Functions of fatty acid binding proteins

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Summary. Cytosolic fatty acid binding proteins (FABP) belong to a gene family of which eight members have been conclusively identified. These 14–15 kDa proteins are abundantly expressed in a highly tissue-specific manner. Although the functions of the cytosolic FABP are not clearly established, they appear to enhance the transfer of long-chain fatty acids between artificial and native lipid membranes, and also to have a stimulatory effect on a number of enzymes of fatty acid metabolism in vitro. These findings, as well as the tissue expression, ligand binding properties, ontogeny and regulation of these proteins provide a considerable body of indirect evidence supporting a broad role for the FABP in the intracellular transport and metabolism of long-chain fatty acids. The available data also support the existence of structure- and tissue-specific specialization of function among different members of the FABP gene family. Moreover, FABP may also have a possible role in the modulation of cell growth and proliferation, possibly by virtue of their affinity for ligands such as prostaglandins, leukotrienes and fatty acids, which are known to influence cell growth activity. FABP structurally unrelated to the cytosolic gene family have also been identified in the plasma membranes of several tissues ($FABP_{pm}$). These proteins have not been fully characterized to date, but strong evidence suggests that they function in the transport of long-chain fatty acids across the plasma membrane.
Key words. Fatty acid binding protein; carrier proteins; long-chain fatty acid; liver; intestine; myocardium; adipose tissue; fatty acid metabolism; cell growth.

Introduction

Long-chain fatty acids play a vital role in the organism. Apart from providing a major source of energy, fatty acids are the precursors of eicosanoids, covalently modify the structure of certain proteins, and are essential constituents of the membrane lipids that maintain cellular and organelle integrity. The very property that makes long-chain fatty acids well suited to be components of membranes, i.e. their acyl chain hydrophobicity, complicates for the organism the task of transporting them from sites of intestinal absorption, hepatic synthesis, and lipolysis, to sites of utilization. A variety of mechanisms have evolved in higher organisms to deal with fatty acid transport. In intestine and liver, long-chain fatty acids are largely esterified to triglycerol, and are secreted into and transported through the lymph and blood vascular compartments as chylomicrons and very low density lipoproteins respectively; in the adipocyte, fatty acids are released from the stored triacylglycerol by lipolysis and are transported in plasma bound with high affinity to albumin. Investigation of mechanisms of intestinal long-chain fatty acid absorption led to the discovery, in the enterocyte and several other tissues, of what proved to be a family of cytosolic fatty acid-binding proteins (FABP), which were originally identified based upon their affinity for long-chain fatty acids.[133].

Although FABPs have been extensively characterized, their exact function is not established. Based upon their tissue expression, binding affinities, regulation, in vitro effects on fatty acid transfer and upon enzymes, it has been postulated that these proteins play an important role in the transport and metabolism of long-chain fatty acids. However, there is also growing evidence that they may have more diverse functions, including a role in the modulation of cell growth.

Several reviews have been published that comprehensively cover the earlier literature[3, 5, 61, 63, 161, 174]. The aim of this article is to focus on the more recent data and evolving concepts regarding the FABP.

The cytosolic FABP family

The cytosolic fatty acid-binding proteins are widely distributed, low molecular weight (14–15 kDa) proteins belonging to a gene family that also includes the cytosolic retinoid-binding proteins. To date, eight members of this gene family have been conclusively identified and characterized to varying extents; two additional less well characterized proteins may also be members. Their nomenclature is generally based upon the tissue in which they were first identified, although a uniform terminology has not yet been established (see table). Liver FABP (L-FABP) is present in the liver[118, 133] and in enterocytes[10, 66], making up 2–5% of cytosolic protein in these tissues[8]. Intestinal epithelium contains another abundant FABP designated intestinal FABP (I-FABP)[131]. A third major cytosolic FABP, heart FABP (H-FABP), was initially purified from heart muscle[36, 53, 177], but has since been found to have a widespread tissue distribution[8, 42, 66]. It has been identified in skeletal muscle[116], brain, ovary, testis, kidney[8], aortic tissue[150] and lactating mammary gland of rats[85]. FABP have also been isolated from lung[71, 72] and placenta[45], but have not been structurally characterized. A fourth member of the FABP gene-family is aP2 (also designated adipocyte lipid-binding protein or ALBP, and p422). It

Members of the cytosolic FABP gene family

	Synonyms and abbreviations	Tissues	References
Liver FABP	L-FABP Hepatic FABP (h-FABP) Z-protein Heme-binding protein (HBP) Early target protein; p14.	Liver, small intestine	3, 5, 32, 63, 65, 100, 133, 158, 174, 181
Intestinal FABP	I-FABP Gut FABP (g-FABP).	Small intestine	3, 5, 10, 131
Heart FABP	H-FABP Myocardial FABP (M-FABP) Cardiac FABP (c-FABP)	Heart, vascular endothelium, skeletal muscle, ovary, testis, brain, mammary gland	60, 61, 77
Myelin P2	mP2	Peripheral nerve myelin	84, 98, 122
Adipocyte FABP	aP2 Adipocyte lipid-binding protein (ALBP) p422 Adipocyte FABP (A-FABP).	Adipose tissue, 3T3-L1 cells	15, 16, 17, 78, 109
Mammary-derived growth inhibitor[a]	MDGI	Lactating bovine mammary glands	19, 20, 21, 99
Gastrotropin[a]		Terminal ileum	184
Cellular retinol binding protein I	CRBP I	Liver, lungs, testes, kidney	33, 167, 169
Cellular retinol binding protein II	CRBP II	Small intestine	33, 46, 102, 169
Cellular retinoic acid binding protein	CRABP	Widespread tissue distribution, but is absent in jejunal and ileal mucosa	33, 168

[a] Not conclusively established as members of the FABP gene family.

was initially identified in rat and human adipose tissue [70], and is also expressed during differentiation of 3T3-L1 adipocytes [16, 17]. aP2 bears close homology to H-FABP, and to myelin P2 protein. The latter is also a member of the FABP family [122], and is present in peripheral nerve myelin [98].

Several, less well characterized proteins appear to be structurally related to the cytosolic FABP, but their place within the gene family has not been definitively established. Among them, a putative growth regulating factor, the mammary derived growth inhibitor (MDGI), purified from lactating mammary gland epithelium [19, 20], has been found to have significant amino acid sequence homology (84 %) with H-FABP, although its gene sequence is yet to be described. More recently, a 13 kDa polypeptide termed fibroblast growth regulator (FGR), has been isolated from the conditioned medium of Swiss 3T3 cells [79, 80]. FGR cross-reacts with antisera to both MDGI and H-FABP [21], but its amino acid sequence is not known. These putative growth regulating factors are discussed below (see 'Role in cell growth and differentiation'). Another possible member of FABP family is a 14,054 Da protein isolated from the ileal mucosa, termed gastrotropin [184]. Porcine gastrotropin has a 35 % amino acid sequence homology with rat L-FABP, but differs from other FABP in that it has little affinity for fatty acids, although it binds bile salts and bilirubin. More surprisingly, this protein which constitutes < 0.1 % of terminal ileal cytosolic protein, has been detected in the blood and, similar to MDGI and FGR, appears to act extracellularly to produce growth-factor-like effects. These include trophic changes in the gastric mucosa and the stimulation of gastric acid secretion. Several retinoid binding proteins, including cellular retinoic acid binding protein and cellular retinol binding proteins I and II also belong to the FABP gene family [33, 46, 102, 167–169].

Cellular FABP not structurally related to the FABP gene family

Recently, other cellular fatty acid-binding proteins have been described that are not structurally related to the FABP family. A 15.5 kDa fatty acid binding protein has been isolated from kidney [55, 56, 97]. This protein is distinct from H-FABP, which is also present in renal tissue, and appears to be a member of the male-specific α-2_u-globulin family [93].

Knudson and co-workers have identified a 9.9 kDa cytosolic acyl-CoA-binding protein (ACBP) in bovine and rat liver [94, 114, 115, 119]. It was initially discovered as an impurity in L-FABP preparations derived from bovine liver [119], and its amino acid sequence shows no homology with L-FABP [114]. Acyl-CoA derivatives (C_8-C_{18}) are bound by ACBP with high affinity in a ratio of 1 mol fatty acyl-CoA/1 mol protein [94], but it does not bind non-esterified fatty acids [94, 115]. ACBP has been found in a wide range of tissues, with the highest concentrations present in liver cytosol, where it makes up 5–6 µg/mg of protein [94]. Its role in fatty acid metabolism is not clear, but its ligand specificity suggests that it serves as an acyl-CoA carrier.

A 40 kDa plasma membrane FABP ($FABP_{pm}$) has been isolated from liver plasma membrane[166], and has subsequently also been identified in the plasma membranes of many tissues (see below). $FABP_{pm}$ binds long-chain fatty acids with a high degree of specificity, and considerable evidence suggests a role for this protein in the cellular uptake of long-chain fatty acids.

Phospholipid transfer proteins (PLTP), isolated from both leaves and seeds of plants, have been shown to possess fatty acid binding activity. PLTP are basic proteins (pI ~ 9.0) with molecular weight of ~ 9 kDa, and their characterization to date suggests that they are not structurally related to the cytosolic FABP occurring in the animal kingdom. (For a detailed review see Arondel and Kader in this issue).

Properties and regulation of cytosolic FABP: functional implications

Individual FABP have specific tissue profiles, and some evidence exists for differences in their binding affinities for fatty acids, and other hydrophobic ligands. These proteins also exhibit disparate responses to dietary and pharmacological manipulations. These lines of evidence, albeit indirect, suggest that members of the FABP family perform distinct and specific roles in fatty acid metabolism suited to the needs of different tissues.

1) Structural and phylogenetic relationships

FABP have not been reported in prokaryotes, or lower eucaryotes, e.g. yeast. However, FABP have been identified in the tissues of all vertebrates studied[51, 87, 90, 110, 177], including avian species[37, 73, 155]. Elasmobranchs (shark) have abundant FABP in the liver, which has 68 % amino acid sequence homology with rat H-FABP, but only 38 % with rat L-FABP[7]. This suggests that mammalian L-FABP evolved relatively recently from an ancestral protein that remains structurally conserved in the H-FABP of the mammalian FABP family.

In mammals, primary structures of the three major FABP are highly conserved among different species[18, 67, 172], and virtually all of the amino acid differences are conservative substitutions, with the overall relative hydropathy profile being strikingly preserved[67, 172].

Comparison amongst different members of the FABP gene family shows that the NH_2 terminal region coded by the first exon is the sequence most conserved among different FABP[67]. The ranking of sequence homology between H-FABP and other paralogous members of the FABP family is of the order: MDGI (~ 84 % homology) > aP2 (62 % homology) > myelin P2 > CRABP > CRBP II > I-FABP > L-FABP (36 % homology)[5, 19, 67, 77]. The differences that do exist in the primary structures of the FABP, especially in the carboxy-terminal regions, could account for the different binding properties exhibited among the members of the gene family.

Recent crystallographic evidence, on the other hand, suggests that in spite of disparate primary sequences, the tertiary structures of FABP may exhibit overall similarity. Both I-FABP and myelin P2 proteins possess a 'clam shell-like' structure, with two parallel β-sheets orthogonally oriented to each other, with the hydrophobic ligand bound within a pocket formed by the β-clam[84, 146–148]. Variations in the size, depth, and hydrophobicity of the ligand-binding site may account for the differences in the binding properties among the different FABP. It is notable that the 'β-clam' configuration also appears to be common to many extracellular hydrophobic ligand-binding proteins, which otherwise are unrelated, e.g. bovine milk lactoglobulin, serum retinol binding protein, and bilin-binding protein[84, 148].

2) Tissue localization

The cytosolic FABP each display a characteristic pattern of tissue-expression. They are abundantly expressed in tissues that are either subject to large fluxes of fatty acids or those which have high demands for long-chain fatty acids as energy substrates. In these tissues FABP constitute between 2–5 % of soluble proteins[8] with estimated cellular concentrations in 0.2–0.4 mM range[27].

In the intestinal epithelium, both L-FABP and I-FABP are expressed most abundantly in the jejunum with concentrations declining along the length of the intestine. Also, immunohistochemical methods[157, 170] have shown that both proteins are present predominantly on the tips of intestinal villi and are not detectable in the intestinal crypts. Proximal jejunum and the tips of intestinal villi are exposed to much higher amounts of dietary fat than are the ileum and the crypts, respectively, and these gradients – 'horizontal' (proximal to distal) and 'vertical' (villus to crypt) according to the terminology of Sweetser et al.[171] – in expression of L-FABP and I-FABP are thus consistent with the concept that these proteins have a role in fatty acid transport and metabolism.

A role for H-FABP specifically in fatty acid β-oxidation is supported by the tissue distribution of this protein. Thus, myocardium, in which fatty acid β-oxidation is the predominant source of energy[123, 152], expresses the highest levels of H-FABP[8, 42]. Slow-twitch red muscle fibers (STR) e.g. soleus, which have a low glycogenolytic capacity, have the next highest H-FABP levels (60 % of the heart levels), and fast-twitch white fibers, which possess high glycogenolytic, and low oxidative capacity, have the lowest H-FABP content among the different types of skeletal muscle fibers[42, 116]. The higher FABP content in STR muscle, which depends upon fatty acid oxidation as a predominant source of energy, suggests a relationship between the expression of H-FABP in muscle and fatty acid β-oxidation. Consistent with this, Kaufman et al.[88] have reported that chronic low-frequency stimulation of a red fast-twitch muscle (tibialis anterior, possessing intermediate levels of H-FABP) for 3–4 weeks increased H-FABP levels to those of soleus,

and almost to those of the heart and was accompanied by an increase in the activity of 3-hydroxyacyl-CoA dehydrogenase. However, the presence of immunoreactive H-FABP in lactating mammary glands[85] or in the brain[11] is difficult to explain on this basis as the former utilizes fatty acids predominantly for the synthesis of milk triacylglycerols, and the brain does not utilize fatty acids as a preferred source of energy. Thus, although its tissue distribution is generally consistent with a role for H-FABP in fatty acid β-oxidation, the evidence is also compatible with other functions, in addition to β-oxidation. It is also entirely conceivable that the immunoreactive H-FABP present in mammary tissues[85] represents the closely related MDGI[21].

Immunocytochemical and subcellular fractionation studies, in which attempts have been made to define the subcellular localization of FABP, have yielded some surprising and often conflicting results. In light microscopic studies, both L-FABP and I-FABP in small intestine were cytoplasmic, with staining being more intense in the apical portions of the villus cells[157]. Another study using immunoelectron microscopy with protein A-gold labeling showed L-FABP in the hepatocyte cytoplasm, endoplasmic reticulum, associated with the outer mitochondrial membranes, and in the euchromatin regions of the nucleus[23]. The presence of L-FABP immunoreactivity in nuclei was also reported by Vinores et al.[183] and was subsequently found to represent cross-reactivity of L-FABP antibodies with histone H3[120].

Using protein A-gold immunochemistry Fournier et al.[50] detected H-FABP in all compartments of the cell, including mitochondria, myofibrils and nuclei, as well as intercellular space. Gradients were identified between cellular compartments, with the highest concentrations present in the mitochondria and myofibrils. Borchers et al.[22] have also recently reported the presence of H-FABP in both mitochondria as well as nuclei, utilizing protein A-gold labeling, Western blotting, and quantitative enzyme-linked immunoassay (ELISA). However, there was a discrepancy between the intensity of H-FABP staining overlying the mitochondria and the relatively small amounts (0.0183 ± 0.0035 µg/mg protein) quantified by ELISA. Other workers have not been able to identify H-FABP in the mitochondria by ELISA or by immunoblotting techniques[42, 135]. None of the FABP display a known mitochondrial targeting sequence, and the nature of the intramitochondrial H-FABP observed by some workers remains to be clarified. It may conceivably represent cross-reactivity of the anti-H-FABP antibody with an intramitochondrial protein other than H-FABP.

3) Comparative ligand-binding properties

FABP bind a variety of hydrophobic and amphipathic ligands with varying degrees of affinity. Long-chain fatty acids are the major endogenous ligands of L-FABP, with fatty acids of chain lengths $C_{16}-C_{20}$ having dissociation constants (K_d) in the range of 0.4–0.9 µM[3]. In the liver, endogenous long-chain fatty acids associated with L-FABP account for 60% of total cytosolic long-chain fatty acids[132], with a substantial enrichment of L-FABP in unsaturated fatty acids, especially 18:2 and 20:4 fatty acids compared with the whole liver homogenate[27, 132]. Acyl-CoA derivatives of fatty acids bind to L-FABP with lesser affinity compared to long-chain fatty acids.[3, 189]. Others have suggested that the acyl-CoA binding site on L-FABP may be separate from the long-chain fatty acid-bindings site(s)[29]. On the other hand, it is also possible that the acyl-CoA binding by L-FABP may be partly, or wholly, due to ACBP contaminating L-FABP preparations. Other lipids reported to bind to L-FABP include lysophosphatidylcholine[28], phosphatidylserine[30] and monoglycerols[137], but not cholesterol and squalene[3, 189]. Certain carcinogens[91], prostaglandin E1[48] and lipoxygenase metaoblites of arachidonic acids, 15-HPETE and 5-HETE[140], are also reported to bind to L-FABP. Indeed 15-HPETE and 5-HETE exhibited 16 and 7 times greater affinity, respectively, for L-FABP than oleate[140]. However, Wilkinson et al.[189] were unable to show competitive displacement of fluorescent fatty acid from L-FABP by prostaglandin E_1. Retinoid and retinyl ester binding to L-FABP has also has been reported with K_d of 20–30 µM and 1.4 µM, respectively[57, 182]. Among the non-lipid ligands, heme binds with the highest affinity[3, 175, 182, 189]. Several organic anions can serve as ligands for L-FABP, including bilirubin, bromosulfophthalein[87, 92, 100, 133, 185] and bile acids[175]. L-FABP also binds bromosubstituted fatty acids, tetradecylglycidic acid, and flavaspidic acid[137].

Studies of the binding stoichiometry of L-FABP have produced differing results depending upon the methodology used. Most binding assays show one mol of fatty acid bound per mol of protein[3, 25, 60, 61], although evidence for binding of two fatty acids in close proximity to one another[151], or three fatty acids to different affinity sites on the L-FABP molecule[58] has also been presented. ^{13}C-NMR spectroscopic studies also suggest that L-FABP has a high capacity for fatty acids with up to three mol bound per mol of protein[34, 35]. Thus, it would appear that L-FABP has the ability to bind more than one fatty acid/FABP molecule, but the exact number of sites and their relative affinities are not established with certainty. Studies on the ligand affinities of H-FABP have provided less consistent results with variable binding affinities for oleate, palmitate and arachidonate being reported in the literature[60, 61, 149]. H-FABP has been reported to have one[61] or two[134] fatty acid binding sites per protein molecule, although H-FABP does bind fatty acids less avidly than L-FABP[134, 141, 162]. Burrier et al.[29] could not detect binding of long-chain acyl-CoA derivatives of long-chain fatty acids, although others have reported H-FABP affinities for palmitoyl-CoA and palmitoyl-carnitine that were comparable to those for palmitic acid[136]. However, H-FABP did not bind other amphipathic compounds, e.g. bilirubin, BSP, heme or prostaglandins[136].

The ligand-binding affinities of I-FABP have not been studied as extensively as those of L- and H-FABP. Lowe et al.[104] expressed rat L-FABP and I-FABP in *E. coli*, and quantified the bacterial endogenous fatty acids bound to the two proteins. Both saturated and unsaturated fatty acids were associated with L-FABP, but only saturated fatty acids were found in association with I-FABP. In studies employing Lipidex, L-FABP had significantly higher affinity than did I-FABP for unsaturated long-chain fatty acids, but their affinities for saturated fatty acids were similar. In ^{13}C-NMR spectroscopic studies, Cistola et al.[34,35] found, in keeping with the crystallographically determined structure of I-FABP[146–148], that the long-chain fatty acid carboxylate binding location is in the solvent-inaccessible interior of the protein molecule, and does not show pH-dependence of fatty acid binding. For L-FABP, in contrast, the carboxylate group of the bound fatty acid appeared to be located near the protein/solvent interface, and fatty acid-protein association was highly pH dependent, even within the physiological pH range.

Myelin P2 is reported to bind oleic acid, retinoic acid, and retinol[178], and limited ligand-binding properties of murine adipocyte lipid-binding protein (ALBP; p422) have also been reported[109]. Oleic acid and retinoic acid, but not retinol, were bound with K_d of 3 and 50 μM, respectively, at ratios of 1 mol per mol of protein.

4) Regulation

a) cis- and trans-acting factors. Analysis of transgenic mice pedigrees into which various lengths of the upstream non-coding region of rat L-FABP gene linked to a reporter gene (human growth hormone; hGH) had been introduced, showed that nucleotides − 4000 to + 21 of the rat L-FABP gene can direct regional- and temporal-specific expression of hGH reporter in the intestine in fetal transgenic mice[74]. Studies in adult transgenic mice showed that a short promoter sequence from nucleotides − 586 to + 21 was sufficient to produce a horizontal (proximal-to-distal), but not a vertical (villus-to-crypt), intestinal gradient for the reporter gene, which was also inappropriately expressed in the colon and in renal epithelial cells. Although the colonic and renal expression could be suppressed by a long promotor construct (containing L-FABP gene nucleotides − 4000 to + 21), inappropriate expression of hGH in crypt cells still occurred[171]. In liver, the short promoter construct could direct appropriate expression of the reporter gene in hepatocytes[171] and could also reproduce the periportal-to-centrizonal (acinar zones I to III) gradient in L-FABP expression normally present in liver[6].

In the case of I-FABP, the presence of a long promoter (nucleotides −1178 to + 28) was sufficient to direct intestine-specific expression of an hGH reporter in transgenic mice, and duplicated both the horizontal and vertical gradients seen for I-FABP in the small intestine[173]. A short promotor (nucleotides − 227 to + 28) was effective in producing the villus-to-crypt gradient, but the proximal-to-distal distribution was abnormal, with ileal levels of hGH mRNA two orders of magnitude less compared to transgenic animals possessing the long promotor. Thus, the cis-acting elements responsible for determining the proximal-to-distal gradient noted for L-FABP and I-FABP are progressively being defined. The trans-acting factors which may interact with these FABP promoter regions remain unknown[64].

Recent studies in the 3T3-L1 cell line have identified cis-regulatory elements and trans-acting factors involved in the differentiation-associated gene-expression of aP2[38,47,139,191]. aP2 gene is activated in a coordinate fashion with other genes, the protein products of which are also involved in effecting the changes in lipid metabolism seen during adipocyte differentiation. Both positive and negative regulatory elements have been identified, including two 'fat specific elements' (FSE), FSE1 and FSE2. FSE1 is a 14-base sequence present in multiple copies in the 5′ upstream region of three genes (adipsin, aP2 and glycerophosphate dehydrogenase) that are activated during the differentiation of 3T3 cells[47]. FSE2, a 21-base sequence 120 bases upstream from the transcription start site, is present in a single copy only in the aP2 and glycerophosphate dehydrogenase genes[47,81]. FSE2 has been shown to bind a trans-acting protein complex which includes the protein product of c-fos and the transcription factor AP-1[47,139]. FSE2 appears to serve as a negative regulatory element in the preadipocyte stage[47]. A glucocorticoid regulatory element (GRE) and a cAMP-responsive element have also been identified in the first 858 base pairs of the 5′-flanking region of the aP2 gene[38,191]. cAMP-mediated activation of the aP2 gene is apparent only during the confluent-preadipocyte stage, and probably occurs by removal of the suppressive effects of a negative regulatory element[191]. Thus, the molecular mechanisms of aP2 activation during differentiation of 3T3 cells are gradually being elucidated, and may also provide information regarding in vivo aP2 regulation and adipocyte differentiation.

b) Developmental. The tissue-specific FABP have distinct ontogenic profiles, which provide indirect evidence consistent with a role for FABP in fatty acid metabolism. L-FABP mRNA and I-FABP mRNA are first detected in the small intestine at day 19 of the 21-day gestation period. Their concentrations rise 3-fold within 24 h of birth and continue to rise during the suckling phase, peaking to 8 times the prepartum levels[66]. During transition from suckling to weanling stage, when the high fat diet of the suckling period is replaced by a carbohydrate-rich low-fat diet, there is a 50 % fall in L-FABP mRNA levels in the intestine, with I-FABP mRNA levels showing less marked reduction. Liver L-FABP mRNA exhibits similar induction during the developmental period, although the fluctuations are not as pronounced. These developmental changes have been shown for the protein product as well[156]. Comparison of the developmental changes in

L-FABP mRNA abundance with mRNA levels of a number of enzymes of cholesterol biosynthesis show that while L-FABP mRNA levels in rat liver rise after birth [66], mRNA abundance of HMG-CoA reductase, HMG CoA synthase and prenyl-transferase, which are highest in the fetal life, undergo a precipitous fall at birth [101]. Thus, the increases in L- and I-FABP occur at a time when the neonatal intestine and liver are adapting to an increased influx of fatty acids, and when the metabolic apparatus in the liver is shifting from lipogenesis and cholesterol synthesis to ketogenesis [76]. In addition, at least in the case of L-FABP, the early transition from fat to carbohydrate (lipogenic diet) feeding is associated with an ebb in mRNA expression.

H-FABP developmental regulation has also been recently described [77]. H-FABP mRNA is detectable by day 19 of fetal life, with a 3-fold rise up to the first post-partum day, followed by a gradual increase until the onset of the weanling stage. This correlates with the shift from predominantly anaerobic glycolytic metabolism in fetal heart [190] to fatty acid oxidation as the preferred energy source in the adult heart [123]. The rise in H-FABP level in the developing heart also parallels the β-oxidation capacity of the myocardium, and correlates with carnitine palmitoyltransferase activity [186]. Overall, the changes seen during development in FABP abundance are consistent with a role in fatty acid metabolism, with a particularly marked correlation with the capacity of tissues to utilize fatty acids as energy substrates.

c) Dietary. L-FABP, both in the liver and small intestine, increases modestly in response to a high fat diet. Adult rats, fed regular laboratory chow ($\sim$ 4–6 % fat) show a declining proximal-to-distal gradient, as well as a villus-to-crypt gradient, for both L-FABP and I-FABP in the small intestine [15, 131]. In rats fed a high fat diet, there is a 30 % increase in L-FABP in jejunum and a 50 % increase in the ileum with loss of the horizontal gradient [5], whereas I-FABP increases only in the ileum [131]. Diets low in fat result in a decline in both I-FABP and L-FABP levels in the proximal small intestine [5, 131]. These studies suggest that in proximal intestine, which is exposed to the highest concentration of luminal fat, expression of I-FABP is maximal even when the animal is ingesting a relatively low fat laboratory chow diet. In distal intestine, in contrast, expression of both I- and L-FABP are relatively low on this diet, and are induced to a greater extent than in jejunum as the load of dietary fat is increased. Diets rich in fat also induce modest increases in H-FABP [50]. Thus, it appears that dietary fatty acids exert a positive effect on FABP abundance, and may be responsible for the gradients in FABP expression in intestine as well as the liver [6].

Recent studies in fetal mice, however, suggest that the proximal-to-distal gradient in intestinal L-FABP and I-FABP levels is constitutively expressed, and may not be secondary to luminal nutrients. The gradient is first noted at day 17 of gestation [74], at a time when the exposure of intestinal mucosa to luminal fat is presumably low. It is nevertheless probable that there is some fat present in the intestinal lumen even in the fetus, e.g. from swallowed amniotic fluid, shed cells, secretions, etc., and a role for luminal fatty acids in modulating the expression of the FABP intestinal gradient remains a possibility.

d) Induction of L-FABP by peroxisome proliferators. Peroxisome proliferators (PP) are a group of structurally diverse chemicals which on administration to rodents cause an increase in the number and volume of peroxisomes in liver [75]. These agents include the fibrate class of hypolipidemic drugs, e.g. clofibrate, bezafibrate and nafenopin, and unrelated chemicals such as acetylsalicylic acid and phthalate esters [75]. Administration of peroxisome proliferators also results in smooth endoplasmic reticulum proliferation and cytochrome P-452 induction [75], as well as a 2–3-fold increase in L-FABP protein and mRNA abundance in the liver and intestine [10, 49, 89]. In contrast, I-FABP concentration is minimally increased [5], and H-FABP concentrations in the heart are unaffected by hypolipidemic agents [135].

Peroxisome proliferators share certain structural features with the long-chain fatty acids. Both are predominantly hydrophobic molecules, with a carboxylic acid moiety. After entering the hepatocyte, peroxisome proliferators are in part converted to their CoA esters [26], and are thus initially processed in a similar fashion to fatty acids. This suggests that peroxisome proliferators may act as fatty acid analogues, and may provide a model for the natural inducers of L-FABP, i.e. putatively fatty acids. A protein receptor for peroxisome proliferators in the cytosol may mediate the effects of these agents at the genomic level, perhaps at a highly specific 'peroxisome proliferator domain' [187]. However, while a nafenopin-binding protein (receptor) in hepatocytes has been described [96], others have not been able to confirm this finding [117]. Further elucidation of the mechanisms of induction of L-FABP and peroxisomes by peroxisome proliferators is of considerable interest, as this may provide insights into the physiological regulation L-FABP and peroxisomes.

Putative functions of FABP

1) Fatty acid transport and metabolism

a) Uptake from plasma: role of $FABP_{pm}$. Movement of unbound fatty acids through the plasma membrane has been conventionally viewed as a simple physical diffusional process [126, 127]. There is, however, increasing evidence suggesting that a significant portion of fatty acid uptake may be mediated by a specific transporter in a number of tissues, similar to that which exists in *E. coli*. The *E. coli* transporter consists of a 43 kDa inner membrane protein that acts as a long-chain fatty acid receptor, and possibly also as a translocase [129]. This protein is part of a complex system responsible for delivering long-chain fatty acids to an acyl-CoA synthase loosely bound to the inner surface of the cytoplasmic membrane.

Oleate-agarose affinity chromatography was used to isolate a 40 kDa protein, the plasma membrane fatty acid binding protein ($FABP_{pm}$), from the plasma membrane fraction of rat liver [166]. Immunologically similar proteins have subsequently been purified from the plasma membranes of jejunal enterocytes [163], cardiac myocytes [159], and adipocytes [153]. $FABP_{pm}$ is entirely distinct from the cytosolic FABP, although surprisingly, its N-terminal amino acid sequence was found to be identical to the N-terminal sequence of mitochondrial glutamate oxaloacetate transaminase (mGOT). Furthermore, $FABP_{pm}$ was found to have mGOT enzymatic activity and to cross-react with antibodies to this enzyme [14]. However, Stremmel and co-workers have been unable to confirm this sequence homology and immunologic identity between $FABP_{pm}$ and mGOT [164]. Incubation of hepatocytes with an anti-$FABP_{pm}$ antibody results in a 60–70 % inhibition of saturable oleate uptake, and offers the most compelling evidence for a physiological role for $FABP_{pm}$ in this process [165]. Similar antibody inhibition of fatty acid uptake has been demonstrated for cardiac myocytes, enterocytes and adipocytes [153, 159, 163]. Trypsin or pronase treatment results in loss of the saturable component of oleate uptake, as does heat treatment of plasma membranes, providing further evidence of a role for $FABP_{pm}$ in the fatty acid uptake process. Hepatocyte $FABP_{pm}$ has a high affinity for oleate and other long-chain fatty acids but does not bind cholesterol esters, phosphatidylcholine, or non-fatty acid organic anions, e.g. bilirubin, BSP, or bile salts [165, 166]. In view of this high degree of ligand specificity of $FABP_{pm}$, it is puzzling that in the jejunum, antibody to $FABP_{pm}$ resulted in a 36–53 % inhibition of the absorption rate of monoacylglycerol, cholesterol and lysophosphatidylcholine in addition to fatty acids [163].

Long-chain fatty acid uptake by hepatocytes has been demonstrated to proceed, at least in part, via a Na^+-coupled, electrogenic process [165, 188], although Na^+-coupling of oleate uptake has not been confirmed by others in either hepatocytes [160], or in adipocytes [153]. Thus, although there is strong evidence for a function of $FABP_{pm}$ in the cellular uptake of fatty acids in a variety of tissues, the exact mechanism of fatty acid transport across the plasma membrane still remains to be determined. After binding to $FABP_{pm}$, long-chain fatty acids may translocate to the inner aspect of the plasma membrane by flip-flop, or via a specific transport system, with subsequent movement into the cytoplasm. $FABP_{pm}$ conceivably represents but one component of a transport mechanism for fatty acids that may involve additional proteins, analogous to the transport system for long-chain fatty acids in *E. coli*.

b) Uptake from plasma: role of cytosolic FABP. It has been suggested that fatty acids bind to FABP after the fatty acids have desorbed from the plasma membrane, rather than by direct interaction of FABP with the plasma membrane [111]. The concentration of FABP within the cytosol approximates 0.2–0.4 mM [27, 132], and the binding sites available exceed cytosolic concentrations of long-chain fatty acids by at least 1–2 orders of magnitude [179]. Thus, FABP may ultimately promote the net movement of fatty acids into the cell by limiting their reassociation with the plasma membrane and efflux from the cell.

A role for L-FABP in the hepatic uptake of fatty acids has been suggested largely based upon the results of earlier studies that found a correlation between L-FABP abundance and the rate of fatty acid uptake by hepatocytes. Treatment of rats with clofibrate, which induces L-FABP, increases the uptake of long-chain fatty acids [142]. Similarly, L-FABP levels are higher in female hepatocytes which, compared with male hepatocytes, show more rapid long-chain fatty acid uptake [130]. However, similar types of evidence also fail to support a role for L-FABP as a major determinant in the uptake of fatty acids from plasma. Although oleate uptake by isolated hepatocytes from obese Zucker rats is greater compared to their lean littermates [108], L-FABP concentrations in hepatocytes were the same in both lean and obese animals [107]. Studies of the uptake of a fluorescent fatty acid analogue, which exhibits marked affinity for L-FABP, also suggest that L-FABP does not determine fatty acid uptake in the liver [4, 9]. Thus, antegrade perfusion of liver of male rats with the fluorescent fatty acid analogue showed that the fatty acid uptake occurred in a declining portal-to-centrizonal gradient. This correlated with the declining portal-to-centrizonal gradient for L-FABP in male rat livers [6]. However, reversal of the direction of perfusion resulted in uptake that was greater in centrizonal than in periportal hepatocytes, i.e. opposite to the L-FABP gradient. Since the fatty acid concentration in the sinusoid normally exhibits a periportal-to-centrizonal gradient, it is possible that the usual gradient of L-FABP expression results from a parallel fatty acid uptake gradient which exists under normal physiological conditions [6].

c) Translocation. The next step in which FABP has been considered to play a role after entry of fatty acids into the cytosol is the translocation to enzymes involved in their metabolism. Since the diffusion coefficient for monomeric fatty acids is about three times that estimated for L-FABP [27], the net flux of unbound fatty acids would be more efficient than that of fatty acids bound to FABP if intracellular concentration gradients for both were identical. However, the estimated concentration of FABP is at least an order of magnitude greater than that of monomeric fatty acids [131], and it is likely that fatty acids bound to FABP diffuse along a steeper concentration gradient than do fatty acid monomers [27]. Thus, transport in the bound state may constitute the more efficient mechanism of transport for fatty acids. Tipping and Ketterer [176], elaborating on this theoretical model, proposed that a protein the size of FABP, with its moderate binding affinity, and high intracellular concentration, would

be expected to enhance the rate of intracellular flux of fatty acids by at least an order of magnitude or more, and thus considerably increase the efficiency of the hepatocyte in metabolizing these ligands. However, others have argued, on the basis of the rapid rates of hydration of fatty acids bound to lipid vesicles, that transfer between the plasma membrane and organelles could be entirely accounted for by diffusion in the aqueous phase in the absence of carrier proteins[43].

Several in vitro experimental systems have been used in order to define the role of FABP in mediating the transfer of fatty acids between artificial membranes, or from artificial membranes to microsomes. The in vitro effect of FABP on the transfer of long-chain fatty acids from phospholipid vesicles to microsomes has been found to depend upon whether unilamellar vesicles (ULV) or multilamellar vesicles (MLV) are used as the donor membranes. Noy and Zakim[128] used the rate of acyl-CoA formation as a measure of the rate of fatty acid transfer from vesicles. They found that the rate of acyl-CoA formation was extremely rapid when ULV were used as the long-chain fatty acids donors for a microsomal acyl-CoA synthase assay. Addition of L-FABP or albumin actually reduced the rate of acyl-CoA formation presumably by competing with the enzyme for substrate. On the other hand, others found the rate of microsomal acyl-CoA formation to be approximately an order of magnitude less when MLV were used as long-chain fatty acids donors, and the addition of L-FABP doubled this rate[111]. In experiments utilizing ULV as fatty acid donors, collisional interaction between ULV and microsomes was evident, with an appreciable transfer of ULV phospholipid to microsomal membranes[111]. The effects of L-FABP on fatty acid translocation therefore appear to depend on the type of phospholipid vesicle used, and the extent to which either may accurately reflect in vivo conditions is unclear. Using equilibrium dialysis, McCormack and Brecher[111] noted that L-FABP enhanced the transfer of long-chain fatty acids across a polycarbonate membrane from a compartment containing donor liposomes to a compartment containing microsomes. This effect was also evident with albumin, but not with myoglobin, ovalbumin, or other proteins that do not bind fatty acids. These studies were further extended by Peeters et al[138] who examined the transfer properties of H- and L-FABP by equilibrium dialysis, as well as in a separated monolayer system. They noted that both L-FABP and H-FABP facilitated the transfer of long-chain fatty acids from one compartment or monolayer to the other. In the equilibrium dialysis experiments, the rate of transfer of oleic acid from mitochondria to ULV separated by a polycarbonate membrane was more rapid with L-FABP than with H-FABP. However, Reers et al.[141] found that bovine H-FABP donated anthroyloxypalmitic acid (A 16:0) to ULV during gel filtration, whereas L-FABP removed this fatty acid derivative from vesicle membranes. Recent studies of the transfer kinetics of anthroyloxy-fatty acids from H- and L-FABP to membranes showed that transfer from H-FABP is 10-fold faster than from L-FABP (Storch, J., and Bass, N. M., unpublished data). Collectively, these studies provide strong support for a significant difference in the binding properties of L- and H-FABP for fatty acids which may determine differences in the functions performed by these two proteins.

Fournier et al.[50–52] have presented evidence interpreted as suggesting that H-FABP undergoes a concentration-dependent self-aggregation with differing affinity for fatty acids among the various self-aggregated forms. These workers speculate that H-FABP could serve as regulator of myocardial fatty acid β-oxidation, with two of its self-aggregated forms mediating the translocation of acylcarnitines into the mitochondria[52]. Others have not been able to demonstrate self-aggregation of H-FABP using ciruclar dichroism spectroscopy[82,134]. Thus, the self-aggregation properties of H-FABP, and the role of multimeric forms in mitochondrial β-oxidation, are interesting but unsettled issues at present.

d) Utilization and synthesis. The presence of different forms of FABPs, and their tissue-specific expression, suggests that these proteins may also have specific roles in cellular fatty acid metabolism. Small intestinal epithelium is the only tissue in which two different FABP (L- and I-FABP) are both abundantly expressed. The significance of this peculiarity of FABP expression in the intestine is unknown but two theories have been advanced. The first theory is that L- and I-FABP are required for the compartmentation of long-chain fatty acids entering the enterocyte from either the bloodstream or the intestinal lumen[3,59]. A second theory, advanced by Cistola et al.[34], proposes that the single fatty acid binding site on I-FABP is required primarily for the transport in the enterocyte of fatty acids absorbed from the lumen. The less lipid-specific and more capacitous binding properties of L-FABP, on the other hand, would enable it to transport up to 2–3 moles of fatty acids, monoglycerols, lysophospholipids and bile salts. This would not only obviate the need for separate carrier proteins for monoacylglycerols, lysophospholipids and bile salts, but would also provide the enterocyte with enough cytosolic fatty acid binding sites to convey the large amounts of fatty acids absorbed from the lumen during feeding. This hypothesis does not, however, specifically address the mechanism of the observed intracellular metabolic compartmentalization[59], and it is unclear how it would be reconciled to the predominantly ileal transport of bile acids where FABP concentrations are low.

FABP have been shown to stimulate the activities of a large number of enzymes involved in fatty acid metabolism. These include enzymes in the fatty acid synthesis, oxidation, and esterification pathways (see Bass[3,5] for comprehensive review). Recently, this stimulatory effect was also demonstrated for the lipogenic enzyme glucose-6-phosphate dehydrogenase (G6PDH)[45]

and for phosphatidic acid synthesis[23]. The mechanism of in vitro enzyme stimulation by FABP is not well understood. Specific targeting of substrate bound to FABP to the active site of enzymes is one, albeit entirely speculative, possibility. For the most part, binding of inhibitors of enzyme activity by FABP has been thought to be the most likely explanation for this effect. For example, binding of long-chain fatty acyl-CoA by L-FABP may account for the stimulation of long-chain acyl CoA synthase[29] (via removal of product inhibition), as well as acetyl-CoA carboxylase[106] and G6PDH[44,45] (via removal of allosteric inhibition) activities by FABP. Other studies suggest a similar importance for the heme-binding properties of L-FABP in its effect upon enzyme activities in vitro[69].

2) Protective function

High levels of unbound long-chain fatty acids and fatty acyl-CoA may exert detrimental effects on many aspects of cellular structure and function by producing alterations in membrane fluidity and by direct non-specific interactions with proteins (see also 'Role in cell growth and differentiation'). It has been hypothesized that FABP protects vital cellular functions by binding intacellular long-chain fatty acids and their derivatives. Since FABP are present intracellularly in concentrations considerably higher than those of long-chain fatty acids, they are well suited to serve this function[5]. Long-chain unsaturated fatty acids act as inhibitors of the brain synaptosomal Na^+-dependent amino-acid uptake systems, probably by altering membrane fluidity[144]. This inhibition is reversed both by exogenous L-FABP, as well as by an FABP (probably H-FABP) present in the brain[11].

L-FABP also prevents or reverses the inhibition by acyl-CoA on acetyl-CoA carboxylase[106], acyl-CoA:cholesterol acyltransferase[69], microsomal hydroxymethyl-glutaryl-CoA reductase[69], and the mitochondrial adenine nucleotide transporter[2], but it is possible that these effects reflect primarily the presence of acyl-CoA binding proteins[94].

Significant accumulation of long-chain fatty acids and acyl-CoA to toxic levels is noted in the myocardium during prolonged ischemia. It has been suggested that H-FABP may serve a protective function by binding these fatty acids and their derivatives. A recent study challenged this hypothesis on the basis that H-FABP released from myocardium during ischemia-reperfusion injury and after calcium paradox in an isolated rat heart had no endogenous fatty acids associated with it[62].

3) Role in cell growth and differentiation

There has been a recent spate of interest in a potential role for the FABP in the regulation of cell growth and differentiation. L-FABP has long been known to bind carcinogens, including aminoazo dyes[91]. Sorof and coworkers[158] have identified a 14 kDa polypeptide (p14) which is markedly increased both during mitosis and regeneration in normal livers, as well as during neoplastic proliferation. This protein has been found to be the early target protein of the activated metabolites of 2-acetylaminofluorene and 3'-methyl-4-dimethylaminoazobenzene following treatment of rats with these carcinogens. Recently p14 has been conclusively identified as L-FABP[12]. The significance of L-FABP binding of carcinogens and the relationship of the alterations in the abundance of this protein during hepatocyte proliferation to the processes of cell growth and neoplasia are unknown. Prostaglandins and leukotrienes have been implicated in the modulation of cell growth[31], and have been reported to bind to L-FABP with high affinity[48,140]. There is evidence that cis-unsaturated long-chain fatty acids, e.g., arachidonate and oleate can activate protein kinase C[112,121,154,180] which plays a vital role in signal transduction of several growth factors[124,125,145]. Protein kinase C activation is associated with increased proliferation in many cell types[41,95]. Intracolonic instillation of unsaturated fatty acids has been shown to activate protein kinase C as well as to cause an increase in both mucosal ornithine decarboxylase activity as well as [^{3}H]thymidine incorporation into DNA[40]. Thus, there is increasing evidence that fatty acids may play a role in cellular growth, proliferation, and differentiation and it is conceivable that the FABP, by virtue of their affinity for long-chain fatty acids, could serve as potential modulators of this activity.

L-FABP has recently been identified as one of the cytosolic selenium-binding proteins[1]. The molecular basis of selenium binding to L-FABP is not clear, but is considered to differ from the selenylation of glutathione peroxidase. Selenium has an inhibitory effect on tumorigenesis in mice, rats, and hamsters[113]. It has also been shown to reversibly inhibit proliferation and prevent tumorigenesis in epithelial tissues from many organ systems, raising the possibility that L-FABP may modulate the effects of selenium on cell growth and proliferation[113].

Recently, a 14.5 kDa growth inhibitor, isolated from lactating bovine mammary glands (mammary-derived growth inhibitor, MDGI) which suppresses mouse mammary adenocarcinoma cell proliferation at subnanomolar concentrations[19,99], was found to have 84% sequence homology with rat H-FABP[19]. MDGI also binds long-chain fatty acids noncovalently[20]. Another growth inhibitor, fibroblast growth regulator (FGR), found in the conditioned medium of density-inhibited 3T3 cells[79,80], appears also to be related to MDGI and to mammalian H-FABP[21]. The physiological relevance as well as the mechanism of growth modulation exhibited by MDGI and FGR is unknown. A cell surface receptor has not been identified for these polypeptides. An alternative explanation for their effects could be the binding of a hydrophobic growth-potentiating ligand present in the medium. Levels of murine aP2 rise markedly during differentiation of 3T3 cells, compatible with a role in cellular growth and differentiation for this protein as

well. Purified aP2 binds oleic acid as well as retinoic acid; exposure of 3T3 cells to high concentrations (10^{-6} M) of the latter results in marked morphological changes[109]. Lane and co-workers[15, 78] have shown that aP2 in 3T3-L1 cells is phosphorylated by the insulin receptor tyrosine kinase. Exposure of cells to phenylarsine oxide, a potent inhibitor of glucose transport in adipocytes, in the presence of insulin results in the accumulation of phosphorylated aP2[15], suggesting that aP2 is an intermediate in the signal transduction pathway of insulin-stimulated glucose transport in 3T3-L1 cells. It is interesting that H-FABP, myelin P2 and MDGI, which are closely related to aP2, also possess the tyrosine kinase phosphorylation site that is phosphorylated in aP2[78].

Conclusions

It seems likely that cytosolic FABP may have more diverse roles in cellular function than was earlier appreciated. The structure of these proteins and their ligand-binding and transfer properties are being further characterized by methods discussed in this review. Introduction of FABP genes into organisms and tissues that normally lack them and definition of the effect of FABP expression on fatty acid metabolic pathways, as well as on cellular growth and differentiation, is one possibility for future work. Johnson et al.[83] recently reported the disruption of the aP2 gene by homologous recombination in mouse embryonal stem cells. Embryonal stem cells engineered in this manner may subsequently be reintroduced into mouse blastocysts to form chimeric mice. These and other innovative approaches more broadly applied to the FABP, offer considerable promise for the eventual elucidation of the functions of these diverse, abundant, and as yet enigmatic proteins.

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