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Human Interstitial Retinoid-binding Protein

GENE STRUCTURE AND PRIMARY SEQUENCE*

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Interstitial retinoid-binding protein (IRBP) is synthesized and secreted by rod photoreceptor cells into the interphotoreceptor matrix and is known to bind retinoids and fatty acids. We have used cDNA clones encoding human IRBP to isolate a 15-kilobase genomic fragment that encompasses the complete human IRBP gene. The IRBP gene spans more than 11 kilobases and is interrupted by three introns, all of which are positioned near the 3′-end of the coding sequence. The 3741-base pair coding region of IRBP appears to have been generated by quadruplication of an approximately 900 base pair long ancestral gene. The deduced amino acid sequence predicts a mature protein of 1,230 residues (calculated molecular weight 133,000). The protein sequence can be aligned into four homologous segments, each consisting of about 300 residues. Sequence similarity between segments is as high as 60% when conservative substitutions are taken into account. Two putative *N*-linked glycosylation sites are located in highly conserved domains in the center of the first and second segment of IRBP. A domain consisting of 41 residues at the COOH-terminal end of the third segment has 15 matching residues (38%) with an intradiscal loop of rhodopsin, a retinal-binding protein in rod photoreceptors.

Vitamin A (retinol) is an essential component of the visual cycle in the vertebrate retina. The 11-cis conformer of vitamin A aldehyde (retinal) serves as a chromophor of the visual pigment rhodopsin in photoreceptor cells (1). After photoreception and bleaching, 11-cis retinal is regenerated extracellularly in the pigment epithelium (2, 3). Regenerated retinal must be returned from the pigment epithelium to the photoreceptors. Interstitial or interphotoreceptor retinoid-binding protein (IRBP)[1] has been suggested to serve as a vehicle for transfer of retinoids (4–6). Bovine IRBP was shown to contain bound retinol, whose amount increased when eyes were illuminated prior to isolation of IRBP. Binding studies have shown that each mole of IRBP can bind 2 mol of all-trans retinol (7) with an association constant of 10^6 M^{-1}. In addition to all-trans retinol, bovine IRBP binds retinal, retinoic acid, cholesterol, fatty acids, and tocopherol indicating multiple functions for this complex protein. Recent studies (8) suggest that IRBP may function as a "buffer" protein for retinoids and may not participate actively in their transport.

IRBP is a large, soluble, single-subunit glycolipoprotein (9–11). In humans, the molecular weight of mature, glycosylated IRBP has been determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to be approximately 135,000 (12). In bovine, the molecular weight was shown to be 145,000 (7). The shape of the molecule appears to be elongated, as judged by the relation of its Stokes' radius to its molecular mass, its sedimentation properties (13), and by its appearance in electron-microscopical specimens (14).

Our goal is to understand the regulation of IRBP gene expression in mammalian photoreceptors, the structure and function of IRBP, and its possible involvement in retinal disorders. Evidence has been presented that rod photoreceptor cells synthesize and secrete IRBP into the interphotoreceptor matrix (15–17). IRBP is also expressed in cells that are not differentiated photoreceptors, such as mammalian pinealocytes (18), and transformed cells of human retinoblastoma (19, 20). Partial cDNA sequences for bovine and human IRBP have been described (21–23). Human IRBP cDNA sequences were used to map the gene to chromosome 10p11.2 → q11.2 (23), and to identify two-allele *Bgl*II (24), and *Sty*I (25) restriction fragment length polymorphisms. The *Bgl*II restriction fragment length polymorphism has established linkage between the multiple endocrine neoplasia, type 2a locus and the IRBP gene (26).

In this paper, we used human cDNA probes to isolate a 15-kb genomic clone HGL-3 that contains the complete human IRBP gene. The gene structure including exon/intron arrangement, transcription start site, and complete exon sequences are described. In addition, unusual features of the predicted IRBP peptide are discussed.

EXPERIMENTAL PROCEDURES

RNA Isolation and Analysis—Human eyes (Lion's Eyes of Texas Eye Bank, Houston, TX) were dissected within 8 h post-mortem, the retinas and pigment epithelium-choroid were separately removed, frozen in liquid nitrogen, and stored in RNase-free microcentrifuge tubes. Total retina RNA was isolated by the extraction procedure of Chirgwin *et al.* (27). Polyadenylated RNA was selected by oligo(dT)-cellulose chromatography (28).

Isolation of cDNAs and Gene Fragments Encoding Human IRBP—Human IRBP cDNA clones H4 (American Type Culture Collection No. 59198) and H12 (Fig. 1) were isolated as described earlier (22) from a human retina cDNA library in λgt10 (generous gift of J.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) JO4637.

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[1] The abbreviations used are: IRBP, interphotoreceptor retinoid-binding protein; kb, kilobase pairs; bp, base pairs.

Nathans, The Johns Hopkins University). Both H4 and H12 had inverted complementary sequences at the 5′ end due to cloning artifacts. These are 325 bp in H4 and 20 bp in H12. H20 and H18 were isolated from a human retina cDNA library. The double-stranded cDNA for this library was prepared according to Gubler and Hoffman (29), ligated to *Eco*RI linkers, and inserted into the unique *Eco*RI site of λgt10 (30). Both H18 and H20 were free of 5′ artifacts. A human genomic library in EMBL3 was purchased from Clonetech, Palo Alto, CA. This library was screened with H4, H18, and H20 cDNA probes, and three overlapping clones, each with 15–17-kb insertions, designated as HGL-1, HGL-2, and HGL-3, were isolated. HGL-3 was further characterized (Fig. 1) and shown to span the entire coding region.

DNA Isolation and Sequencing—Plasmid DNA was isolated in a small scale according to a modified procedure of Birnboim and Doly (31) and in a larger scale by cesium chloride density gradient centrifugation (32). Bacteriophage λ-DNA was isolated from confluent plates (33) or lytic cultures (32). Plasmid DNA was sequenced by the dideoxynucleotide chain termination method (34) using the cyclone method in M13 vectors (35) or the double-stranded sequencing method (36) in puc19 vectors. Universal M13 and synthetic (Genetic Designs, Inc., Houston, TX) oligonucleotide primers (GL-1 to GL-31, Fig. 2) were used to prime T7 DNA polymerase ("sequenase," United States Biochemical Corp., Cleveland, OH).

Transcription Start Site—To determine the transcription start site, S1 nuclease protection and primer extension experiments were carried out. For S1 nuclease protection (37), an 822-bp *Ava*II fragment of the genomic fragment S3 (Fig. 1) that spans the 5′ end of IRBP mRNA was used. Briefly, the fragment was end-labeled with [γ-^{32}P]ATP (Du Pont-New England Nuclear) and T4 polynucleotide kinase (Bethesda Research Laboratories). The denatured fragment was hybridized to 2 μg of human retina poly(A) mRNA in 80% formamide, and the hybridization products digested with S1 nuclease (Pharmacia LKB Biotechnology Inc). The size of the protected fragment was determined on an 8% polyacrylamide-urea sequencing gel adjacent to the sequencing ladder generated by priming denatured S3 fragment with an antisense primer, whose 5′ end was identical with that of the *Ava*II fragment. For primer extension (38), poly(A) mRNA (2 μg) and end-labeled antisense primers (G1-1, G1-2, G1-3, and G1-23, see Figs. 2 and 3) hybridizing to mRNA near AUG were annealed and extended by avian myeloblastosis virus reverse transcriptase (Promega Biotech, Madison, WI). The sizes of the extended fragments were determined as described above. In some primer extension experiments, dideoxynucleotides were added as chain terminators (39) and RNA sequences determined.

RESULTS AND DISCUSSION

Isolation and Characterization of Human IRBP Clones—We have used labeled H4, H18, and H20 cDNA inserts (see Fig. 1) to isolate three overlapping genomic clones (HGL-1 to HGL-3), one of which, HGL-3, contained the complete IRBP gene including transcription start site, all introns, and a putative polyadenylation signal. In addition to the IRBP gene sequences, HGL-3 contained flanking sequences that extend >2 kb upstream from the translation initiation codon and at least 450 bp downstream from the translation termination codon. To obtain a complete sequence of the exons, to map the exon/intron junctions, and to estimate the length of the introns, the 15-kb *Sal*I insert of HGL-3 was digested with *Pst*I, *Sst*I, and *Eco*RI generating a series of overlapping genomic fragments. The *Pst*I fragments P1-P13, the *Sst*I fragments S2-S7, and the *Eco*RI fragments R1-R4 were subcloned into pUC vectors and completely or partially sequenced. The orientation of fragments, partial restriction maps, coding and flanking regions of the gene, and the distribution of introns and exons in the gene are illustrated in Fig. 1*A*.

The 5′ end of the gene, including transcription start site and translation initiator ATG, was contained in the 2.5-kb fragment S3. An 822-bp *Ava*II 5′ end-labeled subfragment of S3 was used for S1 protection experiments to determine the transcription start site (see "Experimenal Procedures"). The 1.3-kb genomic fragment S5 contained the end of exon 1, the junction of which was sequenced with an exon 1 specific

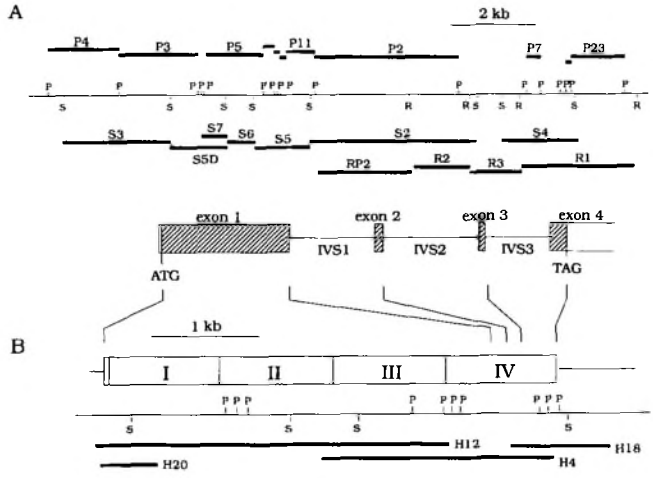

FIG. 1. **Map of human IRBP gene and its mRNA.** *A*, restriction map, orientation of genomic fragments, and distribution of introns and exons of the IRBP gene. The extent of fragments excised from the 15-kb genomic clone HGL-3 with *Sst*I (*S2–S7*), *Eco*RI (*R1–R4*), and *Pst*I (*P1–P13*) is indicated by *bars*. RP2 is an *Eco*RI subfragment of P2. Symbols for restriction enzymes are *P*, *Pst*I; *S*, *Sst*I; *R*, *Eco*RI. The structure derived from sequence analysis of the fragments is illustrated as follows: the *hatched boxes* represent exons, the *open boxes* represent untranslated regions, and *lines* represent introns or flanking regions. The initiation codon, ATG, and the translation termination codon, TAG, are indicated below the *boxes*. *B*, structure of IRBP mRNA derived from cDNA clones. The quadruplicated mRNA segments are depicted as *I–IV*, and the extent of the four overlapping cDNA fragments H20, H12, H4, and H18 is indicated by *bars*. The *box* 5′ to the first quadruplicated segment represents sequences translated into a signal peptide. Scales in *A* and *B* are in kb.

primer (GL-10, see Fig. 2) and an antisense intron 1 primer located about 250 bp 3′ of the junction (not shown). The 3.4-kb fragment S2 harbored exon 2, the position of which was mapped with overlapping fragments R2, P2, and an *Eco*RI subfragment of P2, termed RP2 (Fig. 1*A*). The junctions of exon 2 were sequenced with exon 2-specific sense and antisense primers (Fig. 2). Exon 3 was identified similarly in the genomic fragment R3 which overlaps with fragments S2 and S4. Fragments S4 and R1 carried the beginning of exon 4 and the translation stop codon. R1 harbored both translation stop and a putative polyadenylation signal. All cDNA clones were 5′- and 3′-truncated but were overlapping and covered the complete coding sequence of IRBP (Fig. 1*B*).

IRBP Gene Structure—The IRBP gene spans more than 11 kb and is interrupted by three introns. Fig. 2 shows a 4290-bp composite sequence of the exons, constructed from overlapping sequences of cDNA and genomic clones, and a translated amino acid sequence. In addition to exon sequences, the positions and terminal 12 bases of introns are shown. Except for cloning artifacts at their 5′ ends, cDNA sequences of clones H4, H12, H18, and H20 were consistent with exon sequences of genomic clones. Exon boundaries in genomic fragments were localized by alignment of sequences with those of cDNA clones. The large exon 1 extending from the transcription start site to the first intron junction consists of 3176 bp. It contains more than three quarters of the structural gene. The central exons 2 and 3 are small (192 and 143 bp, respectively) and embedded in approximately 6 kb of intron sequences. The length of the three introns are estimated to be 2.1 kb (intron 1), 2.0 kb (intron 2), and 1.3 kb (intron 3) (Fig. 1). Exon 4 contains the COOH terminus of IRBP and a polyadenylation signal (see below). The exact length of exon 4 has not been determined but is presumed to be longer than 960 bp.

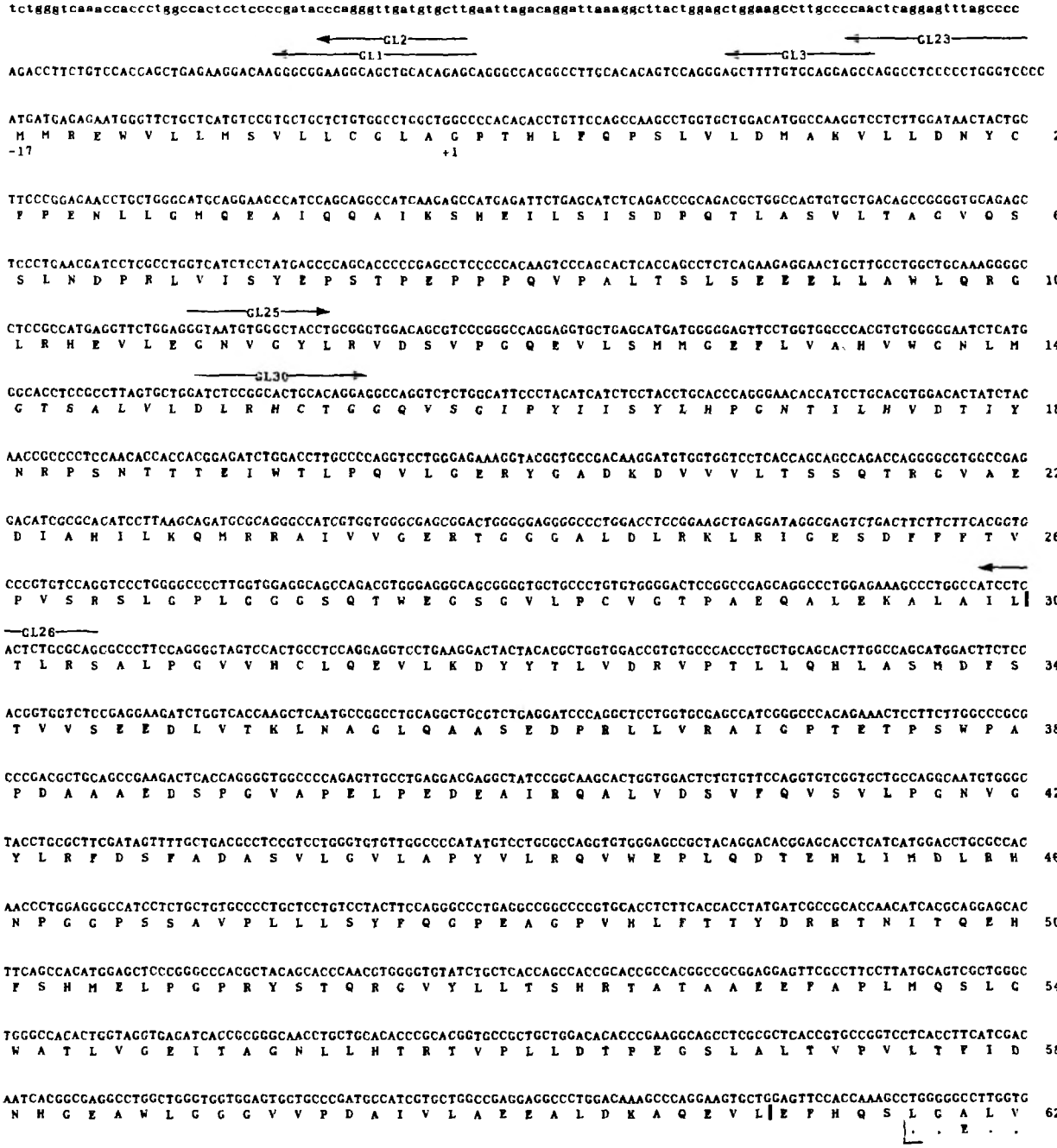

FIG. 2. **Nucleotide sequence, predicted amino acid sequences, and intron/exon junctions of the human IRBP gene.** *Numbers* on the left side indicate nucleotide positions starting with position 1, the transcription start site. Intron (*IVS*) sequences are depicted in *lowercase letters*, only the first or last 12 nucleotides of each intron are shown. Synthetic primers used for DNA sequencing and primer extension experiments are *overlined*. The direction of primer extensions are indicated by *arrows*. Putative polyadenylation signals AATAAA in the 3′-flanking sequence are *underlined*. *Numbers* on the right side indicate positions of amino acids starting with +1, the NH_2-terminal glycine of the mature protein. Amino acids are depicted in *single-letter symbols* underneath their respective codons. The signal peptide is located at positions −17 to −1. Position 618 to the COOH-terminal end shows an alignment with published tryptic peptide sequences (marked by *brackets*) or amino acid sequences deducted from cDNA fragments of bovine IRBP (22, 23). Boundaries of the four homologous segments (see Figs. 1, 4, and 5) are indicated by *vertical bars*.

The 5′ end of the IRBP gene was determined by the following two independent methods (Fig. 3); S1 nuclease protection and extension of synthetic primers. In the S1 nuclease mapping experiment, we annealed a 822-bp 5′ end-labeled *Ava*II fragment of S3, which is a 5′ proximal genomic fragment of HGL-3, to poly(A) mRNA of the human retina and digested with S1 nuclease. For priming cDNA synthesis, we selected a series of synthetic oligonucleotides 17–21 nucleotides in length, and complementary to mRNA near AUG. The primer GL-23 used for the extension experiment shown in Fig. 3 was selected to extend upstream from the *Ava*II site. Its major extension product and the lower of the two major fragments protected by S1 nuclease were of the same length (Fig. 3, *lanes 1* and *2*). The results demonstrate that the major transcription start site is located at an adenine (position +1) 119 bp upstream of the *Ava*II site, or 122 bp upstream of

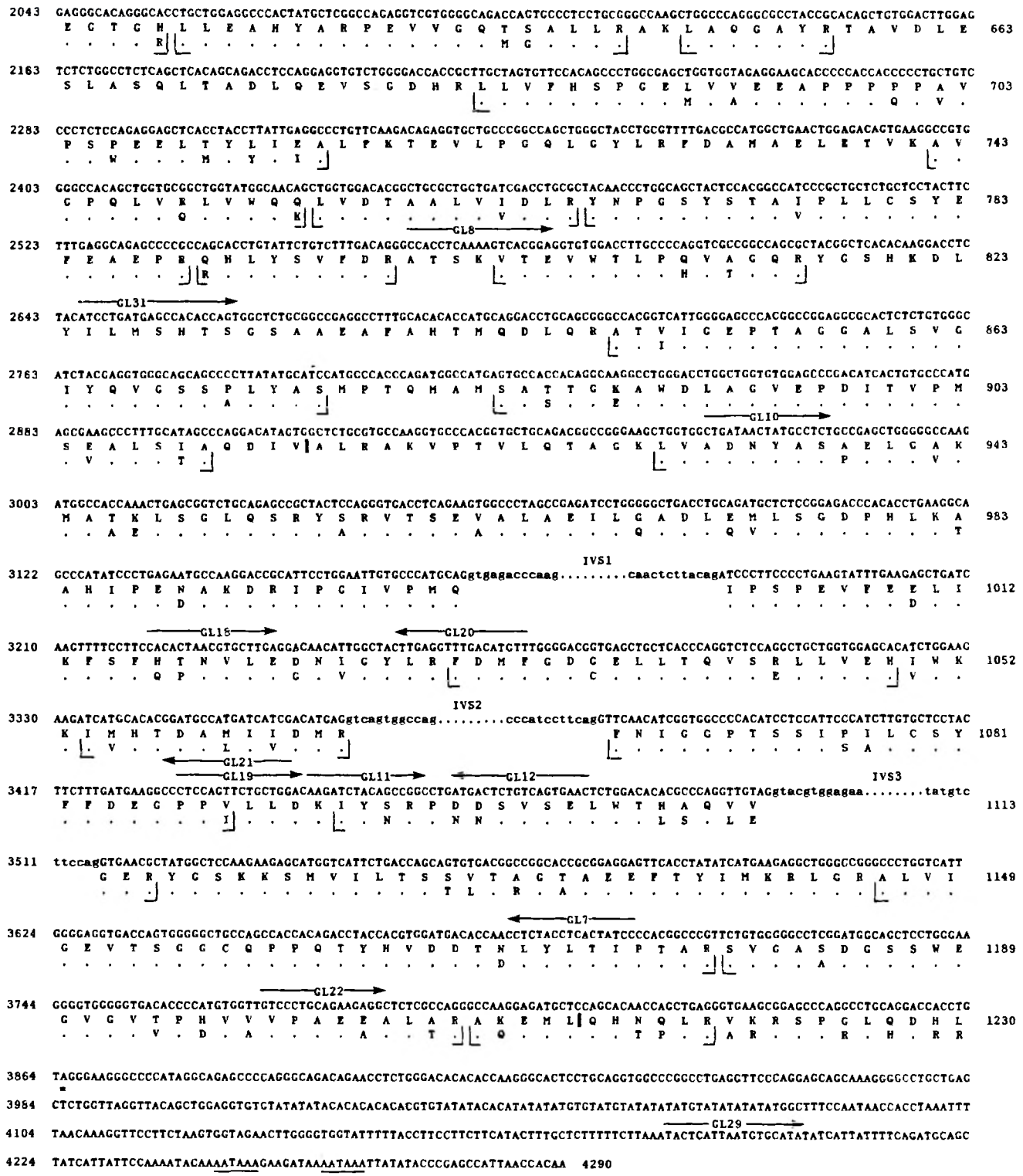

FIG. 2—*continued*

ATG. Minor alternate sites were located 6 bp upstream, and 7, 15, and 18 bp downstream of this site (Fig. 3). Three larger extension products of the *Ava*II primer (upper part of *lane 2* in Fig. 3) are considered background for the following two reasons: 1) extensions with primers GL-1, GL-2, or GL-3 (Fig. 2) which prime upstream from GL-23 do not yield corresponding extension products. 2) There are no bands of the same mobility in lane 1, the S1 nuclease protection experiment. The mRNA sequence obtained when the primer extensions were carried out in the presence of dideoxynucleotides followed precisely the gene sequence up to position +1 indicating the absence of introns in the 5′-flanking region (results not shown). No suitable "TATA" or "CAAT"-box sequences could be located within conventional distances (40) upstream from the transcriptional start sites (Fig. 2). Work is in progress in this laboratory to identify the IRBP promoter by its ability to express the bacterial chloramphenicol acetyltransferase gene in retinoblastoma cell lines.

Northern blot analysis of human retinal poly(A) mRNA with H4 cDNA as a probe revealed an IRBP mRNA of

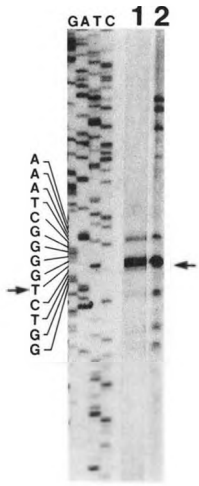

FIG. 3. **Determination of the transcription start site in the IRBP gene.** *Lane 1* displays the products of S1 nuclease digestion after hybridization of a 5′ end-labeled 822-bp *Ava*II fragment of S3 to 2 μg of human retina mRNA. *Lane 2* displays the cDNA extension products generated by priming 2 μg of human retina mRNA with a synthetic, 21-nucleotide, antisense primer extending upstream from the *Ava*II site. At the left, labeled with GATC, is a sequence ladder generated by priming denatured S3 fragment with the same 21-nucleotide primer. The *arrow* indicates the major transcription start site (position +1). For more details, see text and "Experimental Procedures." The sequencing gel contained 8% acrylamide. Exposure was for 72 h.

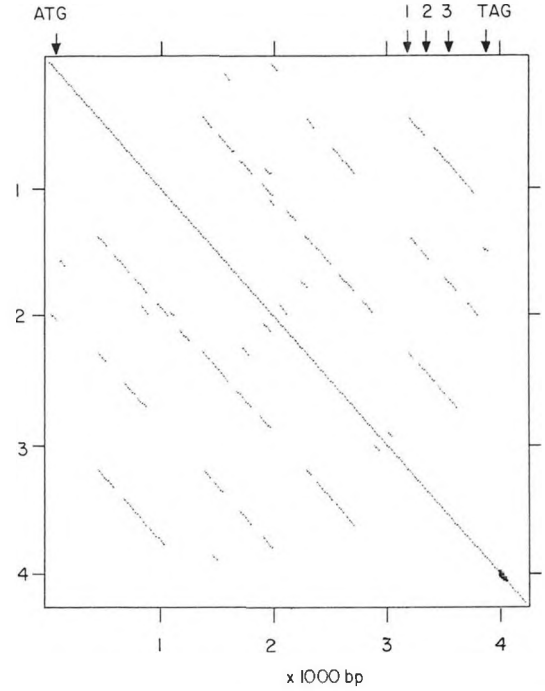

FIG. 4. **Internal quadruplication of the IRBP gene sequence.** Dot matrix analysis (Microgenie release 5, Beckman Instruments) of the IRBP cDNA sequence with itself starting at the transcription start site and extending to the polyadenylation signal. The sequence used for analysis is the composite gene and cDNA sequence of Fig. 2 in which the introns were manually spliced out. The parameters were set at a window of 30 nucleotides with 60% stringency, or a dot was scored when 18 of 30 residues matched. The position of exon/intron junctions are indicated by *arrows*.

approximately 5200 bp (23). The distance between the transcription start site and the polyadenylation signals located 300 bp downstream from translational stop codon TAG is only 4400 bp (excluding introns). To account for the additional 800 bp, we assume the observed polyadenylation signals are followed by other signals further downstream. Large 3′-untranslated sequences of 1700–2000 bp have been previously observed in bovine IRBP mRNA (22) and bovine and human opsin mRNA (41).

Quadruplication of the IRBP Gene—The IRBP cDNA sequence was compared with DNA and protein sequences contained in GenBank (release 56) and Protein Identification Resources (release 17) data banks. The comparison yielded no gene or cDNA sequences with significant similarity to IRBP. Dot matrix analysis (Fig. 4) of the IRBP mRNA with itself, however, strongly suggests that the gene arose by quadruplication of an ancestral sequence of approximately 900 bp. The four gene segments reveal a high degree of similarity (Fig. 4) preserving several domains of the expressed protein (see below). Rat serum retinol-binding protein which has been shown to be the product of gene duplication shows a similar close relationship between the two segments. In serum retinol-binding proteins, exons correspond to discrete tertiary structural elements (42). In the IRBP gene, all intron junctions were found in the last quadruplicated segment. This arrangement of introns generates one large exon containing the first three segments of IRBP and parts of the fourth, one exon containing the COOH-terminal sequences, and two small exons exclusively containing sequences of the fourth segment. It is difficult to comprehend that this arrangement of exon/intron junctions reflects the borders of building blocks, as observed in rat serum retinol-binding protein (42), or in the retinal-binding protein rhodopsin (41), where intron positions coincide with membrane boundaries, but a final answer has to await more information about the tertiary structure of IRBP.

Primary Sequence of IRBP and Internal Homologies—The first translation initiation codon, ATG, was detected 122 bp downstream from the transcription start site, followed by a coding sequence of 3741 bp. No in-frame stop codon was found between transcriptional and translational start. The NH_2 terminus of mature IRBP is defined as Gly at amino acid position 1, consistent with the peptide sequence GPTHLFQPSL found in mature human (12) and rhesus monkey IRBP (43). The 17-amino acid signal peptide preceding the mature NH_2 terminus glycine is rich in hydrophobic residues and ends in an alanine which is most commonly found at this position (44). The predicted mature human IRBP peptide has a calculated molecular weight of 133,000, significantly smaller than calculated for bovine IRBP (145,000), and in approximate agreement with the observed sodium dodecyl sulfate-gel mobility of 135,000 (12).

Tryptic COOH-terminal peptide sequences (Fig. 2) of bovine IRBP (22, 23) and partial amino acid sequences derived from bovine cDNA clones were approximately 85% homologous to the human sequence. The COOH terminus itself has not been determined by peptide sequencing. The gene and cDNA sequences predicted a COOH-terminal sequence of 17 residues, counted from the end of segment 4, or about 30 residues shorter than the bovine IRBP sequence. This truncated COOH terminus in human IRBP accounts for part of the difference in molecular weight to its bovine counterpart.

As indicated by the quadruplication of the gene sequence (Fig. 4), analysis of the primary sequence of IRBP reveals that this protein consists of four homologous segments of approximately equal length. An alignment of the four segments is shown in Fig. 5. For optimal alignment, several gaps had to be introduced. Of the amino acids aligned, up to 36% were identical. The similarity increased to approximately 60% when conservative substitutions were also considered. Hydro-

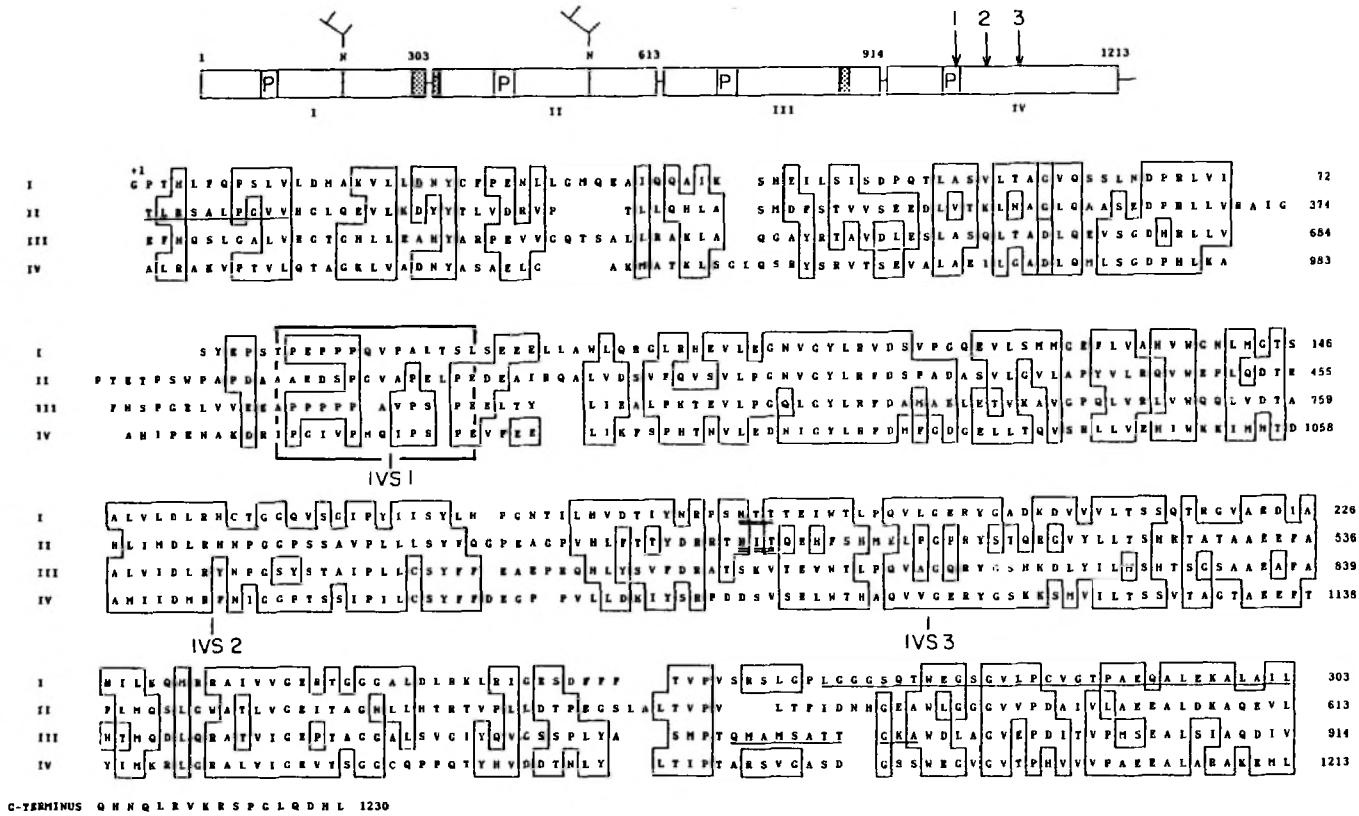

FIG. 5. **Comparison of the four repeated segments of human IRBP.** *A*, simplified diagram of the primary structure of IRBP. The *numbers* denote the last amino acid of each segment. Positions of putative *N*-linked glycosylation sites in segment I and II are marked with a branched structure. Proline-rich regions in all four segments are indicated by a *boxed P*. Areas with homology to rhodopsin are *dotted*. *B*, alignment of the four segments of IRBP. Domains containing identical or similar residues ("conservative substitutions") are *boxed*. The following residues are considered conservative substitutions (52): I = L = V = M; D = E; H = R = K; S = T. The putative glycosylation sites, N-X-T, in segments I and II are *double underlined*. Sequences with similarities to rhodopsin at the end of segments I and III are *underlined*. The proline-rich region is *boxed*. The positions of introns (*IVS*) are indicated by *arrows*.

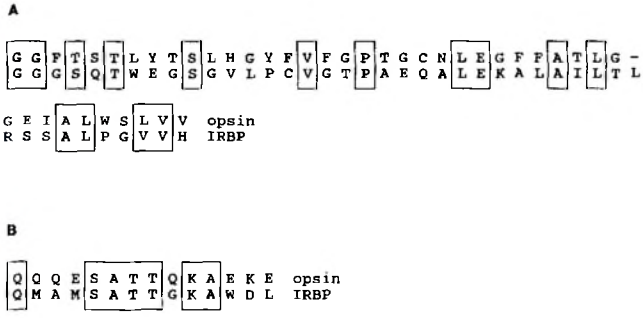

FIG. 6. **Sequence similarities between the retinal-binding protein rhodopsin and the interstitial retinoid-binding protein IRBP.** *A*, sequence (position 89–130) of transmembrane helices 2, 3, and their interconnecting loop of human opsin, aligned with a partial sequence of segments 1, 2 (position 273–315) of IRBP. *B*, 14 residues of opsin's cytoplasmic loop connecting transmembrane helices 5 and 6 (position 236–250), aligned with a short sequence of IRBP (position 789–803.

phobicity plots (45) do not identify α-helical transmembrane domains, consistent with the existence of IRBP in the extracellular interstitial matrix, and do not indicate extensive internal hydrophobic domains, consistent with another retinol-binding protein, serum retinol-binding protein (see below) in which the retinol-binding site is formed by a β-barrel core consisting of several antiparallel β-strands (42). The charge distribution in IRBP appears to be uniform, with a large excess of negatively charged glutamic and aspartic acid residues (144 acidic and 83 basic residues), in agreement with the acidic pI of the protein (7).

Bovine IRBP has five putative *N*-linked glycosylation sites NXT(S) (46), at least one of which must be glycosylated in the mature protein (47). The human sequence displays only two putative glycosylation sites, located in well-conserved domains in the central part of segments I and II (Fig. 5). Each of the four segments of human IRBP has a region rich in proline (Fig. 5), condensed to five adjacent prolines in segment III, and four adjacent prolines in segment 1 (interspersed with a Glu). The proline-rich structure in segment 3 is located near the center of the elongated molecule and may be responsible for formation of a "hinge" structure of IRBP as observed in electron microscopical studies (14).

Sequence Comparison and Local Homologies to Other Retinoid-binding Proteins—Mammalian serum and cellular retinoid-binding proteins, such as rat serum retinol-binding proteins (42), rat cellular retinol and bovine retinal-binding proteins (48, 49) and chicken purpurin (50) have been well characterized. Serum retinol-binding proteins transport retinol from the liver to various retinol-dependent tissues, and rat cellular retinol and bovine retinal-binding proteins may serve as carriers of retinoids between various intracellular locations. Purpurin is a chicken neuroretina adhesion and cell survival protein found in the chicken retina interphotoreceptor matrix, also known as a retinol-binding protein, and shows

50% sequence homology to human serum retinol-binding proteins. Binding of retinoids to IRBP is approximately 10–100-fold weaker than observed for cellular and serum retinol-binding proteins indicating that IRBP may have a function different and distinct from these proteins which bind retinol tighter and more specifically. There is no significant sequence similarity of IRBP with serum and cellular retinol-binding proteins, or with any other protein sequence deposited in the GenBank or Protein Identification Resources data banks. We noticed, however, two short segments with sequence similarity in rhodopsin, the photoreceptor molecule in rod outer segments and a retinal-binding protein, and IRBP (Fig. 6). First, the intradiscal (extracellular) loop connecting membrane-spanning helices 2 and 3 of rhodopsin and the COOH-terminal end of IRBPs segment I share 15 matching residues in a 41-residue long segment. The sequence of this loop in rhodopsin has been precisely conserved in all mammalian opsins, but its function is unknown. Second, eight matches in a 14-residue segment of the cytoplasmic loop 5/6 of opsin and the COOH-terminal segment of segment III have been found. Loop 5/6 of rhodopsin has been implicated in ROS G-protein binding, and serine 240 in opsin is known to be phosphorylated by opsin kinase (for review, see Ref. 51). The significance of these similarities is not known.

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Addendum—Since submission of this manuscript, a paper by S.-L. Fong and C. D. B. Bridges entitled *Internal Quadruplication in the Structure of Human IRBP Deduced from Its Cloned cDNA* has been published ((1988) *J. Biol. Chem.* **263,** 15330–15334). The authors report a peptide of 1262 residues (M_r 136,600), in contrast to our 1,230 residues (M_r 133,000), for mature human IRBP, as deduced from overlapping cDNA clones. The discrepancy is in part due to their COOH-terminal peptide sequence which deviates from ours at position 1228–1233, and in addition is 29 amino acids longer than ours. The differences are caused by insertion of a C at position 3728 and a G at position 3747 (Fig. 2 on p. 15332) resulting in a double frame-shift. Our sequence lacks both nucleotides, ending the open reading frame at the stop codon at position 3750–3752. Our sequence was obtained from the same cDNA clone (H18) described by Fong and Bridges and is in agreement on both strands with sequences obtained from a genomic fragment (S4). However, a COOH-terminal peptide sequence of human IRBP which would match either sequence is currently not available.

Another major discrepancy is a 28-residue peptide (position 982–1010 in Fig. 2, p. 15332) which does not match our sequence. Our corresponding sequence is 3 amino acids shorter and is virtually identical (except for 2 residues) with a deduced partial bovine sequence published by Liou *et al.* (22) and a bovine IRBP sequence published by Borst *et al.* (53). The strong homology between our sequence and the bovine IRBP sequence in the region of interest adds weight to our sequence being correct. Other minor differences in the two IRBP sequences are Fong and Bridges' position 448(R) *versus* Liou *et al.* (22) 448(W) and position 973(E) *versus* 973(Q).

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