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Purification, Characterization, and cDNA Cloning of ABP-2 (Arylphorin Gene-Specific Binding Protein-2) That Specifically Binds to the ABP-1-Binding Sequence in the Arylphorin Gene of *Sarcophaga peregrina*[1]

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Previously, we demonstrated that ABP-1 (arylphorin gene-specific binding protein-1), which is suggested to be the transcriptional activator of the arylphorin gene of *Sarcophaga peregrina*, is present in NIH-Sape-4 cells, which do not express arylphorin. As well as ABP-1, these cells were found to contain another protein (ABP-2) that probably binds to the same sequence as that to which ABP-1 binds [Adachi, N., Kubo, T., and Natori, S. (1993) *J. Biochem.* 114, 55-60]. We purified ABP-2 from a nuclear extract of NIH-Sape-4 cells and compared its DNA-binding activity with that of ABP-1. Both ABP-1 and ABP-2 were found to bind to the same sequence in the arylphorin gene with the same affinity and stability, but an ABP-2-specific hypersensitive site was detected by DNase I footprinting analysis. Analyses of proteolytic fragments suggested that both ABP-1 and ABP-2 have Zn fingers showing high similarity with that of AEF-1, a transcriptional repressor of the *Drosophila melanogaster* alcohol dehydrogenase gene that binds to a sequence very similar to that binding ABP-1 and ABP-2. We isolated a candidate cDNA for ABP-2, and the protein it encoded contained nine Zn fingers and regions rich in alanine, glutamine, serine/threonine, glycine, histidine, and asparagine.

Key words: ABP-1, ABP-2, arylphorin gene, *Sarcophaga peregrina*, transcription factor.

During the final larval instar of holometabolous insects, storage protein is synthesized by the fat body and secreted into the hemolymph (*1*). It is suggested that this storage protein plays no important role in larval life, but is used as a source of amino acids and energy for constructing adult tissues during metamorphosis (*2*). Two classes of storage protein are known. One is called arylphorin, which is relatively rich in aromatic amino acids (*3-10*), whereas the other is rich in methionine (*11*).

The arylphorin gene of the flesh fly, *Sarcophaga peregrina*, is known to be strictly regulated according to the developmental stage (*12, 13*). The *Sarcophaga* arylphorin gene is activated soon after ecdysis to the third instar, and mRNA synthesis persists until the early pupal stage (*12*). The content of arylphorin mRNA at this stage is very high, accounting for more than 50% of total fat body mRNA (*14*).

In previous studies on the regulation of expression of the arylphorin gene, we identified a protein called arylphorin gene-specific binding protein-1 (ABP-1), which has affinity for the sequence ACCACAACA located in the 5′-upstream region of the arylphorin gene (*15, 16*). The timing of the appearance of ABP-1 in the fat body coincides with that of activation of the arylphorin gene, suggesting that ABP-1 is a transcriptional activator of the arylphorin gene. We purified ABP-1 to near homogeneity from a nuclear extract of the fat body of 46-h-old third instar larvae, the stage at which its content in the fat body becomes maximal, and confirmed that the purified protein stimulated transcription of the arylphorin gene *in vitro* (*16*).

Subsequently, we found that ABP-1 is also present in NIH-Sape-4 cells, an embryonic cell line of *Sarcophaga* that does not express the arylphorin gene (*17*). When *in vitro* transcription was carried out using a nuclear extract of NIH-Sape-4 cells, the arylphorin gene was found to be transcribed in this system, and addition of ACCACAACA interfered with its transcription (*17*). These results suggested that ABP-1 is active in NIH-Sape-4 cells, but transcription of the arylphorin gene is suppressed in these cells by some unknown mechanism. Unlike the fat body, NIH-Sape-4 cells were found to contain another protein, called ABP-2, that also had affinity for ACCACAACA (*17*), suggesting that ABP-2 is a negative transcription factor of the arylphorin gene. This paper reports the purification, characterization and cloning of a candidate for ABP-2 cDNA.

MATERIALS AND METHODS

Cells—The embryonic cell line NIH-Sape-4 established

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Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate.

from *S. peregrina* was cultured in M-M medium (*18*) at 25°C as described previously (*19*). Briefly, cells were inoculated at a density of 5×10^5/ml into 4,000 ml of M-M medium and cultured in a spinner flask with constant stirring at 45 rpm for about 5 days. The cells were harvested when their density reached 4×10^6/ml.

Preparation of Nuclear Extract—A nuclear extract was prepared from NIH-Sape-4 cells essentially as described by Dignam *et al.* (*20*). A sample of 1 g of nuclei (wet weight) was extracted with 1.2 ml of 20 mM HEPES/NaOH buffer, pH 7.6, containing 1.5 mM $MgCl_2$, 0.2 M $(NH_4)_2SO_4$, 0.2 mM EDTA, 0.5 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 25% glycerol for 40 min at 4°C with gentle stirring. Then the mixture was centrifuged at $32,000\times g$ for 30 min, and the resulting supernatant was dialyzed against the 20 mM HEPES/NaOH buffer, pH 7.6, containing 0.1 M KCl, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, and 20% glycerol for 5 h. The dialysate was centrifuged at $32,000\times g$ for 20 min, and the supernatant was used as the nuclear extract.

Electrophoresis Mobility Shift Assay—The reaction mixture (20 μl) consisted of 10 mM HEPES/NaOH, pH 7.9, 10% glycerol, 0.1 mM EDTA, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 0-4 μg of Poly(dI)-Poly(dC), 2 μg of bovine serum albumin, 3,000 cpm of ^{32}P-labeled probe DNA (10^4-10^5 cpm/pmol), and the nuclear extract or ABP-2. The reaction mixture was kept on ice for 30 min, then subjected to electrophoresis in 5% polyacrylamide slab gel, which had been pre-run for 1.5 h at 200 V at 4°C in a solution of 45 mM Trizma base, 45 mM boric acid, and 1 mM EDTA. Electrophoresis was carried out for 1.5 h at 360 V at 4°C. The gel was then dried and examined by autoradiography or in an image analyzer.

To assess the stability of the binding complex of ABP-1 or ABP-2 and probe DNA, purified protein was first incubated for 30 min on ice. Then a 100-fold excess of cold probe DNA was added, and the mixture was kept on ice for 0 to 60 min before being subjected to electrophoresis.

As a probe, 5′-ACATAGACCACAACAACACAAG-3′, which corresponds to the sequence −261/−240 of the arylphorin gene (*12*), was annealed with 3′-TCTGGTGTTG-TTGTGTTCTGTA-5′ (wild-type DNA) and labeled by end-filling with Klenow fragment of DNA polymerase I and four deoxyribonucleotides containing [α-^{32}P]dCTP. As a competitor, a mutant DNA was synthesized in which the "A" at positions −255, −252, and −244 of the wild-type DNA was replaced by C, T, and T, respectively, and the "C" at position −248 was replaced by A.

Preparation of DNA Affinity Columns—Sequence-specific DNA affinity columns for isolation of ABP-2 were prepared by conjugating the wild-type or mutant DNA used in the electrophoresis mobility shift assay with CNBr-activated Sepharose 4B (Pharmacia) by the method of Kadonaga and Tjian (*21*).

Purification of ABP-2—The nuclear extract of NIH-Sape-4 cells (1,260 mg protein) was applied to a column of heparin-Sepharose (2.5×4 cm) equilibrated with buffer 1 (10 mM HEPES/NaOH, pH 7.9, containing 0.1 M KCl, 10% glycerol, 0.5 mM EDTA, 5 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM DTT, 1 mM PMSF, 1 μg/ml leupeptin, and 0.7 μg/ml pepstatin). The column was washed with 500 ml of buffer 1, then adsorbed material was eluted stepwise with 150 ml of buffer 1 containing 0.3, 0.4, and 1 M KCl, respectively. Each fraction was examined by electrophoresis mobility shift assay. Both ABP-1 and ABP-2 were recovered in the 0.4 M KCl fraction. Triton X-100 was added to this fraction at a final concentration of 0.1%, and it was dialyzed against 5,000 ml of buffer 2 (20 mM Tris/HCl, pH 9.4, containing 10% glycerol, 1 mM DTT, 1 mM PMSF, and 0.1% Triton X-100) for 5 h.

The dialysate was applied to a column of Q-Sepharose (2.5×3 cm) equilibrated with buffer 2. The column was washed with 90 ml of buffer 2, then adsorbed material was eluted stepwise with 75 ml of buffer 2 containing 0.1, 0.2, and 1 M KCl, respectively. ABP-1 and ABP-2 were separated at this step: ABP-2 was recovered in the flow-through fraction, and ABP-1 in the 0.1 M KCl fraction.

The fraction containing ABP-2 was applied to an affinity column of mutant DNA (1.5×3 cm) equilibrated with buffer 2. The column was then washed with 25 ml of buffer 2, and adsorbed material was eluted with a linear gradient of 0 to 0.5 M KCl in buffer 1 containing 0.1% Triton X-100. ABP-2 was eluted with 40-100 mM KCl. This fraction was applied to an affinity column of wild-type DNA (1.5×1.2 cm) equilibrated with buffer 1 containing 0.1% Triton X-100. The column was washed with 200 ml of the same buffer, and adsorbed material was eluted stepwise with 50 ml of 0.4 and 1 M KCl, respectively. ABP-2 was recovered in the 0.4 M KCl fraction. This fraction was diluted with buffer 1 to adjust the concentration of KCl to 0.1 M. Affinity chromatographies were repeated with this diluted fraction. ABP-2 was almost pure on recovery from the second affinity column of wild-type DNA.

Protein was determined by the method of Bradford (*22*) with bovine serum albumin as a standard. SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli (*23*), and the gel was stained with silver as described by Oakley *et al.* (*24*).

UV-Crosslinking of Purified Protein to a DNA Fragment—Crosslinking was carried out essentially by the method of Lin and Riggs (*25*). Wild-type DNA (5′-ACATA-GACCACAACAACACAAGACAT-3′) was annealed with 5′-ATGTCTTGT-3′, and labeled DNA containing BrdU was synthesized with the Klenow fragment of DNA polymerase I in the presence of dATP, dCTP, [α-^{32}P]-dGTP, and BrdUTP. This DNA (1 pmol, 3×10^4 cpm/pmol) was incubated with ABP-2 under the same conditions as those for the electrophoresis mobility shift assay, and irradiated with a UV-lamp (Toshiba GL30, 253.7 nm) for 30 min on ice. After irradiation, 10 μl of the reaction mixture was mixed with 1 μl of nuclease solution (5 mM Tris/HCl, pH 7.5, containing 2.5 U/μl DNase I, 0.2 U/μl *Micrococcus* nuclease, 50 mM NaCl, 10 mM $CaCl_2$, 35% glycerol, and 50 ng/μl bovine serum albumin) and incubated for 10 min at 37°C to digest the remaining DNA. The protein crosslinked with the labeled DNA was analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography.

DNase I Footprinting—Footprinting was carried out essentially as described by Carthew *et al.* (*26*). A 179-bp DNA of the 5′-upstream region of the arylphorin gene (−335/−157) was prepared by the polymerase chain reaction (PCR). This DNA was end-labeled using T4 polynucleotide kinase, then digested with *Taq* I to produce a 168-bp DNA in which the 5′-end of the sense strand was labeled. The single-end-labeled probe DNA (3×10^4 cpm)

was incubated with 20 ng of ABP-1 and ABP-2, respectively, under the same conditions as those for the electrophoresis mobility shift assay. Then 0.4 unit of DNase I was added and the mixture was incubated for 1 min at 30°C. The reaction mixture was then promptly subjected to 5% polyacrylamide gel electrophoresis followed by autoradiography to separate the protein:DNA complex and free DNA.

The gel strip containing the protein:DNA complex was excised from the gel and shaken overnight at 37°C in 10 mM Tris/HCl buffer, pH 7.9, containing 1 mM EDTA, 0.06 M NaCl, and 1 μg/ml *Escherichia coli* tRNA. The solubilized DNA was extracted with buffer-saturated phenol, and precipitated with ethanol. The resulting DNA was dissolved in 99% formamide solution containing 0.1% xylene cyanol and 0.1% bromophenol blue, heated at 90°C for 3 min, and applied to a standard 8% polyacrylamide–8 M urea sequence gel. After electrophoresis, the gel was dried *in vacuo* and subjected to autoradiography.

cDNA Cloning of ABP-2—PCR was performed using a cDNA library of NIH-Sape-4 with 5′-CAACTGAGCACGCTGGCCAACCA-3′ and 5′-GACTTTGTCCATGACGTGTAT-3′ as primers, which are located in the AEF-1 Zn finger domain. The PCR products were subjected to Southern blot analysis using 5′-GCAGTTCCGACAGTCCAGCACGCTC-3′ as a probe; this is a sequence located between the 5′ and 3′ PCR primer sequences in the AEF-1 Zn finger domain. We cloned a 135-bp hybridization-positive product and used it as the probe for screening the cDNA for ABP-2.

By screening 1,200,000 clones of a NIH-Sape-4 cDNA library, we obtained 6 positive clones. We partially sequenced 4 clones containing the poly(A) tail and confirmed that they were essentially the same clones. Therefore, we sequenced the clone that contained the longest insert using the dideoxy chain-termination method of Sanger *et al.* (27).

RESULTS

Purification of ABP-2 from the Nuclear Extract of NIH-Sape-4 Cells—As reported before, NIH-Sape-4 cells contained two proteins (ABP-1 and ABP-2) that bind to ACCACAACA located in the 5′-upstream region of the arylphorin gene. Of these, ABP-1 has already been purified. To compare the characteristics of these two proteins, we attempted to purify ABP-2. Purification of ABP-2 was monitored by electrophoresis mobility shift assay. A typical purification is summarized in Table I. At the DNA (wild type)-Sepharose column chromatography step, the specific activity of ABP-2 was increased more than 45,000-fold, and the recovery of activity was 4.4%. At this stage, the protein was almost pure, and its molecular mass was determined to be 28 kDa by SDS-polyacrylamide gel electrophoresis (Fig. 1, lane 2). These results indicated that ABP-2 is a different protein from ABP-1, whose molecular mass is 40–42 kDa (lane 1).

To confirm that the DNA-binding activity detected by electrophoresis mobility shift assay was due to this 28-kDa protein, we performed a UV-crosslinking experiment using purified ABP-2. As shown in Fig. 2, a 28-kDa band was shown to be crosslinked with the ^{32}P-labeled probe DNA (lane 1). UV-crosslinking was completely inhibited by

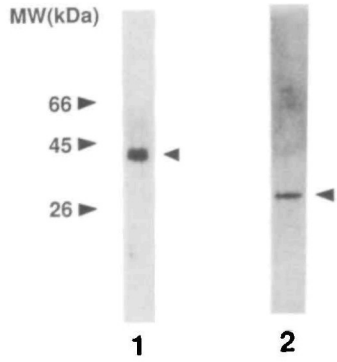

Fig. 1. **Electrophoretic profile of purified ABP-2.** Purified ABP-2 and ABP-1 (each 1 μg) were subjected to SDS-polyacrylamide gel electrophoresis under denaturing conditions and stained with silver. The gel was calibrated with various molecular mass markers, whose positions are indicated in kDa on the left. Lane 1, ABP-1; lane 2, ABP-2.

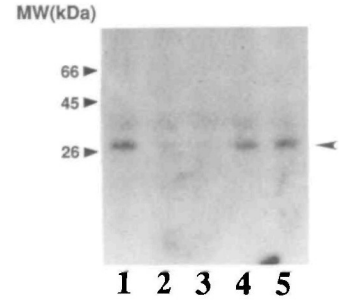

Fig. 2. **UV-crosslinking of ABP-2.** A mixture of purified ABP-2 and probe DNA containing the ACCACAACA sequence was irradiated with a UV-lamp, treated with nuclease, then analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography. Lane 1, control (UV-crosslinking was performed with no competitor DNA); lane 2, with a 20-fold excess of wild-type DNA; lane 3, with a 100-fold excess of wild-type DNA; lane 4, with a 20-fold excess of mutant DNA; lane 5, with a 100-fold excess of mutant DNA. The arrowhead shows ABP-2.

TABLE I. **Summary of purification ABP-2.**

Fraction	Protein (mg)	Total activity (units)[a]	Specific activity (units/mg)	Purification (-fold)	Yield (%)
Nuclear extract	1,260	1,980	1.6	1	100
Heparin-Sepharose	95	1,200	12	7.8	60
Q-Sepharose	0.6	310	500	310	16
DNA (Mutant)-Sepharose	0.2	290	1,500	950	15
DNA (Wild type)-Sepharose	0.06	420	7,300	4,600	21
DNA (Mutant)-Sepharose	0.0034[b]	140	40,000	25,000	7
DNA (Wild type)-Sepharose	0.0012[b]	86	72,000	45,000	4.4

[a]One unit of activity was defined as the amount retarding 1 pmol of probe DNA. [b]Estimated by silver staining.

wild-type DNA (lanes 2 and 3), but not by DNA containing point mutations (lanes 4 and 5). These results indicated that the purified 28-kDa protein was ABP-2, and that, like ABP-1, it is a sequence-specific DNA-binding protein.

Detection of DNA Binding Sequence of ABP-2 for the Arylphorin Gene—The results of the electrophoresis mobility shift assay suggested that ABP-2 binds to the same DNA sequence to which ABP-1 binds. We therefore

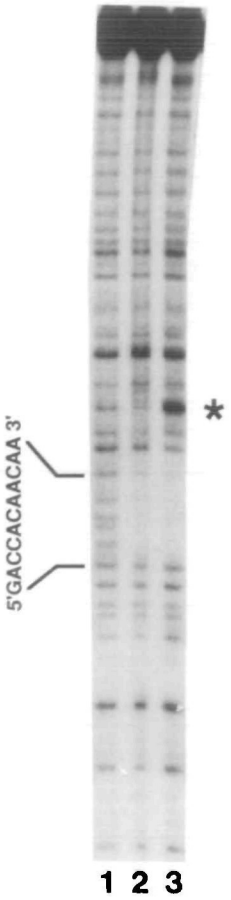

Fig. 3. **DNase I footprinting assay using ABP-1 and ABP-2.** The single-end-labeled probe DNA was incubated with purified ABP-1 and ABP-2, then treated with DNase I. Samples were subjected to electrophoresis in 8% polyacrylamide-8 M urea gel. Lane 1, reaction mixture without protein; lane 2, reaction mixture with purified ABP-1; lane 3, reaction mixture with purified ABP-2. The sequence of the protected region is given on the left. The asterisk shows a hypersensitive site.

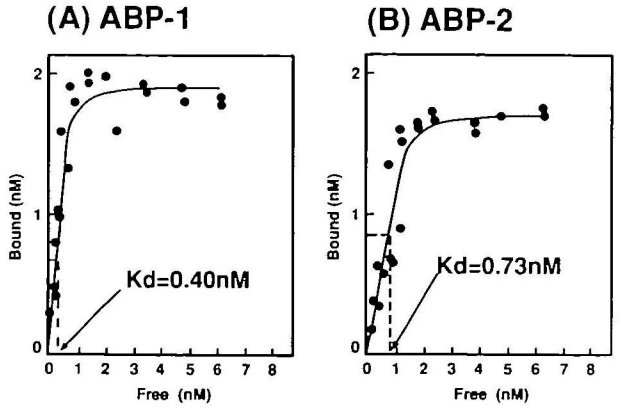

Fig. 4. **Comparison of affinities of ABP-1 and ABP-2 for the arylphorin gene.** Dissociation constants (K_d) of ABP-1 and ABP-2 for the arylphorin gene were determined by electrophoresis mobility shift assay as described in "MATERIALS AND METHODS." (A) and (B) show the K_d values of ABP-1 and ABP-2 for the arylphorin gene calculated by Langmuir plot.

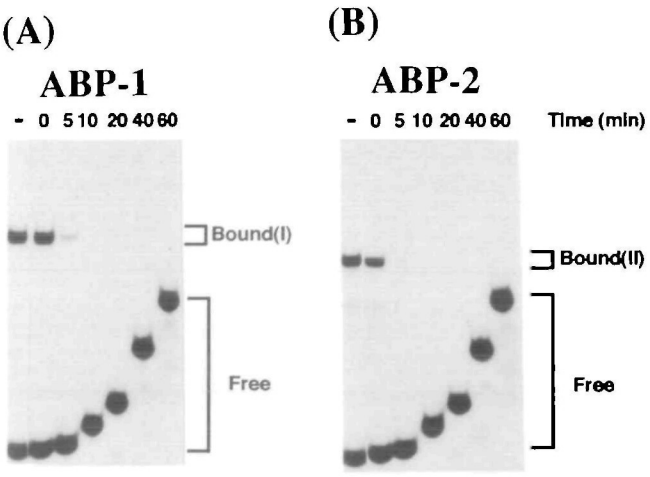

Fig. 5. **Comparison of binding stability of ABP-1 and ABP-2 for the arylphorin gene.** ABP-1 (A) and ABP-2 (B) were incubated with labeled probe DNA for electrophoresis mobility shift assay containing the ACCACAACA sequence. Then the protein-DNA complex was withdrawn at various times after addition of a 100-fold excess of cold probe DNA and subjected to electrophoresis. Electrophoresis was started at zero min. Incubation time after addition of a 100-fold excess of cold probe is shown at the top. Protein-DNA complex is shown as bound (I) (ABP-1), and bound (II) (ABP-2), respectively.

(A)

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  ABP-1:PFEXAVXK
 *AEF-1:PFECVICK

  ABP-1:VYVPVK
  AEF-1:VYVPVK

  ABP-2:HTDERPYK
 *AEF-1:HTGEKPYK

  ABP-2:FRQQSTLINHIK
 *AEF-1:FRQSSTLNNHIK
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(B)

S. peregrina Arylphorin	-255	A CCACAACAACA	-244
D. melanogaster Adh	-534	G CAGCAACAACA	-523
D. melanogaster YP1	-311	T GCACAACTACA	-300
D. mulleri Adh	-2654	A CCACAACAAAA	-2643

Fig. 6. **Partial amino acid sequences and DNA binding sequences of ABP-1 and ABP-2.** (A) Comparison of partial amino acid sequences of ABP-1 and ABP-2 with that of AEF-1. Sequences of four peptides obtained from ABP-1 and ABP-2 are shown with the corresponding sequences of those from AEF-1. Unidentified amino acids are shown as X. Identical residues in both sequences are boxed. Peptide fragments in the AEF-1 Zn finger domain are shown by asterisks. (B) Diagram showing ABP-1 and ABP-2 binding site in the arylphorin gene of *Sarcophaga* and AEF-1 binding sites in the alcohol dehydrogenase genes of *Drosophila melanogaster*, *Drosophila mulleri*, and the yolk protein 1 gene of *D. melanogaster*. The locations of the sites relative to the respective transcription initiation sites are shown on both sides of the sequence.

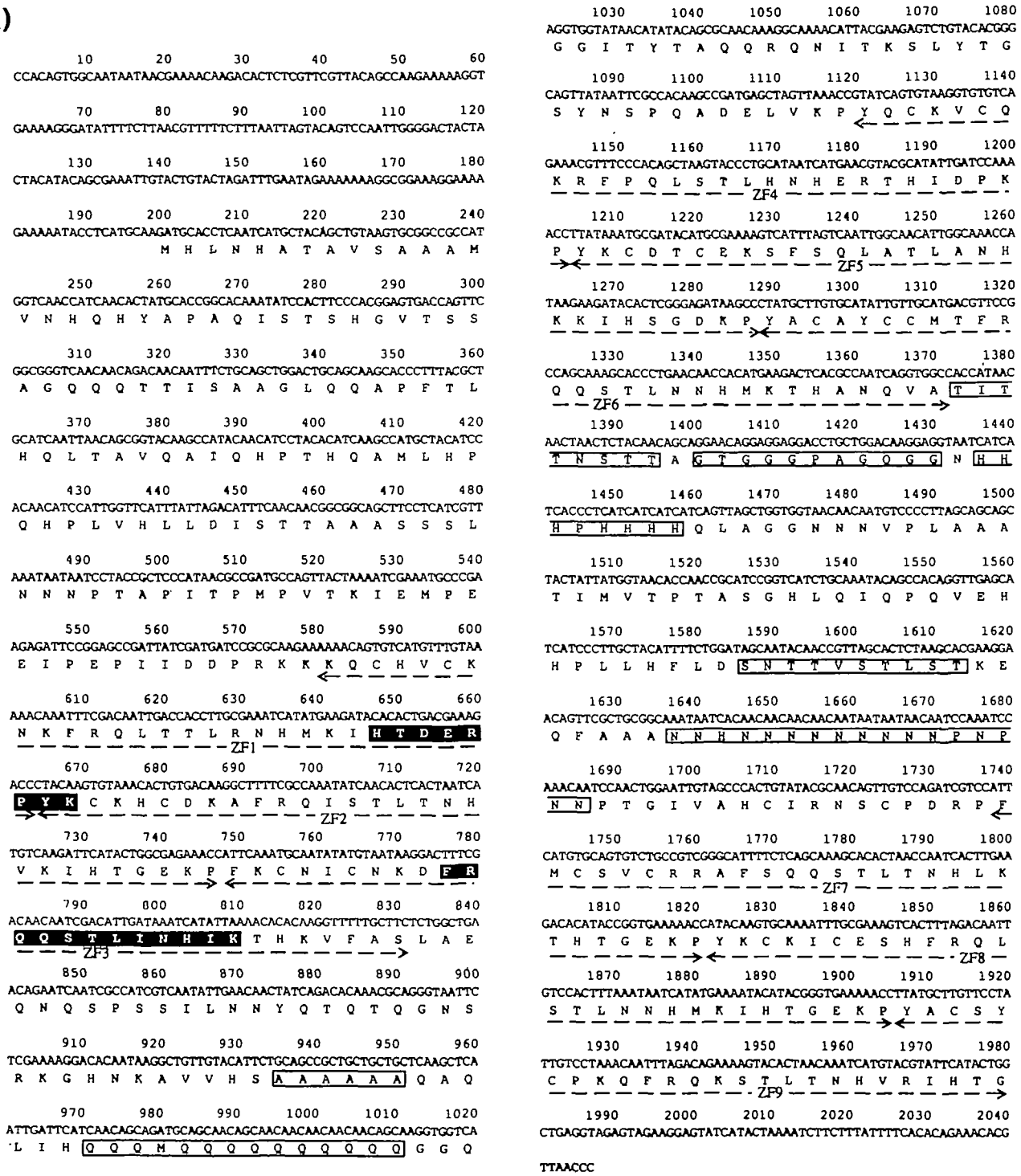

Fig. 7A

performed DNase I footprinting analysis with purified ABP-2 to confirm this. As shown in Fig. 3, the same sequence (ACCACAACA) in the 5′ upstream region of the arylphorin gene was protected by both ABP-1 and ABP-2 (lanes 2 and 3), indicating that they bind to the same sequence of the arylphorin gene. However, as indicated by an asterisk, an ABP-2-specific hypersensitive site was detected. These results suggested that ABP-1 and ABP-2 bind to the same sequence, but induce different conformational changes in the vicinity of the binding site.

Comparison of DNA Binding Activity of ABP-1 and ABP-2—As ABP-1 and ABP-2 were found to bind to the same sequence in the 5′-upstream region of the arylphorin gene, we compared their binding activities. First, we determined the dissociation constants (K_d) of these proteins for the arylphorin gene using the electrophoresis mobility shift assay. As shown in Fig. 4, the dissociation constants of ABP-1 and ABP-2 were calculated to be 0.40

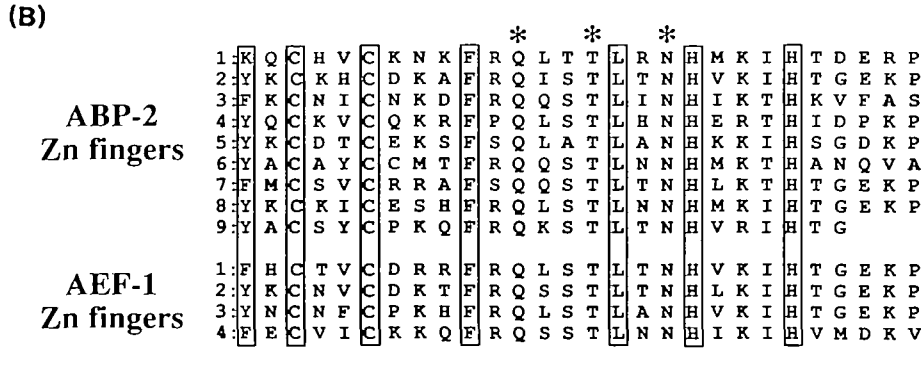

Fig. 7. **Nucleotide sequence of the candidate ABP-2 cDNA and deduced amino acid sequence encoded by it.** (A) Nucleotide sequence of the candidate ABP-2 cDNA. The sequence of the cDNA is shown in the 5′ to 3′ direction. Sequences of two peptides determined from the proteolytic fragments of ABP-2 are shown in closed boxes. The nine Zn fingers are shown by arrows below the sequence. Regions rich in alanine, glutamine, serine/threonine, glycine, histidine, and asparagine are shown in boxes. (B) Zn finger motifs found in the protein encoded by the cDNA and AEF-1. The consensus sequences of the C_2H_2-type Zn fingers are shown in the boxes. Amino acids directly related to the DNA-binding specificity are marked by asterisks.

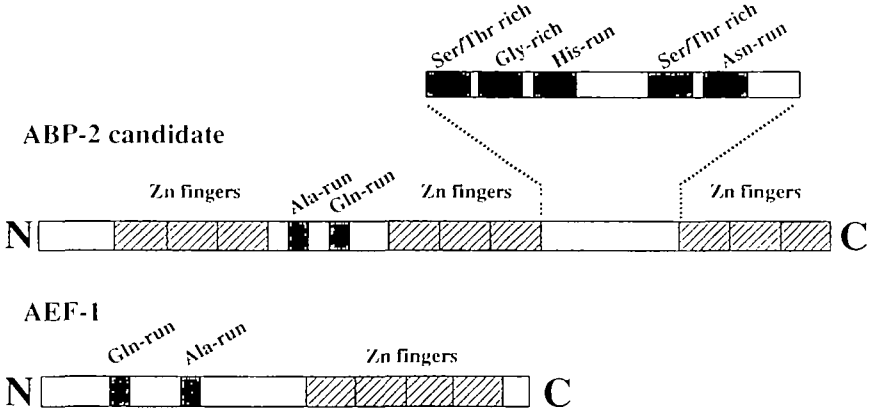

Fig. 8. **Schematic illustration of the functional motifs in the protein encoded by the cDNA.** Functional motifs in the protein encoded by the cDNA and those in AEF-1 are compared.

and 0.73 nM, respectively, indicating that they have almost the same affinity for this gene. Second, we compared the stability of the DNA:protein complex by measuring the dissociation of radiolabeled DNA:protein complex in the presence of a 100-fold excess of cold DNA. As is evident from Fig. 5, both complexes were found to be dissociated rapidly and almost in the same manner, suggesting that ABP-1 and ABP-2 have very similar characteristics in terms of binding to the specific sequence in the arylphorin gene.

cDNA Cloning of ABP-2—To obtain structural information about ABP-1 and ABP-2, we determined their partial amino acid sequences based on peptides obtained by digestion with lysylendopeptidase. As shown in Fig. 6A, the sequences of four independent peptides were found to share significant sequence similarity to the partial amino acid sequences of the Zn finger domain of AEF-1 (*28*). Furthermore, the binding sequences of AEF-1 in the *Drosophila* alcohol dehydrogenase and yolk protein 1 genes were also found to be very simiar to those of ABP-1 and ABP-2 in the *Sarcophaga* arylphorin gene (Fig. 6B). These results suggested that ABP-1 and ABP-2 have Zn finger domains similar to that of *Drosophila* AEF-1. Therefore, we tried to isolate cDNAs for ABP-1 and ABP-2 using probe DNA synthesized according to the sequence of the Zn finger domain of AEF-1.

To prepare the probe DNA, we synthesized two primers for PCR designed using the sequence of the AEF-1 Zn finger domain, and PCR was performed with a cDNA library of NIH-Sape-4 cells. Then we cloned a 135-bp PCR product that could be used as the probe. Using this probe, we finally isolated one clone from the cDNA library. The complete nucleotide sequence of this clone is shown in Fig. 7A with the putative amino acid sequence of the protein encoded by it. This clone contained the sequences of two peptides derived from ABP-2, but not from ABP-1, suggesting that this clone is a cDNA for ABP-2.

This cDNA encoded a protein with 594 amino acid residues. The protein contained three Zn finger domains, and each domain contained three C_2H_2-type Zn fingers whose sequences are shown in Fig. 7B along with those of the four Zn fingers in AEF-1. The three residues marked with asterisks are identical, and these were shown to be critical for the sequence-specific binding of Zn finger (*29, 30*), suggesting that AEF-1 and this protein recognize very similar DNA sequences. Moreover, this protein contained regions rich in alanine, glutamine, serine/threonine, glycine, histidine, and asparagine, which are often found in various transcription factors (*31-35*). Alignments of these motifs in this protein are shown schematically in Fig. 8.

DISCUSSION

Previously we showed that the fat body expressing the arylphorin gene contains ABP-1, whereas NIH-Sape-4 cells that do not express it contain both ABP-1 and ABP-2 (*17*). As ABP-1 stimulated transcription of the arylphorin gene *in vitro* (*16*), and both ABP-1 and ABP-2 were shown to bind to the same sequence in the 5′-upstream region of the arylphorin gene, it was suggested that ABP-1 is an ac-

tivator, whereas ABP-2 is a repressor, of the gene. We purified ABP-2 to homogeneity and compared its binding activity with this sequence to that of ABP-1, and found that the binding activities were almost the same.

In *Drosophila*, it has been shown that the nuclear receptors FTZ-F1α and FTZ-F1β bind to the same DNA sequence with almost the same affinity, and transfection experiments have demonstrated that a 0.5–2-fold excess of FTZ-F1β completely inhibits FTZ-F1α activity (*36*). Possibly, a similar situation may exist for ABP-1 and ABP-2 in NIH-Sape-4 cells. In fact, the amount of ABP-2 in NIH-Sape-4 cells is always 1.5–2-fold higher than that of ABP-1.

We isolated a candidate cDNA for ABP-2 and analyzed it. This cDNA encoded 594 amino acid residues. As the molecular mass of ABP-2 determined by SDS polyacrylamide gel electrophoresis was 28 kDa, the protein encoded by this cDNA is much larger than ABP-2. There are two possible explanations for this. One is that the protein encoded by this cDNA is not ABP-2. Another is that ABP-2 is produced from this protein by partial proteolysis, like the p50 subunit of NF-κB (*37, 38*). As two peptides obtained from ABP-2 are located in the N-terminal region of the protein encoded by this cDNA, the N-terminal half of this protein would correspond to ABP-2 if this latter possibility were correct. In this case, ABP-2 would contain alanine- and glutamine-rich regions. As AEF-1, which binds to a sequence similar to that binding ABP-2, does contain these motifs and is known to act as a transcriptional repressor of the alcohol dehydrogenase gene (*28*), it is possible that ABP-2 is a repressor of the arylphorin gene. Further biochemical and structural analysis of ABP-2 is needed in order to confirm the latter possibility.

However, these proteins may not simply be specific modulators of the arylphorin gene. There will be many other genes whose expressions are regulated by these proteins in *Sarcophaga*, since NIH-Sape-4 cells, which do not normally express fat body proteins, contain these proteins.

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