were determined along the entire etiolated shoot, the root, and within the caryopsis of maize. ABP1 can be detected immunologically in all extracts of the etiolated maize seedling except the tip of the primary root and the endosperm. Within the shoot, but excluding the leaf roll, the highest levels compared on a fresh weight basis are in the apical mesocotyl and basal coleoptile regions, the areas of the most rapid cell elongation and the areas where there is the greatest capacity for auxin-induced growth. The relative abundance of ABP1 compared on a fresh weight basis changed more than fivefold in this organ. When compared on a total protein basis, the relative change in ABP1 abundance was approximately twofold, which is less than the relative change in auxin-induced growth rate along the shoot. Differences in shoot growth rate among varieties of maize were compared with the relative amounts of ABP1 within the apical mesocotyl and basal coleoptile. A statistically significant but not perfect correlation was found between the auxin-induced growth rate of the apical mesocotyl and ABP1 abundance. These results demonstrate a general correlation between the amount of ABP1 and growth along the shoot and within maize hybrid varieties.

The plant hormone auxin regulates cell elongation, cell differentiation, and the establishment and maintenance of polarity (Davies 1987). The auxin receptor mediating any of these major responses has not been identified, although several auxin-binding proteins have been identified (Jones 1994). Auxin-binding protein 1 (ABP1) was identified first by Löbler and Klämbt (1985), and since this time several groups have hypothesized that ABP1 is an auxin receptor that mediates cell elongation (see Jones 1994). This hypothesis is currently being tested.

There are many possible approaches to addressing functionality. For example, the binding affinity of an informative set of ligands can be compared with the ability of these ligands to induce a specific response. Ray et al. (1977) compared the binding affinities of 45 compounds with ABP1 in crude extracts of maize coleoptile. These compounds were auxins and compounds structurally similar to auxins. The binding affinities of these compounds expressed as K_d values correlated with concentrations necessary to induce half-maximal growth expressed as pC_{50} values, although there were some notable exceptions. Another approach for elucidating function is to use transgenic technology to alter the level or activity of ABP1 and to correlate this change with a change in auxin-inducible growth, but this approach is not always technically possible. The

Abbreviations: ABP1, auxin-binding protein 1; NAA, naphthalene-1-acetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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lack of any published reports using this technology since the identification of the cDNA for ABP1 in 1989 (Hesse et al. 1989, Inohara et al. 1989, Tillmann et al. 1989) might suggest that this is not working, is not possible, or is providing equivocal results. Alternative approaches should be employed, some that are simple characterizations of the protein such as its distribution in the plant. This is the approach we use here. The etiolated maize seedling is ideal for this approach since development is separated linearly along the length of the shoot.

Materials and Methods

Chemicals, Antiserum, Plant Materials, and Growth Measurement

All chemicals were purchased from Sigma Chemical Co. Naphthalene-1-acetic acid (NAA) was purified further by recrystallization. Anti-ABP1 serum (NC040109) was prepared as described in Jones et al. (1991) and used at 1:5,000 dilution. The specificity of this antibody is described in Jones et al. (1989, 1991). Maize caryopses (J7710, Jacques Seed Co.; Jubilee, Aztec, Silver Queen, Ferry Morris; Golden Cross Bantam, Page Seed Co.; Merit, Asgrow Seed Co.; Spirit, NK Lawn & Garden Co.) were imbibed for 1 h and then planted in moist vermiculite and grown in the dark for 4.5 days. Whole shoots (3 cm) were then harvested and sectioned into 0.5-cm lengths, three sections above and three sections below the coleoptilar node. Half of the harvested shoots (10–12 sections from each position of the shoot) were preincubated for 3 h in growth buffer (5 mM potassium phosphate, pH 6; 30 mM sucrose; and 80 μM chloramphenicol) and then transferred to growth buffer containing 50 μM NAA for 24 h. The lengths were measured at the indicated times, and growth was expressed as the difference in section length between the 3-h pretreatment and the 24-h treatment of auxin. Sections incubated for 24 h without NAA essentially had the same length as the 3-h preincubated sections. The standard error ranged from 1 to 15% of the average growth. The other half of the sections (10–12 sections each) were extracted, and the extract was analyzed for ABP1 content as described below. The same results were obtained if the tissue was extracted immediately after harvesting the tissue or after a 24-h incubation in the presence of NAA.

Extraction of Proteins from Maize

Harvested shoots from the same experiments where growth was measured were stored with 1 mM phenylmethylsulfonyl fluoride in liquid nitrogen. Prior to immunoblot analysis, an equal volume of SDS sample buffer (6% SDS; 42 mM 2-mercaptoethanol; 375 mM Tris, pH 6.8; 30% glycerol) was added to the tissue, and then the tissue was homogenized at 90 °C by grinding with a small pestle fitted to the microcentrifuge tube. The resulting homogenate was centrifuged for 2 min (12,000 × g), and the supernatant was removed and used for immunoblot analysis.

Auxin Binding Assays, SDS-PAGE, and Immunoblot Analysis

Auxin binding was performed using crude extracts of maize mesocotyl exactly as described by Jones et al. (1989). Auxin binding in crude extracts of tissues taken from the apical and basal 1.5 cm of 3- to 5-cm-long mesocotyls on 4.5-day-old etiolated seedlings were compared. Half-maximal displacement was taken as a first estimate of the dissociation constant (K_d). The range of 10^{-8} to 10^{-5} M 1-NAA was used. SDS-PAGE was performed on 15% polyacrylamide gels. Identification and determination of the relative amount of ABP1 were carried out by immunoblot analysis using anti-ABP1 polyclonal rabbit antibodies. Goat antirabbit immunoglobulin conjugated with alkaline phosphatase was used as the secondary antibody. The amount of ABP1 was determined by measuring the volume of the band recognized by the anti-ABP1 antibodies using a quantitative image analyzer (Molecular Dynamics, Inc.). Each sample, loaded typically twice per blot on typically two blots, was scanned twice. The standard error represents these potential sources of variation. Each experiment was repeated at least once. To determine the absolute quantity of ABP1, each immunoblot of the tissue extracts also contained a series of known amounts of ABP. The ABP1 band in these series was scanned along with the ABP1 band in each extract. The ABP1 signal was compared with the ABP1 series on the same immunoblot to determine absolute ABP1 quantity. Because of differences in transfer and staining, one standard curve cannot be used for another blot. An example of such an internal standard curve for one blot is shown in Figure 1C. Since each blot contained only one series of ABP1, standard error bars are not possible. Therefore, to determine the amount of potential error in this method, a test was performed in which the same amount of ABP1 was loaded in multiple lanes, and single lanes were scanned multiple times. It was found that multiple lanes containing the same amount of ABP1 (loading reproducibility) had a standard error of a maximum of 15% (S.E./average ×100). Scanning the same lane multiple times (scanning reproducibility) had a standard error of 4%.

Quantitative Protein Analysis

The amount of total protein per unit sample was determined using the method described by Bradford (1976) with the following modification described by Konigsberg and Henderson (1983). Briefly, SDS was removed from the samples and the bovine γ-globulin standard prior to Bradford analysis using ion pair extraction by the addition of 50 μl of sample to 1 ml of extraction solution (ES: 5% glacial acetic acid, 5% triethylamine, 90% cold acetone). Protein was precipitated, air dried for 1 h at 65°C, and resuspended in 100 mM sodium hydroxide. Each solution was then subjected to Bradford analysis (Bradford 1976). Duplicate measurements were made.

Results and Discussion

Figure 1A shows the amount of ABP1/g, fresh weight, found at nearly all positions on a 4.5-day-old etiolated maize seedling (J7710) using quantitative immunoblot analysis. The leaf roll contained approximately 500 ng/g, fresh weight, of ABP1 in both the basal and apical halves. The highest levels

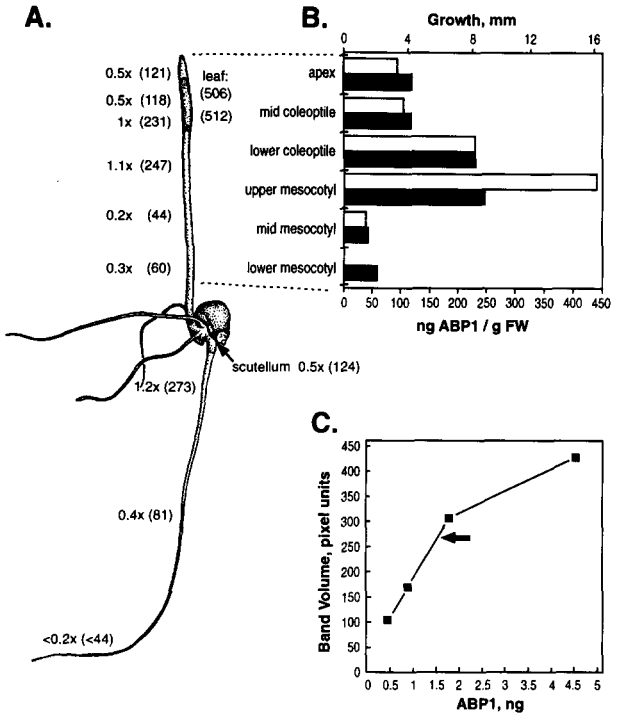

Fig. 1. Relative and absolute amounts of ABP1 in the 4.5-day-old etiolated, maize seedling (hybrid J7710). **(A)** The absolute quantity of ABP1 expressed as ng of protein/g, fresh weight, of tissue is shown in *parentheses* for each location of the seedling. The levels for the primary root tip and the endosperm are at and below the level of detection, respectively. The absolute quantity was determined by comparing the signal on the immunoblot with signals of known quantities of pure ABP1. The numbers shown to the *right* of the absolute values are the relative amounts of ABP1, where the value of one is set for the base of the coleoptile. **(B)** The absolute amount of ABP1 (*closed bars*) is compared with the amount of growth induced by 50 μM NAA (*open bars*) in the same sections of tissue. Growth was recorded after 24 h. ABP1 was measured as described above. **(C)** The signal in the band on the immunoblot is expressed as pixel units from image analysis and is shown for a series of known concentrations of ABP1. The *arrow* indicates the signal obtained for the base of the coleoptile, demonstrating that the quantitation analysis of the shoot is within the linear sensitivity range for the immunoblot. Each blot contained only one series of ABP1 standard with which to compare the amount of ABP1 in the tissue extracts, and therefore the standard error is not shown. However, the error due to loading the gels and scanning the blots is approximately 15 and 4%, respectively, by direct tests of the sources of variability. The values of ABP1 in extracts shown are averages based on two blots each containing two lanes for each sample. Each blot was tested to be sure that the signal was within the linear range of sensitivity by including a series of known amounts of purified ABP1.

of ABP1 in the etiolated shoot were found to be in the coleoptile base and the apical mesocotyl where there is approximately 250 ng/g, fresh weight. This absolute amount is within the range of 10–100 pmol/g, fresh weight, for ABP1 estimated from auxin binding (Ray et al. 1977). The twofold higher amount of ABP1 found in the leaf roll is also consistent with previous estimates based on activity (Ray et al. 1977). ABP1 is present in the secondary roots, but there was essentially no signal detected in the primary root tip. Radermacher and Klämbt (1993) have shown that ABP1 is not detected in roots, but rather that there is another ABP1 isoform with a lower subunit molecular mass and a higher affinity for auxin. Our antiserum does not detect this root isoform of ABP1. ABP1 could not be detected in the endosperm but was detected in the scutellum. Ray et al. (1977) found 12- and 25-fold less auxin binding in the roots and endosperm, respectively, than the coleoptile.

We compared the amount of NAA-inducible growth with the absolute amounts of ABP1 at various positions along the shoot. There was a correlation between the positions of maximum growth and maximum levels of ABP1. The overall relative change in the abundance of ABP1 along the shoot was approximately fivefold.

Figure 2A shows the distribution of ABP1 and total extractable protein along the shoot expressed per g, fresh weight. It is apparent that the highest amount of extractable protein is found in the growing zones of the shoot. Therefore, when the amount of ABP1 is expressed as a function of the total protein, the relative change of ABP1 along the shoot is much smaller than when expressed on a fresh weight basis. Figure 2B shows that the relative change of ABP1 is less than twofold, with the highest amount of ABP1 found in the growing zone of the shoot.

We took advantage of the fact that auxin-inducible growth varies among varieties of maize. Auxin-induced growth of the basal 1 cm of the coleoptile and the apical 1 cm of the mesocotyl of seven varieties of maize was compared with the absolute amount of ABP1/segment. There was a significant correlation (r^2 = 0.72) between the growth and the amount of ABP1 in the mesocotyl. Although there was a positive trend between the growth and ABP1 in the coleoptile, the correlation coefficient was much less (r^2 = 0.42). Figure 3 shows the results for the coleoptile base and mesocotyl apex only, although growth was measured along the entire shoot for each maize variety. The relative growth pattern for each variety was similar to that shown for hybrid J7710 in Fig. 1B (data not shown).

The work presented is a simple balance sheet analysis of the abundance of ABP1 in maize shoots, the first performed for the ABP1 protein using any whole plant. Such an analysis can have practical value such as in determining the optimum source of

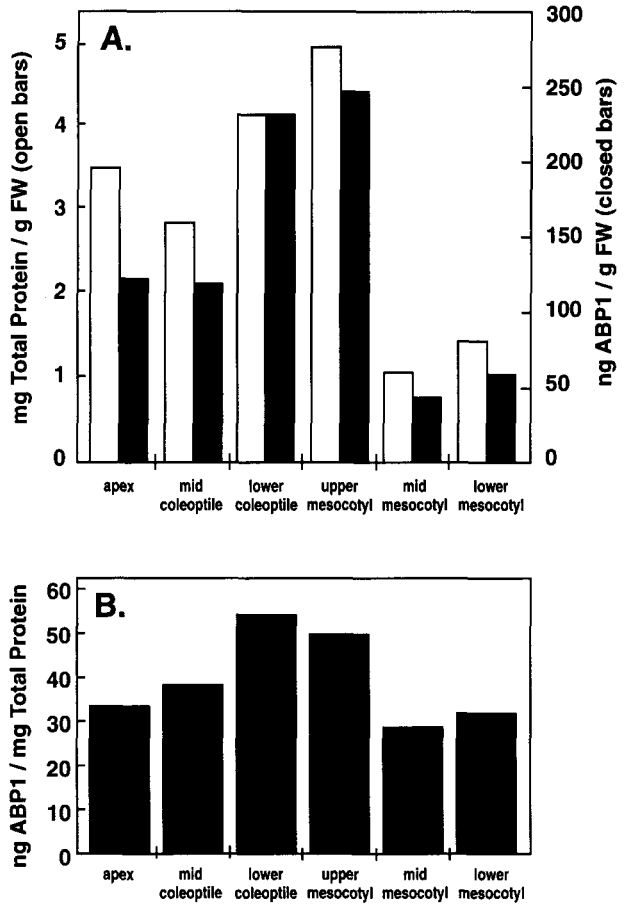

Fig. 2. Comparison of the absolute amounts of ABP1 with the amount of extractable protein at all positions of the shoot. (A) The total amount of ABP1 (*closed bars*) was calculated as described in Figure 1 and is compared with the amount of total extractable protein (*open bars*) determined by a modified Bradford analysis as described in the Materials and Methods section. (B) The amount of ABP1 in ng/mg of protein is shown for each of the positions of the shoot.

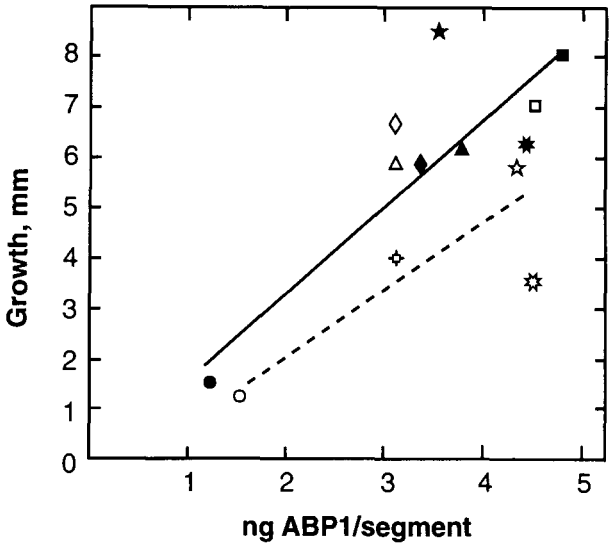

Fig. 3. A comparison of the amount of ABP1 per shoot with NAA-induced growth of the apical mesocotyl (*filled symbols*) and basal coleoptile (*open symbols*) tissue. ABP1 and growth was measured as described. *Asterisks*, Spirit NK; *triangles*, J7710; *square with bars*, Merit; *diamonds*, Jubilee High Yield; *stars*, Sugar Dots; *squares*, Golden Cross Bantam; *circles*, CutiePops.

tissue in purification of ABP1. For example, in this regard, shoots and leaf rolls are ideal, and elimination of caryopsis and roots allows significant enrichment. On the other hand, only minimal enrichment can be made by selecting just the growing zones of the shoot.

Such a balance sheet analysis can also be useful in testing the hypothesis that ABP1 has a role in auxin-regulated growth. We have found that the abundance of ABP1 is highest in zones of the shoot which have the highest capacity for auxin-induced growth. Since there are many proteins that are at their greatest level in growing cells, this correlation between ABP1 abundance and growth does not prove a role for ABP1 in growth but is only consistent with the above hypothesis. Furthermore, since the correlation between ABP1 abundance and growth is not perfect, the growth capacity along the length of this organ is not regulated by ABP1 steady-state levels without assuming that there is a threshold level of ABP1 needed for growth. This suggests that ABP1 abundance is not growth limiting. To test whether activation or deactivation of ABP1 might be the basis by which ABP1 action regulates auxin-induced growth, the auxin-binding affinity for ABP1 in crude extracts taken from the apical and basal regions of the mesocotyl was determined. The K_d values for 1-NAA and the corresponding number of auxin binding sites in crude extracts of the apical and basal regions were 0.17 μM (300 fmol sites/mg of extractable membrane protein) and 0.15 μM (170 fmol sites/mg of extractable membrane protein), respectively. This suggests that the binding affinity is not changing, and that the lower auxin-induced growth capacity in the basal region of the mesocotyl is not the result of a lower affinity of auxin to ABP1. The decrease in site number measured by auxin binding is in agreement with the decrease in ABP1 measured by quantitative immunoblot analysis. The binding affinities determined in this study are similar to reported values (Ray et al. 1977), and the absolute number of sites is consistent with the amount of ABP1 (Fig. 2).

In all cases, the relative abundance of ABP1 (Fig. 1) correlated well with the amount of auxin binding determined previously (Ray et al. 1977). Leaves

have the greatest amount of ABP1, suggesting that ABP1 plays a role in auxin-regulated growth in leaves. Cleland (1964) has shown that auxin is involved in leaf cell expansion. Auxin also seems to regulate other responses in leaf cells. For example, transdifferentiation of mesophyll cells to tracheary elements is auxin mediated (Fukuda 1994). Also, an auxin-induced hyperpolarization of tobacco mesophyll cells has been characterized (Barbier-Brygoo et al. 1991). Moreover, a specific role for ABP1 in auxin-induced hyperpolarization of mesophyl plasma membranes has been proposed (reviewed in Goldsmith, 1993).

Where ABP1 was not found is also important. The lack of ABP1 in the growing tip of the primary root is consistent with the hypothesis that another auxin receptor, one having a greater affinity for auxin, is mediating auxin-regulated root growth. Radermacher and Klämbt (1993) have detected such a putative root isoform. Thus, it may be possible, with sufficient reagents specific to isoforms of auxin-binding proteins, to catalog the locations of each form in order to gain insight into their functional roles.

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