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Cloning of Genes Encoding Auxin-Binding Proteins (ABP19/20) from Peach: Significant Peptide Sequence Similarity with Germin-Like Proteins

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An auxin-binding protein (ABP) was previously isolated from shoot apices of peach trees to homogenity on standard SDS-PAGE. Analysis of low-bis SDS-PAGE and direct peptide sequencing of purified peach ABP demonstrated that the ABP was composed of two types of polypeptides (designated ABP19 and ABP20). Several cDNA and genomic clones which encode peach ABPs were obtained and analysed. We found that there are at least three classes of ABPs in the peach genome. Open reading frames of these ABPs were 627 bp, predicting a 209 amino acid polypeptide of 22 kDa. An N-terminal hydrophobic signal sequence of 18 amino acids and a putative N-glycosylation site at N^{60}-T-T/S were deduced. Homology search analysis revealed that ABP19 is highly homologous to proteins related to the germin family. The deduced amino acid sequence of ABP19 showed very low overall sequence homology with ABP1, an ABP isolated from maize coleoptile, but it contained a small region which shared 40% homology with a putative auxin binding site in ABP1 (BoxA). In addition, the sequence surrounding the region is highly conserved among peach ABPs and the germin family.

Key words: Auxin — Auxin-binding protein (ABP) — Germin-like protein — *Prunus persica* (peach).

It is believed that the auxin signal is perceived by proteins generally referred to as auxin receptors. Based on this hypothesis, numerous attempts have been made to purify auxin-binding proteins (ABPs) that specifically bind auxin. ABP1 isolated from maize coleoptile is the best characterized ABP and is probably involved in mediating rapid auxin effects on cell elongation (Napier and Venis 1991). Some ABPs have been identified as proteins with enzymatic activities such as β-glucosidase (Campos et al. 1992, Brzobohaty et al. 1993), 1,3-β-glucanase (Macdonald et al. 1991), glutathione *S*-transferase (Bilang et al. 1993, Zettl et al. 1994), manganese superoxide dismutase (Feldwisch et al. 1995), and glutathione-dependent formaldehyde dehydrogenase (Sugaya and Sakai 1996).

The investigation of the structures of these ABPs will greatly enhance our understanding of the molecular nature by which auxin signals are perceived and provide information for structural design of new growth regulators. Among the ABPs reported so far, ABP1 is the only protein whose primary structure has been extensively studied (Inohara et al. 1989, Palme et al. 1992, Schwob et al. 1993). Venis et al. (1992) proposed that the domain H^{57}RHSCE contains the structural requirements for the auxin-binding site. They demonstrated that antibodies directed against a synthetic oligopeptide surrounding this domain induced an in vitro auxin response in the absence of auxin. Comparison of deduced amino acid sequences of the ABP1 homologs from four plant species showed a high degree of sequence conservation surrounding the domain (designated BoxA by Jones 1994). A soluble ABP was previously isolated from the shoot apices of peach (*Prunus persica* L. cv. Akatsuki), the most actively growing part of the tree, using 2,4-D-linked-Sepharose 4B chromatography (Ohmiya et al. 1993). The peach ABP has the molecular mass of the subunit (20 kDa) in common with maize ABP1 but has a different dissociation constant (4.1×10^{-5} M) and auxin specificity (2,4-D > PCIB > NAA ≒ IAA). In addition, these peach and maize ABPs differ in organ specificity and regulation of expression (unpublished data), suggesting that they may mediate different auxin responses. In the present paper, we show that the homology between peach ABP and maize ABP1 is relatively low but that a small region in peach ABP shares 40% homology with BoxA in maize ABP1. Additionally, homology search analysis of nucleotide and deduced amino acid sequences of peach ABP revealed that peach ABP shares a significant homology with the members of the germin family.

Materials and Methods

Plant material—Shoot apices of peach trees (*Prunus persica* L. cv. Akatsuki) were harvested at our Institute, washed with distilled water, and stored at −80°C until use.

N-terminal amino acid sequencing of ABP19/20—Low-bis SDS-PAGE was performed on 17% PAGE gels, and the proteins on the gel were electrophoretically transfered to a PVDF filter (Pall Fluortrans, Nippon Genetics Co. Ltd., Tokyo, Japan) according to the method of Hirano (1989). The filter was stained with Coomassie brilliant blue R250. The bands corresponding to ABP19 and ABP20 were cut out and applied to a gas-phase pro-

Abbreviations: ABP, auxin-binding protein; ORF, open reading frame.

The nucleotide sequences reported in this paper have been submitted to the EMBL under accession numbers U79114 (ABP19), U81163 (ABP19′) and U81162 (ABP20).

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tein sequencer (Applied Biosystems model 470A, San Jose, CA, U.S.A.), respectively.

Cloning of ABP20 cDNA—Total RNA was extracted by the method of Logemann et al. (1987), and poly(A)$^+$RNA was subsequently enriched using a column of oligo(dT)-cellulose (Stratagene, La Jolla, CA, U.S.A.) by standard techniques (Sambrook et al. 1989). Double strand cDNA was synthesized from poly(A)$^+$RNA using the cDNA Synthesis System Plus (Amersham, Tokyo, Japan), and cloned into the *Eco*RI site of lambda gt11 by using *Eco*RI-*Not*I-*Bam*HI adaptor (Takara Shuzo, Kyoto, Japan). Recombinant lambda DNA was packaged in vitro using Gigapak Plus (Stratagene) and plated on *E. coli* Y1090$^-$. The cDNA library was screened with anti-ABP19 antibodies and ^{125}I-labeled anti-rabbit IgG essentially as described in Sambrook et al. (1989). The cDNA was subcloned into Bluescript KS$^+$ at the *Eco*RI site and sequenced with an Autocycle Sequencing Kit and ALF DNA Sequencer II from Pharmacia Biotech (Uppsala, Sweden).

Construction of genomic DNA library and cloning of ABP homologs—High molecular weight genomic DNA was isolated from shoot apices of peach as described by Umbeck and Gengenbach (1983), completely digested with *Bam*HI, and size-fractionated on a 5–25% NaCl gradient. Fractionated DNA (approximately 12–16 kb) was treated with calf intestine alkaline phosphatase and cloned into EMBL3 (Stratagene). Recombinant DNA was packaged in vitro using Gigapak Plus (Stratagene) and plated on XLIBlue. The ABP20 cDNA open reading frame (ORF) was labeled with dig according to the manufacture's manual (Boehringer Mannheim, Mannheim, Germany) and used for genomic library screening. Recombinant phage DNAs were double-digested with *Bam*HI and *Sal*I. Genomic DNA inserts were subcloned into Bluescript KS$^+$ at *Bam*HI site. Nested deletions were generated using an Exo/Mung bean deletion kit (Stratagene). DNA sequences were analyzed with the MacVector program (International Biotechnologies, New Haven, CT, U.S.A.). Sequence comparisons were performed using e-mail MPsrch servers (IntelliGenetics Inc., Cambell, CA, U.S.A.) provided by the DNA Information and Stock Center of the National Institute of Agrobiological Resources (Tsukuba, Japan).

RNA and DNA gel blot analyses—Total RNA was denatured at 65°C for 5 min and electrophoresed through a 1.5% agarose gel containing formaldehyde. Transfer of RNA onto a Hybond-N$^+$ membrane (Amersham) and subsequent hybridization were carried out by conventional methods (Sambrook et al. 1989). Coding regions and 3′-untranslated regions of ABP19 and ABP20 were labeled with dig using PCR Dig Probe Synthesis Kit (Boehringer Mannheim) and used as probes.

Genomic DNA was digested with *Bam*HI, *Eco*RI, and *Hin*dIII, separated on a 0.7% agarose gel, and blotted onto Hybond N$^+$ membrane. An *Eco*RI-*Sma*I fragment of ABP20 cDNA was labeled with dig using Dig DNA Labeling Kit (Boehringer Mannheim) and used as a probe.

Immuno-gold electron microscopy—Immuno-gold electron microscopy was performed as described by Ohmiya and Hayashi (1992). The young leaves were taken from the peach shoot, cut into small pieces, and rapidly immersed in 4% (w/v) paraformaldehyde in 0.1 M cacodylate-HCl buffer (pH 7.2) containing 7.5% (w/v) sucrose for 24 h at 4°C. Samples were dehydrated in a graded ethanol series and embedded in EPON 812 (TAAB Lab. Equipment Ltd., Reading, Berks, U.K.). Ultrathin sections were cut with a diamond knife on a Ultrotome NOVA (LKB, Bromma, Sweden) and picked up on nickel grids. Thin sections were incubated with 5% (v/v) normal goat serum in 20 mM Tris-HCl buffer containing 0.15 M NaCl, 0.1% (w/v) gelatin, 0.02% (w/v) sodium azide, and 0.5% (v/v) Tween 20 (TBST) for 20 min. The grids were then incubated with anti-ABP19 antibodies for 1 h, washed with TBST, incubated with colloidal gold-labeled goat anti-rabbit IgG for 1 h, washed with TBST, and finally washed with distilled water. For control staining, sections were incubated with preimmune serum instead of anti-ABP19 antibodies. Samples were observed with JEM 1200 EX (JEOL, Tokyo, Japan) operated at 80 kV.

Results

Direct amino acid sequencing of ABP19 and ABP20—ABP was homogeneous on standard SDS-PAGE gel (Fig. 1A). When the ABP was separated using low-bis SDS-PAGE (Fig. 1B), two types of polypeptides with slightly different mobilities were observed (referred to their molecular mass on low-bis SDS-PAGE gel as ABP19 and ABP20). This is not surprising since resolution of peptide bands on low-bis SDS-PAGE is known to be higher than that of standard SDS-PAGE (Hirano 1989). The difference of mobility between ABP19 and ABP20 may result from differences in molecular mass of the sugar chain in the glycoproteins. N-terminal amino acid sequences of each polypeptide have shown that they are highly homologous, suggesting that they are ABP homologs. It is assumed that the peach ABP is a pentameric protein, because the molecular mass of the purified ABP was estimated to be about 100 kDa.

Nucleotide sequencing of cDNA encoding ABP20—Fifteen positive clones were isolated by the cDNA library screening. Three clones with approximately 1.2 kb inserts were chosen for sequencing analyses, because a full length cDNA which encodes ABP19/20 was estimated to be 1.2 kb from northern analysis (Fig. 2). The nucleotide se-

Fig. 1 Direct peptide sequencing of the N-terminal amino acids of ABP19 and ABP20. A: Laemmli's SDS-PAGE. B: Low-bis SDS-PAGE. Purified peach ABP was subjected to low-bis SDS-PAGE and electroblotted. The bands corresponding to ABP19 (lower) and ABP20 (upper) were isolated and sequenced using a gas-phase protein sequencer. Sequences are shown on right. X: residue not detected. Molecular masses of standard proteins are shown on left in kilodaltons.

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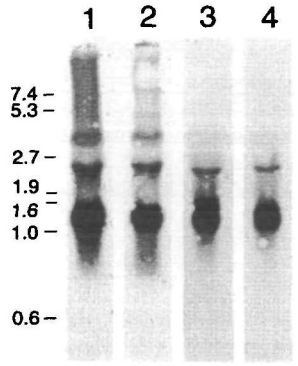

Fig. 2 RNA gel blot analysis. Sixteen μg of total RNA from peach shoot apices were loaded and allowed to hybridize with dig-labeled ABP19 coding region (lane 1), ABP20 coding region (lane 2), ABP19 3′-untranslated region (lane 3), and ABP20 3′-untranslated region (lane 4).

quence and deduced amino acid sequence of one representative clone is shown in Fig. 3. The nucleotide sequence contained an ORF of 627 bp, predicting a 209 amino acid polypeptide of 22 kDa. The deduced amino acid sequence from 19 to 32 was identical to the N-terminal amino acid sequence of ABP20 as determined by gas-phase amino acid sequencing (Fig. 1B). The alignment also indicated that there is an 18 amino acid leader peptide that is absent from the mature protein and the consensus sequence for *N*-glycosylation (N^{60}-T-S).

Cloning and sequencing of ABP homologs—Partial nucleotide sequences of the rest of the 12 positive cDNA clones have revealed that all of them represented the same transcript class, i.e., ABP20, a minor component of purified ABP. A cDNA clone encoding ABP19 was not obtained for some unknown reason. Therefore, we switched our strategy for ABP19 screening from a cDNA library to a genomic library.

Eight positive clones were obtained by the genomic library screening, and a clone encoding ABP19 was chosen by partial nucleotide sequencing of ABP ORF. The nucleotide sequence of the ABP19 ORF contained in the clone is shown in Fig. 4. The deduced amino acid sequence from 19 to 37 was identical to N-terminal amino acid sequence of ABP19 as determined by gas-phase amino acid sequencing

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AAGAAAAGAAAGCTTGCAGCCACCACACACCTATTGCCCCAGGCAACGATGATTTTCCCT  12
                                                M  I  F  P    4

ATCTTGTTCACATTTTTTCTCCTCCTCTCCTCCTCCAATGCGGCTGTGCAAGACTTCTGT  72
I  L  F  T  F  F  L  L  L  S  S  S  N  A  A  V  Q  D  F  C   24

GTTGCAGACTTAGCAGCTCCTGAAGGCCCTGCAGGCTTCTCTTGCAAAAAGCCTGCAAGT 132
V  A  D  L  A  A  P  E  G  P  A  G  F  S  C  K  K  P  A  S   44

GTTAAAGTAAATGATTTTGTGTTCTCGGGCCTGGGCATTGCTGGTAACACCAGTAACATC 192
V  K  V  N  D  F  V  F  S  G  L  G  I  A  G  N  T  S  N  I   64

ATCAAAGCTGCAGTCACCCCTGCATTTGTTGCTCAGTTTCCTGGTGTGAATGGCCTCGGC 252
I  K  A  A  V  T  P  A  F  V  A  Q  F  P  G  V  N  G  L  G   84

ATTTCCATCGCCCGTCTAGACTTGGCGGTTGGCGGAGTTGTCCCATTTCACACACACCCT 312
I  S  I  A  R  L  D  L  A  V  G  G  V  V  P  F  H  T  H  P  104

GGAGCTTCAGAAGTCCTAATTGTCGCCCAAGGGACAATCTGCGCCGGGTTTGTTGCCTCA 372
G  A  S  E  V  L  I  V  A  Q  G  T  I  C  A  G  F  V  A  S  124

GATAACACACCTTATCTGCAAACTCTTGAGAAGGGTGACATTATGGTTTTCCCACAGGGG 432
D  N  T  P  Y  L  Q  T  L  E  K  G  D  I  M  V  F  P  Q  G  144

CTGTTGCACTTCCAAGTCAATGGAGGTGAGGCTCCAGCCCTTGCATTTGCTAGCTTCGGG 492
L  L  H  F  Q  V  N  G  G  E  A  P  A  L  A  F  A  S  F  G  164

AGTGCAAGCCCGGGTCTCCAAATTCTGGACTTTGCTTTGTTCAAAAACGATTTGCCTACC 552
S  A  S  P  G  L  Q  I  L  D  F  A  L  F  K  N  D  L  P  T  184

GAAGTAATAGCTCAGACTACTTTCCTTGATGCTGCTCAGATAAAGAAACTGAAGGGTGTT 612
E  V  M  A  Q  T  T  F  L  D  A  A  Q  M  K  K  L  K  G  V  204

CTTGGTGGTACTAATTAATTAAGGTCTCTCTCTTGAGATGCCAAAAAGCAATGTCACTAG 672
L  G  G  T  N  *                                              209

AAGATTGTCTCTTTCTTTTTCGGTCCATGTCATGTCCATGTCCATTTTGGTTTTCTTTCC 732

TGTGTTTGAGAAGGCCTGTAACTGCAGGATTAATTAGAGTTCAGGTCAGTGGCTGTTTAA 792

TTGTATGATTGTAGAAGTTTTAATGTGTGTGTGCTTAATGTTGAAGGATTTTAGTAGACT 852

ATATTATGCCCCCATAATATCTTCTCCCATGTCCTCAAGCCTCACATTTTCAGACTCTCA 912

AAGATTGTCTCTTTCTTTTTAAAAAAAAAAAAAAAAAAAAA
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Fig. 3 Nucleotide sequence of ABP20 cDNA (top) and deduced amino acid sequence (bottom). The putative signal peptide is underlined, and a consensus sequence for N-glycosylation is boxed.

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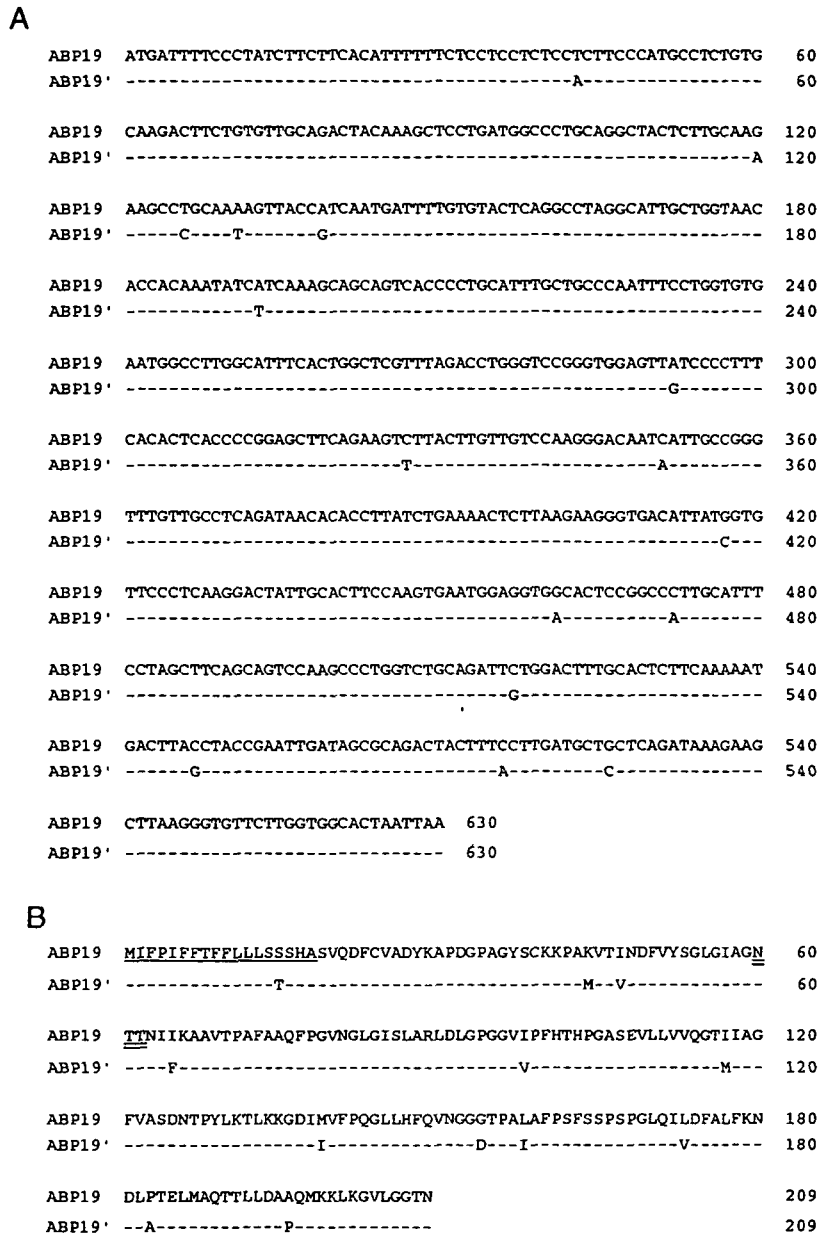

Fig. 4 Nucleotide sequences of genomic DNA of ABP19 and ABP19′ coding regions (A) and deduced amino acid sequences (B). The putative signal peptide is underlined, and a consensus sequence for N-glycosylation is double-underlined. Identical nucleotides and amino acids are indicated by dashes in ABP19′.

(Fig. 1B).

ABP ORFs were amplified from phage DNA of genomic positive clones using two sets of primers generated from the sequences of either ABP19 (ABP19F: 5′-CTGGTACCCTCAGCCATCATG-3′, ABP19R: 5′-CTCTTCGGGAGCTCAATAGACC-3′) or ABP20 (ABP20F: 5′-CAGGCAATCATGATTTTCCCTA-3′, ABP20R: 5′-GAGAGAGCTCAATTAATTAGTACCAC-3′). All the PCR products obtained were approximately 650 bp in length, suggesting that the ABP genes do not contain introns. Among the eight clones used, six generated a 650 bp product with either ABP19F/R or ABP20F/R primers. The remaining two clones generated a 650 bp product only with ABP20F/R primers. All of the sequences of PCR products generated with ABP20F/R primers were identical to that of the ABP20 cDNA. Of 6 PCR products amplified with ABP19F/R primers, one of the sequences was identical to ABP19, and the others had 97.5% similarity to ABP19 (designated

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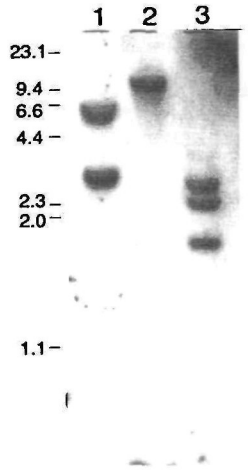

Fig. 5 Genomic DNA gel blot analysis. Ten μg of peach genomic DNA were digested with *Eco*RI (lane 1), *Bam*HI (lane 2), and *Hind*III (lane 3). Fragments were allowed to hybridize with dig-labeled ABP20 cDNA. Numbers at left indicate markers in kilobases.

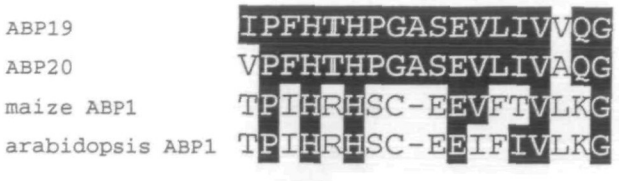

Fig. 6 Sequence comparison of BoxA with the corresponding sequences of ABP19, ABP20, maize ABP1 (Inohara et al. 1989), and arabidopsis ABP1 (Palm et al. 1992).

ABP19′). Over the 209 amino acids, ABP19′ differed from ABP19 and ABP20 at 12 and 26 amino acids, respectively.

The deduced amino acid sequences of three peach ABP homologs share three common structure features: (1) the ABPs are composed of 209 amino acids with N-terminal signal sequences of 18 amino acids, (2) the calculated molecular mass of the entire protein is 22 kDa and that of the mature protein is 20 kDa, and (3) there is a possible glycosylation site at N^{60}.

RNA and DNA gel blot analyses—Northern blot analysis was performed using 3′-untranslated region of either ABP19 and ABP20 as probes (Fig. 2). We found that there is little difference in the level of transcripts between ABP19 and ABP20 in shoot apices of peach. It was therefore expected that positive clones encoding either ABP19 or ABP20 may be obtained by immunoscreening of the cDNA library. Only clones encoding ABP20, however, were actually obtained and the reason has not yet been elucidated.

Among three ABP homologs, only one *Hind*III site existed at 3′-end of the ORF in ABP19. Southern blot analysis was performed using the 5′-region which does not contain a *Hind*III site (Fig. 5). Three bands in the lane of *Hind*III digestion on the Southern blot indicated that there are

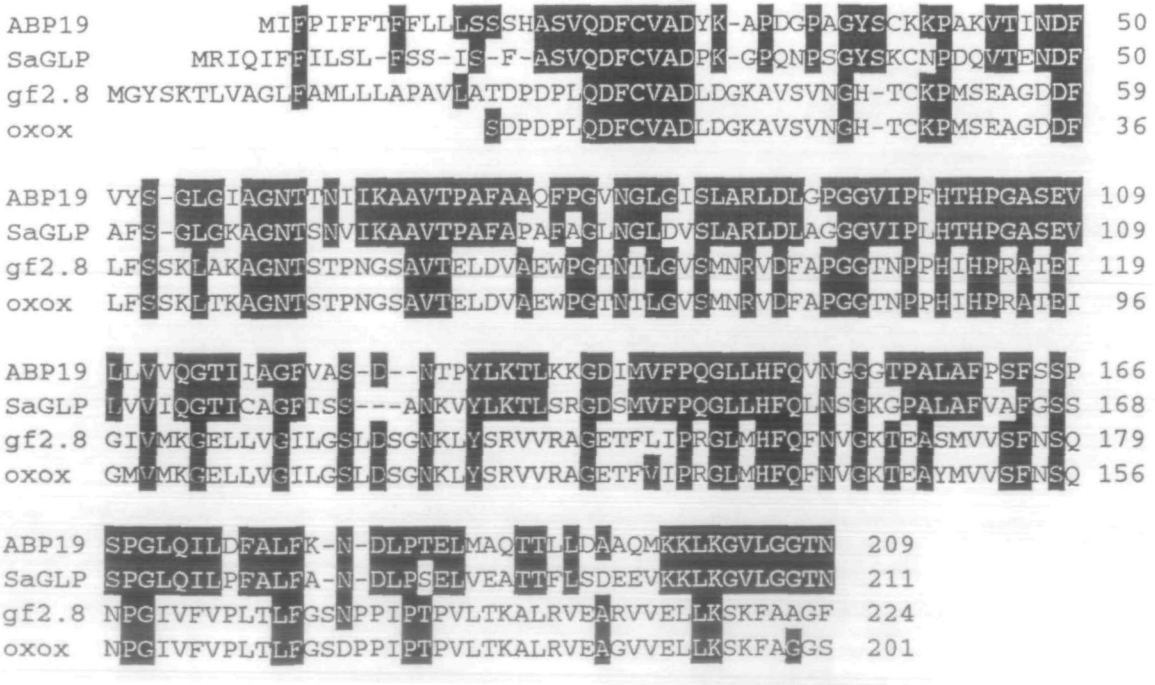

Fig. 7 Alignment of amino acid sequences of ABP19 with SaGLP (Heintzen et al. 1994), gf2.8 (Lane et al. 1991), and oxox (Lane et al. 1993). Gaps introduced to optimize the alignments are marked by dashes.

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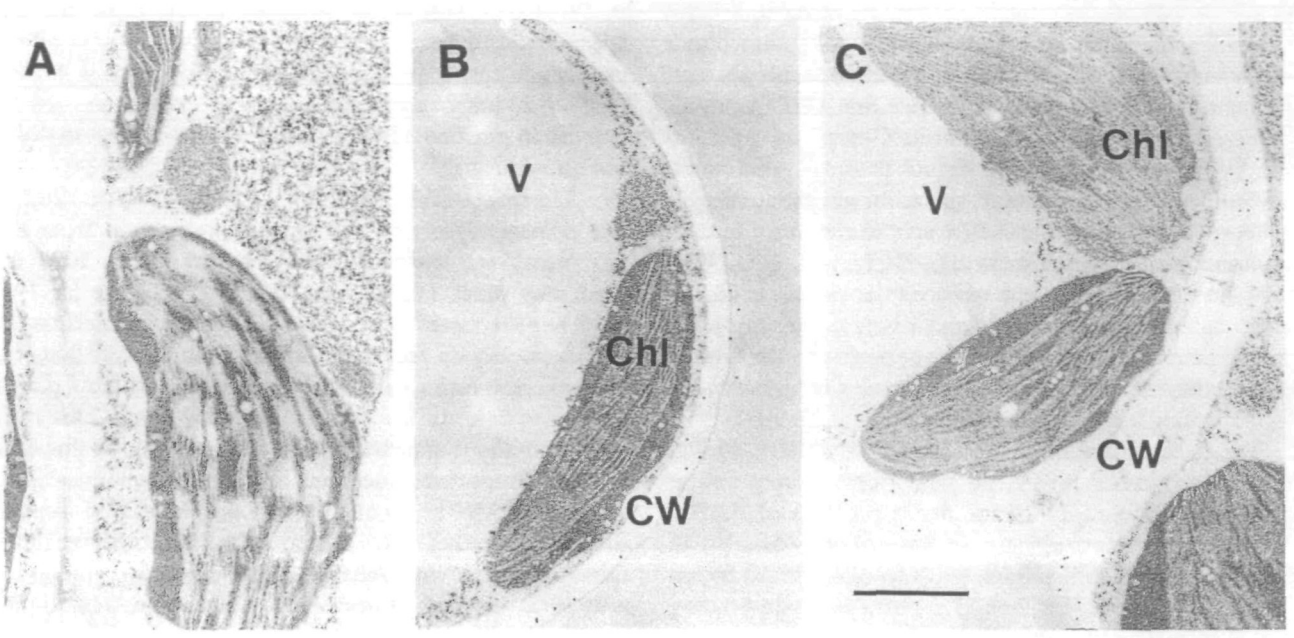

Fig. 8 Subcellular localization of ABP19/20 in leaf cells. A, Control staining of a palisade parenchyma cell. B and C, A spongy parenchyma cell (B) and a palisade parenchyma cell (C) stained with anti-ABP19 antibodies. Bar = 1 μm; × 15,000. Chl, chroloplast; CW, cell wall; V, vacuole.

at least three ABP homologs in peach genome.

Homology of peach ABPs with maize ABP1—The homology between the entire ABP19 protein and the ABP1 from maize was relatively low (< 5%). However, there was a region (98–115) which shares 40% homology (Fig. 6). In particular, proline, glutamic acid, valine, glycine, and two histidines are conserved among peach ABPs and maize ABP1 homologs. This region in ABP1 coincided with BoxA, a putative binding site for auxin. There are several regions that are highly conserved among ABP1 homologs (Jones 1994). None of them except BoxA, however, share homology with peach ABPs.

Homology to germin-like protein—Comparison of the nucleotide sequence and deduced amino acid sequence of ABP19 with available databases revealed that ABP19 is highly homologous to proteins related to the germin family. The most striking homology was found with a germin-like protein from mustard (SaGLP, Heintzen et al. 1994). The nucleotide sequence of ABP19 precursor protein shares 67.7% homology with that of SaGLP precursor (Fig. 7). Sequence homology increases to 71.9% when compared to mature protein. Homology between ABP19 and germin from monocotyledons such as barley (oxox: 22.1%; Lane et al. 1993) and wheat (gf2.8: 21.9%; Lane et al. 1991) was relatively low. The region surrounding a potential N-linked glycosylation site (N^{60}-T-T/S) and the beginning of the mature protein (Q^{21}DFCVAD) are extensively conserved among peach ABP homologs and the germin family.

Subcellular localization of ABP19/20—Fig. 8 shows immuno-gold localization of ABP19/20 in cells of developing leaves of peach shoot. The control staining showed a very low level of randomly distributed gold particles over the sections. In cells of both palisade and spongy parenchyma, the cell wall appeared to be a predominant location site of ABP19/20. The level of label in the plasma membrane was low in all cell types. This may be partly due to poor preservation of membrane structure since, during the tissue fixation procedure for immunocytochemistry, an osmium fixation step was omitted for preservation of antigenicity (Ohmiya and Hayashi 1992). There was a very low level of labeling, almost background level, of other cellular compartments such as the nucleus, cytoplasm, and vacuole.

Discussion

Analyses of peach ABP clones obtained from both cDNA and genomic libraries have revealed that the peach ABP consists of a small multigene family with at least three classes of ABP. The nucleotide sequences of these homologs contained an ORF of 627 bp, predicting a 209 amino acid polypeptide of 22 kDa. It is assumed that peach ABPs are glycosylated since it contained a putative glycosylation site at N^{60} and is retained on a conA-Sepharose column (Ohmiya et al. 1993). In addition, peach ABPs have an N-terminal stretch of hydrophobic amino acids. These results suggest that the cell wall and/or plasma membrane is a possible location site for ABP19/20. Immuno-gold elec-

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tron microscopy revealed that ABP19/20 is associated within the cell wall. Jones and Herman (1993) have shown that most of maize ABP1 is located in the endomembrane system, including the ER. Despite the fact that ABP1 contains an ER-retention signal (KDEL) at its C-terminus (Inohara et al. 1989), some ABP1 was found at the plasma membrane and within the cell wall space. In addition, there is considerable evidence that ABP1 acts at the outer face of the plasma membrane (discussed by Barbier-Brygoo 1995). These data indicate that the extracellular matrix is an important cellular compartment functioning as a primary site of auxin action. This hypothesis is supported by the finding that the concentration of endogenous auxin is higher in the apoplast than in the symplast (Tsurusaki et al. 1997).

The deduced amino acid sequence of ABP19/20 was compared to that of ABP1, an ABP isolated from maize. Although the homology of the entire proteins of ABP19 and ABP1 was relatively low (<5%), there was a small region that shared 40% homology. Interestingly, the region in ABP1 coincided with BoxA, a putative auxin-binding site of ABP1. The idea that BoxA plays an important role in auxin-binding was first proposed by Venis et al. (1992), based on previous studies using chemical modification which demonstrated that histidine, cysteine, tyrosine or lysine (Venis 1977), and arginine (Nave and Benveniste 1984) are necessary for auxin-binding. BoxA is rich in charged residues and contains four of these five amino acids. Venis et al. (1992) showed that antibodies directed against a synthetic BoxA induced an in vitro auxin response in the absence of auxin. Comparison of the deduced amino acid sequences of ABP1 from three dicots and one monocot showed a high degree of conservation in BoxA (Jones 1994). Recently, Sugaya and Sakai (1996) isolated a protein with auxin-binding activity using antibodies specifically raised against the hexapeptide (HRHSCE) in BoxA. These results suggest that the structure of BoxA may be conserved among proteins which have auxin-binding activity.

Comparison of the nucleotide sequence and deduced amino acid sequence of ABP19 with available databases revealed that ABP19 is highly homologous to proteins related to the germin family. There was a low homology between ABP19 and germin from monocotyledons such as barley (oxox; Lane et al. 1993) and wheat (gf2.8; Lane et al. 1991). Germin is a cell wall protein whose synthesis is induced during germination of wheat embryos. Lane et al. (1993) have found that germin is highly homologous in amino acid sequence to barley oxalate oxidase. Degradation of oxalate releases Ca^{2+} and H_2O_2, both of which are thought to be necessary for the cross-linking reaction of cell wall polysaccharides and to be allied with cell wall expansion and lignification during germination (Lane 1994). It is worthy of note that germin from wheat (gf2.8) is a cell wall protein and contains an auxin responsive element in its 5′-flanking region (Lane et al. 1991). It is therefore speculated that germin has a role in auxin-mediated cellular expansion during germination. A question arises whether ABP19/20 has oxalate oxidase activity. We examined this activity in purified ABP19/20 but did not detect any (data not shown).

The most striking homology was found with a germin-like protein. Two types of germin-like protein from long day plant *Sinapis alba* (SaGLP; Heintzen et al. 1994) and short day plant *Pharbitis nil* (PnGLP; Ono et al. 1996) have been reported. They were isolated as transcripts that are specifically up-regulated during photoperiodic flower induction, suggesting that these germin-like proteins play an important role on the induction of flowering. This raises the possibility that peach ABP is also involved in flower induction. Important questions are whether changes in the amount of ABP19/20 affect the flower induction in peach shoot and whether auxin promotes or inhibits flower induction in concert with ABP19/20. The precise function of ABP19/20 has not yet been elucidated and answers to these questions will provide us further insight into the role of ABP19/20 in flowering.

High conservation was exhibited in the domain 101 through 108, the region homologous to BoxA in ABP1, among proteins in the germin family and peach ABP homologs. We speculate that this domain may play an important function in ligand-binding. Considering the fact that both auxin and oxalate, a substrate of oxalate oxidase, have a carboxylic acid, the region may play an important function in carboxylic acid binding. Jones (1994) pointed out that a carboxylic acid binding site could be formed by the positively charged cluster of H-R-H or by R alone. Both peach ABPs and proteins of germin family, however, do not contain an arginine residue between two invariant histidines (H-T-H or H-I-H). We speculate that the H-X-H structure is essential for carboxylic acid binding. Since ABP1 has a lower dissociation constant (higher affinity) for auxin-binding than peach ABP, the arginine residue may help auxin bind strongly to proteins by providing a positively charged site.

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