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COMPLEX STRUCTURE AND REGULATION OF THE ABP/SHBG GENE

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Summary—Extracellular androgen-binding proteins (ABPs) are thought to modulate the regulatory functions of androgens and the *trans*-acting nuclear androgen receptor. Testicular ABP and plasma sex hormone-binding globulin (SHBG), which is produced in the liver, are encoded by the same gene. We report here that the ABP/SHBG gene is also expressed in fetal rat liver and adult brain. Immunoreactive ABP was localized in the brain and fetal liver and mRNAs were identified in both tissues by northern blot hybridization. Analysis of brain and fetal liver cDNA clones revealed alternatively processed RNAs with sequence characteristics suggesting the encoded proteins could act as competitors of ABP/SHBG binding to cell surface receptors. One cDNA represented a fused transcript of the ABP/SHBG gene and the histidine decarboxylase gene that was apparently formed by a *trans*-splicing process. Gene sequencing experiments indicate that tissue-specific ABP/SHBG gene promoter–enhancer elements are utilized in testis, brain and fetal liver. These data demonstrate that the structure, RNA transcript processing and likely regulation of the ABP/SHBG gene are very complex.

INTRODUCTION

The expression of androgen-binding protein (ABP) has been extensively used as a model to study Sertoli cell regulation and function [1–3 and cited references in 3]. To decipher the mechanisms of hormonal regulation in the Sertoli cell, we have cloned the cDNA encoding rat and mouse ABP [4–6] and characterized the rat gene [7]. Later the single ABP gene locus (*Shbg*) was localized to chromosome 10 of the rat [8] and chromosome 11 of the mouse [9]. Concurrent with our studies, the human sex hormone-binding globulin (SHBG) gene was characterized by Hammond *et al.* [10] and Gershagen *et al.* [11] and found to be the same gene that encodes ABP. Our gene studies [7] identified a putative testicular transcription start site and 5′-regulatory region of the rat gene. These data indicated that the entire region encoding testicular ABP (8 exons) was located on <3 kb of DNA. We now know that the gene is much more complex than initially thought. Studies of ABP/SHBG gene expression in fetal rat liver [8] and adult rat brain [12] have revealed an unexpected complexity of the gene.

EXPERIMENTAL PROCEDURES

Most of the methods used in these experiments have been described previously [3–9]. Other procedures are referenced in the text.

RESULTS AND DISCUSSION

In most species including man, the ABP/SHBG gene is expressed in liver throughout life (male and female), but in the rat no SHBG protein or mRNA is detectable in adult liver [4]. However, Carreau [13] and Gunsalus *et al.* [14] reported the presence of an immunoreactive ABP-like protein in the serum of developing male and female embryos, suggesting it was synthesized in the liver. We have followed up this study and demonstrated the transient expression of immunoreactive ABP and ABP mRNA in fetal liver [8]. Characterization of the fetal liver mRNAs by cDNA cloning revealed the presence of testicular-like ABP mRNA that encoded a protein identical to testicular ABP and two alternatively processed mRNAs that encoded unique proteins. One cDNA (Fig. 1, FLABP2) represented a mRNA with an alternate exon 1 sequence and lacked

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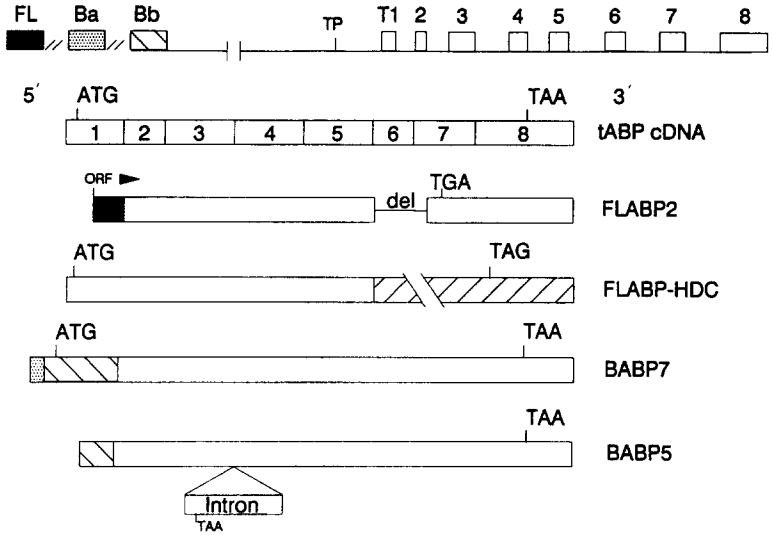

Fig. 1. Diagrams of the rat ABP/SHBG gene and cDNA clones from testis, brain and fetal liver. Top: diagram of rat ABP gene. T1–8 represent testicular exons 1–8. The TP region is the identified testicular promoter region [7]. Bb is a localized exon sequence found in the brain; brain exon Ba has not been located and is upstream at an unknown site. Likewise, the location of the fetal liver exon 1 sequence is unknown. The distance from the beginning of T1 to the end of T8 is 3.2 kb. Brain exon Bb is located 4 kb upstream from T1. Diagrams of cDNA clones. tABP cDNA represents full-length testicular ABP cDNA (1400 bp) with sequences representing exons indicated. The start and termination points of translation are indicated by Met (ATG) and termination (TAA) codons. Diagrams below with open bars are identical to testicular ABP cDNA. FLABP2 and FLABP–HDC represent alternatively processed fetal liver transcripts. Clone FLABP2 was identical to testicular ABP except it contained an alternate exon 1 and was missing an exon 6 sequence. It encodes a truncated protein terminating at TGA. ORF, open reading frame. The translation start site in FLABP2 has not been identified. Clone FLABP–HDC represented a fusion transcript of the AJBP gene and the HDC gene. The HDC domain (□) is 2.3 kb. The cDNA encodes a 98,000-M_r fusion protein. TAG, translation termination signal. Clones BABP7 and BABP5 represent brain transcripts with alternate 5′-sequences. Clone BABP5 also differed from testicular AJBP cDNA, containing the full sequence of intron 3. BAJBP5 cDNA encoded a protein slightly larger than testicular ABP with a unique N-terminal sequence, whereas, BABP5 cDNA encoded a truncated protein with translation terminating within the intron 3 sequence at TAA. The unique sequence at the 5-terminus of BABP5 cDNA was identical to the sequence in BABP7, but truncated.

testicular exon 6. This mRNA encodes a truncated protein with an N-terminal amino acid sequence unique from the testicular ABP sequence. It does not encode a region of ABP/SHBG that has been identified to be in the steroid-binding domain [15] and therefore the encoded protein would not be expected to bind androgens. However, the exact location of the steroid-binding domain has not been elucidated. Other investigators have implicated different regions of ABP/SHBG in steroid-binding [16, 17, 18]. Two other laboratories [10, 11] have identified in human testis a similar transcript that lacks exon 7. The encoded human protein would also not be expected to bind steroid.

The location of the alternate fetal liver exon 1 sequence is not known, but it originates from a promoter, distinct from the testicular promoter, that is >4 kb upstream from the testicular start site (Fig. 1; top diagram, FL). Similarly, an alternate testicular exon 1 has been located upstream in the human ABP/SHBG gene [10]. The sequence of this exon has no similarity to the fetal liver alternate exon sequence or the alternate rat brain sequence (see below).

The other characterized fetal liver cDNA represented a fusion mRNA of the ABP/SHBG gene and the histidine decarboxylase (HDC) gene. Clone FLABP–HDC (Fig. 1) contains the first five exon sequences of the ABP/SHBG gene and all of the HDC cDNA except the sequences representing exon 1. This cDNA encodes a protein (M_r 98,000) containing ABP and HDC domains, including the ABP signal peptide, the cell surface receptor-binding domain of ABP [19] and all but 14 N-terminal amino acid residues of histidine decarboxylase. The data indicate that this transcript was formed by a *trans*-splicing process; the ABP and HDC domains were joined at donor and acceptor RNA splice junctions and the ABP and HDC genes are located on rat chromosomes 10 and 3, respectively. 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; (2) the fetal liver hepatocyte is the primary location of immunoreactive ABP and HDC; (3) hybridization experiments identified a 4.4 kb mRNA that hybridized with ABP and HDC cDNA; (4) PCR experiments indicate that a fusion transcript analogous to FLABP–HDC cDNA is synthesized in fetal liver and (5) an immunoreactive protein consistent with the size ($M_r = 93{,}000$) of the protein encoded by the fusion DNA was identified in fetal liver by western blot analysis.

We have also found that the ABP/SHBG gene is expressed in rat brain [12]. Immunoreactive ABP was present in neuronal cell bodies throughout the male and female brain as well as fibers in and around the median eminence [12]. The highest concentration of immunoreactive cell bodies was found in the supraoptic and paraventricular nuclei. Likewise, a 1.7 kb mRNA was present in all brain regions examined, whereas a 2.3 kb species was only present in the hippocampus, cortex and striatum. Analysis of cDNA clones representing brain ABP mRNAs revealed amino acid sequence differences in brain and testicular ABPs. Two ABP-related cDNAs were isolated from a λ gt10 rat brain cDNA library and characterized. The structure of two brain ABP cDNA clones compared with testicular ABP cDNA is diagrammed in Fig. 1. The DNA insert in λ gtllBABP7 was identical to testicular ABP cDNA, except that exon 1 DNA was replaced by an alternate sequence. Since the first Met codon (ATG) follows an in-frame stop codon (TGA), it appears to be the site of translation initiation. The deduced amino acid sequence of the alternate brain cDNA is in the same reading frame as testicular ABP exons 2–8 and encodes a protein slightly larger than the testicular ABP subunit precursor (414 amino acid residues vs 403 residues). Analysis of the ABP gene revealed that a portion of brain exon 1 sequence is located 4 kb upstream of the putative testicular transcription start site (Fig. 1; top diagram, Bb), confirming the identity of the alternate sequence. The remaining 5′-cDNA sequences are located upstream at an unknown location (Fig. 1; top diagram, Ba). In the gene this sequence is flanked by consensus donor and acceptor splicing signals. Another brain cDNA clone (BABP5) contained a portion of the alternate exon 1 sequence of BABP7 (nucleotide residues 55–159) and also contained the full sequence of intron 3 (Fig. 1). This cDNA could represent either an incompletely processed RNA transcript or a translatable ABP mRNA. In support of the latter possibility, the size of this cDNA is consistent with the size of the major 2.3 kb mRNA species found in the hippocampus, striatum and cortex. This transcript encodes a truncated version of ABP (155 residues) and includes only the amino acid sequence encoded by testicular exons 2 and 3. The encoded protein does not contain the total putative steroid-binding domain of ABP/SHBG [15].

These studies of ABP/SHBG gene expression in testis, fetal liver and brain have revealed that the rat ABP/SHBG gene is extremely complex. Perhaps each exon 1 sequence represents transcription from a unique promoter, with each promoter controlled by tissue specific regulatory elements. The utilization of two ABP gene promoters (major and minor) in the testis was indicated by findings from primer extension studies [7]. Although it appears unlikely we can not rule out the possibility that transcripts from all 3 tissues originate from a common upstream promoter and the observed tissue-specific differences are due to alternative processing. Based on the complexity of the ABP/SHBG gene and RNA transcript processing, it would appear that regulation of the gene is also complex. Past studies demonstrating different regulatory properties of testicular ABP and liver SHBG and differential FSH regulation of Sertoli cell transcripts supports this idea [3].

The function of ABP in the testis and epididymis is not well understood, but it is thought that ABP aids in the concentration of androgens in the epididymis, where high levels are required for epididymal function. In light of the recent identification of a cell surface receptor for SHBG and likely ABP, the possible functions of ABP and SHBG are being re-evaluated [20]. Likewise, the function of ABP gene products in the developing rat and in the adult brain are not known, but based on our present knowledge we can speculate on possible functions of ABP.

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 [21]. 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. Also, high androgen levels are required the last week of gestation and immediately after birth for normal development of the male brain [22]. During this time of development (birth to 6 days of age) ABP gene expression initiates in the brain (our unpublished results). 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 [20]. 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 C-terminus; encoded by exons 7 and 8). In support of this idea, William Rosner *et al.* [19] have demonstrated that a peptide encoded by human ABP/SHBG exon 3 binds with high affinity to the human ABP/SHBG membrane receptor.

The properties of the proteins encoded by all of the characterized alternate ABP/SHBG mRNAs suggest they may have a common function. One property characteristic of all of the alternate proteins is the amino acid sequence encoded by exons 2 and 3, the identified receptor-binding domain [19]. If the encoded proteins retain the receptor-binding capacity 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 [21].

Our studies support the existence of a fetal liver ABP/HDC fusion protein that could have a specific function. If the 93,000 M_r 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 receptor-binding region with the protein trafficking system 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 [8] the possibility exists that it is secreted from liver. Whether or not this protein is secreted, the enzymatically active HDC domain of the fusion protein could be directed to a site that is not accessible by normal HDC.

The finding of ABP/SHBG gene expression in the brain suggests that like the testis, the brain may need an androgen carrier protein. Similarities in organization of the blood supply in brain and testis indicate a common need for ABP in both tissues. Because testis and brain contain blood–tissue barriers formed by tight cellular junctions, both tissues must synthesize functionally important proteins that are not available from blood (e.g. transferrin and ceruloplasmin). This similarity suggests that ABP may function in cerebrospinal fluid (CSF) in the same manner ABP functions in epididymal fluid. In addition, the presence of immunoreactive fibers in the median eminence raises the possibility that ABP is secreted into the portal blood, where it could have a direct effect on endocrine function in the pituitary. One specific function of ABP in the brain may be to regulate the metabolism of testosterone. For example, the high affinity-binding nature of ABP could act to modulate the aromatization of testosterone to estrogens. ABP may function in the brain as an androgen carrier protein, however, in view of the widespread presence of ABP and ABP mRNA in the brain, the protein may have a much broader yet unknown function.

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