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Review

New insights into the structure and function of fatty acid-binding proteins

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Abstract. Fatty acid-binding proteins (FABPs) are members of a superfamily of lipid-binding proteins, and occur intracellularly in vertebrates and invertebrates. This review presents recent findings on the diversity of these FABPs and their proposed roles in fatty acid (FA) metabolism and other cellular processes. Special attention is paid to the structural features of the different mammalian FABP types and the physiological role of these proteins in FA transport, cell growth and differentiation, cellular signalling, gene transcription and cytoprotection. Additionally, data on FABP knockout mice and the implication of FABP in medicine are discussed.

Key words. Fatty acid-binding protein; fatty acid transfer; site-directed mutagenesis; gene transcription; cancer; diagnostic marker; signal transduction.

Introduction

The uptake and biosynthesis of both water-soluble and -insoluble metabolites is essential for every living cell. The solubility and the translocation of hydrophobic ligands is facilitated in intra- and extracellular fluids by lipid-binding proteins. The structure of three families of proteins which bind fatty acids (FAs), albumin, lipocalins and fatty acid-binding proteins (FABPs) has been described [1–3]. Many of the proteins of these families bind FAs as their main ligand, but other proteins with quite different structures also have affinity for FAs [e.g. fetuin, adipose differentiation-related protein, heat shock protein, caveolin 1, plasma membrane FABP, fatty acid transporter (FAT), fatty acid transport protein (FATP), glutathione S-transferase and sterol-carrier protein-2 [3–5]]. Together with lipocalins and avidins, the FABPs form part of the superfamily of the calycins with a β barrel structure [2].

The presence of cytoplasmic proteins that associate non-covalently with FAs has been appreciated for almost three decades [6]. Although molecular cloning and structural studies have increased our knowledge of the evolutionary and cellular diversity of FABPs, evidence for their physiological role has been equivocal for many years. Only recently have studies on knockout mice provided direct proof for the importance of FABPs in the uptake and transport of long-chain FAs and their interaction with other transport systems and enzymes. Numerous reviews on structural and functional aspects of the FABP family have appeared over the last 10–15 years [7]. We will focus mainly on recent data on the structure of FABPs, their role in FA uptake and transport and their involvement in the modulation of several cellular processes including signal transduction, gene expression, growth and differentiation.

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Family of FABPs

On a structural basis, the family of FABPs also involves the retinoid-binding proteins [8]. These comprise three types of cellular retinol-binding protein (CRBP) and two types of cellular retinoic acid-binding protein (CRABP) which will not be discussed here. The intracellular or cytoplasmic FABPs form a group of at least nine distinct protein types. They are 14- to 15-kDa proteins of 126–134 amino acids, and are named after the first tissue of isolation or identification (table 1). Some tissues contain several types, either in different cell types (brain, kidney, stomach) or in the same cell type (enterocyte). Heart (H-)FABP is the most widely distributed FABP. It is found in heart, skeletal and smooth muscle, mammary epithelial cells, aorta, distal tubules of the kidney, lung, brain, placenta and ovary.

The members of the FABP family show an amino acid sequence similarity of 22–73 % (fig. 1), but their three-dimensional structures remain highly conserved. The amino acid sequence of the human T-FABP is not known. On the basis of the amino acid sequence, the family can be divided into three groups: one containing H-FABP, brain (B-)FABP, myelin (M-)FAB; adipocyte (A-)FABP and epidermal (E-)FABP; a group containing ileal lipid-binding protein (I-LBP) and liver (L-)FABP; and intestinal (I-)FABP. I-LBP and L-FABP have a four-residue gap in the C-terminal part that is conserved in the other FABP types. This feature is probably related to their different binding characteristics. L-FABP and I-LBP bind more bulky, hydrophobic ligands, such as lysophospholipids, prostaglandins, bile acids, eicosanoids and some drugs [9–11]. Unlike L-FABP, which can bind two FAs simultaneously, I-LBP has no affinity for FAs [12]. I-FABP differs from both groups of FABP types in the conformation of its bound ligand (bent instead of U shaped) [13, 14].

Table 1. Tissue occurrence of FABP types.

FABP type	Abbreviation	Tissue
Liver	L	liver, intestine, kidney, stomach
Intestinal	I	intestine, stomach
Heart	H	heart, kidney, skeletal muscle, aorta, adrenals, placenta, brain, testes, ovary, lung, mammary gland, stomach
Adipocyte	A	adipose tissue
Epidermal	E	skin, brain, lens, capillary endothelium, retina
Ileal	IL	Intestine, ovary, adrenals, stomach
Brain	B	brain
Myelin	M	peripheral nervous system
Testicular	T	testis

The indication of an FABP type in a tissue does not mean its presence in all cell types of that tissue; the FABP type may be limited to specific cells or may be present at certain developmental stages.

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H-FABP  MVDA..FLGT WKLVDSKNFD DYMKSLGVGF ATRQVASMT. .KPTTIIEKN  45
B-FABP  MVEA..FCAT WKLTNSQNFD EYMKALGVGF ATRQVGNVT. .KPTVIISQE  45
M-FABP  .SNK..FLGT WKLVSSENFD DYMKALGVGL ATRKLGNLA. .KPTVIISKK  45
A-FABP  MCDA..FVGT WKLVSSENFD DYMKEVGVGF ATRKVAGMA. .KPNMIISVN  45
E-FABP  MATVQQLEGR WRLVDSKGFD EYMKELGVGI ALRKMGAMA. .KPDCIITCD  47
I-FABP  MA....FDST WKVDRSENYD KFMEKMGVNI VKRKLAAHDN LK..LTITQE  43
L-FABP  MS....FSGK YQLQSQENFE AFMKAIGLPE EL..IQKGKD IKGVSEIVQN  43
I-LBP   MA....FTGK FEMESEKNYD EFMKLLGISS DV..IEKARN FKIVTEVQQD  43

H-FABP  GDILTLKTHS TFKNTEIS.F KLGVEFDETT ADDRKVKSIV TLD.GGKLVH  93
B-FABP  GDKVVIRTLS TFKNTEIS.F QLGEEFDETT ADDRNCKSVV SLD.GDKLVH  93
M-FABP  GDIITIRTES TFKNTEIS.F KLGQEFEETT ADNRKTKSIV TLQ.RFSLNQ  93
A-FABP  GDVITIKSES TFKNTEIS.F ILGQEFDEVT ADDRKVKSTI TLD.GGVLVH  93
E-FABP  GKNLTIKTES TLKTTQFS.C TLGEKFEETT ADGRKTQTVC NFT.DGALVQ  95
I-FABP  GNKFTVKESS AFRNIEVV.F ELGVTFNYNL ADGTELRGTW SLE.GNKLIG  91
L-FABP  GKHFKFTITA GSKVIQNE.F TVGEECELET MTGEKVKTVV QLEGDNKLVT  92
I-LBP   GQDFTWSQHY SGGHTMTNKF TVGKESNIQT MGGKTFKATV QME.GGKLVV  92

H-FABP  LQKWD...GQ ETTLVRELID G.KLILTLTH GTAVCTRTYE KEA         132
B-FABP  IQKWD...GK ETNFVREIKD G.KMVMTLTF GDVVAVRHYE KA.         131
M-FABP  VQRWN...GK ETTIKRKLVD G.KMVAECKM KGVVCTRIYE KV.         131
A-FABP  VQRWN...GK STTIKRKRED D.KLVVECVM KGVTSTRVYE RA.         131
E-FABP  HQEWD...GK ESTITRKLKD G.KLVVECVM NNVTCTRIYE KVE         134
I-FABP  KFKRTD.NGN ELNTVREIIG D.ELVQTYVY EGVEAKRIFK KD.         131
L-FABP  TF.....KN. .IKSVTELNG D.IITNTMTL GDIVFKRISK RI.         126
I-LBP   NF.....PN. .YHQTSEIVG D.KLVEVSTI GGVTYERVSK RLA         127
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Figure 1. Alignment of amino acid sequences of the members of the FABP family. All sequences are for human proteins. Positions of well-conserved amino acids (identical residues present in at least five molecules) are in bold. For human T-FABP, the sequence is unknown (LBP, liquid-binding protein).

The more recent FABP members to be identified are B-FABP and E-FABP. The human B-FABP amino acid sequence shows 67 % homology with H-FABP, whereas E-FABP is 48 % homologous with H-FABP. B-FABP is exclusively expressed in the central nervous system where its main function is possibly the binding and transport of polyunsaturated FAs, like docosahexaenoic acid (DHA). These FAs are essential during early postnatal development when cellular differentiation, active synaptogenesis and photoreceptor membrane biogenesis take place [15–17]. E-FABP, also known as psoriasis-associated (PA-)FABP [18], keratinocyte FABP [19] or DA11 [20], was first recognized as a psoriasis-associated FABP, one of the gene products whose expression is highly upregulated in human psoriatic skin. Later, the same protein was identified in many other normal tissues such as adipocyte, tongue epithelia, lens, retina, testis, lung and mammary gland [21–23]. The presence of one or more disulphide bridges is a unique feature of E-FABP that may be physiologically relevant [24, 25].

Phylogenetic analyses have shed light on the molecular relationships between the members of the FABP family and their evolution [reviewed in ref. 26]. The presence of FABPs has been established in many non-mammalian tissues, but the pattern of tissue expression is different from mammals [7]. We will largely omit discussion of the non-mammalian FABPs, since recent reviews have elaborated extensively on this subject [7, 26]. The occurrence of a specific basic L-FABP in chicken, catfish and iguana liver is remarkable. FABPs from the same mammalian tissue of different species show greater amino acid similarity and identity than observed between FABPs isolated from different tissues from the same species [4, 9, 27]. Molecular information supports the hypothesis that

FABPs have developed distinct binding sites in order to perform specific functions within the different tissues in which they are expressed.

Gene structure and regulation of expression

Screening of genomic libraries with specific cDNAs identified the genes of eight FABP types (fig. 2). The T-FABP gene structure is not known for any species. The overall organisation of the genes is identical: four exons and three introns for all members of the FABP family, FABPs, CRBPs and CRABPs. The exon/intron positions are similar in all genes, but the intron length is variable. The CRBP-encoding genes have relatively large introns (not shown). Several cis-acting elements in the 5′ promoter region and various trans-acting nuclear factors that may influence transcription have been identified. The structure and regulation of most FABP genes have been reviewed [4, 28]. The structure of the H-FABP gene has been elucidated for mouse [29], rat [30], human [31] and pig [32]. The M-FABP gene has been described in human [33] and mouse [34], the I-FABP gene in human [35], the L-FABP gene in rat [36], the A-FABP gene in mouse [37], the E-FABP gene in mouse [38, 39], and the B-FABP gene also in mouse. The I-LBP gene has been elucidated for mouse [40] and rabbit [41]. Recently, a concise promoter region of the H-FABP gene was found which dictates tissue-appropriate expression [42]. A bile acid-responsive element was identified in the I-LBP gene [43] and a repeated heptad sequence with suppressor and activator functions in the L-FABP gene [44].

Chromosomal mapping of the FABP genes (FABP1–9) shows a dispersed pattern of loci among the genomes (table 2). Within the different species, the genes are located on different but comparable chromosomes. Synteny exists between the regions in which the H-FABP-encoding genes are located [29, 32, 45, 46]. The A-FABP- and I-LBP-encoding genes (FABP4 and FABP6) are also located in chromosomal segments with a syntenic origin

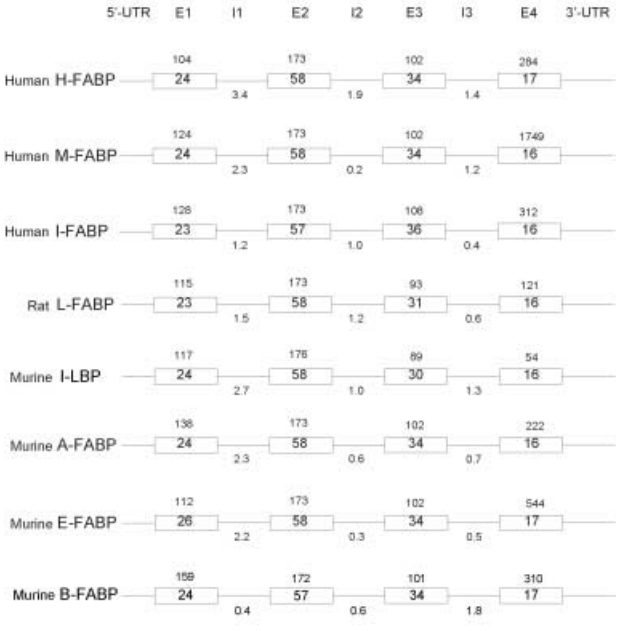

Figure 2. Comparison of the structures of FABP genes. E, exon; I, intron; UTR, untranslated regions of exon 1 and exon 4. The numbers in the boxes indicate the number of amino acids encoded within each exon. The intron lengths are in kilobase pairs, the exon lengths in base pairs. The gene structures were derived from refs 31, 33, 35, 36, 40, 37, 38 and 218.

[47–50]. The human and mouse A-FABP, M-FABP and E-FABP genes are mapped on the same chromosome, A-FABP and M-FABP even in the same subregion (8q21.3–q22.1) [33, 50].

Polymorphisms of the FABP genes have been detected in human, cattle, pig and mouse (table 3). One of the polymorphisms appeared to cause a structural and physiological defect. Substitution of Thr54 by Ala in exon 2 of the human I-FABP (FABP2) gene in the Pima Indian population appeared to be associated with an increase in body mass index, percent body fat and non-insulin-dependent diabetes mellitus (NIDDM) [51]. The threonine-containing protein had a twofold greater affinity for long-chain FAs and the Thr/Thr and Thr/Ala individuals had increased fat oxida-

Table 2. Chromosomal location of genes of members of the FABP family.

FABP type	Human gene	Mouse gene	Chromosome (region)			
			Human	Mouse	Rat	Pig
Liver FABP	FABP1	Fabp1	2(p11)	6	4	–
Intestinal FABP	FABP2	Fabpi	4(q28–q31)	3	2	–
Heart FABP	FABP3	Fabph	1(p32–p33)	4	5q36	6
Adipocyte FABP	FABP4/AP2	Fabpa/Ap2/Albp	8(q21)	3	-	4
Epidermal FABP	FABP5/KLBP	Fabpe/Klbp/Mal1	8	3(3A1-3)	-	–
Ileal FABP	FABP6/ILBP	Ilbp	5(q23–q35)	11	-	–
Brain FABP	FABP7	Fabpb	6(q22–q23)	10	-	–
Myelin FABP	FABP8/PMP2	Fabpm/Pmp2	8(q21.3–q22.1)	3	-	–

All human, mouse and rat gene locations have been retrieved from the human genome database (GDB), mouse genome database (MGD) and rat genome database (RGD), respectively. The pig gene assignments are described by Gerbens et al. [32, 296]

Table 3. Natural occurring polymorphisms identified in FABP genes.

FABP type	Gene	Species	Polymorphism	Position	Reference
I-FABP	FABP2	human	$[TTA]_n$ repeat	intron 2	297
			Ala54Thr	exon 2	51
			A ↔ C	exon 4	298
			G ↔ A	3′ UTR	
		cattle	*Taq*I RFLP	unknown	299
H-FABP	FABP3	pig	*Hae*III RFLP	intron 2	32
			*Hinf*I RFLP	5′ UTR	
			*Msp*I RFLP	intron 2	
			$G[T]_6$	intron 2	300
		cattle	duplication	exon 3	61
		human	Asn98Asp	exon 2	31
			Lys53Arg	intron 3	301
			$[CA]_n$ repeat		
A-FABP	FABP4	pig	$[CA]_n$ repeat	intron 1	296
M-FABP	FABP8	mouse	$[CA]_n$ repeat	3′ UTR	marker D3Mit130

tion rates in vivo and showed insulin resistance compared to Ala/Ala individuals [51, 52]. Caco-2 cells transfected with Thr54 I-FABP-encoding cDNA showed higher long-chain FA transport and triacylglycerol secretion than cells expressing Ala54 I-FABP cDNA [53]. In general, this polymorphism was shown to exert an effect on energy metabolism [reviewed in ref. 54]. Ethnic differences may partly account for the disparate findings on the Ala54Thr polymorphism [55–59].

Two isoforms of bovine H-FABP were reported, with Asp or Asn at position 98 [60]. The heterogeneity was caused by distinct mRNA species [61], but whether this is due to different genes, alternative splicing or RNA editing is not yet clear. Phelan et al. [31] reported a Lys53Arg substitution in human H-FABP which had a low frequency in a Swedish population and showed no association with breast cancer incidence.

Data on the presence of isoforms of L-FABP are conflicting. On the one hand, L-FABP was suggested not to exist as isoforms but that the two fractions of rat L-FABP isolated after proteolytic cleavage represent native conformers [62]. On the other hand, two isoforms differing both in structure and ligand binding were detected by circular dichroism, time-resolved fluorescence spectroscopy and binding/displacement of fluorescent ligands [63, 64]. The two isoforms (I and II) differ at residue 105, being Asn in the former and Asp in the latter [64]. Isoform II probably has a more open conformation than isoform I, thus allowing the binding of a greater variety of ligands. These characteristics suggest that rat L-FABP isoforms may accomplish different functions, similar to the distinct L-FABP types in non-mammalian species [64, 65]. Three charged isoforms of rat L-FABP were attributed to modifications or mutations of Cys69 [66]. Besides single amino acid substitutions, the different isoforms of L-FABP may also be explained by bound ligands, protein conformation or post-translational S-thiolation and/or acetylation [reviewed in ref. 67].

Structure of FABPs

Crystallography [14, 24, 68–72] and/or nuclear magnetic resonance (NMR) [73–77] studies have revealed the tertiary structure of I-FABP, H-FABP, A-FABP, M-FABP, L-FABP, E-FABP, B-FABP, L-FABP and I-LBP. Figure 3 shows the structure of H-FABP, I-FABP and L-FABP. Structural properties of H-FABP, A-FABP and L-FABP have been reviewed recently [78–80]. All FABP types show similar structural features. They are composed of ten antiparallel β strands (βA-βJ) that form a β barrel. The bound ligand is found within the barrel in a central internal water-filled cavity. The interior of the cavity is determined by the sidechains of both hydrophobic and polar amino acids, and is variable between the different FABP types. These buried amino acids probably determine the volume of the cavity and the binding specificity. Internal water molecules within the cavity are assumed to contribute to the protein stability. Certain internal water molecules are well ordered and highly conserved in homologous proteins. In FABPs, these water molecules function in the displacement of FA and maintain the electrostatic interactions inside the binding cavity. Holo H-FABP contains at least 13 ordered water molecules [81]. I-FABP was recently reported to have a cluster of internal water molecules located within its FA-binding cavity (~20 in the apo and 6–8 in the holo form), and a single 'structural' internal water molecule (w135) located in a pocket close to the external surface [82]. The latter water molecule is present in the wedge formed by the loop between βD and βE and the peptide group of Trp82 and forms three H bonds with the protein backbone.

The overall tertiary structures of M-FABP, I-FABP, A-FABP and H-FABP types are very similar (fig. 3) [13]. The apo and holo structure are comparable for I-FABP and A-FABP based on their X-ray diffraction analysis [68, 70]. The FA is bound in a bent conformation in I-FABP. The three-dimensional structure of human H-FABP with bound FA was resolved and refined by X-ray diffraction to 2.1-Å resolution [69]. The FA appeared to bind in a U-shaped conformation. The carboxylate group of the FA binds within the protein cavity and interacts with the side chains of Tyr128 and Arg126 and several ordered water molecules. In this network of hydrogen bonds, Arg106 and Thr40 are indirectly involved in FA binding. The inner cavity of H-FABP is connected to the external solvent through a small opening, formed by side chains of Val25, Thr29, Phe57, Lys58, Ala75 and Asp76. A positive charge on the edge of the opening (Lys58) has been postulated to be the driving

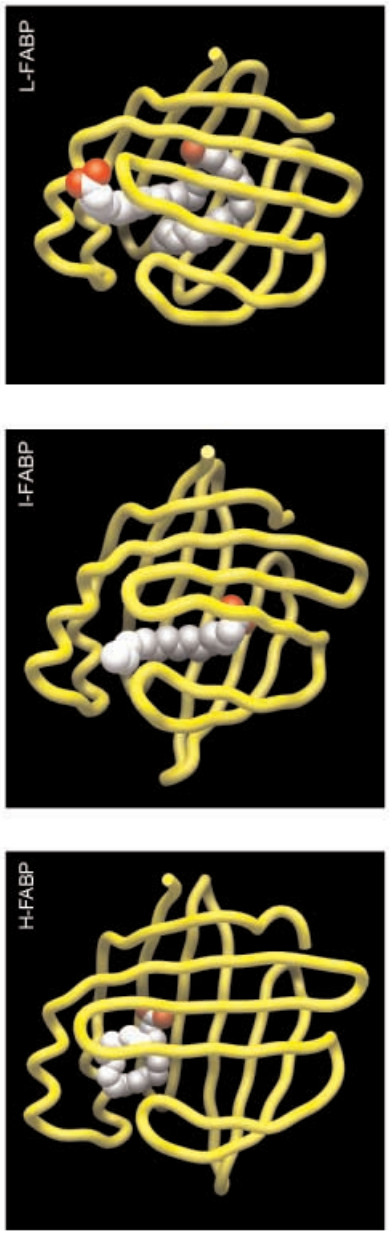

Figure 3. Graphical representation of the three-dimensional backbone structures of FABPs. Panels show H-FABP, I-FABP and L-FABP with a bound oleate molecule. Oleic acid is represented as a space-filling model in red (carboxylate group) and white. Figures were produced using GRASP (C. Lücke, Frankfurt, Germany).

force that attracts the negatively charged FA [81]. A second gap is present between strands βD and βE. Internal solvent molecules are thought to exit the protein via this second gap when a FA ligand enters the portal. However, this area does not represent a real opening, since the space is filled with solvent molecules. Dynamic simulation led to the hypothesis that this region could undergo a zip-like movement to widen the first, small aperture [83, 84]. This movement would not disrupt the hydrogen-bonding network of the barrel.

Although high-resolution X-ray studies on complexes of H-FABP with oleate, elaidate and stearate showed that these FAs are bound in a similar fashion [81], the recently published solution structure of human H-FABP [77] suggests a selected-fit mechanism in FA binding, depending on the chain length of the ligand. This behaviour appears to be especially pronounced in H-FABP, possibly due to its more rigid backbone structure compared with other FABP types.

The structure of M-FABP (P_2) is highly comparable with the structures of H-FABP and A-FABP [71, 85]. Although M-FABP contains two cysteine residues, no disulphide bridge is present. M-FABP is able to bind both FAs and retinoids according to Uyemura et al. [86], but this result could not be confirmed [87].

Recently, the crystal structures of E-FABP [24] and B-FABP [74] became available. Although these FABP types are very similar to H-FABP, M-FABP and A-FABP, they both display unique features. Human E-FABP contains six cysteine residues. A disulfide bridge is formed between Cys120 and Cys127, which contributes to the stability of the protein. The presence of cysteine pairs in E-FABP may also function to relieve oxidative stress in the epidermis and other tissues by thiol-disulphide interchange reaction [25]. Mouse B-FABP is capable of binding DHA [88]. The three-dimensional structure of B-FABP in complex with oleate shows that the hydrocarbon tail assumes a U-shaped conformation (as in H-FABP, A-FABP and M-FABP) whereas in the complex with DHA, the hydrocarbon tail adopts a helical conformation. The binding specificity of B-FABP appears to be the result of the non-conserved amino acid Phe104, which interacts with double bonds present in the lipid hydrocarbon tail [72]. Richieri et al. [89] observed, however, a weaker affinity of human B-FABP for polyunsaturated FAs than for saturated and monounsaturated FAs.

The three-dimensional structure of porcine I-LBP was resolved by high-resolution NMR and appeared similar to other FABP types but highly flexible, with a relatively weak hydrogen-bonding network [76]. NMR data support the hypothesis that the shortened β strands G and H found only in I-LBP and L-FABP produce a wider opening [90]. This flexibility probably allows a bile acid, which is larger and more rigid than an FA, to enter the internal protein cavity. Recently, the solution structure of I-LBP in complex with glycocholate was resolved [76]. The bile acid appeared to be bound inside the protein, with the steroid moiety penetrating deep into the water-accessible internal cavity. The carboxylate tail of the ligand protrudes from the proposed bile acid portal into the surrounding aqueous solution. The non-polar face of the steroid moiety interacts with the hydrophobic residues of β strands C, D and E. The polar face makes contact with the side chains of Tyr97, His99, Glu110 and Arg121 of βH, I and J.

The crystal structure of rat recombinant L-FABP in the presence of oleic acid was completed to 2.3-Å resolution [14]. Two FA molecules are bound within the central cavity (fig. 3). The carboxylate of one FA interacts with Arg122 and is shielded from free solvent. It has an overall bent conformation. The more solvent exposed carboxylate of the other FA is located near the helix-turn-helix that caps one end of the β barrel, while the acyl chain lies in the interior. The primary and secondary oleate-binding sites appear to be totally interdependent, mainly because favourable hydrophobic interactions form between both aliphatic chains. As in other FABP types, Arg122 appeared to be important for the binding of the first FA [11, 91]. Serine residues at positions 39 and 124 also play a role in ligand binding. Structural data on L-FABP confirm the presence of βG and βH strands shorter than the average for the FABP family. The decreased number of intra-main chain hydrogen bonds girding the portal region suggests a greater degree of possible motion in L-FABP.

All FABP structures determinated to date by NMR or crystallography showed two α-helical regions [13]. To investigate the structural and functional role of this conserved helical domain of FABPs, a helix-less variant of rat I-FABP was engineered by deleting residues 15–31 and inserting a Ser-Gly linker after residue 14 [92]. Circular dichroism measurements and NMR spectra indicated that this I-FABP variant (named I-FABP HL) has a high β sheet content and a β clam topology similar to that of the wild-type protein [92]. Triple-resonance three-dimensional NMR revealed that the backbone conformation of I-FABP HL is nearly superimposable with the β sheet domain of wild-type I-FABP, and that deletion of the α-helical domain creates a very large opening that connects the interior cavity with the exterior solvent [93]. I-FABP HL was less stable to guanidine treatment than wild-type I-FABP. The FA-protein interactions of I-FABP HL were similar to those of the wild-type I-FABP at the carboxylate end of the FA, but not at the methyl end. Ligand association rates for the helix-less variant and the wild-type protein were comparable, but the dissociation rate was 16-fold lower for the wild-type protein [94]. These data indicate that the α helices of I-FABP are not required to maintain the integrity of the FA-binding cavity but may serve to regulate the affinity of FA binding. FA transfer studies showed that in the absence of the α-helical domain, effective collisional transfer of FA to phospholipid membranes does not occur, indicating that the α-helical region of FABP is essential for interaction with membranes [95, 96].

Structure-function relationship of FABPs

Site-directed mutagenesis has been used to study more systematically the structure-function relationship of FABPs. Conserved residues in the amino acid sequence alignment are putative targets for mutagenesis. Target amino acids are chosen on the basis of the putative importance of their side chains in FA binding or for the maintenance of protein conformation, based on the elucidated three-dimensional structure.

Various single amino acid mutants of different FABP types have been studied for ligand specificity and affinity, and protein stability. The highly conserved Arg126 residue appeared to be important for binding affinity and ligand specificity of A-FABP, since mutation to Gln caused a strong decrease in affinity for *cis*-parinaric acid [97]. Mutation of Arg106, Arg126 or Tyr128 markedly affected FA binding and conformational stability of H-FABP [98]. Replacement of Arg106 by Gln in rat I-FABP caused a 20-fold increase in the dissociation constant for oleic acid with a 6-fold decrease of the enthalpic contribution to the free energy of binding [99]. For rat L-FABP also, mutation of Arg122 indicated a major role of this amino acid in ligand binding and structural integrity [11, 91].

Hydrophobic amino acid residues are thought to play a role in ligand affinity and/or specificity by forming Van der Waals contacts with the acyl chain of the bound FA. Amino acid residues Phe16 and Phe57 of H-FABP are both located near the putative FA entry site and make Van der Waals contacts with the bound ligand. The phenyl ring of Phe16 may form a key determinant in FA specificity and affinity of H-FABP [69], since it may change its orientation upon ligand binding [13]. The importance of Phe16 in ligand binding was confirmed by studies on H-FABP mutants [98, 100]. Results of mutations of Phe57 in B-FABP and H-FABP indicate that this residue is not a major contributor to the FABP/ligand interaction [88, 98]. In A-FABP, however, Phe57 appeared critical for the formation of the FA/A-FABP complex and the stability of the protein, but was not involved in determination of selectivity for ligands [101, 102]. Phe57 displayed a greater mobility in A-FABP relative to H-FABP [103]. Mutation of Gly33 of B-FABP, which is adjacent to the opening of the binding pocket, to a bulkier amino acid could prevent access of the ligand to the binding pocket by partially occupying the volume normally used by the ligand [88].

All members of the FABP family contain nine turns, eight of which are between antiparallel β strands. Seven of these turns contain a glycine residue. The turn between β strands D and E is of particular interest, since no hydrogen bonds are present between the main chain atoms of these two strands. A series of single, double and triple mutations within this turn in I-FABP resulted in a large decrease in oleic acid binding and loss of stability [104]. These data indicate that this region may be involved in the last stage of the protein-folding process. I-FABP mutants that lack Leu64 appeared to have a lower conformational stability and a high rate of refolding [104]. Systematic mutation of the glycine residues located in the turns between the β

strands of I-FABP showed that mutations in any of the three turns connecting the last four C-terminal strands slow the folding and decrease stability, whereas for most of the other turn mutations, no apparent correlation was observed between stability and refolding rates [105]. The α helices of I-FABP are not required to maintain the integrity of the binding cavity, since a helix-less variant of I-FABP showed similar β sheet and β clam topology and FA-binding characteristics as the wild-type protein [92, 94].

Acetylation and substitution of external lysine residues of H-FABP and A-FABP located on the β2 turn and helices αI and αII did not change the binding affinity for FA, but changed the transfer rate of 2-(9-anthroyloxy)palmitate (2-AP) to phospholipid membranes [106–108]. A lysine to isoleucine mutation in the non-portal βA strand of A-FABP also decreased the 2-AP transfer rate, suggesting that not only the portal region but also other distinct regions are involved in electrostatical interactions between FABPs and membranes [108].

Studies on mutants of I-FABP and A-FABP revealed binding affinities ranging from about 200-fold smaller to 30-fold larger than the wild-type proteins [109, 110]. Reduced rates of binding were generally, but not exclusively, associated with sites within the portal region [110]. In A-FABP, Ala substitutions for Arg106 and Arg126, which interact with the FA carboxylate, reduced affinities by about 100-fold, but in I-FABP, R106A has a 30-fold higher affinity. An explanation for the latter is that the loss in enthalpy due to the elimination of the favourable interaction between the FA carboxylate and Arg106 is compensated for by an increase in entropy [109, 110]. Enthalpy and entropy measurements appear to provide more insight about the FA-FABP interaction than affinity measurements alone [111].

FA uptake and transport

Most eukaryotic cells are capable of taking up long-chain FAs to be used for a variety of cellular processes. Extracellularly (in the blood), FAs are transported mainly in complexes with albumin which has three high-affinity binding sites [1]. FA uptake by the eukaryotic cell may occur both by passive diffusion and by protein-mediated binding and translocation mechanisms. Diffusion of FAs across the plasma membrane is a fast process, also called 'flip-flop' [112–114]. It is driven by an inwardly directed FA gradient, with an extracellular FA concentration in 10- to 20-fold excess over the intracellular content [115, 116].

Hamilton and Kamp [117] suggest plasma membrane FA transporters function indirectly by increasing the FA partitioning into the membrane or sequestering FA to a membrane-bound enzyme, thereby enhancing metabolism [117]. Recently, Stump et al. [118] found that oleic acid uptake by rat adipocytes is the sum of saturable (facilitated) and linear (flip-flop) processes. Over 90 % of the transport occurs via the saturable pathway when the oleic acid bovine serum albumin (BSA) ratio is within the physiological range. In contrast to data reported by Hamilton and Kamp [117], they found that rate constants for saturable transmembrane influx were faster than those for non-saturable uptake. The discrepancies between the data of the different groups are possibly due to the different methods used (tracer kinetic studies versus intravesicular acidification rate measurements).

Protein-facilitated FA uptake has been shown in cardiac myocytes [119, 120], adipocytes [121, 122] and hepatocytes [123, 124]. Several membrane-associated FA transporters have been identified during the last decade [5]. Plasma membrane FABP ($FABP_{PM}$, [125]), FAT (CD36, [126]) and FATP [127] act as long-chain FA transporters [128].

Intracellularly, FAs are bound by FABPs, which are considered to be important carriers for intracellular FAs. They increase FA solubility and facilitate transport of FA from the plasma membrane to sites of FA oxidation (mitochondria, peroxisomes), to sites of FA esterification into triacylglycerols (TGs) or phospholipids, or to the nucleus, possibly for regulatory functions (fig. 4). Several studies have demonstrated FA transfer between FABPs and membranes by use of radiolabelled or fluorescent FAs, or by NMR [3, 4, 129–131]. In hepatocytes and adipocytes, uptake of photoactivatable radiolabelled FAs was accompanied by labelling of FABPs [132, 133]. FA transfer by FABPs has been studied with the help of fluorescent anthroyloxy-labelled fatty acids (AOFAs) and phospholipid vesicles [131]. AOFA transfer from different FABP types to phospholipid membranes occurs by distinct mechanisms [reviewed in ref. 134]). AOFAs are

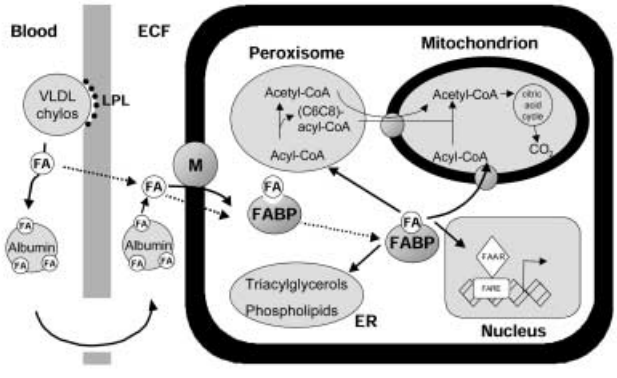

Figure 4. Schematic representation of the role of FABPs in FA uptake and intracellular FA trafficking. FA, fatty acid; VLDL, very low density lipoprotein; chylos, chylomicrons; LPL, lipoprotein lipase; ECF, extracellular fluid; M, membrane-associated FA transport protein; ER, endoplasmic reticulum; FAAR, fatty acid-activated receptor; FARE, fatty acid-responsive element. FABP can also be present in the nucleus (see text).

transferred from I-FABP, H-FABP and A-FABP by collisional interaction with an acceptor membrane, since their rate of transfer increases linearly with the concentration of acceptor vesicles. In contrast, L-FABP transfers AOFA to membranes in an aqueous diffusional manner involving an initial and obligatory release of ligand to the aqueous environment. The transfer rates differ markedly between different FABP types. With 12-(9-anthroyloxy)oleic acid, the transfer rate is A-FABP > H-FABP ≫ I-FABP ≫ L-FABP [131]. When membranes contain anionic phospholipids, the AOFA transfer rate from H-FABP and A-FABP increases, suggesting that positively charged amino acid residues on the FABP surface are involved in the interaction between FABP and membrane. The interaction between A-FABP and membranes appeared to be electrostatic, since binding was dependent on the molar concentration of anionic phospholipid, and decreased when acetylated A-FABP was used [135]. When surface lysine residues of H-FABP and A-FABP were neutralized by acetylation, the transfer rate decreased markedly and the transfer mechanism changed to aqueous diffusion instead of collisional interaction [106, 107, 136]. Mutation of single lysine residues showed that the helix-turn-helix domain, and especially Lys21 in H-FABP and A-FABP, is critical for interaction with anionic acceptor membranes [107, 108]. Deletion of the α-helical domain of I-FABP altered the regulation of AOFA transfer to acceptor membranes, making the normally collision-mediated process more characteristically diffusion mediated [95]. Transfer of AOFA from phospholipid membranes to different FABP types also appeared to occur by different mechanisms. As with transfer from FABP to membranes, the process is diffusion mediated for L-FABP and collisional for I-FABP [137].

The technique of fluorescence recovery after photobleaching (FRAP) was used to measure the intracellular transport of a fluorescent fatty acid [12-N-methyl-(7-nitrobenzo-2-oxa-1,3, diazol)aminostearate; NBD-stearate] [138, 139]. Cytoplasmic transport of NBD-stearate could be inhibited by α-bromo-palmitate [138]. Transfection of L-cell fibroblasts with L-FABP or I-FABP cDNA increased NBD-stearate uptake and cytoplasmic transport [139]. The latter appeared to be dependent on FABP concentration and binding activity [140, 141], which is consistent with the proposition that binding proteins enhance diffusive transport by reducing ligand binding to immobile intracellular membranes [142]. Recently, a kinetic model of intermembrane ligand transport was developed in which diffusional transfer of ligand between membrane and protein is assumed [143]. This model was tested by using the stopped-flow technique to monitor transfer of 12-anthroyloxy stearate (12-AS) between model membrane vesicles. The 12-AS transfer rate was shown to decline asymptotically with increasing concentrations of BSA or L-FABP and was linearly correlated with the concentration of I-FABP, in agreement with the data of Storch et al. [131].

Experimental data of different investigators led to paradoxical conclusions about intermembrane FA transport by L-FABP. Most studies support a diffusional mechanism of FA transfer [143–147]. Other investigators have shown that the binding of L-FABP to anionic lipid vesicles results in ligand (DAUDA) release, consistent with a collisional transport mechanism [148]. The decrease of the rate of intermembrane 12-AS transfer with increasing FABP concentration supports, however, a predominantly diffusional mechanism of L-FABP-mediated transport [143]. FRAP analyses with NBD-stearate support the concept that BSA and L-FABP enhance FA diffusion [138], while vesicle transfer studies with 12-AS indicate that BSA and L-FABP inhibit intermembrane FA translocation [143]. These contrasting observations may be due to the fact that both NBD-stearate and 12-AS carry a different attached fluorescent group which may alter the physical properties of the FA molecule. Another explanation is that FRAP analyses do not distinguish between membrane- and protein-bound ligands and wrongly assume steady-state conditions [143].

In a study of FA transfer from immobilized liposomes to rat liver or heart mitochondria, FABPs stimulate transfer but no preference for any FABP type was observed [149]. The transfer rate was higher from positively charged liposomes than from neutral or negatively charged liposomes. I-LBP did not transfer FA. The significance of intracellular bile acid-binding proteins for bile acid transport remains to be established [150]. I-LBP appears to bind preferentially conjugated bile acids in contrast to L-FABP [12, 151]. A correlation was found between the presence of L-FABP in bile and both bile flow and bile acid release [152].

Function of FABPs in modulation of signal transduction and gene transcription

FAs, their CoA and carnitine esters and other lipid mediators, such as eicosanoids and lysophospholipids, may directly or indirectly influence various cellular processes by their interactions with enzymes, membranes, ion channels, receptors or genes [3, 129, 153–155]. By modulation of the concentrations of unesterified FA and their derivatives, FABPs have an indirect effect on these parameters. Besides FAs, L-FABP also binds lysophospholipids and eicosanoids [9]. H-FABP was recently shown to bind cytochrome P450 monooxygenase and lipoxygenase pathway products [156]. Distinct FABPs may differentially affect processes that can be influenced by FAs. Examples of such processes are given below.

FAs (in particular unsaturated FAs) are able to act as second messengers involved in the transduction of external

signals because their concentrations are rapidly and transiently altered in response to the binding of specific agonists to plasma membrane receptors. They may substitute for the classical second messengers of the inositide phospholipid and the cyclic AMP signal transduction pathways [153–155]. FAs inhibit growth factor-induced diacylglycerol kinase α activation in vascular smooth muscle cells and increased levels of FAs may contribute to chronic protein kinase C activation associated with diabetes [157]. Very recently, Collett and co-workers [158] showed that n-6 and n-3 polyunsaturated FAs differentially modulate oncogenic Ras activation in colonocytes. FA, especially polyunsaturated FAs, such as arachidonic acid and linoleic acid, directly regulate Na^+, K^+, Ca^{2+} and Cl^- ion channels [159–163].

FAs play a role in the transcription of genes, especially those genes which encode proteins involved in lipid metabolism, e.g. acyl-CoA synthase [164], acyl-CoA oxidase [165, 166], stearoyl-CoA desaturase [165, 167] and carnitine-palmitoyl transferase [168]. The transcription of FABP and FATP genes is also promoted by FAs [164, 169–174]. Recently, FA regulation of gene transcription was reviewed by Duplus et al. [175]. In mammals, the expression of many genes has been shown to be modulated by FAs in a positive or negative manner. The control of hepatic lipogenic enzymes is an example of negative regulation. Clarke and Jump [165] reported that polyunsaturated FAs of the n-6 and n-3 families, in contrast to saturated and monounsaturated FAs, inhibit transcription of a number of hepatic lipogenic and glycolytic genes by a mechanism that does not involve peroxisome proliferator-activated receptors (PPARs), a subfamily of the nuclear hormone receptors [176–180]. Up to now, three types of PPAR have been described: PPARα, PPARβ [also known as FA-activated receptor (FAAR) or PPARδ] and PPARγ. Their differential tissue distribution suggests that they have specific roles in different organs. PPARα is predominantly expressed in the liver and brown adipose tissue and plays an important role in FA catabolism [181]. PPARγ is highly expressed in adipocytes where it is involved in the regulation of adipose differentiation and adipogenesis [182]. PPARδ or FAAR displays a high level of expression in lipid-metabolizing tissues, such as adipose tissue, small intestine, heart and skeletal muscle and could regulate the expression of genes implicated in FA uptake and activation [183]. It also mediates the effects of long-chain FAs on post-confluent cell proliferation [184]. Naturally occurring (FAs, leukotrienes, prostaglandins) and synthetic (fibrates, glitazones) molecules that are ligands for these nuclear receptors control the transcriptional activity of PPARs [185, 186].

Intriguingly, the expression of FABPs is regulated by FAs and other PPAR ligands. Wy14,643 (a synthetic activator of PPARα) induces L-FABP, I-FABP and H-FABP mRNA in the respective mouse tissues [187]. Wy14,643 was also shown to enhance the binding activity of PPARα/RXRα to peroxisome proliferator responsive elements of acyl CoA oxidase and L-FABP genes [188]. Activation of PPARδ by FA induced transcription of genes encoding FAT, A-FABP and PPARγ in 3T3C2 fibroblasts, resulting in lipid accumulation and adipocyte differentiation [189]. FAs are involved as signal-transducing molecules in the differentiation of preadipose to adipose cells and induce A-FABP expression [169–171, 190]. A sunflower oil-enriched diet specifically increased L-FABP mRNA and protein in duodenum and proximal jejunum, but did not affect I-FABP levels [191]. Targeted disruption of the gene encoding PPARα fully abolished the hepatic induction by fibrates of the gene encoding L-FABP [181]. Interestingly, analysis of long-chain FA- and fibrate-mediated effects on L-FABP mRNA levels in wild-type and PPARα null mice showed that PPARα in the intestine does not constitute a dominant regulator of L-FABP gene expression [192]. Instead, PPARδ can act as a fibrate/FA-activated receptor, and L-FABP is a PPARδ target gene in the small intestine [192]. Bile components appear to regulate I-LBP expression in the ileum [193].

FABPs have been detected in the nucleus of hepatocytes [194, 195], heart myocytes [196], locust myocytes [197] and astrocytes [198]. Recently, the role of L-FABP in FA transport to the nucleus was examined using fluorescein-conjugated L-FABP [199]. L-FABP appeared to interact directly with rat liver nuclei in a specific, ligand-dependent manner. An interaction was observed between L-FABP and a 33-kDa nuclear protein, which was enhanced in the presence of oleic acid. These data indicate that L-FABP is involved in communicating the state of FA metabolism from the cytosol to the nucleus through an interaction with lipid mediators that are involved in nuclear signal transduction [199]. A direct role for L-FABP in the regulation of gene expression was reported by Wolfrum et al. [200]. They found co-localization of L-FABP and PPARα in the nucleus of mouse primary hepatocytes. L-FABP interacted with PPARα and PPARγ but not with PPARβ and RXRα by protein-protein contacts. With all ligands applied, a strict correlation of PPARα and PPARγ transactivation with intracellular concentration of L-FABP was observed. Together these findings strongly suggest nucleus-directed signalling by FA and hypolipidemic drugs where L-FABP may act as a cytosolic gateway for these PPARα and PPARγ agonists [200].

Data from FABP knockout mice

The generation of knockout mice a couple of years ago was an important step forward in understanding the physiological role of FABPs. Table 4 gives an overview of

Table 4. Effects of FABP loss in knockout mice.

Knockout	Characteristics	Reference
H-FABP	exercise intolerance localized cardiac hypertrophy	201
	reduced oleate/palmitate uptake increased glucose oxidation	202
A-FABP	normal phenotype (compensation by E-FABP)	203
	protection from insulin resistance at induced obesity	205
	altered cytokine production and less accumulation of cholesterol in macrophages	208
I-FABP	normal phenotype	210
	hyperinsulinaemia gender-specific body weight gain	210
I-LBP	normal phenotype normal bile acid pool size	211
E-FABP	normal phenotype upregulation of H-FABP altered water permeability of the skin	212

FABP knockouts generated to date and their phenotypical and physiological characteristics. Mice lacking H-FABP exhibit a severe defect in peripheral long-chain FA utilization. The heart is unable to take up efficiently plasma long-chain FAs which are normally its main fuel, and switches to glucose usage [201]. H-FABP deficiency is only incompletely compensated, causing acute exercise intolerance and, at old age, a localized cardiac hypertrophy [201]. Furthermore, in resting and contracting cardiac myocytes from H-FABP null mice, both uptake and oxidation of palmitate are markedly reduced [202]. In resting H-FABP-deficient cardiac myocytes, glucose oxidation is increased by 80%. Taken together, these findings provide evidence that H-FABP plays a crucial role in the uptake and oxidation of long-chain FAs, in fuel selection and in metabolic homeostasis.

Mice lacking A-FABP (or aP2) are healthy, develop apparently normal adipose tissue, and exhibit only minor alterations in their steady-state lipid metabolism [203]. This lack of significant effect of A-FABP deficiency was potentially the consequence of the compensatory upregulation E-FABP mRNA [204, 205], which is otherwise present at only low levels in adipose tissue [19]. There was no difference in the rate of FA influx or esterification in adipocytes of wild-type and A-FABP null mice, but basal lipolysis was approximately 40% decreased in A-FABP null mice [204]. A-FABP null mice with induced obesity failed to express tumour necrosis factor-α in adipose tissue and were significantly protected from hyperinsulinemia and insulin resistance compared with wild-type mice, suggesting that adipocyte FA metabolism is a critical component of the mechanisms leading to systemic insulin resistance in obesity [203, 205]. The role of A-FABP in pathogenesis of type 2 diabetes may involve the regulation of hyperinsulinaemia and insulin resistance through its impact on both lipolysis and insulin secretion [206]. Experiments with obese A-FABP null mice indicated that A-FABP deficiency not only improves peripheral insulin resistance but also preserves pancreatic β cell function and has beneficial effects on lipid metabolism [207]. Recently, the expression of A-FABP in macrophages was demonstrated [208]. A-FABP appeared to play a significant role in the biological responses of macrophages, and contributes to the development of atherosclerosis. Apolipoprotein E-deficient mice also deficient for A-FABP showed protection from atherosclerosis, and A-FABP-deficient macrophages exhibited alterations in inflammatory cytokine production and a reduced ability to accumulate cholesterol esters when exposed to modified lipoproteins [208]. Oxidized low-density lipoprotein appears to induce the expression of A-FABP mRNA and protein in human macrophages [209]. These data indicate distinct actions of A-FABP in adipocytes and macrophages and could provide a new therapeutic target for the prevention of atherosclerosis.

I-FABP null mice were used to study the role of I-FABP in the uptake of dietary FAs [210]. I-FABP null mice appeared viable but display alterations in body weight and are hyperinsulinemic. Male I-FABP null mice had elevated plasma triacylglycerols and weighed more regardless of the dietary fat content. In contrast, female I-FABP null mice gained less weight in response to a high fat diet. These findings led to the idea that I-FABP is not essential for dietary fat absorption but may rather function as a lipid-sensing component of energy homeostasis that alters body weight gain in a gender-specific fashion [210]. I-LBP knockout mice showed the same phenotype and bile acid pool size as wild-type mice [211].

E-FABP-deficient mice were viable and showed no macroscopical aberrations compared to wild-type mice, but H-FABP gene expression was upregulated and may compensate for the lack of E-FABP [212]. The basal transepidermal water loss of the E-FABP null mice was, however, decreased compared to wild-type mice, and these animals were not able to recover this loss when the lipid barrier was disrupted. These results indicate that E-FABP may be involved in the formation of the water permeability barrier of the skin.

Expression of FABPs and their role in growth, differentiation and cytoprotection

The expression of FABP mRNA and the synthesis of FABPs are dependent on cell differentiation and postnatal development for various FABP types in different tissues [4, 213], e.g. I-FABP and L-FABP in intestine [214], L-FABP in liver [215] and H-FABP in various

tissues [216]. For brain, more recent data are available [reviewed in ref. 17]). The brain contains three different types of FABP: H-FABP [47], B-FABP [217, 218] and E-FABP [20, 219]. The genes for these brain FABP types show a spatiotemporally differential expression in developing and mature brain [219, 220]. The expression of H-FABP becomes evident in rat brain after birth, with a gradual increase, and is confined to the grey matter, suggesting that its mRNA is neuron specific [220]. In mouse brain, H-FABP levels are detectable after fetal day 19 and increase until post-natal day 14, but become lower in the adult brain [221]. In contrast to H-FABP, the expression of B-FABP and E-FABP is dominant in the pre-natal and peri-natal mouse and rat brain [219, 220]. E-FABP mRNA and protein are expressed at high levels during neurogenesis, neuronal migration and terminal differentiation of neurons [219, 222].

Hormones and physiological changes have a particular effect on the expression of L-FABP [9, 213, 223]. The H-FABP content of heart was markedly changed by exercise and testosterone [129, 213]. Bacterial lipopolysaccharide downregulated H-FABP in rat heart and muscle and L-FABP in liver; cytokines only downregulated L-FABP in liver [224]. In contrast to L-FABP, I-FABP expression is limited to fully differentiated human Caco-2 cells and can be more easily regulated by lipids, hormones and cytokines [225].

Members of the FABP family also appear to be connected with the modulation of cell growth. L-FABP modulates the mitogenesis of liver and hepatoma cells [215]. L-FABP cDNA transfection of hepatoma cells increased the efficacy of the utilization of unsaturated FAs, especially linoleic acid, leading to a higher proliferation rate [226]. Furthermore, maintenance of membrane integrity and preservation of morphology were promoted in L-FABP cDNA-transfected hepatoma cells, although others found an increase in plasma membrane fluidity in L-FABP cDNA-transfected L-cell fibroblasts [227]. Two classes of carcinogenic peroxisome proliferators are activated by L-FABP, resulting in cell multiplication in hepatoma cells [228]. L-FABP expression in rat enzyme-altered foci is determined by the initiating carcinogenic regime [229]. Embryonic stem cells transfected with cDNA encoding L-FABP showed morphological changes, a reduced level of stage-specific embryonic antigen-1, and localization of L-FABP in both the cytosol and the nucleus [230]. These findings suggest that L-FABP may play a role in regulating embryonic stem cell differentiation by acting in the nucleus as well as the cytoplasm.

Other FABP types have also been implicated in some cases in the regulation of growth and differentiation. Bovine mammary-derived growth inhibitor (MDGI), which was identified as a mixture of H-FABP and A-FABP [231], and H-FABP caused specific growth inhibition and terminal differentiation of mammary epithelial cells [232, 233]. H-FABP cDNA transfection caused a modest anti-proliferative activity in human breast cancer cells [234]. In addition, in vivo tumorigenicity of transfectants expressing H-FABP was reduced [234]. Of interest is that human H-FABP was mapped to chromosome 1p33–p35, a common region of loss in sporadic breast cancer [31]. Furthermore, MDGI appeared to be a potent inhibitor of bovine, mouse and human mammary epithelial proliferation in cell and organ culture [233, 235, 236]. Evidence for decreased H-FABP expression was found in human breast cancer [234]. MDGI-derived peptide P108 inhibits tumour growth of breast cancer cell lines in nude mice [237]. Together, these observations led H-FABP to be considered a tumour suppressor [reviewed in ref. 238]). Differentiation-promoting effects of MDGI/H-FABP were observed in pluripotent mouse embryonic stem cells [239], mammary epithelial cells [233] and cardiac myocytes [240]. Expression of bovine H-FABP in yeast caused inhibition of growth [241], but L6 muscle cells transfected with H-FABP cDNA did not show changes in growth or differentiation compared to mock-transfected cells [242]. Interestingly, the expression of H-FABP in C_2C_{12} muscle cells appeared to be differentiation dependent [243]. Cultured human and rat muscle cells also showed an increase in H-FABP at differentiation [242, 244]. The increasing H-FABP content upon differentiation may relate to increasing FA oxidation that has been reported for differentiated L6 cells [245]. Transfection of L6 cells with A-FABP cDNA increased the proliferation rate and blocked the fusion of these cells, simultaneous with changes in their phospholipid composition [242]. Transfection of MDCK cells with either H-FABP, L-FABP or A-FABP cDNA did not influence proliferation [246]. Some FABP types inhibited cell-free protein synthesis in a reticulocyte lysate, whereas others had no effect [247]. The inhibition was not influenced by delipidation, and for H-FABP mutants was not related to their affinity for FAs.

In some cases, FABPs could be used as tumour markers (table 5). The development of liver and gut cancer appears to be related to changes in FABP content. The FABP content decreases with further dedifferentiation and progression of the tumour. Generally, the level of L-FABP and I-FABP decreased in rat and human colon carcinoma [248, 249]. In human hepatic tumours and rat hepatoma, the L-FABP staining decreased and showed mosaicism [250, 251]. Furthermore, L-FABP expression is suitable for use as a new pre-surgical prognostic marker for patients undergoing hepatic surgery for colorectal cancer metastases [252].

A-FABP synthesis was only detected in lipoblasts in lipoblastoma and liposarcoma, but not in other benign adipose tissue tumours and malignant connective tissue or epithelial tumours [253]. In contrast, A-FABP expression by normal bladder urothelium was lost at various

Table 5. FABP as tumour markers.

FABP type	Human type	Species	Change
L-FABP	hepatoblastoma	H	↓
	hepatocellular carcinoma	H, R	↓
L-FABP, I-FABP	small intestinal and colonic adenocarcinoma	H, R	↓
	colorectal adenoma	H, R	↓
H-FABP	ductal carcinoma mammary gland	H	↓
A-FABP	bladder transitional cell carcinoma	H	↓
	lipoblastoma, liposarcoma	H	+
B-FABP	glioma	H	+
E-FABP	papilloma	M	+
	pancreatic adenocarcinoma	H	↑
	prostate carcinoma	H	↑
	bladder carcinoma	H	↑

H, human; R, rat; M, mouse; ↓, decrease; ↑, increase; +, presence.

stages of carcinoma progression [254]. In addition, low-grade tumours contained more A-FABP than their high-grade counterparts. Protein abundancy and mRNA levels of A-FABP correlate in non-invasive and invasive bladder transitional cell carcinomas, and the loss of A-FABP was not compensated by an increase in E-FABP, as is the case in A-FABP knockout mice [255].

B-FABP expression correlates with glial fibrillary acidic protein (GFAP) in a subset of human malignant glioma cell lines [256]. Its expression is regulated by differential phosphorylation of nuclear factor I (NFI), which is able to bind to two NFI-binding sites in the B-FABP gene [257]. E-FABP expression is increased in drug-resistant human adenocarcinoma of the pancreas and was suggested to be part of a mechanism of sequestration or removal of cytotoxic drugs [258]. The E-FABP gene is overexpressed in most human prostate carcinomas and some squamous carcinomas of the bladder and skin [259–261]. The increased E-FABP expression may be involved in the malignant dissemination of some human cancers, and the E-FABP gene is suggested to be a metastasis-inducing gene [261]. Studies on rat mammary epithelial cells (Rama 37 cells) demonstrated that E-FABP induces metastasis by up-regulating the expression of the vascular endothelial growth factor gene, which is one of the most potent stimulating factors for angiogenesis [262].

The presence of FABPs has also been suggested to provide protection against high intracellular FA concentrations and to prevent their toxic effect on membranes and cells [9]. H-FABP was suggested to bind FAs accumulating under pathophysiological circumstances [263]. H-FABP could also protect the heart by scavenging free radicals [264] and by inhibiting a β-adrenergic response as observed in cultured neonatal rat heart cells [265]. The presence of FABPs would enable a more rapid exchange of FAs among subcellular and intercellular sites, to counteract local FA accumulation [266]. Overexpression of E-FABP in chemoresistant pancreatic cancer cell lines was suggested to be part of a mechanism of sequestration or removal of cytotoxic drugs [258]. No significant differences in the effects of chemical anoxia or high extracellular oleic acid concentrations were observed between non-transfected, mock-transfected or FABP cDNA-transfected (MDCK) cells [246]. Therefore, no definitive conclusion can be drawn about the putative cytoprotective effect of FABPs.

FABPs as diagnostic marker for tissue damage

Because of their small size, high solubility, and tissue specificity, FABPs are supposed to be good candidates as biochemical markers for tissue injury under experimental and pathological conditions. The FABP type and its serum concentration may provide information about the nature and extent of tissue damage. Human H-FABP can be determined in extracellular fluids such as plasma and urine [267–270]. FABP released after acute myocardial infarction is quantitatively recovered in plasma, making it a useful biochemical plasma marker for the estimation of infarct size in humans [269, 271, 272]. The diagnostic sensitivity was significantly higher for H-FABP than for myoglobin [273]. In addition, the differences in myoglobin and FABP content in heart and skeletal muscles and their simultaneous release upon muscle injury allow the plasma ratio of myoglobin/FABP to be applied for discrimination of myocardial from skeletal muscle injury [274]. In contrast to these data, results obtained by others [275] indicate that H-FABP does not demonstrate the sensitivity and specificity necessary to detect acute myocardial infarction significantly earlier than do existing markers. Plasma concentrations of H-FABP are also markedly elevated in patients with chronic renal failure [276]. Therefore, caution must be taken when using H-FABP as a marker for early diagnosis of myocardial infarction, in case of renal insufficiency.

L-FABP is released from liver in various liver diseases [277]. I-FABP is released into the circulation in the acute phase of intestinal ischemia and is therefore a potential biochemical marker to facilitate the early detection of mesenteric ischemia [278–282]. Studies on neonates suffering from necrotizing enterocolitis indicated that the sensitivity of the I-FABP marker may be limited to late stages of this disease. L-FABP seems to be a more sensitive marker, since it could already be detected at stage I [283]. Acute intestinal allograft rejection could be detected earlier with I-FABP as a serum marker compared to other markers [284, 285] although this was contested by others [286].

FABPs as targets for immunotherapy or chemotherapy against parasites

Various parasites are not able to synthesize FAs and therefore need FAs from their host to develop and/or survive. Molecules similar to FABPs appear to be essential, and have been found in the perivitelline fluid of the parasitic nematodes *Ascaris suum* [287] and *Ascaridia galli* [288]. The blood flukes *Schistosoma mansoni* [289] and *S. japonicum* [290, 291] and other parasitic platyhelminths such as *Fasciola hepatica* and *Echinococcus granulosus* [292] contain FABPs. The structure of an allergen of the mite *Blomia tropicalis* was partially similar to *Schistosoma* and mammalian FABP [293]. The potential discontinuous epitopes not present in the mammalian (host) FABPs may give possibilities to develop vaccines against these, for the parasite, essential proteins. FABPs have therefore been studied as candidate molecules for immunotherapy [294, 295]. Specific drugs may also be developed based on this information.

Conclusions

Despite the large amount of data on the three-dimensional structure, FA-binding characteristics and tissue occurrence of FABPs, the physiological role of these proteins has not been completely resolved. Their specific occurrence in certain tissues or cells possibly results from adaptation to specific cellular needs. FABPs undoubtedly play an essential role in cellular FA transport and utilization. FABPs are indirectly involved in FA-mediated regulation of gene expression, sometimes via PPARs. More investigations are, however, necessary on these aspects and also on the interaction of FABPs with membrane lipids, with FA transporters in the plasma membrane, acceptor proteins of FAs in intracellular membranes and with enzyme systems involved in FA metabolism. Extended studies on knockout mice and transgenic cell lines transfected with FABP-encoding cDNAs will certainly help in reaching a better understanding of the physiological significance of distinct FABP types in different tissues.

Application of the knowledge about FABPs also has potential in medicine. The role of FABPs in normal and pathological growth and cell signalling certainly deserves attention. Deficiency or malfunctioning of FABPs may play a role in the pathology observed in cancer, diabetes, obesity and atherosclerosis. FABPs may be suitable targets for intervention in these cases. Since FABPs are released in the blood and/or urine after tissue damage, they may be used as diagnostic markers e.g. in cases of cardiac infarction, intestinal ischaemia or renal failure. The release of specific FABP types may be indicative of the origin and severity of the damage. Finally, knowledge on FABPs of parasites may help to develop better vaccines and drugs against these organisms, which are hostdependent for their FAs.

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