Intestinal fatty acid binding protein (I-FABP) is believed to participate in the uptake, intracellular metabolism, and/or transport of long chain fatty acids within enterocytes. The 15.1-kDa rodent protein is a member of a family of low M<sub>c</sub> cytoplasmic proteins that have evolved to bind different ligands. We have now determined the nucleotide sequence of the gene encoding human I-FABP and defined the primary structure of its protein product. The human I-FABP gene spans 3382 nucleotides and contains 4 exons (103 or 128, 173, 108, and 312 base pairs) interrupted by 3 introns (1194, 1023, and 444 base pairs). The 132-residue rat and human I-FABPs have 82% amino acid sequence identity. Blot hybridization studies of RNAs prepared from a variety of adult rhesus monkey tissues as well as human intestine and liver indicate that I-FABP mRNA is confined to the intestine. I-FABP mRNA was not detectable in a number of cultured human enterocyte-like cell lines, suggesting it may be a sensitive marker for differentiating villus-associated, small intestinal lining cells. Given the similar patterns of tissue-specific expression exhibited by the rat and human genes, we compared their 5′ nontranscribed regions. Optimal alignments of the two sequences disclosed 64% identity among the 260 nucleotides immediately 5′ to the start site of transcription. Matrix plots revealed a 14-nucleotide long repeated sequence (5′-TGAACTTGAACCTT-3′) in the 5′ nontranscribed region of both genes as well as in a comparable region of another family member that is expressed in enterocytes. Given their distributions and high levels of expression, the I-FABP and homologous liver FABP (L-FABP) gene were defined in mice and humans. The mouse genes were mapped using restriction fragment length polymorphisms and recombinant inbred strains. The I-FABP gene is located on mouse chromosome 3 between the amylase 1,2 (Amy 1,2) and alcohol dehydrogenase 3 (Adh-3) loci while the L-FABP gene is on mouse chromosome 6 within 3 centimorgans of the lymphocyte antigen-2 (Ly-2) locus. Mouse L-FABP may be identical to the major liver protein-1 (Lyp-1) which is encoded by a gene situated within a centimorgan of Ly-2. Human gene mapping studies were carried out using a panel of mouse-human somatic cell hybrid clones as well as in situ hybridization to metaphase chromosomes. The I-FABP gene is located in the q28-q31 region of human chromosome 4 while the L-FABP gene resides in the p12-q11 region of human chromosome 2. These findings extend the regions of syntenic homology that exist between these human and mouse chromosomes.

The cytoplasm of the rat small intestinal epithelial lining cell (enterocyte) contains two abundant fatty acid binding proteins (FABPs). These proteins have been named intestinal (I-) and liver (L-) FABP based on their initial sites of isolation (1, 2). Both are remarkably abundant, representing 2–3% of the enterocyte's cytoplasmic protein mass (3). The mRNAs encoding I- and L-FABP account for 2–3% of the translatable RNA sequences in adult rat intestinal epithelium (4). Expression of the I-FABP gene is confined to the gastrointestinal tract (5, 6). Gradients in I-FABP gene expression exist along the horizontal axis of the gut with relatively low levels detected in stomach and colon (5, 7). Concentration gradients also exist within the small intestinal villus, i.e. increasing from crypt to villus tip (7). Rat L-FABP mRNA is present in high concentrations in hepatocytes as well as enterocytes (1−8). Given their distributions and high levels of expression, the I- and L-FABP genes represent potentially useful models for defining how "regional" expression is achieved in the gastrointestinal tract.

Three homologous FABPs have been identified in the rat to date, I-, L-, and a heart (H-) FABP (9–11). Each has a distinct pattern of tissue-specific and developmental regulation (5, 6, 12). Numerous roles have been proposed for the FABPs (reviewed in Refs. 13–15). They include (i) facilitating the uptake of fatty acids into cells and their transport to organelles or their delivery to specific metabolic pathways; (ii) protecting cell membranes and enzymes from the effects of high concentrations of free fatty acids and their acyl-CoA esters; and (iii) serving as useful models for defining how "regional" expression is achieved in the gastrointestinal tract.
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derivatives; and (iii) maintaining a large intracellular pool of fatty acids for rapid mobilization. A direct, systematic comparison of the ligand binding characteristics of all 3 rodent FABPs has yet to be reported. However, both I- and L-FABP have recently been efficiently expressed in Escherichia coli and their ability to bind endogenous (E. coli) as well as exogenous fatty acids studied (16). Both proteins bind long chain (C16-C20) fatty acids noncovalently with Kd values varying from 1–3 μM. They do not exhibit appreciable affinity for medium chain (C6–C14) fatty acids. E. coli-derived L-FABP appears to bind unsaturated fatty acids with greater affinity than saturated acyl chains while I-FABP binds both forms with similar affinity (16). At present it is unclear why the enterocyte possesses two abundant FABPs. I-FABP may be involved in targeting fatty acids to the 2-monoyacylglycerol pathway for triacylglycerol resynthesis (16).

The three FABPs are similar in size (127–133 residues). They belong to a family of cytoplasmic proteins which contains 8 known members: the FABPs, two proteins which bind all-trans-retinol (cellular retinol binding protein and cellular retinol binding protein II; Refs. 17 and 18), a protein which binds all-trans-retinoic acid (cellular retinoic acid binding protein; Ref. 19), an intestinal specific protein referred to as p2 (20–22), and the P2 protein of peripheral nerve myelin (23–25). The rodent L-FABP, CRBP II, and p2 genes all contain 3 introns. Each intron is located at a comparable position (22, 26, 27). The relationship between exonic domains and protein structure should be testable now that several family members have been crystallized (L-FABP, CRBP, and I-FABP; Refs. 28–30).

We have now isolated the human I-FABP gene in order to address several questions. Does this gene exhibit the same pattern of intestine-specific expression in primates as in rodents? If so, are there any conserved sequences in the 5' nontranscribed regions of the rat and human genes which could potentially confer enterocyte-specific expression? Finally, given the similarities in their organization, what are the linkage relationships between the homologous I-FABP, L-FABP CRBP, and CRBP II genes in mice and humans?

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

Isolation and Characterization of I-FABP Genomic Clones—When a human genomic library was screened with a 32P-labeled rat I-FABP cDNA (2), a single probe-positive recombinant phage was isolated and named λ-HIFABP. Southern blots of restriction endonuclease digests of X-HIFABP were probed with a full-length rat I-FABP cDNA as well as oligodeoxynucleotides derived from the 5' and 3' nontranslated regions of this rodent mRNA. The results (data not shown) suggested that λ-HIFABP contains all of the sequences present in (rat) I-FABP mRNA plus additional genomic DNA that extends 3.7 kb upstream and 7 kb downstream from the gene's exonic boundaries. There were no inconsistencies between the restriction map of the human I-FABP gene displayed in Fig. 1A and the results obtained from Southern blot analysis of human lymphocyte DNA (data not shown) implying that I-FABP is a single copy gene in the human genome.

The rat I-FABP cDNA was also used to screen a rat genomic library. A single clone was isolated and named λ-RIFABP. Based on Southern blot analysis, we concluded that this recombinant phage contains exons 1 and 2 of the rat I-FABP gene as well as 11 kb of DNA 5' to the first exon (Fig. 1B).

The sequence of the human I-FABP gene is shown in Fig. 2. Primer extension studies indicate that the gene has two sites of transcription initiation, each used with equal efficiency (data not shown). One start site occurs 38 nucleotides upstream from the initiator methionine codon. A second start site is located 25 nucleotides further upstream producing a 5' nontranslated domain of 61 nucleotides (Fig. 2). A "TATA box" is present 23–26 nucleotides upstream from each of the two transcription start sites (these are underlined in Fig. 2). The rat gene also contains these two TATA box elements (panel B of Fig. 3). Primer extension analysis employing rat small intestinal RNA revealed that there are also two transcription start sites in the rat gene. However, in contrast to the results obtained in the human, the larger rat primer extension product was only ~20% as abundant as the smaller (cDNA) species. The biological significance of these observations is presently unclear.

5' nuclease protection experiments (see "Experimental Procedures") indicated that the human gene has a 3' nontranslated region of 263 nucleotides. The canonical hexanucleotide polyadenylation signal, AATAAA, is located 16 nucleotides upstream from the inferred poly(A) addition site (Fig. 2).

Alignment of the previously published rat I-FABP cDNA sequence (2) with our partial rat I-FABP genomic clone allowed us to conclude that it contains exon 1 (103–128 bp depending upon the start site) and exon 2 (173 bp). These are separated by an intron of 1259 bp.3

The human I-FABP protein and cDNA sequences have not been published. Nonetheless, it was possible to define the intron/exon boundaries of the human I-FABP gene by exploiting the high degree of sequence identity between the orthologous (61) rat and human proteins (see below) as well as the nucleotide sequence conservation between the rat and human genes. The human I-FABP gene contains 4 exons: 105 or 128, 173, 108, and 312 bp. These exons are interrupted by 3 introns (1194, 1023, and 444 bp). All intron/exon junctions conform to the "GT-AG" dinucleotide rule of Breathnach and Chambon (62).

The 4 exons of the human I-FABP gene contain 721 nucleotides. When Northern blots of human and rat small intestinal RNA were sequentially probed with cloned I-FABP exonic sequences, an mRNA of ~900 nucleotides was noted in each tissue (data not shown). Taking into account the average size of the poly(A) tail in eukaryotic mRNA (150–200 nucleotides), the predicted and observed sizes of human I-FABP mRNA are in close agreement.

The deduced sequence of human intestinal fatty acid binding protein was aligned with the primary translation product of rat I-FABP mRNA using the ALIGN algorithm (63, 64). The results (Fig. 4) indicate that 108 of the 132 residues are identical (82%). Most of the 24 substitutions are conservative whether defined in an evolutionary sense by the mutation data matrix scoring system (Refs. 62 and 64) or by the proportions of this paper (including "Experimental Procedures," Tables I-III, and Figs. 7-9) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 87M-1669, cite the authors, and include a check or money order for $4.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

3 To conserve space we have not listed the nucleotide sequence of our partial rat I-FABP genomic clone in this paper. The sequence is available from the authors upon request and has been submitted to the GenBank/EMBL Data Bank. The defined nucleotide sequence includes 1178 bp of 5' nontranscribed region, exon 1 (103 or 128 bp depending upon the transcription start site used), intron 1 (1259 bp), exon 2 (173 bp) and the first 300 bp of intron 2.
**FIG. 1.** Restriction map and sequencing strategy for the human and rat I-FABP genes. Panel A, the recombinant phage λ-HIFABP was isolated from a human genomic library. A 3.2-kb BamHI fragment and a 3.9-kb BamHI/EcoRI fragment were subcloned into M13mp18 and 19 for sequence analysis. In addition, a 7.8-kb EcoRI fragment was subcloned into pUC13 to aid in restriction mapping and to provide overlapping sequence data for the M13 subclones. Panel B, the recombinant phage λ-RIFABP was isolated from a rat genomic library. Two EcoRI fragments that hybridized to a full-length rat I-FABP cDNA were subcloned into M13 for sequence analysis. To provide overlapping sequence data, a synthetic oligonucleotide was used to obtain sequence data from a 4.6-kb HindIII fragment cloned into M13. I-FABP exons are indicated by solid bars. The length and direction of each sequencing reaction are shown by an arrow. Arrows beginning with open circles represent dideoxy sequencing reactions primed with synthetic oligodeoxynucleotides. Arrows beginning with a bar indicate reactions that used a universal 17-nucleotide M13 primer (Collaborative Research). Arrows beginning with closed circles represent sequencing reactions carried out on templates generated using a rapid deletion system marketed by International Biotechnology Inc. (see "Experimental Procedures"). Both universal and synthetic oligodeoxynucleotides were used to prime these latter reactions. The EcoRI site designated by an asterisk is derived from EcoRI linkers added during construction of this genomic library.

physical-chemical properties of the amino acids. For example, 16 of the 24 substitutions involve residues with similar relative hydrophathy according to the scale of Kyte and Doolittle (65). The structural significance of these substitutions should become more apparent once the tertiary structure of (E. coli-derived) rat I-FABP is known (30).

The locations of the introns in the human I-FABP gene are indicated in Fig. 4. The figure illustrates two points. First, the
peptide specified by exon 3 is the least conserved, having only 72% identity between the two species. This fits with previous observations that the NH2-terminal halves of family members have been more highly conserved through the course of evolution than their COOH-terminal halves (19, 20, 26, 27). Second, the introns are identically positioned in all family members whose genes have been sequenced. Crystallographic studies should indicate whether these exonic boundaries "punctuate" structural or functional domains in these proteins.

Tissue Specific Expression of the I-FABP Gene—The small intestine is the primary site of I-FABP synthesis in rats (5, 6). To determine if I-FABP mRNA has a similar tissue distribution in the rhesus monkey, a dot blot was constructed from the start sites are underlined. A single Alu repeat (60) flanked by 7-bp direct repeats is located 57 bp 3' to exon 4 and has also been underlined. The human I-FABP also contains a (ATT)n sequence at the 3' end of intron 2. This sequence appears in numerous entries in GenBank. Its significance is unknown at present.

Fig. 2. Nucleotide sequence of human I-FABP gene and flanking protein.

The two transcription initiation sites determined by primer extension analysis are indicated by rightward pointing arrows. The start site closest to the AUG start codon is numbered +1. The leftward pointing arrow starting at position +70 represents the position of the synthetic oligodeoxynucleotide used for these primer extension studies. TATA boxes located 23–26 nucleotides upstream from the start sites are underlined. A single Alu repeat (60) flanked by 7-bp direct repeats is located 57 bp 3' to exon 4 and has also been underlined. The human I-FABP also contains a (ATT)n sequence at the 3' end of intron 2. This sequence appears in numerous entries in GenBank. Its significance is unknown at present.
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FIG. 3. Analysis of the 5' nontranscribed regions of the orthologous human and rat I-FABP genes. Panel A displays the results obtained when the T-MATRIX program (35) was used to compare sequences upstream from the AUG initiation codons of the rat and human I-FABP genes. The threshold for plotting was set so that only spans having at least 13 of 15 identities would be displayed. Arrows point to conserved sequences that are also aligned in panel C. Where two numbers accompany an arrow, the first number designates the rat I-FABP DNA sequence while the second number refers to the matched human I-FABP gene sequence. Panel B, alignment of the nucleotides -281 to +40 of the rat and human I-FABP genes generated by NUCALN (40) using a gap penalty of +7, and a k-tuple of 3. The two start sites of transcription in each gene are indicated by arrows. Sequences resembling the consensus sequence for the TATA box (5'-TATA(A/T)A(A/T)-3') are underlined. Two other highly conserved elements found in many eukaryotic promoters, the "CAAT box" and the CCGCCC binding site for the transcription factor SP1, were not discernible in either the rat or human gene sequences. Panel C, homologous sequences in the 5' flanking sequences of three genes which are expressed in small intestinal enterocytes. Methods used for identifying these sequences are described under "Results and Discussion."
regions of the small intestine (Fig. 5B). However, since these RNAs were prepared from different individuals, we could not determine the relative abundance of I-FABP mRNA along the horizontal axis of the human gut. A single preparation of human liver RNA did not contain detectable levels of this mRNA (Fig. 5).

I-FABP mRNA was not detected in several cultured human cell lines which have many of the features of small intestinal enteroctyes (Fig. 5B). Caco-2 cells are derived from a human colon adenocarcinoma (45, 46). Following growth to confluence they differentiate assuming several of the phenotypic characteristics of the small intestinal lining cell. There is polarization of cells. Tight junctions are formed. An apical brush border appears that contains several enterocytic hydro-

Analysis of the 5′ Flanking Regions of the Rat and Human I-FABP Gene — Given its efficient expression within the small intestine, the I-FABP gene represents a potentially attractive model system for defining cis acting elements that confer enterocyte-specific expression. Our strategy for attempting to identify candidate sequences in this gene was based on the following considerations. First, conserved sequences between species could represent important regulatory elements. We had sequence data from the 5′ nontranscribed regions of the rat and human I-FABP genes. Second, I-FABP is a member of a gene family whose membership includes a
gene of known structure which is also specifically expressed in enterocytes (i.e. the cellular retinol binding protein II gene). Another family member, whose nucleotide sequence is known, specifies a protein expressed in enterocytes as well as hepatocytes (i.e. the L-FABP gene). These gene sequences could be searched to see if they retained conserved elements initially identified in the orthologous I-FABP genes. Finally, other rodent and human gene sequences could serve as negative and positive controls: these include family members which are not expressed in the intestine (the adipocyte-specific aP2 sequence) as well as nonhomologous genes which are expressed in the enterocytes and/or hepatocytes (the apolipoproteins).

The T-MATRIX program (35) was used to initially compare the 5’ flanking sequences of the human and rat I-FABP genes (Fig. 3A). A long main diagonal was observed indicating considerable sequence conservation between those 260 nucleotides situated immediately upstream from the start site of transcription. When these regions are aligned using the NUCALN algorithm, it is also apparent that they exhibit high (64%) nucleotide sequence identity (Fig. 3B). A dramatic reduction in sequence homology begins immediately upstream from this point and extends through the remaining ~1000 nucleotides of defined upstream sequence (Fig. 3A).

The T-MATRIX plot shown in Fig. 3A also reveals another set of conserved sequences. Several related sequences offset from the main diagonal are evident even when the threshold for plotting was set such that only spans exhibiting 13 of 15 identities were exhibited. When these sequences are aligned, a common sequence 5’-TGAACTTTGAACTT-3’ could be readily defined (panel C of Fig. 3). This 14-nucleotide sequence is made up of two direct 7-nucleotide repeats. Several copies of this sequence are located at comparable sites in the 5’ flanking regions of the orthologous I-FABP genes (summarized in Fig. 3C).

The rat CRBP II gene has a sequence located at positions -602 to -615 which matches 11 of the 14 nucleotides in the consensus 14-mer. In addition, an adjacent sequence spanning nucleotides -616 to -629 matches 10 of the 14 nucleotides (Fig. 3C). These CRBP II sequences are in the opposite orientation to the I-FABP consensus and overlap a 7-nucleotide sequence (5’-CTGTGAC-3’) which is directly repeated 4 times between nucleotides -567 and -634 of the rat CRBP II gene (27).

If one includes matches of the opposite orientation, sequences matching 11 of the 14 nucleotides in the consensus would be expected to occur by chance once every 10,820 bp of a randomized sequence. Several other sequences matching 10 of the 14 nucleotides in the consensus were found in the defined 5’ nontranscribed region of the rat CRBP II gene. However, a match of this degree would be expected to randomly occur every 980 nucleotides.

The nonhomologous human and rat apolipoprotein A-I genes which are expressed in hepatocytes and enterocytes also contain sequences in their 5’ nontranscribed domains which matched at least 11 of the 14 nucleotides in the consensus (Fig. 3C). Interestingly, the matching sequence in the human apoa-I gene consists of three copies of the fundamental 7-
between Amy 1,2 and Adh-3 on mouse chromosome 3 (Fig. 6). Although the gene encoding the mouse adipocyte-specific aP2 gene is also located on mouse chromosome 3 (12), it is separated from the Fabpi allele by at least 64 centimorgans (see Fig. 6).

Southern blots of mouse DNAs were subsequently probed with a full-length rat L-FABP cDNA. Three different restriction enzymes each defined an RFLP (Table II). Based on these RFLPs, we identified three alleles of the mouse L-FABP gene. These were named Fabpl, Fabplb, and Fabpl'. Eighteen AKR/J X C57L/J RI strains (AKXL) were screened and no crossovers were observed with either the T-lymphocyte antigen 2 (Ly-2) locus or the immunoglobulin light chain gene cluster (Ly-3). These data also show that Fabpl and Ly-2, the major liver protein 1 (Lup-1) locus, and Fabplb, the major liver protein 2 (Lup-2) locus, are located within 3.0 centimorgans of one another at the 95% confidence level (53).

The major liver protein 1 (Lup-1) locus is situated within 1 centimorgan of Ly-2 (see Fig. 6). This locus specifies an abundant liver cytosolic protein with a molecular mass of 16-18 kDa (76, 77). Electrophoretic variants of this abundant cytosolic protein, i.e., a fast and slow moving species, have been reported among inbred strains (76). Inheritance is autosomal and codominant (76). The metabolic consequences of this variation have not been analyzed to date. Based on our L-FABP gene mapping results, Lup-1 may encode the liver fatty acid binding protein. Proof of this hypothesis will require isolation and sequencing of the mouse hepatocyte gene.

Chromosomal Localization of the Human Intestinal and Liver FABP Genes—The chromosomal location of the human I-FABP gene was initially determined by examining DNA isolated from a panel of mouse-human somatic cell hybrids. When Southern blots of normal human cellular DNA were digested with EcoRI and probed with a 300-bp BamHl I-FABP fragment, no recombinants were observed between the human chromosome and mouse chromosomes 3 and 4. By contrast, all other chromosomes showed 3 or more recombinations (Table IV). Correlation of the presence of the 10-kb band with the human chromosome content of panel members revealed that the human I-FABP (I-FABP) gene resides on human chromosome 4. There were no clones discordant for co-segregation of I-FABP and chromosome 4. By contrast, all other chromosomes showed 3 or more discordancies (Table IV).

The human I-FABP exonic probe was hybridized to human metaphase chromosomes prepared from 120 lymphocytes in order to further define the location of this gene. Fig. 8A depicts the distribution of grains over these metaphase chromosomes. Twenty-seven of the 133 grains (20%) appeared over the distal portion of the long arm of chromosome 4. Peak grain density occurred in the q28-q31 region (Fig. 8B). These results are consistent with the data obtained from our somatic cell hybrid panel and indicate that I-FABP resides in the q28-q31 region of human chromosome 4 (Fig. 6).

Chen et al. (79) have recently used somatic cell hybrids to localize the human L-FABP gene to the short arm of chromosome 2. We have extended their analysis in the following way. Southern blots containing EcoRI digests of human DNA and mouse DNA, as well as DNA isolated from somatic cell hybrid panel members were probed with a full-length cloned.
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TABLE IV

Segregation of the I-FABP and L-FABP genes with human chromosomes in mouse-human somatic cell hybrids

| Hybrid clone | I-FABP | L-FABP | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X | Y |
|-------------|--------|--------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 84-2        | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-3        | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-4        | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-5        | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-20       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-27       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-7        | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-13       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-21       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-25       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-26       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-30       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-34       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-35       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-37       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-38       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| 84-39       | +     | +     | + | + | + | + | + | + | + | + | + | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |

Number of discordant clones

| I-FABP | 7 | 10 | 6 | 0 | 7 | 4 | 4 | 12 | 8 | 8 | 6 | 8 | 3 | 10 | 9 | 5 | 6 | 7 | 5 | 11 | 6 | 12 | 14 |
|--------|---|---|---|---|---|---|---|----|---|---|---|---|---|----|---|---|---|----|---|----|---|----|---|----|---|----|
| L-FABP | 5 | 0 | 9 | 10 | 12 | 8 | 6 | 11 | 6 | 11 | 7 | 12 | 6 | 6 | 9 | 6 | 7 | 11 | 6 | 5 | 9 | 9 | 8 | 4 | 7 |

*+* indicates that a 10-kb EcoRI fragment derived from the human I-FABP gene was detected in Southern blots of DNA prepared from this hybrid clone (see Fig. 7). 

**-** indicates that the 2.5- and 2.0-kb EcoRI restriction fragments derived from the human L-FABP gene were not detected Southern blots of DNA prepared from this hybrid clone. 

The mapping data also indicate syntenic homologies between the region of mouse chromosome 6 containing the Fabpl allele and a portion of human chromosome 2 which contains L-FABP (Fig. 6). The mouse Fabpl alleles are closely linked to a T-lymphocyte membrane glycoprotein locus (Ly-2) and to the immunoglobulin light chain gene cluster (Iglk). The human L-FABP gene is also linked to the IGK gene cluster (IGK) as well as to a locus specifying a T-cell antigen (Leu72). The human Leu-2/T8 membrane glycoprotein is the orthologous homologue of the mouse Lyt-2 T-cell membrane glycoprotein and exhibits sequence similarities to immunoglobulin variable regions (81).

Thus, with the exception of the closely linked CRBP and CRBP II genes on mouse chromosome 9 and human chromosome 3 (77), all the other family members appear to be dispersed in the genomes of these two mammalian species. At the same time, these studies emphasize the remarkable similarities in the organization of the genes comprising this dispersed family. Given the similar sizes of their comparably positioned exons, this family should prove quite amenable to phylogenetic analysis. However, further insights into the evolution of these small, abundant cytoplasmic hydrophobic ligand binding proteins will require isolation and characterization of their homologues in other (lower) eukaryotes.

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The Intestinal Fatty Acid Binding Protein Gene


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RNA blot hybridizations - RNA dot blots were constructed using a technique described in theboilerplate research laboratory and methods outlined in our references 4 and 9. Four different concentrations of each RNA sample (0.5, 1, 2, and 5 pg), each including in 10% neomycin solution were added to each tissue RNA preparation prior to denaturation so that the total amount of marker RNA was identical. 14 C-labeled mouse RNAs were used as internal controls. Five hybridization reactions were performed on each blot, each with a different restriction fragment which includes sequences derived from exon 3 of the human I-FABP gene. This probe was labeled with [32P]ATP in a specific activity of 1,000 cpm/μg with an avidin nitrocellulose primer and the kinase fragment of DNA polymerase I (3). Hybridization and washing conditions are detailed in ref. 39. The labeling efficiency of each restriction fragment and the amount of radioactivity bound to each fragment were determined by photometric densitometry. The radioactivity of the hybridized fragments was determined by scintillation counting. The intensity of the radioautographs was normalized to the intensity of the 26 S rRNA band and 35 S-labeled 18 S ribosomal control. The data were obtained from a single representative gel and are shown in the figure. The data were obtained from a single representative gel and are shown in the figure.
The Intestinal Fatty Acid Binding Protein Gene

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Group I: AKR/1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32

Group II: C57BL/6J, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32

Group III: C57BL/6J, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32

* Southern blots of spleen DNA digested with the indicated restriction endonucleases were probed with a full-length rat L-FABP cDNA using conditions described in ref. 37.

**bx** and **bx** indicate that these have not yet been determined.