Alternative Processing of Androgen-binding Protein RNA Transcripts in Fetal Rat Liver

IDENTIFICATION OF A TRANSCRIPT FORMED BY Trans SPlicing*

(Received for publication, April 4, 1990)

Patrick M. Sullivan*,a, Peter Petrusz*, Claude Szpirer**, and David R. Joseph*a,*,b

From the Departments of Pediatrics, aBiology, aPhysiology, and aAnatomy, Laboratories for Reproductive Biology, University of North Carolina, Chapel Hill, North Carolina 27599 and the bDepartment of Molecular Biology, Universite Libre de Bruxelles, B-1640 Rhode-St-Genese, Belgium

Androgens and their nuclear receptor regulate genes necessary for development of the male phenotype, a process that is thought to be modulated by extracellular androgen carrier proteins. Two of these carrier proteins, testicular androgen-binding protein (ABP) and plasma sex hormone-binding globulin (SHBG), are encoded by the same gene, but differ in glycosylation and possibly amino acid sequence. To investigate ABP-SHBG gene expression in fetal rat liver, we analyzed RNA transcripts and expressed protein. These studies demonstrated a transient expression of ABP in hepatocytes during the time of testosterone-dependent differentiation of the Wolffian duct. Analysis of cDNA clones derived from fetal rat liver cDNA libraries identified two cDNAs encoded by the ABP-SHBG gene that represented alternatively spliced RNAs. One cDNA had an alternate exon 1, suggesting the function of another promoter in fetal liver. This cDNA also lacked testicular exon 6 DNA, an alteration that implicates the encoded protein in regulatory functions. The other cDNA represented a fused transcript of the ABP-SHBG gene (exons 1–5) and the histidine decarboxylase (HDC) gene, encoding a M, 93,000 precursor protein. The two domains were joined at splice junctions of the ABP-SHBG and HDC genes, which were localized to rat chromosomes 10 and 3, respectively. Our results indicate that the joining of the two domains was by a trans (donor and acceptor)-splicing mechanism. Data from Northern hybridization experiments suggest the fusion transcript is present in fetal liver RNA.

Polymerase chain reaction experiments with fetal liver cDNA further support the existence of an ABP-HDC fusion transcript, as well as the alternate mRNA. Moreover, a M, 93,000 immunoreactive protein was transiently expressed in fetal liver during the time of ABP and HDC gene expression. Expression of the fusion cDNA in COS cells yielded HDC activity and the predicted size protein (M, = 93,000) on Western immunoblots.

Androgens and their receptor play an important role in male sexual differentiation and function. The trans-acting androgen receptor (AR') binds the male sex steroids, testosterone and dihydrotestosterone (DHT), and regulates the genes necessary for male sexual differentiation and development (1). Androgens and the receptor are also necessary for the maintenance of spermatogenesis, sperm maturation, and accessory sex gland function (2). Extracellular androgen carrier proteins are thought to modulate androgen action by binding androgens in blood and luminal fluids of the male reproductive tract (3, 4). Also, these proteins are known to interact with a specific external membrane receptor (5, 6). Although the importance of this interaction is not known, evidence has recently been presented that suggests steroid carrier proteins may actually be hormones themselves (3).

Androgen-binding protein (ABP) is a testicular Sertoli cell secretory protein that binds testosterone and dihydrotestosterone with high affinity (7). ABP acts as a carrier of androgens in the seminiferous tubule and epididymis, but its precise function is not well understood (4, 8, 9). A related blood steroid carrier protein, sex hormone-binding globulin (SHBG), has almost identical physicochemical properties as ABP, differing in glycosylation and possibly amino acid sequence (4, 10). Rat ABP and human SHBG (homodimers) subunits share 69% identity of their 373-amino acid residues (11). In man there is a single gene encoding both ABP and SHBG (12). Likewise, in rat there is one ABP-SHBG gene (13). SHBG is synthesized in the adult liver of some species, including man (10, 14, 15), monkey (16), and rabbit (17), but the rat liver does not produce detectable SHBG protein or mRNA (18, 19). However, Gunsalus et al. (20) and Carreau (21) reported the presence of an immunoreactive ABP-like protein in the serum of developing male and female rat embryos, suggesting it was synthesized in the liver. In this manuscript we report that the ABP gene is expressed transiently during rat liver development. Studies of fetal liver ABP RNA transcripts revealed cDNA clones that represented

* This work was supported by United States Public Health Service Grants HD-21744 (to D. R. J.) and 5-P30-HD-18968 (Laboratories for Reproductive Biology, to F. S. French) and grants from the Andrew Mellon Foundation and The North Carolina Biotechnology Center. Work in Brussels was supported by the Belgian program on interuniversity attraction poles initiated by the Belgium State, Prime Minister’s Office, Science Policy Programming. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) M38759.

1 This work represents partial fulfillment of the requirements for a Ph.D. degree in the Department of Biology.

* Senior Research Associate of the FNRS (Belgium).

To whom correspondence and reprint requests should be addressed.

The abbreviations used are: AB, androgen receptor; ABP, androgen-binding protein; SHBG, sex hormone-binding globulin; HDC, histidine decarboxylase; DHT, dihydrotestosterone; RIA, radioimmunoassay; ID element, brain identifier repetitive DNA element; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase(s).
alternatively processed mRNAs. One of the cDNAs represented a RNA transcript that was apparently formed in a trans-splicing mechanism. Furthermore, the characteristic features of both alternate transcripts suggest that the encoded proteins have functions different from testicular ABP. Henceforth, the fetal liver protein will be referred to as ABP, but SHBG would be equally appropriate.

**EXPERIMENTAL PROCEDURES**

**Preparation and Screening of cDNA Libraries**—Preparation of the oligo(dT)-primed fetal rat liver (16 days post-conception) cDNA library (λgt11) has been described (22). A random-primed library was prepared by the same technique, except random hexamers replaced oligo(dT) in the synthesis of first strand cDNA. The unamplified libraries (3 × 10⁸ recombinants) were screened by in situ plaque hybridization on *Escherichia coli* Y1088, with 3²P-labeled ABP cDNA as previously described (23). Positive recombinants were plaque purified, recombinant XDNA was prepared by the method of Kassel (24), and the DNA inserts were isolated by electrophoresis.

**Transient Expression in Monkey Kidney Cells**—The DNA inserts were excised by restriction enzymes from λgt11 or pCMV5, a modified pCMV plasmid containing an EcoRI cloning site (25). The pCMV plasmids were from David Russel and Matthew Lawrence, University of Texas Southwestern Medical Center, Dallas. These plasmid vectors contain the promoter-enhancer of the cytomegalovirus immediate early gene, an additional EcoRI site 570 bp from the 5' end of the human growth hormone gene, the SV40 origin of replication, and a polylinker region for the insertion of cDNAs. Recombinants with the correct orientation for expression were identified by restriction endonuclease analysis and the plasmid DNA was purified by CsCl-density gradient ultracentrifugation. A polylinker region for the insertion of cDNAs. Recombinants with the correct orientation for expression were identified by restriction endonuclease analysis and the plasmid DNA was purified by CsCl-density gradient ultracentrifugation.

**Preparation and Screening of cDNA Libraries**—Preparation of the oligo(dT)-primed fetal rat liver (16 days post-conception) cDNA library (λgt11) has been described (22). A random-primed library was prepared by the same technique, except random hexamers replaced oligo(dT) in the synthesis of first strand cDNA. The unamplified libraries (3 × 10⁸ recombinants) were screened by in situ plaque hybridization on *Escherichia coli* Y1088, with 3²P-labeled ABP cDNA as previously described (23). Positive recombinants were plaque purified, recombinant XDNA was prepared by the method of Kassel (24), and the DNA inserts were isolated by electrophoresis.

**Transient Expression in Monkey Kidney Cells**—The DNA inserts were excised by restriction enzymes from λgt11 or pCMV5, a modified pCMV plasmid containing an EcoRI cloning site (25). The pCMV plasmids were from David Russel and Matthew Lawrence, University of Texas Southwestern Medical Center, Dallas. These plasmid vectors contain the promoter-enhancer of the cytomegalovirus immediate early gene, an additional EcoRI site 570 bp from the 5' end of the human growth hormone gene, the SV40 origin of replication, and a polylinker region for the insertion of cDNAs. Recombinants with the correct orientation for expression were identified by restriction endonuclease analysis and the plasmid DNA was purified by CsCl-density gradient ultracentrifugation.

**Preparation and Screening of cDNA Libraries**—Preparation of the oligo(dT)-primed fetal rat liver (16 days post-conception) cDNA library (λgt11) has been described (22). A random-primed library was prepared by the same technique, except random hexamers replaced oligo(dT) in the synthesis of first strand cDNA. The unamplified libraries (3 × 10⁸ recombinants) were screened by in situ plaque hybridization on *Escherichia coli* Y1088, with 3²P-labeled ABP cDNA as previously described (23). Positive recombinants were plaque purified, recombinant XDNA was prepared by the method of Kassel (24), and the DNA inserts were isolated by electrophoresis.

**Transient Expression in Monkey Kidney Cells**—The DNA inserts were excised by restriction enzymes from λgt11 or pCMV5, a modified pCMV plasmid containing an EcoRI cloning site (25). The pCMV plasmids were from David Russel and Matthew Lawrence, University of Texas Southwestern Medical Center, Dallas. These plasmid vectors contain the promoter-enhancer of the cytomegalovirus immediate early gene, an additional EcoRI site 570 bp from the 5' end of the human growth hormone gene, the SV40 origin of replication, and a polylinker region for the insertion of cDNAs. Recombinants with the correct orientation for expression were identified by restriction endonuclease analysis and the plasmid DNA was purified by CsCl-density gradient ultracentrifugation.

**Preparation and Screening of cDNA Libraries**—Preparation of the oligo(dT)-primed fetal rat liver (16 days post-conception) cDNA library (λgt11) has been described (22). A random-primed library was prepared by the same technique, except random hexamers replaced oligo(dT) in the synthesis of first strand cDNA. The unamplified libraries (3 × 10⁸ recombinants) were screened by in situ plaque hybridization on *Escherichia coli* Y1088, with 3²P-labeled ABP cDNA as previously described (23). Positive recombinants were plaque purified, recombinant XDNA was prepared by the method of Kassel (24), and the DNA inserts were isolated by electrophoresis.

**Transient Expression in Monkey Kidney Cells**—The DNA inserts were excised by restriction enzymes from λgt11 or pCMV5, a modified pCMV plasmid containing an EcoRI cloning site (25). The pCMV plasmids were from David Russel and Matthew Lawrence, University of Texas Southwestern Medical Center, Dallas. These plasmid vectors contain the promoter-enhancer of the cytomegalovirus immediate early gene, an additional EcoRI site 570 bp from the 5' end of the human growth hormone gene, the SV40 origin of replication, and a polylinker region for the insertion of cDNAs. Recombinants with the correct orientation for expression were identified by restriction endonuclease analysis and the plasmid DNA was purified by CsCl-density gradient ultracentrifugation.

**Preparation and Screening of cDNA Libraries**—Preparation of the oligo(dT)-primed fetal rat liver (16 days post-conception) cDNA library (λgt11) has been described (22). A random-primed library was prepared by the same technique, except random hexamers replaced oligo(dT) in the synthesis of first strand cDNA. The unamplified libraries (3 × 10⁸ recombinants) were screened by in situ plaque hybridization on *Escherichia coli* Y1088, with 3²P-labeled ABP cDNA as previously described (23). Positive recombinants were plaque purified, recombinant XDNA was prepared by the method of Kassel (24), and the DNA inserts were isolated by electrophoresis.

**Transient Expression in Monkey Kidney Cells**—The DNA inserts were excised by restriction enzymes from λgt11 or pCMV5, a modified pCMV plasmid containing an EcoRI cloning site (25). The pCMV plasmids were from David Russel and Matthew Lawrence, University of Texas Southwestern Medical Center, Dallas. These plasmid vectors contain the promoter-enhancer of the cytomegalovirus immediate early gene, an additional EcoRI site 570 bp from the 5' end of the human growth hormone gene, the SV40 origin of replication, and a polylinker region for the insertion of cDNAs. Recombinants with the correct orientation for expression were identified by restriction endonuclease analysis and the plasmid DNA was purified by CsCl-density gradient ultracentrifugation.

**Preparation and Screening of cDNA Libraries**—Preparation of the oligo(dT)-primed fetal rat liver (16 days post-conception) cDNA library (λgt11) has been described (22). A random-primed library was prepared by the same technique, except random hexamers replaced oligo(dT) in the synthesis of first strand cDNA. The unamplified libraries (3 × 10⁸ recombinants) were screened by in situ plaque hybridization on *Escherichia coli* Y1088, with 3²P-labeled ABP cDNA as previously described (23). Positive recombinants were plaque purified, recombinant XDNA was prepared by the method of Kassel (24), and the DNA inserts were isolated by electrophoresis.

**Transient Expression in Monkey Kidney Cells**—The DNA inserts were excised by restriction enzymes from λgt11 or pCMV5, a modified pCMV plasmid containing an EcoRI cloning site (25). The pCMV plasmids were from David Russel and Matthew Lawrence, University of Texas Southwestern Medical Center, Dallas. These plasmid vectors contain the promoter-enhancer of the cytomegalovirus immediate early gene, an additional EcoRI site 570 bp from the 5' end of the human growth hormone gene, the SV40 origin of replication, and a polylinker region for the insertion of cDNAs. Recombinants with the correct orientation for expression were identified by restriction endonuclease analysis and the plasmid DNA was purified by CsCl-density gradient ultracentrifugation.

**Preparation and Screening of cDNA Libraries**—Preparation of the oligo(dT)-primed fetal rat liver (16 days post-conception) cDNA library (λgt11) has been described (22). A random-primed library was prepared by the same technique, except random hexamers replaced oligo(dT) in the synthesis of first strand cDNA. The unamplified libraries (3 × 10⁸ recombinants) were screened by in situ plaque hybridization on *Escherichia coli* Y1088, with 3²P-labeled ABP cDNA as previously described (23). Positive recombinants were plaque purified, recombinant XDNA was prepared by the method of Kassel (24), and the DNA inserts were isolated by electrophoresis.

**Transient Expression in Monkey Kidney Cells**—The DNA inserts were excised by restriction enzymes from λgt11 or pCMV5, a modified pCMV plasmid containing an EcoRI cloning site (25). The pCMV plasmids were from David Russel and Matthew Lawrence, University of Texas Southwestern Medical Center, Dallas. These plasmid vectors contain the promoter-enhancer of the cytomegalovirus immediate early gene, an additional EcoRI site 570 bp from the 5' end of the human growth hormone gene, the SV40 origin of replication, and a polylinker region for the insertion of cDNAs. Recombinants with the correct orientation for expression were identified by restriction endonuclease analysis and the plasmid DNA was purified by CsCl-density gradient ultracentrifugation.
autoradiography. Hybridizations of DNA and RNA blots with \(^3P\)-labeled oligonucleotides were done according to Lathe (48). To ensure that the oligonucleotides were appropriate probes, positive and negative DNAs were hybridized as controls, except for the oligonucleotide representing an exon 7 deletion, for which no positive control was available. The sequence of the oligonucleotide specific for the ABP exon 7 deletion was CCAAGACCAAGAGAGGACTC. Other oligonucleotides are described in "Results."

For nucleotide sequencing, restriction endonuclease fragments were subcloned in bacteriophage M13mp18 or M13mp19 (49). DNA sequences were determined by the dideoxy chain termination method (50, 51), with \(^3P\)-labeled deoxyadenosine 5'-[\(\alpha\)-thio]triphosphate (Amersham Corp.). Oligonucleotides were synthesized based on the nucleotide sequences obtained and used as primers to walk down the cDNAs on both strands. DNA homologous with testicular ABP cDNA was sequenced on at least one strand and non-homologous DNA was sequenced on both strands. Sequences were compared with each other and the Genetic Sequence Data Bank (GenBank) and the National Biomedical Research Foundation (NBRF) Protein Identification Resource with Microgenie computer program (Beckman Instruments).

ABP RIA was done with the kit supplied by the National Hormone and Pituitary Program (27). Specific binding of \(^3H\) dihydrotestosterone in tissue extracts was determined using the dextran-coated charcoal method (28). The preparation of extracts and modifications of the assay for ABP have been previously described (38). Briefly, cell extracts were incubated with 6 nM \[^3H\]DHT for 1 h at 0°C, charcoal treated for 2 min to remove unbound DHT, and the radioactivity in the supernatant fluid was determined by liquid scintillation spectrometry. Specific binding was calculated as previously described (28).

Pregnant Sprague-Dawley rats were obtained from Charles River Labs (Wilmington, MA). Rats were decapitated and the embryos were excised and aged by length and skeletal formation. Tissues were excised and frozen in liquid nitrogen. Protein extracts of frozen tissues were prepared by disruption in a glass homogenizer and processed as described above for COS cells. Protein concentrations of tissue extracts were determined after precipitation with 6% trichloroacetic acid, 0.15 mg/ml sodium deoxycholate.

**RESULTS**

**Immunoreactive ABP and Dihydrotestosterone Binding in Fetal Liver**—ABP radioimmunooassay of fetal rat liver extracts revealed an immunologically related protein. Soluble liver extracts from 16-, 18-, and 21-day embryos contained 11, 8.6, and 2.3 ng of ABP/mg of protein, respectively, whereas the adult rat caput epididymis and testis contained 292 ng/mg and 25 ng/mg, respectively. The amount of ABP in the insoluble fraction was not determined. These declining ABP levels in liver coincide with the decreasing fetal serum levels of immunoreactive ABP from 16 to 21 days after conception (21). A polyclonal antibody against purified ABP (36) was used to localize ABP in fetal liver by immunocytochemistry. The rabbit antiserum reacted with hepatocytes in fetal liver, but because insufficient purified rat ABP was available for blocking experiments specificity could not be demonstrated. Therefore, based on the amino acid sequence of rat ABP we generated antibodies with synthetic peptides. One of these peptides (peptide B) yielded an antibody in rabbit serum with a high titer against ABP that reacted with the ABP-containing cells and ducts of the testis and epididymis. Fig. 1A shows immunostaining of the epididymal lumen and epithelial cells lining the lumen. These findings are in agreement with the well known pathway of ABP biogenesis where ABP is synthesized in the testis, secreted into the seminiferous tubule lumen and transported to the epididymis where it internalized by the epithelium (36). Pretreatment of the antibody with peptide B blocked staining of the epididymal and testicular structures. Immunocytochemical localization of fetal liver ABP with this antibody demonstrated the presence of immunoreactive protein in the hepatocytes of fetal liver at 15-day post-conception (Fig. 1, B and C). Asymmetric staining of the cytoplasm suggests that ABP is secreted from the hepatocytes in a polar fashion. Furthermore, pretreatment of the antibody with serum with peptide B blocked the staining in hepatocytes.

The intensities of the specific hepatocyte staining were lower at 16 and 17 days than at 15 days post-conception, as were the percent of stained cells (Fig. 1, D–F), consistent with the decline of RIA-determined ABP after 16 days of development. Western blot analysis of the insoluble (14,000 x g pellet) fetal liver protein detected a signal corresponding in size to that of the ABP subunit size (M, = 43,000) (see below, Fig. 9). No M, = 43,000 signal was observed in the fetal liver soluble fraction or in either fraction of adult liver protein. The immunoreactive fetal liver protein (M, = 43,000) was present at 16-days post-conception, with much lower amounts at 15 and 17 days of gestation. We do not know the subcellular location of the insoluble ABP.

Specific \(^3H\)DHT binding was determined for protein extracts of fetal liver at various times of development. At each age (16–20 days post-conception) specific binding was detected at a level approaching the lower limit of the assay (approximately 5 fmol of DHT/mg of protein). This level of specific binding corresponds to 0.4 ng of ABP/mg of protein, assuming one molecule of DHT bound/dimer of ABP. Under the conditions of assay (ligand occupied AR and 1 h incubation at 0°C) little AR should be detected, however, it cannot be ruled out that all or part of the activity is due to AR. Nevertheless, much less steroid-binding activity was detected than the levels of ABP measured by RIA, suggesting that a large fraction of the immunoreactive ABP in the liver does not bind DHT. Perhaps a non-steroid-binding form of ABP is not secreted, remaining in the liver (see discussion of alternative RNA processing below).
Cloning and Characterization of Fetal Liver ABP RNA Transcripts

We have previously described the testicular cDNA and gene structure of rat ABP and presented evidence that ABP and SHBG are encoded by the same gene (13, 23). Because human liver SHBG and testicular ABP are regulated very differently we proposed that alternate promoters and regulatory elements are utilized in these tissues (13). To compare testicular and fetal liver ABP RNA transcripts in the rat, we prepared 16-day fetal liver cDNA libraries (oligo(dT)- or random-primed) and screened them for ABP cDNA. Four homologous fetal liver cDNAs were isolated and sequenced. The structure of each clone compared with testicular ABP cDNA is shown diagrammatically in Fig. 4. Clone FLABP1 was a partial cDNA identical in sequence to testicular ABP cDNA; except that it lacked 400 bp at the 5'-end, whereas clone FLABP5 (from a random-primed library) encoded nearly all of the testicular ABP protein, lacking only 9 nucleotide residues (3 amino acid residues) at the 3'-end of the amino acid coding region. Isolates FLABP1 and FLABP5 did contain the coding region in the correct reading frame for the proposed COOH-terminal steroid-binding domain (52).

Clones FLABP2 and FLABP-HDC represented alternately processed transcripts of the ABP gene (Fig. 4). Clone FLABP2 was identical to testicular ABP cDNA except exon 1 was replaced by an alternate sequence and exon 6 was totally missing. The lack of exon 6 causes a change in reading frame, resulting in a termination codon 18 bp into exon 7 (Fig. 5A). This altered mRNA encodes a 245-residue protein that does not contain the amino acids encoded by exons 7 and 8 of the ABP gene, which include the proposed steroid-binding domain (52). Recently, Hammond et al. (12) and Gershagen et al. (53) have identified human testicular ABP transcripts with exon 7 deletions, which also result in removal of the steroid-binding domain. To investigate exon deletions in rat testicular transcripts, we prepared oligonucleotides specific for exon 6 (oligo FL3-X6, Fig. 5A, underlined) and exon 7 (see "Experimental Procedures") deletions. Hybridization with 20 unique testicular ABP cDNA clones and 30 uncharacterized cDNA isolates determined that none contained exon 6 or 7 deletions. Thus, it appears that the lack of the amino acids encoded by exons 6 or 7 do not contribute to the subunit size heterogeneity of testicular ABP, which is primarily due to glycosylation (55, 56).

The sequence of clone FLABP2 also differed from

![Fig. 2. Tissue distribution of ABP mRNA. Poly(A)+ RNA (10 μg) from various rat tissues was analyzed by Northern blot hybridization with 32P-labeled ABP cDNA as probe. Lane 1, adult adrenal; lane 2, fetal liver (16 days after conception); lane 3, adult liver; lane 4, adult kidney; lane 5, 26-day testis; lane 6, adult testis; lane 7, adult submandibular gland; lane 8, adult spleen. The RNA samples are flanked by 32P-labeled single-stranded DNA molecular weight markers (M); the sizes are indicated to the right of the autoradiogram. The size of the major hybridizing species is indicated at the left.](image-url)

![Fig. 3. ABP mRNA during fetal liver development. Pregnant Sprague-Dawley rats were decapitated, and the embryos were excised and aged by length and skeletal formation. Total RNA (20 μg) from liver at each age was analyzed by Northern blot hybridization. The size of the hybridizing mRNA species is indicated at the left.](image-url)

![Fig. 4. Diagrams of fetal liver cDNA clones. The open bar at the top of the figure represents testicular ABP mRNA with the translation start (ATG) and termination (TAG) signals indicated. The sequences encoded by testicular exons 1-8 are marked below. The vertical dashed lines indicate boundaries at exon junctions in the ABP gene. Open bars in FLABP1, FLABP2, FLABP5, and FLABP-HDC are identical to testicular ABP. The solid line (del) in FLABP2 represents a deletion of exon 6. Stippled and hatched bars represent DNA that is unrelated to testicular ABP cDNA. The hatched area of FLABP-HDC is 2.3 kb and identical to FLHDC. FLHDC encodes all of rat HDC. An exon junction in FLHDC DNA is indicated by exon junk. ORF signifies an open reading frame from the 5' terminus to the termination codon. Translation initiation (ATG) and termination (TAG, TGA, and TAG) codons are indicated.](image-url)

D. R. Joseph and Y.-M. Wang, unpublished results.
testicular ABP sequence in exon 1 (Fig. 5B). The unique 5'-end of clone FLABP2. The first 60 nucleotide residues contain no Met or stop codons and are unique from testicular ABP cDNA. The remaining sequence is identical to ABP cDNA, except for the deletion of exon 6 (see A above). The deduced amino acid sequence of residues 1–60 has no homology with any known protein in the data base. The complementary sequence of oligo FL35P is underlined. Oligo 3545, which was used for PCR, represented nucleotide residues 29–48.

The presence of each of the cloned forms of fetal liver ABP mRNA was supported by PCR analysis of the cDNA. cDNA was prepared from liver of developing rats (15–16 day) and subjected to PCR with various combinations of oligonucleotides. To test for the presence of each cDNA, the following oligonucleotides were used (the location and amplified region of each clone is diagrammed in Fig. 6): testicular ABP cDNA exon 1, oligos 570 and 569–2, theoretical 1025-bp product; fetal liver alternate exon 1, oligos 3545 and 569, theoretical 600-bp product; fetal liver exon 6 deletion, oligos 1856 and 569–2, theoretical 516-bp DNA with testicular cDNA or 380 bp with exon 6 deleted. Each set of oligonucleotides was tested with the appropriate cloned cDNA and found to yield the predicted size fragment after amplification with PCR and each amplified cDNA hybridized with ABP cDNA after blotting. The oligonucleotides were then used to amplify fetal liver cDNA. In each case the reaction yielded the predicted size product as visualized by ethidium bromide staining (data not shown). To confirm the nature of the products, the gel was blotted with a nylon membrane and hybridized with ABP cDNA. Fig. 6A demonstrates that each stained band hybridized with ABP cDNA. Oligos 570 and 569–2 yielded the predicted 1025-bp DNA, demonstrating the presence of the testicular form of ABP mRNA in fetal liver. With oligos 3545 and 568, a 600-bp DNA was amplified, demonstrating the presence of the alternate exon 1 sequence in fetal liver ABP mRNA. Oligos 1856 and 569–2 yielded 516- and 380-bp species, the expected DNAs if cDNAs with and without exon 6 sequences were present. The nature of the high molecular weight hybridizing DNA in each lane is unknown. No hybridization was observed when fetal liver cDNA was omitted from the reaction. Amplification of fetal liver cDNA with oligos 3545 and 569–2 yielded a heterogeneous, high molecular weight, reaction product without discrete bands.

The 5'-730 bp of clone FLABP-HDC was identical to the testicular ABP cDNA sequence representing exons 1–5 (13, 20); however, beginning at the end of exon 5 (15 bp 5' from EcoRI site), the remaining 2.5 kb was not homologous with

---

**Fig. 5.** Nucleotide and amino acid sequence of clone FLABP2. A, nucleotide sequence and deduced amino acid sequence of clone FLABP2 around the exon 5–7 junction. The effect of the exon 6 deletion on the deduced amino acid sequence of FLABP2 as compared with testis ABP (ABP) is shown. Nucleotide and amino acid residue numbers are displayed at the ends of each sequence. The testicular ABP amino acid sequence terminates at residue 403. A dashed line denotes uninterrupted nucleotide sequence or amino acid sequence. The complementary sequence of oligo FL35P is underlined. B, nucleotide sequence and deduced amino acid sequence at the 5'-end of clone FLABP2. The first 60 nucleotide residues contain no Met or stop codons and are unique from testicular ABP cDNA. The remaining sequence is identical to ABP cDNA, except for the deletion of exon 6 (see A above). The deduced amino acid sequence of residues 1–60 has no homology with any known protein in the data base. The complementary sequence of oligo FL35P is underlined. Oligo 3545, which was used for PCR, represented nucleotide residues 29–48.

**Fig. 6.** PCR of fetal liver cDNA. The diagram describes the location of oligonucleotides used for PCR and the theoretical product size from each clone. The open bar represents testicular ABP cDNA with the exon sequences indicated (see Fig. 4). Oligonucleotides are indicated above or below their location on each fetal liver cDNA and are defined in the legends to Figs. 5 and 7 and under "Experimental Procedures." Oligos 570, 1856, and 3545 are based on testicular ABP cDNA, except for the deletion of exon 6. Each set of oligonucleotides was tested by gel electrophoresis and Southern blot hybridization with ABP cDNA as probe. Autoradiography was for 0.5 h with Kodak XAR film and intensifying screens.

Lane 1, oligos 570 and 569–2; lane 2, oligos 3545 and 568; lane 3, oligos 1856 and 569–2. B and C, identification of the fusion transcript in fetal liver RNA by PCR of the cDNA. Primers for amplification of the fusion transcript were oligos 1856 and 53–6 (Figs. 6 and 7, theoretical 572-bp product). Products were analyzed by agarose gel electrophoresis and blot hybridization with an oligonucleotide specific for the fusion junction of the ABP and HDC domains of FLABP-HDC DNA (oligo 1210). After transfer of the DNA from the gel to a nylon membrane the DNA was hybridized with 3P-labeled oligo 1210 (Fig. 7, legend) in 5X SSC, 30% formamide at 25 C for 16 h. The filter was washed in 2X SSC and the final stringent wash was in 0.2 SSC at 25 C for 20 min. B, amplification of fetal liver cDNA with oligos 1856 and 53–6, hybridization with oligo 1210. Lane 1, 16-day embryo; lane 2, 17-day embryo, C, control samples, hybridization with oligo 1210 as probe. Lane 1, PCR of FLABP-HDC DNA with oligos 1856 and 53–6; lane 2, PCR of FLABP5 DNA with oligos 1856 and 569–2 (gel electrophoresis demonstrated amplification of the expected 516-bp DNA). The size of each major hybridizing species is indicated.
ABP cDNA (Fig. 4). Fig. 7 shows the total nucleotide sequence of clone FLABP-HDC (2975 bp) with the deduced amino acid sequence. The cDNA encodes a protein of 98,000 molecular weight, with the NH₂-terminal region identical to testicular ABP (240 amino acid residues), including the signal peptide. The remaining amino acid sequence (643 residues) has no sequence identity with ABP. Comparison of the deduced amino acid sequence of the 3'-2.3 kb of FLABP/HDC with the National Biomedical Research Foundation Protein Data Bank revealed extensive homology with the amino acid sequence of aromatic L-amino acid decarboxylase (56). Further cloning studies with expression of the full-length cDNA homologue in COS cells, and genetic linkage analysis demonstrated that the L-amino acid decarboxylase-related domain encodes most of the cDNA for HDC (EC 4.1.1.22) (22, HDC catalyzes the formation of histamine, a biogenic amine involved in numerous physiological processes, including neurotransmission, inflammation, vasomotor control, and gastric acid secretion). Amino acid residue 241 of FLABP-HDC corresponds to rat HDC residue 15, the remaining residues being identical to one of the allelic forms of HDC (22). This region of homology includes the putative pyridoxal phosphate-binding site of HDC (Lys-434, Fig. 7, underlined). In summary, FLABP-HDC cDNA encodes the signal peptide of ABP, likely the membrane receptor-binding domain of ABP (see “Discussion”) and all but 14 NH₂-terminal amino acid residues of HDC.

Identification of RNA Splice Junction in Rat HDC Gene—In clone FLABP-HDC the end of the ABP nucleotide sequence (residue 730) occurs at an exon splice junction (donor) of the ABP gene (Fig. 8). To determine if the beginning of the HDC domain of FLABP-HDC (residue 731) also was at a splice junction, we began analysis of the HDC gene. Southern blot hybridization of rat DNA with the 3'-2.3-kb EcoRI fragment of FLABP-HDC DNA as probe revealed hybridizing EcoRI fragments of 3.0, 10, and 17 kb (data not shown). A phage Charon 4A genomic DNA library (57) was constructed with partially EcoRI-digested rat DNA (10-21 kb) and screened unamplified by in situ plaque hybridization with the HDC domain of clone FLABP-HDC (2.3-kb EcoRI fragment) and an oligonucleotide probe specific for the sequence adjacent to the potential splice site (Fig. 7, oligo b3-7, complemented by residues 761-777, underlined). Two positive recombinants were identified which contained 10- and 3-kb inserts (each with one stuffer fragment of Charon 4A DNA). Sequence analysis of the clones with oligo b3-7 as primer revealed that the 10-kb DNA contained the identical sequence of FLABP-HDC upstream to residue 731, the beginning nucleotide of FLABP-HDC cDNA.
the HDC domain (see Fig. 7). Juxtapositioned 5′ to the first residue of the homology was a consensus acceptor splice sequence, TCCCTCTGGCTTGGCTGCTCAG (Fig. 8). Thus, the ABP and HDC domains of FLABP-HDC cDNA appear to be joined at donor and acceptor splice sites of the two genes. No ID repetitive element (see "Discussion") was observed by sequence analysis in the intron within 500 bp of the splice junction. However, hybridization with cDNA probes representing ID elements (46, 47) revealed that the 10-kb genomic DNA fragment did contain at least one ID element, with location(s) relative to HDC DNA unknown. Further upstream at an unidentified location the 10-kb genomic DNA contained the exon encoding the initiating methionine and the putative transcription start site (unpublished results). This sequence was identified by direct sequence analysis of the 10-kb DNA and primer extension of poly(A) RNA with an HDC exon 1 oligomer.

Chromosomal Localization of Rat HDC and ABP Genes—To further evaluate the mechanism by which the FLABP-HDC transcript was formed, the chromosomal locations of the rat ABP and HDC genes were obtained by analysis of cell hybrids. Rat-mouse cell hybrids generally discard some of the rat chromosomes, making it possible to use karyotyped hybrids for mapping genes (31, 32). Rat and mouse DNA was digested with a number of restriction endonucleases and analyzed by Southern blot hybridization with ABP and HDC cDNA as probes. Autoradiography revealed polymorphisms with ABP cDNA (EcoRI) and HDC cDNA (BamHI) that could be used for mapping studies (see details under "Experimental Procedures"). Blot hybridization analysis of DNA from 13 mouse-rat hybrids, with ABP cDNA and HDC cDNA, revealed that eight hybrids contained the rat ABP gene and seven contained the rat HDC gene (Table I). Of the 13 hybrids, 7 were discordant for ABP and HDC DNA, clearly demonstrating that the two genes are located on different chromosomes. Comparison of the rat chromosomal content of the hybrid cells with the rat ABP DNA content demonstrated that the presence or absence of ABP DNA was correlated with the presence or absence of chromosome 10, whereas, the presence of HDC DNA appeared to be correlated with chromosome 3. With ABP cDNA no discordant hybrids were found for chromosome 10, whereas, at least 3 of 13 hybrids were discordant for the other chromosomes. The data also suggest that the HDC gene is on chromosome 3, but because of the many low discordant numbers (2 to 3) of the other chromosomes, the assignment of the HDC gene to rat chromosome 3 should be considered as provisional. In support of the chromosomal locations of rat ABP-SHBG (Shbg) and HDC (Hdc) genes, the mouse loci are located on chromosomes 11 and 2, respectively (22, 58). Mouse chromosomes 11 and 2 are partially homologous to rat chromosomes 10 and 3, respectively (59).

Biological Significance of the FLABP-HDC RNA Transcript—Clone FLABP-HDC appeared to represent a fusion RNA transcript of two unique genes. Because of the unusual nature of the mRNA we designed experiments to test its biological significance.

To determine if the fetal liver produced an ABP-like protein corresponding to the size of the theoretical fusion protein, we analyzed fetal liver protein on immunoblots with ABP antisera. Fig. 9A demonstrates that an immunoreactive protein of size M, = 43,000 was present in the insoluble protein (14,000 x g pellet) from liver extracts of developing rats at 15-17 days of gestation (lanes 3-5). It was also observed that a M, = 93,000 immunoreactive protein was present in the insoluble fetal liver protein (Figs. 9, A and B, short and long exposure, lane 4). The estimated size of this immunoreactive species is in close agreement with the predicted size of the fusion protein encoded by FLABP-HDC cDNA, minus the signal peptide (95,000 molecular weight). Like the M, = 43,000 protein species, the maximum immunoreactivity of the M, = 93,000 species was at 16 days. No immunoreactive protein migrating as M, = 93,000 was present in the soluble fetal liver protein or in either protein fraction from adult liver (not shown), brain (not shown), testis (Fig. 9, lane 1), or epididymis (lane 2). As expected, immunoreactive ABP (M, = 43,000) was observed in the soluble fraction of testis and epididymis (not shown). The major band of immunoreactivity migrating as a M, = 60,000 protein corresponds to a major protein band on stained gels; this reactivity appears not to be related to ABP. Immunoblot experiments with the antisera against ABP peptides A and B have proven not to be sensitive enough to detect either the M, = 43,000 or 93,000 species in fetal liver protein. These data demonstrate that a protein with some of the properties of the ABP-HDC fusion protein is transiently expressed in fetal liver. Whether this immunoreactive protein represents the fusion protein remains to be determined. Obviously, parallel studies with HDC antisera would have aided in the characterization of this protein. However, the requisite antibody against HDC is not available.

The properties of the protein encoded by FLABP-HDC cDNA were determined by expression of the cDNA in COS cells. ABP cDNA, HDC cDNA, and FLABP-HDC cDNA were cloned into the expression vector, pCMV, yielding the recombinants pCMVABP6, pCMVHDC-18, pCMVFABP-HDC, pCMV5FLABP-HDC, and pCMV5FLABP-HDCrev (construction details are described under "Experimental Procedures"). COS 7 cells were transformed with the purified recombinant DNAs, and the cell extracts were assayed for ABP and HDC activity. pCMVABP yielded a high level of DHT binding activity in the medium, indicating that ABP is secreted, and pCMVHDC-18 yielded high HDC activity in the soluble and insoluble fraction (14,000 x g pellet) of the cell extract (22). Surprisingly, over 90% of the HDC activity was located in the insoluble fraction; in a control study 95% of the fetal liver HDC activity was found in the soluble fraction. After sonication of the COS cell-insoluble fraction, all of the HDC activity remained in the soluble fraction (14,000 x g pellet), suggesting that most of the HDC in COS cells is in a membrane fraction or possibly stored in inclusion bodies as insoluble protein. Neither pCMVFABP-HDC DNA nor pCMV5FLABP-HDC DNA yielded detectable cellular or secreted steroid binding activity. However, both FLABP-HDC DNA constructs yielded HDC enzymatic activity (1% of the level obtained with cells transformed with pCMVHDC-18) in the insoluble fraction of COS cell extracts, but not in the soluble cell fraction or medium.

Fig. 10 displays autoradiograms of Western immunoblot analyses of transformed COS cells with ABP antisera. Transformation with pCMVABP9 DNA yielded immunoreac-
transformed with the other recombinants; only nonspecific protein revealed immunoreactive bands. The 93,000 molecular weight protein was found in the insoluble fraction of COS cells (14,000 g pellet) transformed with the FLABP-HDC DNA recombinants (Fig. 10C, lanes 2 and 3). In addition, smaller bands of reactivity were present that likely resulted from proteolysis. Our conclusion that the 93,000 molecular weight peptide B (see above) represents secreted ABP; a lower level of reactivity was present that likely resulted from proteolysis. Our conclusion that the 93,000 molecular weight protein is encoded by FLABP-HDC DNA is supported by the long exposure of the autoradiogram, 15-h exposure. The size of the testicular ABP, which is a homodimer composed of identical subunits, could appear as a discrete band of reactivity (Mr 93,000) was observed in the 14,000 g pellet fraction from cells transformed with the FLABP-HDC DNA recombinants (Fig. 10C, lanes 2 and 3). Thus, the immunoreactive protein encoded by FLABP-HDC DNA is not secreted by COS cells even though translation should initiate with the ABP signal peptide. Perhaps the HDC domain contains a sequence that targets the protein from the rough endoplasmic reticulum to another cellular compartment. The cellular location of HDC is not known, although a fraction of HDC is thought to be membranous in the adult brain.

Northern hybridization was used to determine if a fetal liver RNA species may contain both the HDC and ABP domains. The 2.3-kb 3' EcoRI fragment (HDC) of clone FLABP-HDC and ABP cDNA were used to probe a Northern blot (Fig. 11). This 2.3-kb fragment contains HDC cDNA and 15 bp of ABP cDNA but does not hybridize with ABP mRNA or cDNA. After hybridization with ABP cDNA the blot was stripped of radioactivity and autoradiographed to confirm that stripping was complete. The RNA blot was then hybridized with HDC cDNA (Fig. 11). The HDC cDNA probe hybridized with a major 3.2-kb mRNA species and a minor 4.4-kb species in fetal liver poly(A) RNA (lane 1). No hybridization was seen with poly(A) RNA from adult rat liver or testis (lanes 2 and 3). The gene content of each hybrid was determined by Southern blot hybridization with ABP and HDC cDNA probes. The presence or absence of the rat genes is indicated by + or −, respectively; ND = not done. The rat chromosomes, the symbols mean: + = chromosome present in at least 60% of the metaphases; (−) = chromosome present in 10-20% of the metaphases; + = chromosome absent.

**Table I**

| Hybrids | Rat genes | Rat chromosomes |
|---------|-----------|-----------------|
|         | ABP | HDC | X | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| LB150-1 | + | + | − | − | + | − | − | − | + | − | + | − | + | + | − | + | + | − | + | + | (+) | (−) | (+) | (+) | (+) |
| LB161 | + | + | + | − | + | + | + | + | + | + | − | + | + | − | + | + | + | + | + | + | + | + |
| LB210-1 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| LB251 | + | − | + | + | + | + | + | + | (−) | − | + | + | + | + | + | + | + | + | + | + | + |
| LB330TG3 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| LB330TG6 | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB510-6 | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB600 | + | + | + | + | + | + | + | + | (−) | + | + | + | + | + | + | + | + | + | + | + | + |
| LB630 | − | + | + | + | + | + | + | + | (−) | + | + | + | + | + | + | + | + | + | + | + | + |
| LB780-6 | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB780-8 | ND | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| LB810 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB860 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB1040TG5 | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| LB150 | ND | + | + | (−) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LB210 | ND | − | + | + | + | + | + | + | (−) | + | + | + | + | + | + | + | + | + | + | + | + |
| Discordant hybrids* | | | | | | | | | | | | | | | | | | | | | | | | | | | |

*Hybrids that contained chromosomes at 10-20% (−) were not included as discordant hybrids because they could appear as + or − for the ABP and HDC genes.

**Fig. 9. Immunoblot analysis of rat fetal liver protein.** Proteins from various tissues (14,000 × g pellet) were analyzed as described in the legend to Fig. 10. A, autoradiogram, 15-h exposure. B, 55-h exposure. Lane 1, testis; lane 2, epididymis; lane 3, 15-day fetal liver; lane 4, 16-day fetal liver; lane 5, 17-day fetal liver. The antiserum used for the immunoblots was directed against purified ABP (36).
and 3). As described above, ABP cDNA hybridized with fetal liver mRNA species of 1.7 and 4.4 kb (Fig. 11, lane 1). Thus, ABP cDNA and HDC cDNA hybridize to a 4.4-kb fetal liver mRNA, which may represent the ABP-HDC fusion transcript. However, hybridization of the two cDNAs to two unique 4.4-kb RNAs remains a possibility.

PCR was also utilized to assay for fusion transcripts analogous to FLABP-HDC DNA in fetal liver RNA. cDNA was synthesized from fetal liver poly(A) RNA (16 and 17 days after conception) and subjected to PCR (30 cycles) with oligonucleotides flanking the fusion point of the two domains (see Figs. 6 and 7). After PCR of the single-stranded cDNA with oligonucleotides 1856 and b3-6, gel electrophoresis of the products revealed no stained DNA of the predicted size, whereas PCR of FLABP-HDC cDNA yielded the predicted 572-bp DNA. Although no 572-bp DNA was detected by staining after PCR, Southern blot hybridization analysis of the products from 17-day DNA with ABP cDNA did yield a single band of hybridization migrating in the same position as the major PCR product of FLABP-HDC cDNA (572 bp). No band of hybridization was observed with cDNA from 16-day embryos or in the control PCR reaction with no added DNA. This hybridizing DNA species was identified to be the product of the fusion cDNA by hybridization with an oligonucleotide specific for the ABP-HDC DNA fusion point. Oligonucleotide 1210 (20 residues) consisted of 10 nucleotide residues complementary to ABP cDNA and 10 residues complementary to HDC cDNA. (The sequence and location of this sequence is described in the legend to Fig. 7.) This oligonucleotide hybridized to FLABP-HDC cDNA but did not hybridize to ABP or HDC cDNA under our reaction conditions. Fig. 6 demonstrates that this oligonucleotide hybridized to the 572-bp PCR product of FLABP-HDC cDNA (Fig. 6C, lane 1) and to a similar size DNA species in the PCR products of 17-day fetal liver cDNA (Fig. 6B, lane 2) and a very faint signal from 16-day liver cDNA (lane 1), but not from products amplified from ABP cDNA (Fig. 6C, lane 2). In addition, PCR reactions with oligonucleotides B3-6 and 1856 without added cDNA no hybridization signal at 572 bp was observed. Even after 60 cycles of amplification in the absence of cDNA no signal was present. Positive reactions of the fusion product were obtained with cDNAs from two different RNA preparations derived from liver of 17-day embryos.

**DISCUSSION**

Our studies have demonstrated that the ABP gene is expressed transiently during fetal rat liver development. Immunoreactive protein was primarily localized in hepatocytes of fetal liver, where it is likely synthesized. Most of the ABP synthesized in fetal liver appears to be secreted into the blood (20, 21), however, our studies found a fraction of immunoreactive protein in an insoluble cellular compartment. Analyses of the fetal liver steroid binding activity and cDNA encoding properties of ABP mRNA indicate that fetal liver ABP is capable of specific DHT binding.

During the time of ABP gene expression in the fetal male rat (15–17 days of gestation), the level of testosterone is high; after 19 days of gestation the concentration declines until 3 weeks of life (60). At 15–17 days of gestation high concentrations of testosterone are required in the male fetus for differentiation of the Wolffian duct into the epididymis and seminal vesicle (61, 62). Also, high androgen levels are required during
the last week of gestation and immediately after birth for normal development of the male brain (63). During this time of development (birth to 6 days of age) ABP gene expression initiates in the brain. It is our hypothesis that plasma and brain ABP act as a carrier of androgens to facilitate these developmental processes. Interaction of testosterone-bound ABP with cell surface receptors could target testosterone to specific sites, where androgen is required for differentiation and imprinting. Although it has been assumed that steroids can readily enter cells by diffusion, evidence is accumulating for the role of carrier proteins in cellular uptake of steroids via specific surface receptors (3). Since the most evolutionarily conserved ABP-SHBG amino acid sequence between species is encoded by exon 3 this sequence could be the receptor-binding domain (the steroid-binding domain is at least partly located near the COOH terminus; encoded by exons 7 and 8). In support of this idea, Rosner and co-workers have demonstrated that a peptide encoded by human ABP-SHBG exon 3 binds with high affinity to the human ABP-SHBG membrane receptor.

Clone FLABPB also differed from testicular cDNA by the absence of exon 6, resulting in a deletion of the proposed steroid-binding domain from the encoded protein. Likewise, two other laboratories (12, 53) have identified a human testicular cDNA lacking exon 7, which also results in removal of the putative steroid-binding domain. Since the alternate human and rat transcripts contain testicular exons 2–5, they both would appear to encode proteins with a similar function. If the encoded proteins retain the receptor-binding capacity (see discussion of receptor-binding domain in exon 3 above) without steroid binding they could act as natural antagonists to regulate ABP action. Such an antagonist has been described for the thyroid hormone receptor, where alternative RNA splicing generates a protein with a functional DNA-binding domain, but a non-functional T3-binding domain, yielding a protein with opposing biological activity (66). Likewise, other alternatively processed RNAs have been shown to encode proteins with different functions (67, 68). The concept that ABP and SHBG may have direct regulatory effects at the cell surface has recently been proposed by Rosner and co-workers (3, 69–71). They have presented evidence that human SHBG acts as a hormone via the receptor, increasing intracellular cAMP levels (3, 70). More recently, they have shown that the interaction of SHBG with the cell surface receptor is modulated by bound androgen and have developed a model for ABP-SHBG action (71).

cDNA Clone FLABP-HDC encoded regions of ABP and HDC domains, which were joined at donor and acceptor RNA splice junctions of the two genes. Since the ABP and HDC genes are located on different rat chromosomes, this transcript was apparently formed by a trans splicing process. It is highly unlikely that this cDNA was generated by a cloning artifact. The following findings support the possibility that a representative transcript is expressed in fetal liver. 1) The ABP and HDC genes are transcriptionally active during this developmental period (from 15 days post-conception until birth the fetal liver contains extremely high levels of HDC mRNA). 2) The fetal liver hepatocyte is the primary location of immunoreactive ABP and HDC (72) and is likely the site of HDC and ABP gene transcription. 3) Hybridization studies identified a 4.4-kb poly(A)+ RNA(s) that hybridized with ABP and HDC cDNA. 4) PCR experiments indicate that a fusion transcript analogous to clone FLABP-HDC is synthesized in fetal liver. 5) An immunoreactive protein consistent with the size of the protein encoded by the fusion DNA was identified in fetal liver. 6) The hybridization-reactive 4.4-kb RNA and the immunoreactive M subunit were localized in fetal liver and not adult liver, testis, epididymis, or brain. Our data support a trans-splicing mechanism for formation of the fusion transcript, however, a mechanism involving recombination at the DNA level cannot be ruled out. In vivo trans splicing of RNA transcripts has not been reported in vertebrates. Trans splicing of nuclear pre-mRNAs occurs in trypanosomes and nematodes, but is limited to the splicing of a signal sequence to the 5′-end of the mRNA (73–75). Also, trans splicing appears to be involved in the formation of the Chlamydomonas chloroplast RNAs, a process that affects the region encoding protein (76).

ID elements may have been involved in the formation of the alternatively processed ABP transcripts. Rat ID elements are middle repetitive DNA sequences, containing RNA polymerase III promoters (46, 47), that are expressed in many tissues, with one of the poly(A)+ transcripts (BC1, 160 residues) specifically expressed in brain and another RNA species (T3, 75 residues) in testis (77). ID elements have been shown to be transcribed in fetal liver and not adult liver, testis, epididymis, or brain. Our data support the idea that ID RNAs are associated with the processing of RNA polymerase II transcripts, but strong evidence supporting this hypothesis is lacking. Three of these elements are associated with the rat ABP gene, one in opposite orientation (13). The reversed element is in the 3′-flanking DNA, near the poly(A) addition site, and the other two elements (same orientation) are in the intron between exons 5 and 6, adjacent to the region of the alternative splicing phenomena. Interestingly, the ID elements, in opposite orientation, flank the region of the ABP-SHBG gene missing in the fusion cDNA (exons 6–8). These studies suggest that the expressed RNAs of these elements may be involved in alternative splicing of ABP transcripts. The presence of ID transcripts in tissues expressing ABP RNA transcripts would inhibit intramolecular hybridization and could presumably affect splicing. The recent observation that the human ABP-SHBG gene contains an ALU repetitive element in an intron adjacent to the alternate splice site (12) supports the role of repetitive elements in RNA splicing of the ABP-SHBG gene. Since at least one of these elements is associated with the HDC gene, complementary ID elements could also have been involved in the formation of the FLABP-HDC transcript. In analogy, trans splicing of mRNA precursors has been demonstrated to occur in nuclear extracts with two RNA transcripts constructed to contain complementary sequences in their introns (78, 79). Base pairing between RNAs was thought to bring
the transcripts together for intermolecular splicing. Based on these in vitro studies, Solnick (75) supposed that the most likely candidates for RNAs that undergo trans splicing are those that contain repetitive elements in their introns.

A surprising observation was the localization of most of the immunoreactive fetal liver ABP to the insoluble fraction. As expected, immunoreactive ABP from testis and epididymis was in the soluble fraction. At this time we do not know the subcellular localization of the immunoreactive M, 43,000 and 93,000 proteins, however, they would appear to be membrane-bound or stored in insoluble granules. If the M, 93,000 protein represents the ABP-HDC fusion protein, it should be targeted to the rough endoplasmic reticulum (i.e. the cDNA encodes the ABP signal peptide). Furthermore, interaction of the ABP purified ABP and to Dr. Kay Lund, Dr. Mary Hynes, and Cookie G. Levan for the cytogenetic analysis of cell hybrids used for the pilot experiment. We would also like to acknowledge Dr. Frank French for the antiserum against the ABP signal peptide). Furthermore, interaction of the ABP bound or stored in insoluble granules. If the ABP could direct the protein to a subcellular compartment containing the ABP receptor (e.g. vesicles). Even though the protein is not secreted from COS cells the possibility exists that it is secreted from liver. Secreted or not secreted, the enzymatically active HDC domain of the fusion protein could be directed to a site, not accessible by normal HDC.

The results of this study strongly support the hypothesis that trans splicing of ABP and HDC RNA transcripts occurs in fetal rat liver. Whether this phenomenon has biological significance, creating a functional fusion protein, remains to be determined.

Acknowledgments—We thank David Fenstermacher and Vivian Fischer for excellent technical support. We thank the Laboratories for Reproductive Biology core technicians: Catherina Weaver (RIA), Rachael McNiel (Tissue Culture), and Pat Hicks (Histochemistry). We are indebted to Dr. Frank French for the antiserum against the ABP signal peptide). Furthermore, interaction of the ABP bound or stored in insoluble granules. If the ABP could direct the protein to a subcellular compartment containing the ABP receptor (e.g. vesicles). Even though the protein is not secreted from COS cells the possibility exists that it is secreted from liver. Secreted or not secreted, the enzymatically active HDC domain of the fusion protein could be directed to a site, not accessible by normal HDC.

The results of this study strongly support the hypothesis that trans splicing of ABP and HDC RNA transcripts occurs in fetal rat liver. Whether this phenomenon has biological significance, creating a functional fusion protein, remains to be determined.

REFERENCES

1. Wilson, J. D., and Griffin, J. E. (1989) in The Metabolic Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) pp. 1919-1944, McGraw-Hill, Inc., New York
2. Hamburger, V., and Hamilton, H. (1992) in Methods of Embryology (Greep, R. O., Astwood, E. B., Hamilton, D. W., and Geiger, S. M., eds) pp. 1919-1944, Raven Press, New York
Alternative Splicing of ABP RNA Transcripts

Harley, M. J., Musto, N. A., Cheng, C. Y., and Bardin, C. W. (1987) FEBS Lett. 215, 100-104

Koenig, R. J., Lazar, M. A., Hodin, R. A., Brent, G. A., Larsen, P. R., Chin, W. W., and Moore, D. D. (1989) Nature 337, 659-661

Feener, C. A., Koenig, M., and Kunkel, L. M. (1989) Nature 338, 2408

Kimura, H., Sogawa, K., Sakai, Y., and Fujii-Kuriyama, Y. (1989) Cell 53, 501-508

Schibler, U., Hagenbuehle, O., Wellaeu, P. K., and Pittet, A. C. (1983) Cell 33, 501-508

Koeber, C. A., Koenig, M., and Kunkel, L. M. (1989) Nature 338, 2408

Krause, M., and Hirsh, D. (1987) Cell 49, 753-761

Murphy, W. J., Watkins, K. P., and Agabian, N. (1986) Cell 47, 517-525

Sutton, R. E., and Boothroyd, J. C. (1986) Cell 47, 521-535

Choquet, Y., Goldschmidt-Clermont, M., Girard-Bascou, J., Knick, U., Bennoun, P., and Rocheaix, J.-D. (1988) Cell 52, 903-913

McKinnon, R. D., Danielson, P., Brow, M. A. D., Bloom, F. E., and Sutcliffe, J. G. (1987) Mol. Cell. Biol. 7, 2148-2154

Solnick, D. (1985) Cell 42, 157-164

Konarska, M. M., Padgett, R. A., and Sharp, P. A. (1985) Cell 42, 165-171

53. Gershagen, S., Lundwall, A., and Fernlund, P. (1989) Nucleic Acids Res. 17, 9245-9257

54. Danzo, B. J., and Bell, B. W. (1988) J. Biol. Chem. 263, 2402-2408

55. Kovacs, W. J., Bell, B. W., Turney, M. K., and Danzo, B. J. (1988) Endocrinology 122, 2639-2647

56. Evelth, D. D., Gierz, R. D., Spencer, C. A., Nargang, F. E., Hodgette, R. B., and Marsh, J. L. (1986) EMBO J. 5, 2663-2672

57. DeWet, J. R., Daniels, D. L., Schroeder, J. L., Williams, B. G., Hryb, D. J., Khan, M. S., Romas, N. A., and Rosner, W. (1990) J. Virol. 33, 401-410

58. Joseph, D. R., Adamson, M. C., and Kozak, C. A. (1990) Cytogenet. Cell Genet., in press

59. Szpirer, C., Szpirer, J., Islam, M. Q., and Levan, G. (1988) Curr. Topics Microbiol. Immunol. 137, 33-38

60. Weisz, J., and Ward, I. L. (1988) Endocrinology 106, 306-316

61. Schultz, F. M., and Wilson, J. D. (1974) Endocrinology 94, 979-986

62. Jossen, N. (1970) Arch. d'Anat. Microsc. 59, 37-50

63. Raisman, G., and Field, P. M. (1971) Science 173, 731-733

64. dePagger-Holthuizen, P., Jansen, M., van Schaik, F. M. A., van der Kammen, R., Oosterwik, C., van den Brande, J. L., and Sussenbach, J. S. (1987) FEBS Lett. 214, 259-264

65. Schibler, U., Hagenbuehle, O., Wellaeu, P. K., and Pittet, A. C. (1983) Cell 33, 501-508