The EMBO Journal vol.8 no.9 pp.2463-2467, 1989

cDNA clones of the auxin-binding protein from corn coleoptiles (*Zea mays* L.): isolation and characterization by immunological methods

Ulrich Tillmann[1,4], Gero Viola[1], Bernhard Kayser,[1] Gerhard Siemeister[1], Thomas Hesse[1,2], Klaus Palme[2], Marian Löbler[3,5] and Dieter Klämbt[1]

[1]Botanisches Institut, Universität Bonn, D5300 Bonn 1, [2]Max-Planck-Institute für Züchtungsforschung, D5000 Köln 30, FRG and [3]Division of Plant Molecular Biology, University of California, Berkeley, CA 94720, USA

[4]Present address: National Institutes of Health, Bldg 6, Rm 338, Bethesda, MD 20892
[5]Present address: Department of Biology, UCLA, Los Angeles, CA 90024, USA

Communicated by J.Schell

An auxin-binding protein (ABP) cDNA clone was selected from a λgt11 cDNA library from corn coleoptiles with highly purified $IgG_{anti\ ABP}$. The sequence of 794 bp contains an open reading frame (ORF) of 603 bp, coding for a 22 kd protein. There are indications of a signal peptide of 38 amino acids (von Heijne,G. 1983, *Eur. J. Biochem.*, 133, 17-21). A N-glycosylation site can be deduced and a C-terminal KDEL amino acid sequence is detected. An *Eco*RI fragment containing the beginning portion of the cDNA with about three quarters of the ORF was used to select cDNA clones from an independently produced λgt11 cDNA library of corn coleoptiles. Northern blot analysis with *in vitro* transcribed biotinylated RNA showed a single band of not more than 850 bases. The full-length *in vitro* transcript directed the *in vitro* synthesis of a protein which is precipitated by $IgG_{anti\ ABP}$. Rabbit antibodies raised against a fusion protein detect the ABP as a double band on Western blots. Only the smaller of the two ABP bands is labeled by two different KDEL-specific IgG preparations.

Key words: biotinylated RNA/cDNA sequence/$IgG_{anti\ ABP}$/$IgG_{anti\ KDEL}$/*in vitro* transcription

Introduction

The auxin-binding protein (ABP) solubilized from total membrane preparations of corn coleoptiles has been described as a putative auxin receptor by Löbler and Klämbt (1985a,b). They localized the glycosylated ABP predominantly at the outer epidermis. This finding is in accordance with the reactivity of these cells to auxin-induced cell elongation (Thimann and Schneider, 1938; Kutschera *et al.*, 1987). The application of highly purified $IgG_{anti\text{-}ABP}$ to coleoptile segments and split coleoptiles before auxin incubation caused significant inhibition of the auxin effect. These results led to the interpretation that the ABP molecules which mediate the auxin effect have to be localized at the plasmalemma. Experiments characterizing auxin-binding sites in different vesicle preparations of corn coleoptiles (Dohrmann *et al.*, 1978; Shimomura *et al.*, 1988) and corn mesocotyles (Walton and Ray, 1981), showed a site, called site I, in endoplasmic reticulum (ER) vesicles as the main auxin-binding site. Recently Barbier-Brygoo *et al.* (1989) demonstrated that the auxin effect upon hyperpolarization of tobacco mesophyll protoplasts can be specifically inhibited by application of $IgG_{anti\ ABP(corn)}$. Therefore, the auxin-stimulated H^+-ATPase activity also seems to be mediated by an ABP at the plasmalemma. Proteins translated *in vitro* from poly(A)$^+$ RNA of corn coleoptiles contained pre-ABP as shown by immunoprecipitation (Löbler *et al.*, 1987). These findings encouraged us to screen cDNA libraries for ABP cDNA clones, which would be helpful for further analyses of ABP-mediated auxin effects.

Results

Isolation of a cDNA clone for the ABP

In the initial screening of 250 000 plaques of a corn coleoptile cDNA library, 16 clones were detected that gave positive signals with our highly purified antibody probe. The putative ABP clones were plaque purified and retested in three more rounds of screening at increasingly lower phage densities until all plaques scored positively. The immunostaining signal produced by different clones varied considerably. One clone, designated λgt11.6, consistently exhibited a much stronger immunostaining signal than the others. SDS-PAGE analysis of bacterial lysates showed that λgt11.6 directs the synthesis of a 135 kd β-galactosidase fusion protein which is detected on Western blots by both the purified $IgG_{anti\text{-}ABP}$ (Löbler *et al.*, 1987) as well as by a preparation of IgG that was raised against SDS-denatured ABP (Löbler *et al.*, 1987). The increase in the size of the fusion protein as compared to the β-galactosidase encoded by the parental vector suggested that the cDNA insert of λgt11.6 might encompass the entire protein-coding region of the ABP.

DNA sequencing

Different pUC19-derivatives were constructed for sequencing: pUC350 and pUC450 harbor the two *Eco*RI fragments of λgt11.6, pUC800 contains the entire cDNA, pUC340 contains a fragment from the *Eco*RI linker to the first *Rsa*I site (nucleotide position 343) and pUC184 contains the *Rsa*I fragment (positions 374-558) spanning the internal *Eco*RI site (position 455) of the cDNA. The derived DNA sequence is shown in Figure 1. The longest open reading frame (ORF) starts at the first AUG at base 15 and is deduced to code for a protein of ~22 kd. The next possible translation starting point lies in the same reading frame at base 171 and would code for a polypeptide of ~17 kd. All available data suggest usage of the longest ORF (Kozak, 1984; Löbler *et al.*, 1987). The larger (450 bp) *Eco*RI fragment was used to screen an independently constructed λgt11 cDNA library from corn coleoptile mRNA. The longest insert among the six positive clones was subcloned into the plasmid pBS M13+ resulting in plasmid pBS.ABP which was analyzed by restriction mapping and partially sequenced. These

```
1                                                    M  A  P  D  L  S  E  L  A
1                                          GTCGGGAGCAGGCAATGGCGCCGGATCTAAGCGAACTCGCC
CCTCATTCCACTCCGACATTCACGTGCAGCTGTCGGGAGCAGGCAATGGCGCCGGATCTAAGCGAACTCGCC

10   A  A  A  A  A  R  G  A  Y  L  A  G  V  G  V  A  V  L  L  A  A  S  F  L
42   GCCGCCGCTGCAGCCCGTGGCGCCTACCTCGCCGGCGTCGGTGTCGCGGTCCTCCTCGCTGCCTCCTTCCTC
     GCCGCCGCTGCAGCCCGTGGCGCCTACCTCGCCGGCGTCGGTGTCGCGGTCCTCCT

34   P  V  A  E  S  S  C  V  R  D  N  S  L  V  R  D  I  S  Q  M  P  Q  S  S
114  CCAGTAGCCGAGTCGTCCTGCGTGCGAGATAACTCATTGGTGAGAGACATAAGCCAAATGCCGCAAAGCAGC

58   Y  G  I  E  G  L  S  H  I  T  V  A  G  A  L  N  H  G  M  K  E  V  E  V
186  TATGGGATTGAAGGATTGTCACATATAACAGTTGCTGGTGCGCTCAATCATGGGATGAAGGAGGTGGAAGTG

82   W  L  Q  T  I  S  P  G  Q  R  T  P  I  H  R  H  S  C  E  E  V  F  T  V
258  TGGCTTCAGACAATAAGTCCAGGTCAAAGGACGCCGATCCACAGGCATTCCTGTGAAGAAGTTTTCACTGTC

106  L  K  G  K  G  T  L  L  M  G  S  S  S  L  K  Y  P  G  Q  P  Q  E  I  P
330  CTCAAAGGGAAGGGTACGCTCTTGATGGGATCAAGCTCACTAAAGTACCAGGGCAGCCACAGGAAATTCCT
                                                    GTACCCAGGGCAGCCACAGGAAATTCCT

130  F  F  Q  N  T  T  F  S  I  P  V  N  D  P  H  Q  V  W  N  S  D  E  H  E
402  TTCTTTCAGAATACCACATTTTCAATCCCTGTAAACGATCCACACCAGGTTTGGAATTCTGACGAGCACGAA
     TTCTTTCAGAATACCACATTTTCAATCCCTGTAAACGATCCACACCAGGTTTGGAATTCTGACGAGCACGAA

154  D  L  Q  V  L  V  I  I  S  R  P  P  A  K  I  F  L  Y  D  D  W  S  M  P
474  GATTTGCAAGTTCTTGTGATCATTTCGAGACCACCTGCTAAGATATTTTTATATGATGATTGGAGCATGCCT
     GATTTGCAAGTTCTTGTGATCATTTCGAGACCACCTGCTAAGATATTTTTATATGATGATTGGAGCATGCCT

178  H  T  A  A  V  L  K  F  P  F  V  W  D  E  D  C  F  E  A  A  K  D  E  L
546  CATACAGCCGCGGTACTGAAATTCCCCTTCGTCTGGGATGAGGACTGCTTCGAAGCAGCAAAAGACGAACTC
     CATACAGCCGCGGTAC

     *
618  TAGGTCACAAGTGTTTCCTGCAATGTATCTGCTTCATCCATGATCCTGCTGGACTACTAAAATTCACATGCA

690  CTAGTCGTAATAAAGCCAGTGCGCTTTTCATGTATAATTCTGTATTGTGCTCGCTAAAATAAAATTTGGCAA
                                TTTTCATGTATAATTCTGTATTGTGCTCGCTAAAATAAAATTTGGCAA

762  CGGTTTATGAAAAAAAAAAAAAAAAAAAAAA
     CGGTTTATGAACTTATGCGCTTGTGGAGTTGATCCAATGCTCACCGTTTAAAAAAAAAAA
```

Fig. 1. The complete nucleotide sequence, derived from λgt11.6 cDNA, and the deduced amino acid sequence. Nucleotide sequences of the partially sequenced pBS.ABP are given in addition. Sequences are shown without *Eco*RI linker (GGAATTCC). *Rsa*I and the internal *Eco*RI restriction sites are underlined. The arrow shows the predicted signal sequence cleavage site. The arrowhead indicates the possible N-glycosylation site.

sequences were identical to those of pUC800 with the exception of some additional bases in the noncoding ends of pBS.ABP.

Northern blotting

The Northern blot was carried out by using a biotinylated probe prepared by substituting biotin-11-UTP for UTP in the T3 polymerase transcription mix. Detection of hybrids was carried out with streptavidin-coupled phosphatase and the chromogenic substrates as in the Western blot procedure. Poly(A)$^+$ RNA from 3-day-old etiolated corn coleoptiles was hybridized with run-off transcripts of pBS.ABP DNA linearized with *Sac*I. The mRNA detected has a maximum size estimated to be 850 bases (Figure 2). This suggests that our cDNA is almost full length.

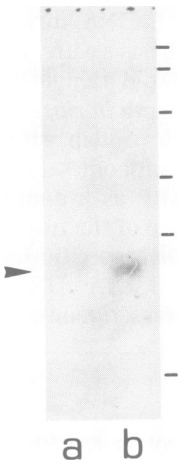

Fig. 2. Northern blot analysis of corn coleoptile poly(A)$^+$ RNA; 5 μg (**lane a**) and 10 μg (**lane b**) of RNA were electrophoresed on a gel containing 1.4% formaldehyde, transferred to a nitrocellulose membrane, and hybridized with biotinylated run-off transcripts from *Sac*I-cleaved pBS.ABP. The arrowhead indicates a hybridization signal localized to a size region of ~0.8 kb. Bars indicate the positions of the mol. wt markers from top: 9.5, 7.5, 4.4, 2.4, 2.4, 0.24 kb.

Characterization of the cDNA translation product

Two strategies were used to test whether the cloned cDNA codes for the ABP. The first was to translate the putative ABP cDNA *in vitro* and characterize the product. Therefore the *Bam*HI−*Hin*dIII digestion fragment of pUC800 containing the whole ABP-cDNA, was cloned into the transcription vector pGem1. This plasmid, pGem.ABP, allows synthesis of sense RNA with SP6 RNA polymerase. The transcription resulted in a single RNA species of ~850 bases, which directed the synthesis of a 22 kd protein in a wheat germ translation system (Figure 3, lane b). This result is consistent with the sequence in Figure 1, if the first AUG is the translation start. The 22 kd protein is immunoprecipitated with highly purified $IgG_{anti\text{-}ABP}$ and ABP, isolated from corn coleoptiles, competes with IgG binding in immunoprecipitations (Figure 3, lanes d−f). Similar results were obtained, using T7 RNA polymerase-mediated transcription of pBS.ABP for *in vitro* translation instead of T3 RNA polymerase (data not shown).

The second strategy was the production of antibodies against the cDNA product. The *Eco*RI fragment of ~450 bp, coding for the N-terminal part of the cDNA

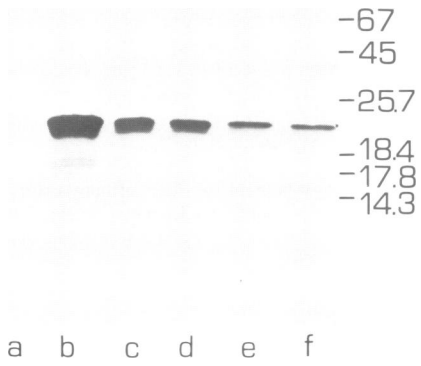

Fig. 3. Analysis of the *in vitro* translation products and immunoprecipitations by SDS−PAGE and fluorography. The wheat germ translation system was directed by run-off transcripts of *Bam*HI-linearized pGem.ABP in the presence of [^{35}S]methionine. **Lane a**, control, no RNA added to the translation system; **lane b**, total translation products; **lane c**, immunoprecipitation of translation products by affinity-purified $IgG_{anti\ ABP}$; **lanes d−f**, same as lane c, but immunoprecipitation completed by increasing amounts of purified ABP from corn coleoptiles (1:2:4). Mol. wt markers given in kilodaltons.

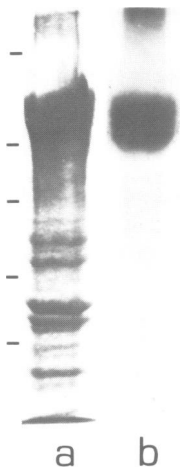

Fig. 4. β-Galactosidase ABP fusion product. A total preparation of *E.coli* proteins obtained after IPTG induction is shown. **Lane a**, Coomassie blue staining after SDS−PAGE; **lane b**, immunostaining after Western blotting with highly purified $IgG_{anti\ ABP}$. Bars indicate mol. wt markers from top: 200, 96, 67, 45, 30, 21.5 kd.

product, was cloned into the *Eco*RI site of the expression vector pUR292 (Rüther and Müller-Hill, 1983). An *Escherichia coli* strain (JM103) was transformed with pUR292.ABP, induced with isopropylthiogalactoside (IPTG), and screened for the production of an ABP β-galactosidase fusion protein. Immunoreactive colonies were detected by immunostaining of colony replicas on nitrocellulose filters with highly purified $IgG_{anti\ ABP}$. One of these colonies was selected to overproduce the fusion protein (Figure 4). The fusion protein showed β-galactosidase activity which was used to assay for the purification of this protein by affinity chromatography (Ullmann, 1984). Rabbits were immunized with this purified fusion protein. The amount of IgG directed against the protein coded by this truncated cDNA in the antiserum was low, nevertheless, it could be demonstrated that the antibody reacts with the ABP from corn coleoptiles (Figure 5).

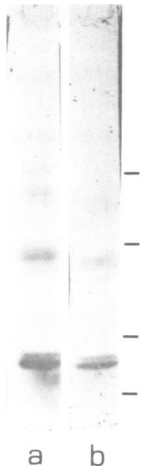

Fig. 5. Reactivity of crude ABP with anti-ABP IgG. A partially purified preparation of solubilized corn coleoptile membrane proteins (0.3 M NaCl eluate of DEAE−cellulose) (Klämbt and Löbler, 1987) was subjected to SDS−PAGE and blotted onto nitrocellulose membrane. Immunodetection was carried out with either IgG from rabbits immunized with corn coleoptile ABP (**lane a**) or with IgG from rabbits immunized with β-galactosidase ABP fusion protein. Bars indicate mol. wt markers from top: 67, 45, 25, 12.5 kd.

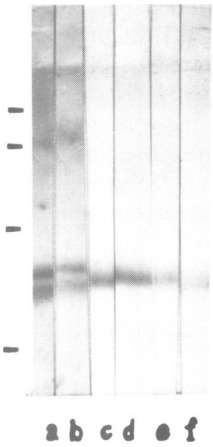

Fig. 6. Immunostaining of ABP Western blots after SDS−PAGE (15%) with different primary antibodies. Results from two independent experiments are shown. **Lanes a** and **b**, staining with $IgG_{anti\ SDS-ABP}$; **lanes c** and **d**, staining with $IgG_{anti\ KDEL}$ affinity selected by the undecapeptide Sepharose (carboxy end of rat BIP); **lanes e** and **f**, staining with $IgG_{anti\ KDEL}$ affinity selected by the octapeptide Sepharose (carboxy end of rat PDI). Bars indicate mol. wt markers from top: 67, 45, 25, 12.5 kd.

A further experiment to prove the identify of the cDNA product with the ABP was the identification of the known tetrapeptide KDEL at the C-terminus at the ABP. Crude $IgG_{anti\text{-}SDS-ABP}$ preparations (Löbler *et al.*, 1987) were incubated overnight with affinity matrices which contained either an octa- or an undecapeptide with a C-terminal KDEL sequence (kindly provided by Steve Fuller). The peptide-bound IgG was eluted at pH 11.0 after extensive washing. The resulting putative $IgG_{anti\text{-}KDEL}$ is shown to react with the smaller of the two ABP bands detected on Western blots with anti-ABP IgG (Figure 6).

Discussion

The immunological data presented here prove that the cDNA we have isolated encodes ABP. The cDNA is the same as

selected by oligonucleotides designed from the amino acid sequence of the purified main component of ABP (Hesse *et al.*, 1989). The differences between the ABP from corn coleoptiles and the *in vitro*-produced cDNA product may give some insight into the fate of the ABP within plant cells. The *in vitro* cDNA-encoded protein (22 kd) as well as the immuno-precipitated ABP from poly(A)$^+$ RNA translations is larger than the ~18 kd peptide core of the corn coleoptile glycoprotein (Löbler *et al.*, 1987; Napier *et al.*, 1988). This finding is consistent with previous results. The difference in size may be due to processing events at the N-terminal signal sequence *in vivo*. The signal sequence prediction method of von Heijne (1983, 1985), which was based on a sample of 118 eukaryotic signal sequences, strongly indicates a cleavage site between the two serine residues at amino acid position 38–39. This modification would result in a molecular mass of the unglycosylated ABP of ~18.5 kd which is in accordance with the experimental data of Löbler *et al.* (1987).

It has to be mentioned that, up to now, all ABP preparations from corn coleoptiles did not contain a homogeneous protein except for the use of analytical methods (Hesse *et al.*, 1989). Even affinity chromatography methods of purification with immobilized auxin analogs result in at least two protein species of slightly different sizes (Figure 5; Shimomura *et al.*, 1986). Other purification procedures are also unable to separate these proteins. Since monoclonal antibodies recognize both proteins (Napier *et al.*, 1988) a close relationship between these proteins is indicated.

It is uncertain whether these findings are due to purification artifacts, catabolic events (Napier *et al.*, 1988), or the result of a controlled process. In our opinion the latter alternative is more plausible. The C-terminal KDEL sequence is known as an ER-sorting element (Munro and Pelham, 1987). Therefore, our sequence data are in accordance with results which show the main auxin-binding activity at ER vesicles (Dohrmann *et al.*, 1978; Walton and Ray, 1981; Shimomura *et al.*, 1988). On the other hand, physiological data indicate the presence of ABP at the plasmalemma (Löbler and Klämbt, 1985b; Barbier-Brygoo *et al.*, 1989). The $IgG_{anti\text{-}KDEL}$, a subset of $IgG_{anti\text{-}ABP}$, are able to recognize the ABP. This is further proof for the identity of the cDNA product and the ABP. But only one of the two purified ABP species is detected, indicating that the other ABP fraction lacks the ER-sorting signal and is secreted to fulfill its function in hormone recognition and signal transformation. It will be necessary in further experiments to elucidate the real origin of the secreted ABP. The different forms of ABP may be genetically encoded or the product of post-translational events. The function of the 'ABP' that resides in the ER has to be analyzed.

Materials and methods

Restriction enzymes, T4 DNA ligase, calf intestinal phosphatase and plasmid pUC19, were purchased from Boehringer Mannheim, FRG. Goat anti-rabbit IgG alkaline phosphatase conjugate and *p*-aminobenzyl 1-thio-β-D-galactopyranosideagarose were obtained from Sigma, Taufkirchen,FRG. Horse hemoglobin, Fast Blue B Salt and β-naphthyl acid phosphate were from Serva, Heidelberg, FRG and cyanogen-activated Sepharose 4B, Protein A Sepharose, *E.coli* JM103 and PD 10 columns from Pharmacia, Freiburg, FRG. Plasmid pGem-1 and bacterial strains *E.coli* Y 1090 and Y 1089 were obtained from Promega Biotech,, Madison, WI, USA. Plasmid pBSM13$^+$ was from Stratagene, San Diego, CA, USA and *E.coli* BNN 93 from the American Type Culture Collection, Rockville, MD, USA. All other reagents were of the highest available purity.

Preparation of affinity matrices

Escherichia coli–Sepharose. The bacterial pellets from cultures of Y 1090 and BNN03 were resuspended in coupling buffer 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.5), subjected to three freeze–thaw cycles and sonicated (six 10 s bursts, Branson sonifier, full setting). After centrifugation (40 000 *g*, 4°C, 1 h) the supernatant was recovered and part of it coupled to cyanogen-activated Sepharose 4B following the manufacturer's protocol. The resulting affinity matrix, *E.coli*–Sepharose, was finally equilibrated with TBE (50 mM Tris, 0.15 mM NaCl, pH 7.4).

ABP–Sepharose. Preparation of an ABP affinity matrix followed the procedures described by Löbler and Klämbt (1985b). 300 μg of highly purified ABP were coupled to 1 g of cyanogen activated Sepharose 4B and finally equilibrated in TBS.

Antibody preparation

Rabbits were immunized with the appropriate antigen as described (Löbler and Klämbt, 1985a). Crude IgG preparations were obtained by three successive ammonium sulfate precipitations (33% saturation) of rabbit sera. These preparations were used for Western blot immunostaining unless otherwise stated.

Purification of antibodies

Total IgG were purified by affinity chromatography on Protein A–Sepharose as recommended by the manufacturer. In order to remove IgG crossreacting with *E.coli* proteins, the purified IgG (30 mg total, 1 mg/ml in TBS) were incubated in batches by shaking with *E.coli*–Sepharose for 2 h at room temperature. After sedimentation of the Sepharose beads the supernatant, containing the nonadsorbed IgG was pumped onto an ABP–Sepharose column (flow rate: 5 ml/h, 4°C), and the flow-through was continuously reapplied over 24 h. The column was then washed with TBS until A_{280} was <0.01. Monospecific $IgG_{anti\ ABP}$ were eluted with ethylene glycol pH 11 (NaOH) after incubation for 2 h at 4°C as described (Löbler and Klämbt, 1985b) and transferred into TBS by gel filtration on Sephadex PD 10 columns.

Preparation of $IgG_{anti\text{-}KDEL}$

Two affinity matrices, one containing eight amino acids of the carboxy end of rat PDI (Edman *et al.*, 1985) and the other containing 11 amino acids of the carboxy end of rat BIP (Bole *et al.*, 1986) coupled to Sepharose 4B, were a generous gift of Steve Fuller, EMBL, Heidelberg, FRG. Both C-terminal sequences end up with KDEL. One milliliter of each matrix was batch incubated with 50 mg of crude IgG from antisera raised against SDS-denatured ABP. After extensive washes in columns the IgG bound to the affinity matrices were eluted with 0.15 M NaCl adjusted to pH 11.0 with NH_4OH. The eluates were immediately transferred into PBS by gel filtration through PD10 columns and used as $IgG_{anti\text{-}KDEL}$ probes.

Isolation of cDNA clones

Amplified cDNA libraries were constructed in λgt11 from poly(A)$^+$ RNA of 3-day-old etiolated maize coleoptiles. Approximately 250 000 phages form this library were screened with purified $IgG_{anti\ ABP}$ essentially as described (Huyng *et al.*, 1985). Horse hemoglobin (0.5%) was used to block nonspecific protein-binding sites on nitrocellulose filters. Nitrocellulose replica were blocked overnight and then incubated with the primary, highly purified antibody at a concentration of 0.5 μg/ml for 3 h at room temperature.

Detection of the bound antibodies was as described for immunostaining of Western blots.

Sequence analysis

The sequence of the ABP-cDNA was determined by the dideoxy nucleotide sequencing technique (Sanger *et al.*, 1977) using the following plasmids.

Plasmid pUC800. λgt11.6 was digested partially with *Eco*RI. The 800 bp fragment was purified by agarose gel electrophoresis, eluted and absorbed to glass beads (Vogelstein and Gillespie, 1979). The recovered fragment was filled in by Klenow reaction and blunt-end ligated into the *Hinc*II site of pUC19 polylinker.

Plasmids pUC350 and pUC450. λgt11.6 DNA was completely digested with *Eco*RI, and the agarose gel purified fragments were cloned into the *Eco*RI-digested, dephosphorylated pUC19.

Plasmid pUC340. pUC450 was *Eco*RI and *Rsa*I (nucleotide position 343) digested. A 340 bp fragment was isolated, blunt-ended and ligated into *Sma*I-linearized pUC19.

Plasmid pUC184. The internal *Rsa*I fragment (positions 374–558), spanning the EcoRI site of the cDNA, was obtained from *Rsa*I-digestion of pUC800 and agarose gel purified. After a Klenow reaction to blunt the cohesive ends,

the *Rsa*I fragment was blunt-end ligated into *Hinc*II-digested, dephosphorylated pUC19.

Construction of pBS.ABP

In order to isolate a full-length clone the λgt11 library was screened with the 5′ *Eco*RI fragment of the cDNA isolated by antibody screening. Of the six clones found, the one with the longest 5′ and 3′ fragment was chosen for subcloning. Phage plate lysate was subjected to CsCl-gradient centrifugation and phage DNA isolated by the formamide method (Thomas and Davis, 1975). Phage DNA was partially digested with *Eco*RI and fragments were separated by agarose gel electrophoresis. The cDNA fragment of ~850 bp was inserted into the *Sma*I site of pBS M13$^+$ vector. The plasmid insert was sequenced from both ends and a subclone, pBS184, containing the blunted *Rsa*I fragment, cloned as described for pUC184, was sequenced completely in both directions.

Northern analysis

Three-day-old etiolated corn coleoptiles were pulverized in liquid nitrogen. The powder was thawed in guanidine hydrochloride and further treated as described by Chirgwin *et al.* (1979). Poly(A)$^+$ RNA was prepared by oligo(dT)chromatography (Maniatis *et al.*, 1982). The poly(A)$^+$ RNA was separated on a 1.4% formaldehyde containing agarose gel, blotted onto nitrocellulose membrane, baked for 2 h under vacuum and hybridized (Zinn *et al.*, 1983) with biotinylated antisense RNA. The biotinylated antisense RNA was synthesized by T3 RNA polymerase with *Sac*I-cleaved pBS.ABP as template according to McCracken (1985) with the following modifications: increased NTP concentrations, biotin-11-UTP 2 mM, ATP, CTP, GTP 1 mM each. The hybridized probes were detected by incubation of the nitrocellulose filters with phosphatase-coupled streptavidin. The wash and color development steps were as described for Western blot immunostaining.

In vitro translation

The template for sense RNA synthesis was pGem.ABP. This plasmid derived from pGem-1 and a *Bam*HI−*Hin*dIII fragment from pUC800 containing the full-length cDNA. Full-length sense RNA was transcribed by SP6 RNA polymerase from *Bam*HI cleaved pGem.ABP according to Melton *et al.* (1984). The wheat germ translation system was prepared according to Anderson *et al.* (1983), and translation was carried out in the presence of [^{35}S]methionine. After incubation, aliquots from the translation mix were applied directly to SDS−PAGE or subjected to immunoprecipitation (Anderson and Blobel, 1983) with highly purified $IgG_{anti\ ABP}$. Highly purified ABP was added together with the $IgG_{anti\ ABP}$ for the competition experiments. Fluorography of dried polyacrylamide gels was carried out at −70°C with Kodak X-Omat AR-films.

Preparation of β-galactosidase−ABP fusion protein

The plasmid pUR292 (Rüther and Müller-Hill, 1983) was used to produce a fusion protein of β-galactosidase with a truncated ABP. *Eco*RI-digested, dephosphorylated pUR292 was ligated with the 5′ *Eco*RI fragment of the cDNA. Transformed, IPTG-induced *E.coli* JM103 colonies were screened for expression of a fusion product, showing immunoreactivity with highly purified $IgG_{anti\ ABP}$. Starting from an immunopositive colony, β-galactosidase−ABP was prepared according to Ullmann (1984), with *p*-aminobenzyl 1-thio-β-*d*-galactopyranoside-agarose as an affinity matrix. After elution β-galactosidase activity continuing fractions were combined, desalted on PD10 columns and stored lyophilized until used for rabbit immunization.

SDS−PAGE, Western blot, immunostaining

SDS−PAGE and Western blot were performed according to Laemmli (1970) and Khyse-Anderson (1984). For immunostaining blotted nitrocellulose membranes were blocked with 0.5% hemoglobin TBS. Incubation with primary antibody in TBS−0.5% hemoglobin lasted for 2 h, followed by four washing steps with TBS, 2× TBS−0.5% Triton X100, TBS−0.5% hemoglobin. Incubation with phosphatase-coupled $IgG_{anti\ rabbit\ IgG}$ was performed in TBS−0.5% hemoglobin followed by washing steps in TBS, 2 × TBS−Triton X100, TBS, borate buffer (60 mM borate, 5 mM $MgSO_4$, pH 9.5) and color development with Fast Blue B and β-naphthyl acid phosphate (0.25 mg/ml each) in borate buffer.

DNA manipulations

λ-DNA was purified according to Arber *et al.* (1983). Recombinant DNA work followed the procedures outlined (Maniatis *et al.*, 1982).

Acknowledgements

The able technical assistance of Uschi Schwabe is gratefully acknowledged. We thank Steve Fuller, EMBL, Heidelberg, for providing us with the KDEL affinity matrices. This work was supported by grants of the Deutsche Forschungsgemeinschaft (Lo 351/1-1) and a DAAD-fellowship to M.L. and a Stipendium des Landes NRW (GrFöG) to G.V.

References

Anderson,C.W. and Blobel,G. (1983) *Methods Enzymol.*, **96**, 111−420.
Anderson,C.W,, Strauss,J.W. and Dudock,B.S. (1983) *Methods Enzymol.*, **101**, 635−644.
Arber,W., Enquist,L., Hohn,B. and Murray,N. (1983) *CSH Monograph.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Barbier-Brygoo,H., Ephritikhine,G., Klämbt, D., Ghislain,M. and Guern,J. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 891−895.
Bole,D.G., Hendershot,L.M. and Kearny,J.F. (1986) *J. Cell. Biol.*, **102**, 1558−1566.
Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry*, **18**, 5294−5299.
Dohrmann,D. Hertel,R. and Kowalik,H. (1978) *Planta*, **140**, 97−106.
Edman,J.C., Ellis,L., Blacher,R.W., Roth,R.A. and Rutter,W.A. (1985) *Nature*, **317**, 267−270.
Hesse,T., Feldwisch,J., Balshüsemann,D., Bauw,G., Puype,M., Vandekerckhove,J., Löbler,M., Klämbt,D., Schell,J. and Palme,K. (1989) *EMBO J.*, **8**, 2453−2461.
Huynh,T.V., Young,R.A. and Davis,R.W. (1985) In Glover,D.M. (ed.), *DNA Cloning. A Practical Approach.* IRL Press, Oxford, UK.
Khyse-Anderson,J. (1984) *J. Biochem. Biophys. Methods*, **10**, 203−209.
Klämbt,D. and Löbler,M. (1987) In Klämbt,D. (ed.), *Plant Hormone Receptors.* Springer-Verlag, Berlin, pp. 261−263.
Kozak,M. (1984) *Nucleic Acids Res.*, **12**, 857−872.
Kutschera,U., Bergfeld,R. and Schopfer,P. (1987) *Planta*, **170**, 168−180.
Laemmli,U.K. (1970) *Nature*, **227**, 680−685.
Löbler,M. and Klämbt,D. (1985a) *J. Biol. Chem.*, **260**, 9848−9853.
Löbler,M. and Klämbt,D. (1985b) *J. Biol. Chem.*, **260**, 9854−9859.
Löbler,M., Simon,K., Hesse,T. and Klämbt,D. (1987) In Fox,J.E. and Jacobs,M. (eds), *Molecular Biology of Plant Growth Control.* Alan R.Liss, New York, pp. 279−288.
Maniatis,T., Fritsch,E. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
McCracken,S. (1985) *Focus*, **7**, 5−8.
Melton,D.A., Krieg,P.A., Rebagliati,M.R., Maniatis,T., Zinn,K. and Green,M.R. (1984) *Nucleic Acids Res.*, **12**, 7035−7056.
Miller,H.J. (1972) *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Munro,S. and Pelham,H.R.B. (1987) *Cell*, **48**, 899−907.
Napier,R.M., Venis,M.A., Bolton,M.A., Richardson,L.I. and Butcher,G.W. (1988) *Planta*, **176**, 519−526.
Rüther,U. and Müller-Hill,B. (1983) *EMBO J.*, **2**, 1791−1794.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl Acad. Sci. USA*, **74**, 5463−5467.
Shimomura,S., Sotobayashi,T., Futai,M. and Fukui,T. (1986) *J. Biochem.*, **99**, 1513−1524.
Shimomura,S., Inohara,N., Fukui,T. and Futai,M. (1988) *Planta*, **175**, 558−566.
Thimann,K.V. and Schneider,C.L. (1938) *Am. J. Bot.*, **25**, 627−641.
Thomas,M. and Davis,R.W. (1975) *J. Mol. Biol.*, **91**, 315−328.
Ullmann,A. (1984) *Gene*, **29**, 27−31.
Vogelstein,B. and Gillespie,D. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 615−619.
von Heijne,G. (1983) *Eur. J. Biochem.*, **133**, 17−21.
von Heijne,G. (1985) *J. Mol. Biol.*, **184**, 99−105.
Walton,J.D. and Ray,P.M. (1981) *Plant Physiol.*, **68**, 1334−1338.
Zinn,K., DiMaio,D. and Maniatis,T. (1983) *Cell*, **34**, 865−879.

Received on April 3, 1989; revised on May 22, 1989