The Human Sex Hormone-Binding Globulin Gene Contains Exons for Androgen-Binding Protein and Two Other Testicular Messenger RNAs

Geoffrey L. Hammond, D. Alan Underhill, Harriet M. Rykse, and Carolyn L. Smith

Departments of Obstetrics and Gynecology and Biochemistry
University of Western Ontario at Victoria Hospital
London, Ontario, N6A 4G5 Canada

When a sex hormone-binding globulin (SHBG) cDNA was used to screen a human testicular cDNA library, three distinct cDNAs were isolated, one of which corresponds to the human SHBG cDNA sequence and probably represents testicular androgen-binding protein. The other two SHBG-related cDNAs each contain unique 5′ regions that diverge from the SHBG cDNA sequence at the same position, and one of them (SHBGr-2) lacks a 208-base pair region within the SHBG cDNA. As a result, this cDNA could potentially encode for a truncated form of SHBG which lacks N-linked carbohydrates and part of the steroid-binding domain. Southern blots of human placental DNA and cloned genomic DNA fragments also indicate that SHBG and its related testicular cDNAs are the products of a single gene. Sequence analysis of the gene indicates that the complete coding region for the SHBG precursor is comprised of 8 exons, which are distributed over 3.2 kilobase (kb) of genomic DNA, and the unique 5′ regions associated with the two SHBG-related testicular cDNAs were identified 1.9 kb upstream from the initiating codon for SHBG. In addition, the deletion within SHBGr-2 is due to the removal of exon 7, and an interesting feature of the gene is that differentially used exons are preceded by *Alu* repetitive DNA sequences. Although the relative abundance of the various SHBG-related mRNAs in the testis has not been established, Northern blot analysis indicates that they are similar in size (1.6 kb) to that of hepatic SHBG mRNA. (Molecular Endocrinology 3: 1869–1876, 1989)

INTRODUCTION

Sex hormone-binding globulin (SHBG) and androgen-binding protein (ABP) are closely related, extracellular steroid transport proteins (1, 2) that are produced by hepatocytes (3) and Sertoli cells (4), respectively. They are both characterized as approximately 90-kDa glycoproteins (5), and direct analysis of human SHBG has revealed a single amino-terminal sequence (6, 7) for a polypeptide of 373 residues (8). Consequently, it is generally assumed that SHBG and ABP are homodimers of approximately 50-kDa subunits, and this has been confirmed immunochemically (6, 9).

Human SHBG and ABP may be distinguished by virtue of their affinities for Concanavalin-A (10), and the purified proteins exhibit minor variations in their peptide maps as well as in the sizes and relative abundance of their respective protomers (2, 11). However, given the degree of similarity that exists between the primary structures of human SHBG and rat ABP (12, 13), these differences in physicochemical properties may be attributed entirely to heterogeneity in carbohydrate composition. It is, therefore, possible that SHBG and ABP are encoded by a single gene, the products of which may be modified by cell-specific differences in transcription, mRNA processing, or posttranslational modification.

A genomic fragment that appears to contain the transcription unit for rat ABP mRNA has already been sequenced (14), but studies of the tissue-specific expression of the rat ABP gene have been limited because adult rat blood does not contain a SHBG-like protein, and Northern blots of adult rat liver RNA are negative when probed with a rat ABP cDNA (13). This is interesting because the hormonal regulation of SHBG and ABP synthesis appears to be quite different. For instance, the plasma concentrations of human SHBG are increased by estrogens and T_4, but are decreased by androgens (15), while rat testicular ABP synthesis is increased by FSH and testosterone (13). During experiments designed to establish the molecular characteristics of human SHBG and ABP and their genes, we unexpectedly isolated a family of three different testicular cDNAs that hybridize with a human SHBG cDNA and have obtained evidence that they represent distinct products of a single gene.

0888-8809/89/1869-1876$02.00/0
Molecular Endocrinology

RESULTS

Comparison of the SHBG-Related cDNAs Isolated from a Human Testicular Library

Three classes of cDNAs were isolated from an adult human testicular cDNA library, and the sequence of one of them (SHBGr-1) is identical to that of the human liver SHBG cDNA probe (16), but is 116 base pairs (bp) shorter at its 5′ end (Fig. 1A). Upon excision from the cloning vector with *Eco*RI, two other types of testicular cDNA (SHBGr-2 and SHBGr-3) could be distinguished by smaller 3′ *Eco*RI fragments of approximately 300 bp when compared with the 3′ *Eco*RI fragment of the SHBG cDNA (Fig. 1A). Sequence analysis revealed that SHBGr-2 is not polyadenylated, and when compared with the SHBG cDNA it differs in two important respects: it lacks a 208-bp region from within the 3′ *Eco*RI fragment, and their sequences diverge from each other 48 nucleotides from the 5′ end of the SHBG cDNA (Fig. 1A). As a result, SHBGr-2 has a unique 5′ region of 96 bp. The sequence of the 5′ *Eco*RI fragment of SHBGr-3 also diverges from the SHBG cDNA in the same position and contains a distinct 5′ sequence of 57 bp (Fig. 1A). The sequence of its 3′ *Eco*RI fragment contains an inversion which appears to have been generated by a loop formation at a palandromic sequence (5′-ATCTTGGCTCAGTCTCCACCTCCAA-GAT-3′) present in all of the SHBG-related cDNAs. Unfortunately, this cDNA was only represented by a single clone.

Analyses of SHBGr-1 and SHBGr-2 indicated that their major reading frames correspond to that associated with SHBG (Fig. 1B). However, due to the out of frame deletion within the 3′ region of SHBGr-2, this reading frame terminates prematurely compared to the coding sequence for SHBG (Fig 1B). As a result, the carboxy-terminal region of SHBGr-2 lacks two of the four cysteine residues in the SHBG molecule as well as the only two consensus sites for N-glycosylation (Fig. 1B). In addition, although the 5′ end of this cDNA does not contain an initiation codon within this reading frame, it encodes for 32 amino acids that are predominantly hydrophobic and which may, therefore, represent a signal peptide (Fig. 1B).

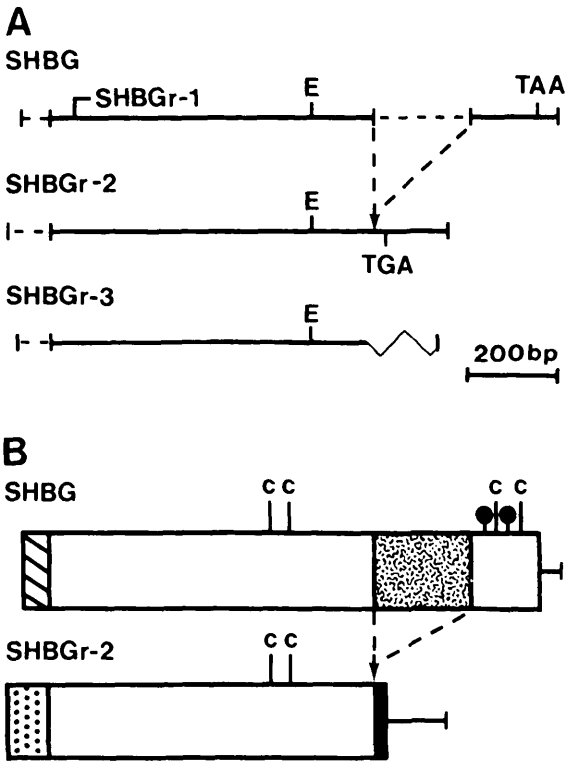

Fig. 1. Schematic Representation of the cDNAs (A) and Predicted Reading Frames (B) for SHBG and the SHBG-Related Gene Products in the Human Testis

A, Common sequences in the SHBG, SHBGr-1, -2, and -3 cDNAs are shown as *solid lines*. The 3′ region deleted in SHBGr-2 is indicated (*dashed lines*), and 3′ sequence inversion in SHBGr-3 is shown as a *jagged line*. The location of the common *Eco*RI restriction site is also indicated (E), as are the stop codons used in major reading frames. B, Major reading frames (*boxed*) for SHBG and SHBGr-2 are shown. The *hatched and stippled boxes* represent the different amino-terminal sequences of SHBG and SHBGr-2, respectively. The out of frame deletion that results in the truncation of SHBGr-2 is *shaded*, and its predicted carboxy-terminus is represented by a *solid box*. The location of cysteines (c) and the two N-linked oligosaccharides (*circles*) are shown.

Southern Analyses of the Human SHBG Gene

When analyzed by restriction endonuclease digestion and Southern blotting, eight different human genomic clones from a pCVI08 cosmid library (17) contained 3.1-kilobase (kb) and 0.8-kb *Eco*RI fragments that hybridized with the 5′ *Eco*RI fragment of the SHBG cDNA. Moreover, *Eco*RI and several other enzymes produced very similar hybridization patterns when human placental DNA and one of these genomic clones (hgSHBG-6) were both analyzed in this way (Fig. 2, A and B). They varied, however, with respect to the size of a single *Eco*RI fragment that hybridized with the SHBG cDNA 3′ *Eco*RI probe (not shown). Since an *Eco*RI site is located close to the 3′ cloning site (*Bam*HI) of the cosmid vector (17), the clone (hgSHBG-6) containing the largest hybridizing *Eco*RI fragment (~4.0 kb) was, therefore, selected for DNA sequencing. Restriction digests of hgSHBG-6 were also analyzed using the SHBGr-2 cDNA 5′ *Eco*RI fragment, and this revealed an additional *Pst*I fragment (~500 bp).

One other genomic clone produced a different restriction pattern when analyzed with the human SHBG cDNA probes. However, subsequent comparisons of the hybridizing fragments with the SHBG gene sequence (see below) indicated that this clone contained only a portion of the 5′ region of the gene, but extended in the 3′ direction to include at least the approximately 13-kb *Eco*RI fragment observed on the Southern blot of human genomic DNA (Fig. 2C).

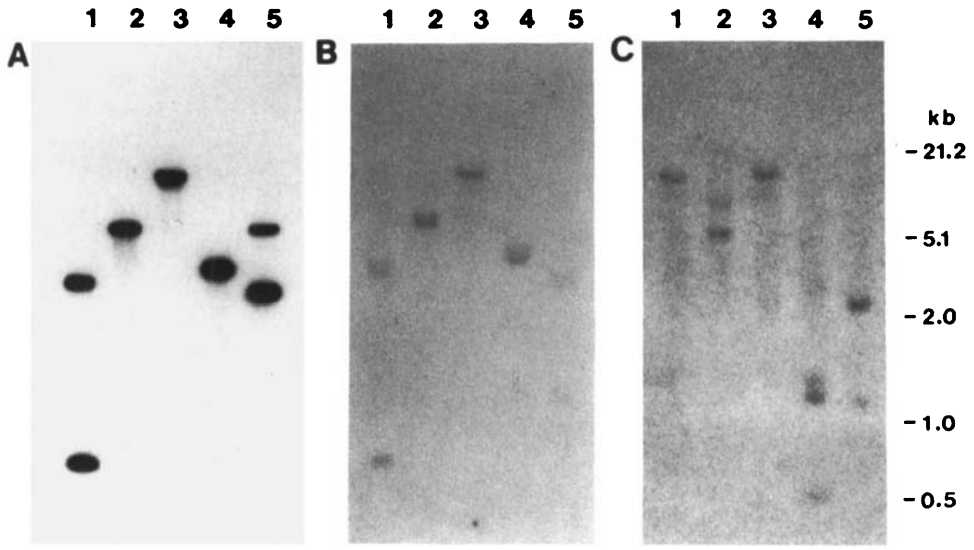

Fig. 2. Southern Analyses of the Human SHBG Gene

Samples of hgSHBG-6 cosmid DNA (A) and placental DNA (20 μg; B and C) were digested with restriction endonucleases, fractionated by electrophoresis on 0.6% agarose gels, and transferred to a nylon membrane and nitrocellulose, respectively. Immobilized DNA restriction fragments were hybridized with either ^{32}P-labeled, SHBG 3′ *Eco*RI cDNA (C) or SHBG 5′ *EcoRI* cDNA (A and B). The resulting signals were visualized by autoradiography for 7 days (B and C) or 60 min (A) at −80 C. Lane 1, *EcoRI*; lane 2, *BamHI*; lane 3, *Hind*III; lane 4, *Pst*I; lane 5, *Pvu*II. The positions of DNA molecular size standards in kilobases are shown on the *right*.

Sequence Analyses of the Human SHBG Gene

We have sequenced 6.1 kb of human genomic DNA located 2.1 kb from the 3′ end of an approximately 40-kb fragment (Fig. 3). This includes eight exons which span 3.2 kb of genomic DNA and comprise the entire coding region for human SHBG. The sequence of the first of these exons has also revealed a potential initiation codon (ATG) for the SHBG precursor polypeptide. The deduced signal peptide is predominantly hydrophobic and contains an unusual repeat of 10 leucines (Fig. 3). The DNA sequence immediately 5′ of this region was examined for possible regulatory elements, and a sequence (5′-GCCCCTGTTTCCT-3′) that is related to a liver-specific enhancer (5′-GCCCCTGTTT-GCT-3′) in the human α_1-proteinase inhibitor gene promoter (18) was identified 115 bp from the proposed initiation codon for the SHBG precursor.

Within the human SHBG gene sequence we have also identified the unique 5′ regions that correspond to the testicular cDNAs SHBGr-2 and SHBGr-3 (Fig. 3), and we have determined that the deletion of 208 bp in the SHBGr-2 cDNA is due to the omission of exon 7. Another interesting feature of the human SHBG gene sequence is that introns preceding exons that are differentially used in the testis contain either single or multiple *Alu* sequences (Fig. 3). A partial restriction map summarizing these structural features of the SHBG gene is illustrated in Fig. 4.

Comparison of SHBG-Related mRNAs in the Human Liver and Testis

A Northern blot of human liver, testicular, and ovarian poly(A)$^+$ RNA was hybridized at high stringency with human SHBG cDNA probes to compare the relative abundance and sizes of SHBG-related mRNAs. The resulting autoradiogram (Fig. 5) demonstrates the presence of hybridizing mRNA species of 1.6 kb in only the liver and testicular extracts. Based on the relative intensities of the signals obtained, the liver appears to contain slightly (~2-fold by densitometry) more SHBG-related mRNA than does the testis. In addition, the relative intensities of these signals did not change during the process of increasing the washing stringency from 0.2 × SSC at 42 C to 0.1 × SSC at 46 C. The human liver RNA also contains an approximately 2.5-kb mRNA that hybridized with the SHBG cDNAs, and this signal persisted even when the blot was washed to high levels of stringency. The identity of this mRNA is unknown, but it might represent incompletely processed SHBG mRNA.

DISCUSSION

The identification of three distinct, SHBG-related cDNAs within an adult human testicular library was unexpected, but the fact that two of them diverged from the human SHBG cDNA sequence in the same position suggested that they were not cloning artifacts. Furthermore, although it is important to note that the two novel SHBG-related cDNAs we have identified have not previously been detected in either human adult (16, 19) or fetal (20) liver libraries, an ABP cDNA with an unusual 5′-terminus has been isolated from an imma-

Fig. 3. Nucleotide Sequence of the Human SHBG Gene

An approximately 6100-bp portion of human genomic DNA spanning the human SHBG gene was sequenced and is numbered in the 5′ to 3′ direction along the *righthand margin*. Exons were identified by comparisons to an SHBG cDNA (16), and consensus splice sites are *overlined*. The corresponding amino acid sequence (single letter code) is shown in *boldface*. The 5′ exons for SHBG (comprising its deduced signal peptide), SHBGr-2 (with deduced amino acids), and SHBGr-3 (*boxed*) are indicated, and remaining exons are number (no. 2–8). The SHBG mRNA polyadenylation site is indicated by a *square bracket* at the end of exon 8. The four *Alu* sequences within the SHBG/ABP gene are *underlined*, and a *dashed underline* denotes the location of a putative liver-specific enhancer element (18).

ture rat testicular library, and its 5′ sequence of 31 bp may be part of a separate exon (14). This sequence does not, however, resemble either of the unique 5′ regions of the human testicular cDNAs and cannot be identified within the human SHBG gene sequence we have obtained so far. Nevertheless, it is possible that these variations in SHBG- or ABP-related cDNAs represent testis-specific mRNA species that are produced in different amounts in immature and adult testes. It also raises the question of whether they are derived from separate genes or are formed by alternative splicing of exons within a single gene; to clarify this it was necessary to isolate and characterize genomic fragments that encode for SHBG and its related testicular mRNA species.

In our initial experiments we screened several bacteriophage λ-human genomic libraries without success because primary signals could not be rescued by further cloning, and this suggested that sequences within the human SHBG gene may limit the propagation of recombinant phage or their bacterial host. Eventually, nine clones were obtained from a human cosmid genomic library (17), and all grew considerably slower than colonies containing other human genomic fragments. Restriction map analyses demonstrated that these clones all contained different genomic inserts that span an approximately 90-kb region of human DNA. Furthermore, comparisons of human placental DNA and one of these clones by Southern analyses revealed restriction fragments of similar size when hybridized with the human SHBG cDNA probes (16). Taken together, these observations indicate that a single gene is not only responsible for the production of SHBG by the liver, but also contains coding sequences for three SHBG-related mRNAs in human testis.

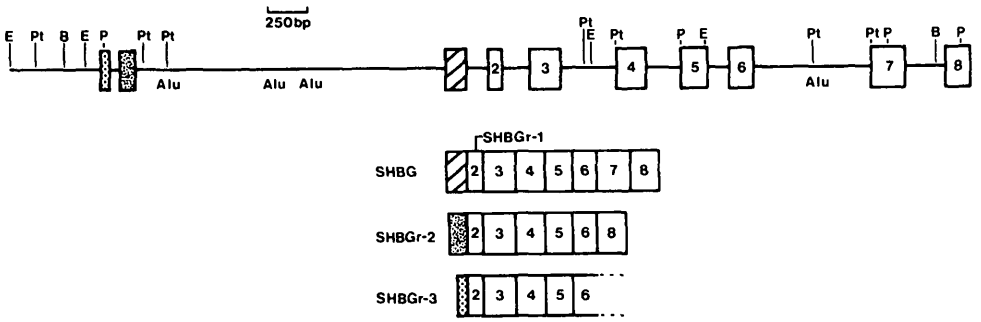

Fig. 4. Partial Restriction Map and Organization of the Human SHBG Gene, and Exon Composition of SHBG and Its Related (SHBGr) Testicular Transcripts

Exons are represented by *boxes*. The SHBGr-1 cDNA is identical to the SHBG cDNA (16), and its 5′-end is located 69 bp from the 5′ splice site of exon 2. Those containing the unique 5′ regions of the SHBG (*hatched*), SHBGr-2 (*shaded*), and SHBGr-3 (*stippled*) cDNAs are indicated with reference to their corresponding transcripts. The positions of *Alu* sequences are shown *below* the gene map. The sequence inversion in SHBGr-3 is indicated by *dashed lines*. Restriction endonuclease sites are shown for *Eco*RI (E), *Bam*HI (B), *Pvu*II (P), and *Pst*I (Pt).

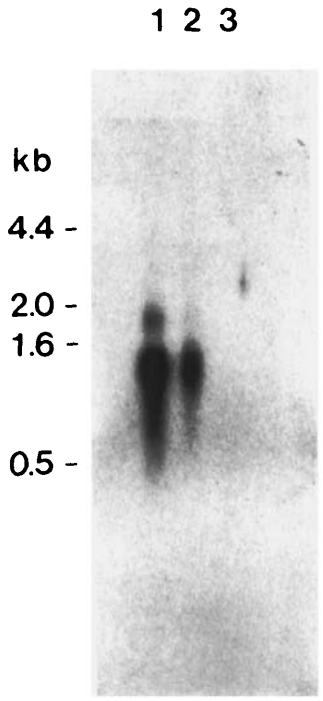

Fig. 5. Northern Blot of SHBG-Related mRNAs in Human Liver and Testis

Poly(A)$^+$ RNA (10 μg) from human liver (lane 1), testis (lane 2), and ovary (lane 3) was subjected to formaldehyde gel electrophoresis (36) and transferred to a nylon membrane by capillary blotting (37). The blot was hybridized with ^{32}P-labeled, human SHBG cDNA probes. The position of denatured *Hin*dIII-digested λDNA molecular size markers in kilobases are shown on the *left*.

Sequence analysis of hgSHBG-6 indicated that the coding sequence for SHBG is distributed over eight exons, spanning 3.2 kb of genomic DNA. When compared with the rat ABP gene (14), the positions of intron/exon junctions are perfectly conserved, but there are notable differences in the sizes and sequences of introns, especially those between exons 5, 6, 7, and 8. The sequence of the human SHBG gene also revealed a potential initiation codon for the SHBG precursor in an optimal context for translation (21), and the deduced signal peptide for human SHBG exhibits extensive sequence identity with that for rat ABP (12). Furthermore, it is preceeded by a similar 5′ noncoding region to that observed in the rat ABP cDNA (12) and gene (14). We also extended our sequence of the 5′ region of the SHBG gene and in doing so located exons for the unique 5′ regions of the SHBG-related testicular cDNAs. Although the 3′ ends of these exons are adjacent to consensus splice sites (22), our cDNA sequences do not allow us to define their 5′ boundaries. There are, however, several reasons to believe that they are not the first exons in the transcription unit(s) for their corresponding mRNAs. First of all, the different cDNA sequences indicate that all three 5′ exons are alternatively used and are, therefore, separate entities. Secondly, the first two exons must be separated by only a small intervening sequence (<55 bp), and if the second one comprises part of an open reading frame, there is no initiation codon within at least 600 bp extending 5′ from its 3′ boundary.

There is evidence for an additional exon associated with a rat ABP cDNA as well as other transcriptional start sites in the rat ABP gene (14). Moreover, there is a general lack of common eukaryotic promoter elements within the proposed 5′ flanking region of the rat ABP gene (14). It is, therefore, interesting that the sequences of the human SHBG and rat ABP genes show considerable similarity in this region. Furthermore, at the point at which they diverge, the SHBG gene contains a sequence that closely resembles an element necessary for liver-specific expression of the human α_1-proteinase inhibitor gene (18). Its absence in the rat ABP gene (14) may, therefore, contribute to a lack of ABP gene expression in the adult rat liver.

Another interesting feature of the human SHBG gene is the presence of *Alu* sequences in the introns preceding differentially used exons. In this context, it is per-

haps pertinent to note that a rat equivalent of the human *Alu* sequence is also present in the intron preceding exon 6 in the rat ABP gene (14), and that this exon has recently been reported to be differentially used in the fetal rat liver (23). Repetitive *Alu*-like DNA sequences have been implicated as a mediator of chromosomal recombination events leading to gene rearrangements (24), and our observations suggest that they may also influence the differential utilization of exons within the human SHBG and rat ABP genes.

Apart from ABP, the products of differential expression of the human SHBG gene in the testis are not known, but it would appear from Northern blot analyses that testicular mRNAs that hybridize with the SHBG cDNA are essentially the same size as the major mRNA species for SHBG in the liver. In addition, the major reading frame within all three testicular cDNAs corresponds to that for SHBG. Although the one that resembles SHBG does not contain the first exon comprising its amino-terminus and signal peptide, this probably represents ABP. On the other hand, the largest of the testicular cDNAs contains a reading frame for a SHBG-related polypeptide with a unique amino-terminal region, which is predominantly hydrophobic and could, therefore, represent a leader sequence. However, when compared with the SHBG sequence (8, 16) this reading frame terminates prematurely because of a deletion of exon 7, and this replaces 118 amino acids from the carboxy-terminus of SHBG with nine different residues. This deleted region contains consensus sites for the only two N-linked oligosaccharide chains associated with SHBG (8, 16, 25) as well as a domain that probably contains at least a portion of the steroid-binding site (16, 26). This may explain why SHBG-related polypeptides have not been identified during the course of ABP isolation from human testicular extracts by steroid affinity chromatography. The third type of SHBG-related cDNA (SHBGr-3) also contains a unique 5′ sequence, but this contains a stop codon that is inframe with the SHBG sequence. Therefore, if this reading frame is used one would predict that the initiating AUG would correspond to the Met-30 in the SHBG sequence (8, 16), and that the resulting polypeptide would lack a leader sequence. However, the 3′ sequence inversion in the cDNA precludes any further comment on the possible nature of its product in the testis.

The fact that the two novel SHBG-related cDNAs we have identified in the adult human testicular library have not previously been identified in immature rat testicular libraries (12, 13) is intriguing. The human testicular library was constructed from mRNA isolated from a healthy 50-yr-old who had not received hormonal treatment, and it is, conceivable that these different SHBG gene products may be produced in different amounts at different stages of testicular development. It will, therefore, be of interest to examine the differential expression of the SHBG gene in the human testis and to determine the biological significance of its various products with respect to developmental changes in testicular maturation and function.

MATERIALS AND METHODS

Materials

Restriction endonucleases, T_4 DNA ligase, RNase-A, and oligo(dT)-cellulose were obtained from Pharmacia (Piscataway, NJ). Sequencing reagents, including Sequenase (T7 DNA polymerase), were purchased from U.S. Biochemical Corp. (Cleveland, OH). Cloning vectors were pBluescript (Stratagene Cloning Systems, La Jolla, CA) and Sequenest transposon deletion vectors from Gold Biotechnology, Inc. (St. Louis, MO). Radiolabeled nucleotides ([^{32}P]dCTP and [^{35}S]dATP) were purchased from New England Nuclear (Boston, MA). The human SHBG cDNA used in these studies was amplified as 5′ (0.64 kb) and 3′ (0.55 kb) *Eco*RI fragments in pBR322, as described previously (16). Both fragments were isolated from 6% polyacrylamide gels by electroelution and labeled with [^{32}P]dCTP using the random primer method (27) to yield specific activities of approximately 10^9 cpm/μg DNA. The hybridization solution used for Northern and Southern blotting experiments contained 5 × Denhardt's solution (0.1% Ficoll 400, 0.1% polyvinylpyrrolidone, and 0.1% BSA), 5 × SSPE (0.75 M NaCl, 50 mM NaH_2PO_4, and 5 mM EDTA), 0.1% sodium dodecyl sulfate (SDS), and 100 μg/ml denatured salmon sperm DNA. The SSC (0.15 M NaCl and 0.015 M sodium citrate) wash buffer contained 0.l% SDS.

Complementary DNA and Genomic DNA Cloning

An adult human testis λgtll library (Clontech Laboratories, Inc., Palo Alto, CA) was screened with an equal mixture of ^{32}P-labeled SHBG 5′ and 3′ *Eco*RI cDNA fragments using standard hybridization conditions (28). Positive plaques were rescreened independently with both probes to ensure that both fragments were represented. Phage DNA from liquid lysates (29) was digested with *Eco*RI and ligated into the *Eco*RI site of pBluescript vectors without further purification. Recombinant plasmids were isolated using the alkaline lysis method (28), digested with *Eco*RI, and examined by 0.8% agarose gel electrophoresis.

A cosmid (pCV108) human genomic DNA library was kindly provided by Dr. Y.-F. Lau (University of California, San Francisco, CA). Approximately 0.5 × 10^6 colonies from the library were grown initially for 6 h at 37 C on 150-mm Lauria broth (LB)/ampicillin agar plates and then transferred on a nitrocellulose filter to LB/chloramphenicol plates to amplify the cosmid copy number (18 h at 37 C). The filters were washed and hybridized with the two ^{32}P-labeled SHBG *Eco*RI cDNA fragments. The nine colonies that hybridized with the probe upon secondary screening grew poorly and required incubation of up to 48 h at 37 C to reappear on the master plate. Positive colonies were propagated in LB/ampicillin medium (18 h at 37 C), and cosmid DNA was isolated using the alkaline lysis method (28).

Southern Hybridization Analysis

Cosmid DNA and human genomic DNA from placenta (30) were digested for 1–16 h at 37 C with restriction endonucleases, and the products were resolved on a 0.6% agarose gel and transferred to either a Zeta-Probe nylon membrane (Bio-Rad Laboratories, Richmond, CA) or nitrocellulose by capillary blotting (31). Duplicate Southern blots were baked (2 h at 80 C), prehybridized in the presence of 50% formamide at 42 C, and hybridized with either ^{32}P-labeled SHBG 5′ or 3′ *Eco*RI cDNAs, under the same conditions. They were then washed with 0.1 × SSC at 42 C and autoradiographed at −80 C against a Cronex HI-PLUS intensifying screen (DuPont, Wilmington, DE).

DNA Sequence Analysis

Appropriate restriction fragments of human testicular cDNAs and genomic DNA were subcloned into pBluescript SK(+) and KS(+) for the production of sequencing templates. Restriction fragments of genomic DNA were also subcloned into Sequenest transposon deletion vectors for the production of overlapping templates (32). In both cases single stranded DNA was sequenced using the dideoxy chain termination method (33).

Northern Hybridization Analysis

Total RNA was extracted from samples of adult human liver, testis, and ovary using lithium chloride-urea (34), and poly(A)$^+$ RNA was isolated (35), subjected to 1% agarose gel electrophoresis in the presence of formaldehyde (36), and transferred to a nylon membrane (Zeta-Probe) by capillary blotting (37). The membrane was baked (2 h at 80 C), prehybridized in the presence of 50% formamide at 42 C, and hybridized with an equal mixture of ^{32}P-labeled SHBG cDNA fragments, under the same conditions. After washing with 0.1 × SSC at 46 C, the Northern blot was exposed for 3 days at −80 C, as described for the Southern blot.

Acknowledgments

We thank Gail Howard for secretarial assistance.

Received August 14, 1989. Revision received August 25, 1989. Accepted August 25, 1989.

Address requests for reprints to: Geoffrey L. Hammond, Ph.D., Department of Obstetrics and Gynecology, University of Western Ontario, 375 South Street, London, Ontario, N6A 4G5 Canada.

This work was supported by a grant from MRC Canada; an OCTRF Research Scholarship (to G.L.H.), a grant from the Arcangelo Rea Family Foundation (to D.A.U.), and an OGS Studentship (to C.L.S.).

REFERENCES

1. Cheng CY, Frick J, Gunsalus GL, Musto NA, Bardin CW 1984 Human testicular androgen-binding protein shares immunodeterminants with serum testosterone- estradiol-binding globulin. Endocrinology 114:1395–1401
2. Cheng CY, Musto NA, Gunsalus GL, Frick J, Bardin CW 1985 There are two forms of androgen binding protein in human testes. J Biol Chem 260:5631–5640
3. Khan MS, Knowles BB, Aden DP, Rosner W 1981 Secretion of testosterone-estradiol-binding globulin by a human hepatoma-derived cell line. J Clin Endocrinol Metab 53:448–449
4. Feldman M, Lea OA, Petrusz P, Tres LL, Kierszenbaum AL, French FS 1981 Androgen-binding protein. Purification from rat epididymis, characterization and immunocytochemical localization. J Biol Chem 256:5170–5175
5. Westphal U 1986 Steroid-protein interactions II. In: Gross F, Grumbach MM, Labhart A, Lipsett MB, Mann T, Samuels LT, Zander J (eds) Monographs on Endocrinology. Springer-Verlag, Berlin and Heidelberg, vol 27:198–299
6. Hammond GL, Robinson PA, Sugino H, Ward DN, Finne J 1986 Physicochemical characteristics of human sex hormone binding globulin: evidence for two identical subunits. J Steroid Biochem 24:815–824
7. Gershagen S, Henningsson K, Fernlund P 1987 Subunits of human sex hormone binding globulin. J Biol Chem 262:8430–8437
8. Walsh HA, Titani K, Takio K, Kumar S, Hayes R, Petra PH 1986 Amino acid sequence of the sex steroid binding protein of human plasma. Biochemistry 25:7584–7590
9. Danzo BJ, Bell BW, Black JH 1989 Human testosterone-binding globulin is a dimer composed of two identical protomers that are differentially glycosylated. Endocrinology 124:2809–2817
10. Hsu A-F, Troen P 1978 An androgen binding protein in the testicular cytosol of human testis. Comparison with human plasma testosterone-estradiol binding globulin. J Clin Invest 16:1611–1619
11. Cheng CY, Musto NA, Gunsalus GL, Bardin CW 1983 Demonstration of heavy and light protomers of human testosterone-estradiol-binding globulin. J Steroid Biochem 19:1379–1389
12. Joseph DR, Hall SH, French FS 1987 Rat androgen-binding protein: Evidence for identical subunits and amino acid sequence homology with human sex hormone-binding globulin. Proc Natl Acad Sci USA 84:339–343
13. Reventos J, Hammond GL, Crozat A, Brooks DE, Gunsalus GL, Bardin CW, Musto NA 1988 Hormonal regulation of rat androgen-binding protein (ABP) messenger ribonucleic acid and homology of human testosterone-estradiol-binding globulin and ABP complementary deoxyribonucleic acids. Mol Endocrinol 2:125–132
14. Joseph DR, Hall SH, Conti M, French FS 1988 The gene structure of rat androgen-binding protein: identification of potential regulatory deoxyribonucleic acid elements of a follicle-stimulating hormone-regulated protein. Mol Endocrinol 2:3–13
15. Anderson DC 1974 Sex-hormone-binding globulin. Clin Endocrinol (Oxf) 3:69–96
16. Hammond GL, Underhill DA, Smith CL, Goping IS, Harley MJ, Musto NA, Cheng CY, Bardin CW 1987 The cDNA-deduced primary structure of human sex hormone-binding globulin and location of its steroid-binding domain. FEBS Lett 215:100–104
17. Lau Y-F, Kan YW 1983 Versatile cosmid vectors for the isolation, expression, and rescue of gene sequences: studies with the human γ-globulin gene cluster. Proc Natl Acad Sci USA 80:5225–5229
18. DeSimone V, Ciliberto G, Hardon E, Paonessa G, Palla F, Lundberg L, Cortese R 1987 *Cis*- and *trans*-acting elements responsible for the cell-specific expression of the human α_1-antitrypsin gene. EMBO J 6:2759–2766
19. Gershagen S, Fernlund P, Lundwall A 1987 A cDNA coding for human sex hormone binding globulin. FEBS Lett 220:129–135
20. Que BG, Petra PH 1987 Characterization of a cDNA coding for sex steroid-binding protein of human plasma. FEBS Lett 219:405–409
21. Kozak M 1986 Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell 44:283–292
22. Breathnach R, Chambon P 1981 Organization and expression of eukaryotic split genes coding for proteins. Annu Rev Biochem 50:349–383
23. Joseph DR, Sullivan PM, Fenstermacher DA, Behrendsen ME, Zahnow CA, Alternative processed androgen-binding protein mRNAs; identification of a fusion transcript with DOPA decarboxylase-like mRNA. 71st Annual Meeting of The Endocrine Society, Seattle WA, 1989, p 71 (Abstract)
24. Lehrman MA, Goldstein JL, Russell DW, Brown MS 1987 Duplication of seven exons in LDL receptor gene caused by *Alu-Alu* recombination in a subject with familial hypercholesterolemia. Cell 48:827–835
25. Avvakumov GV, Matveensteva IV, Akhrem LV, Strel'chyonok OA, Akhrem AA 1983 Study of the carbohydrate moiety of human serum sex hormone-binding globulin. Biochim Biophys Acta 760:104–110
26. Petra PH, Que BG, Namkung PC, Ross JBA, Charbonneau H, Walsh HA, Griffin PR, Shabanowitz J, Hunt DF

1988 Affinity labeling, molecular cloning and comparative amino acid sequence of sex steroid-binding protein of plasma. Ann NY Acad Sci 538:10–24
27. Feinberg AP, Vogelstein B 1983 A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13
28. Maniatis T, Fritsch EF, Sambrook J 1982 Molecular Cloning–A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 200–389
29. Kaslow DC 1986 A rapid biochemical method for purifying lambda DNA from phage lysates. Nucleic Acids Res 14:6767
30. Davis LG, Dibner MD, Battey JF 1986 Basic Methods in Molecular Biology. Elsevier, New York, pp 42–43
31. Southern EM 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517
32. Peng Z-G, Wu R 1986 A simple and rapid nucleotide sequencing strategy and its application in analyzing a rice histone 3 gene. Gene 45:247–252
33. Sanger F., Nicklen S, Coulson AR 1977 DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467
34. Auffray C, Rougeon F 1980 Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. Eur J Biochem 107:303–314
35. Aviv H, Leder P 1972 Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA 69:1408–1412
36. Rave N, Crkvenjakov R, Boedtker H 1979 Identification of procollagen mRNAs transferred to diazolenzloxymethyl paper from formaldehyde agarose gels. Nucleic Acids Res 6:3559–3567
37. Thomas PS 1980 Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77:5201–5205