The Emerging Functions and Mechanisms of Mammalian Fatty Acid–Binding Proteins

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Abstract

Fatty acid–binding proteins (FABPs) are abundant intracellular proteins that bind long-chain fatty acids with high affinity. Nine separate mammalian FABPs have been identified, and their tertiary structures are highly conserved. The FABPs have unique tissue-specific distributions that have long suggested functional differences among them. In the last decade, considerable progress has been made in understanding the specific functions of the FABPs and, in some cases, their mechanisms of action at the molecular level. The FABPs appear to be involved in the extranuclear compartments of the cell by trafficking their ligands within the cytosol via interactions with organelle membranes and specific proteins. Several members of the FABP family have been shown to function directly in the regulation of cognate nuclear transcription factor activity via ligand-dependent translocation to the nucleus. This review will focus on these emerging functions and mechanisms of the FABPs, highlighting the unique functional properties of each as well as the similarities among them.

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INTRODUCTION

Fatty acid–binding proteins (FABPs) are abundant ~15-kDa cytoplasmic proteins expressed in almost all mammalian tissues. FABPs are members of a conserved multigene family that evolved approximately 1000 mya by subsequent duplications of an ancestral gene, thereby generating a large number of tissue-specific homologs. This diversity is unusual in that most other lipid species have a single type of intracellular binding protein with ubiquitous tissue distribution, and it is strongly suggestive of specialized functionality. The mammalian FABP family includes nine FABPs as well as the cellular retinoid-binding proteins. The FABP genes have been identified and show a similar organization of four exons interrupted by three introns, with the intron length varying greatly among the proteins. Various enhancer elements have also been identified; these direct tissue-specific expression. Tissues with high rates of fatty acid (FA) metabolism, such as intestine, liver, adipose, and muscle, have high FABP levels that parallel FA uptake and utilization. Of particular interest is the regulation of the FABPs by their FAs or other ligands—a phenomenon that has been observed for some members of the family.

FABP names are assigned according to the tissue where the FABP was first recognized, and are designated by adding "-type," e.g., liver-type FABP (LFABP), to indicate that the protein may also be expressed in other tissues (**Table 1**). Recently, a numerical nomenclature for the different FABP genes was introduced (47a). Many FABPs are prominently expressed in a single tissue or cell type, but some—particularly heart-type and keratinocyte-type FABPs (HFABPs and KFABPs, respectively)—display broad tissue distribution. In several cell types, more than one FABP type is expressed, suggesting that these proteins have specialized functions.

The members of the FABP family show only moderate primary structure similarity, with amino acid sequence homology varying from 20% to 70% (13, 44). However, extensive X-ray crystallographic and nuclear magnetic resonance (NMR) analyses have shown that these proteins display a striking tertiary structural similarity (78). They fold as a slightly elliptical β barrel comprising 10 antiparallel β strands, with two short α helices located between the first and second β strands (**Figure 1**). The β barrel possesses appreciable structural stability, as it is virtually unaffected by chemical modifications, the presence of bulky fluorescent groups, or targeted mutagenesis (32, 67, 106). The β strands are organized into two nearly orthogonal β sheets that wrap around a solvent-accessible ligand-binding cavity. The cavity is centered at the end of the barrel near the helix-turn-helix motif, which is thought to act as a portal for ligand entry and exit (49, 109). The α-II helix is a key structural element of the putative FA portal and forms long-range interactions with the α-II turn between β strands C and D.

The β barrel cavity is two to three times larger than the volume of the FA, and the structures reveal ordered water molecules in the cavity that are hydrogen bonded to internal polar residues. Most of the FABPs bind only a single FA, with the carboxylate group oriented inward. LFABP has the unique property of binding two FAs and other larger hydrophobic molecules.

Table 1 Long-chain fatty acid–binding members of the fatty acid–binding protein (FABP) family

FABP type[a]	Gene	Expression	Phenotype of KO mice[b]
LFABP	*Fabp1*	Liver, small intestine, kidney	• Defective hepatic and intestinal β-oxidation
			• Decreased intestinal lipid secretion
IFABP	*Fabp2*	Small intestine	• Elevated body weight
			• Elevated plasma TG
HFABP	*Fabp3*	Cardiac and skeletal muscle, brain, mammary tissue, kidney, adrenals, ovaries, testis, placenta, lung, stomach	• Defective muscle FA oxidation compensated by increased glucose utilization
			• No mammary phenotype
AFABP	*Fabp4*	Adipocyte, macrophages	• Protection against diet-induced atherosclerosis
			• Modest decreases in plasma glucose, insulin
			• Double KO with KFABP shows strong protection against insulin insensitivity and hepatic steatosis
KFABP	*Fabp5*	Epidermis, adipocyte, macrophages, mammary tissue, tongue, testis, liver, lung, brain, heart and skeletal muscle, retina, kidney	• Defective transepidermal water loss
			• Double KO with AFABP shows strong protection against insulin insensitivity and hepatic steatosis
BFABP	*Fabp7*	Central nervous system, retina	• Increased anxiety and fear memory
MFABP	*Fabp8*	Peripheral nerve myelin	
TFABP	*Fabp9*	Testis	

[a]Other members of the FABP gene family include *Fabp6*, which encodes the ileal bile acid–binding protein, and genes for the cellular retinol-binding proteins and cellular retinoic acid–binding proteins, which bind retinol/retinaldehyde and retinoic acid, respectively.
[b]See text for details.
Abbreviations: AFABP, adipocyte-type fatty acid–binding protein; BFABP, brain-type fatty acid–binding protein; HFABP, heart-type fatty acid–binding protein; IFABP, intestine-type fatty acid–binding protein; KFABP, keratinocyte-type fatty acid–binding protein; KO, knockout; LFABP, liver-type fatty acid–binding protein; MFABP, myelin-type fatty acid–binding protein; TFABP, testis-type fatty acid–binding protein.

Crystallographic analysis indicates little difference between apo- and holo-FABPs; however, NMR-derived solution structures demonstrate considerable differences (**Figure 1**) (45, 49, 50). Notably, the distal half of the α-II helix and the turn between β strands C and D exhibit large structural differences: Both of these portal domain elements are more disordered in the unliganded state and exhibit diminished long-range interactions. These differences suggest that during ligand exit/entry, the portal region undergoes a conformational change, allowing the FA to pass through the portal.

All FABPs bind saturated and unsaturated long-chain (≥14-C) FAs (LCFAs). Dissociation constants appear to be in the nano- to micromolar range, depending on the technique used to measure the lipid-protein interactions. Considerable variations of the Kd values for a given FABP have been reported, but the relative values for a series of FABPs are in general agreement among the various methods. Techniques that require physical separation of unbound ligand usually lead to the underestimation of binding affinity. Other techniques require ligands with specific reporter groups or very large amounts of protein, and thus do not permit the study of ligands under proper physiological conditions. Analysis of LCFA binding to the FABPs using the acrylodated intestinal fatty acid–binding protein method (104), which does not suffer from these caveats, provides Kd values in the nanomolar range and has led to two important generalizations. First, affinities tend to increase with increasing ligand hydrophobicity. Second, none of the FABPs has a particularly large selectivity for a specific FA ligand (105). Possible exceptions to these generalizations are discussed below.

*Erratum

*This PDF amended on (3 Nov. 2008): See explanation at http://arjournals.annualreviews.org/errata/nutr

The FABPs have been proposed to function, at least in part, as intracellular transporters for FA. Experiments employing a fluorescence resonance energy transfer (FRET) assay, which can directly monitor the kinetics of FA movement between FABPs and membranes, have demonstrated that different FABPs transfer FAs at various rates using distinctly different ligand-transfer mechanisms. These studies divide the FABPs into two groups. Most of the FABPs examined (e.g., intestinal-type, adipose-type, heart-type, keratinocyte-type, and myelin-type FABPs, as well as cellular retinol-binding protein I) collect and deliver their ligands by contact/collision with a membrane; only LFABP and cellular retinol-binding protein II transfer their ligand to and from membranes via aqueous-phase diffusion (126). Thus, FA transfer from the "collisional" proteins involves an intermediate step in which the FABP and the membrane are in physical contact, and direct protein-membrane interactions have in fact been demonstrated under physiologically relevant conditions for several FABPs. "Diffusional" FABPs, however, may function as cytosolic reservoirs for their ligands; alternatively, the diffusional FABPs may transfer ligand via protein-protein interaction. It is hypothesized that FA transfer from collisional FABPs involves targeted interactions of the protein with specific membrane-lipid domains and/or membrane-protein domains (27, 32, 146).

The structural elements underlying the collisional transfer of a FA from FABPs to membranes could have important physiological consequences, as these domains may dictate the FA trafficking patterns within the cell. The importance of electrostatic interactions between cationic surface residues on FABPs and anionic membrane phospholipid headgroups, as well as the lesser contribution of hydrophobic interactions, has been demonstrated for those FABPs that transfer ligand collisionally (29). A net positive surface electrostatic potential across the helix-turn-helix portal region of collisional FABPs (65), together with the amphipathic character of the FABPs' α-I helices in particular, supports the suggestion that this region is important for interactions with membranes. Structure-function analysis using site-directed mutagenesis, chimeric FABPs in which the helical domains of diffusional and collisional FABPs are exchanged, and a helixless variant of the intestinal FABP strongly support the centrality of the α-helical region in determining the ligand-transfer mechanism used by each of the FABPs (27, 28, 32, 47, 66).

In spite of the abundant information about their structure, binding properties, and in vitro lipid transfer mechanisms, the precise physiological roles of FABPs and their mechanisms of action remained unclear for several decades after they were identified. As we discuss below, however, the functions of individual members of the FABP family are being elucidated by a combination of gene knockout mouse models, cell culture studies in which FABP levels are altered, molecule-level analyses of FABP gene regulation (in some cases), and an examination of the physiological correlates of specific human polymorphisms.

Several outstanding reviews cover the identification and biochemical characterization of the FABPs and provide interesting insights into FABP functions. In general, earlier studies ($\geq$8–10 years ago) will not be comprehensively discussed herein; however, the reader is encouraged to examine several excellent summations (13, 14, 38, 81, 129, 139). In the remainder of this review, we focus upon the emerging understanding of the functions of the different FABPs. Only those FABPs that bind primarily LCFAs are discussed. Thus, we do not discuss the cellular retinoid-binding proteins or FABP6, the ileal bile acid transporter. Not surprisingly, given the large number of conserved genes that constitute the FABP family, recent literature identifies a range of functions for these proteins, including unique and overlapping roles in specific tissues. As a consequence of their intracellular effects, FABP functions have also been found to include systemic effects that influence whole-body lipid and energy metabolism.

LIVER-TYPE FABP; FABP1

LFABP is abundantly expressed in liver, where it represents 2%–5% of the total cytosolic protein. It is also highly expressed in small intestine and to a lesser extent in kidney proximal tubule. Whereas in liver LFABP is the only family member expressed at a high level, in proximal small intestine both LFABP and IFABP (intestinal-type FABP) are highly expressed. In humans, LFABP levels are greater than IFABP levels; in rodents, however, they are comparably expressed (96).

LFABP is enriched in the proximal small intestine. This correlation of expression with the site of maximal lipid absorption has long reinforced the hypothesis that LFABP functions in intestinal lipid assimilation. Immunocytochemical localization in rat intestine demonstrated that in fasting animals LFABP was apically localized; however, after fat feeding LFABP was found to be distributed in the entire cytoplasm (6). Experiments evaluating the rate of diffusion of the fluorescent probe N-(7-nitro-2,1,3-benzoxadiazol-4-yl)-stearate in liver and HepG2 cells also provide strong support for a role for LFABP in intracellular transport of FAs (71, 72).

It is likely that the atypical binding properties of LFABP indicate a unique functionality. Both the crystal and solution structures of LFABP show two bound oleate molecules, one of which (as in other FABPs) is completely internalized, with its carboxylic acid moiety interacting with an arginine and two serines. The second oleate represents an entirely different binding mode, with the carboxylate moiety appearing near the protein surface and exposed to solvent (45, 133). Unlike other FABPs, LFABP binds not only LCFAs but also a wide range of other hydrophobic ligands, including single-chain amphiphiles such as lysophospholipids, as well as heme, vitamin K, and several carcinogens (126). The binding affinities of the two LCFA sites are approximately equivalent for saturated FAs, but the internal site has approximately tenfold higher affinity for unsaturated FAs than does the surface-exposed site (103). Binding of the surface ligand appears to depend upon initial binding of the internal ligand (141).

LFABP is considered a cytosolic protein; however, small amounts of the protein are specifically associated with other subcellular compartments. Observations such as these are helping to shed light on LFABP's functions. In vitro studies with model membranes indicate that under certain conditions, notably low ionic strength, LFABP can associate with phospholipid vesicles (40; B. Corsico, unpublished observations). However, it is likely that in vivo LFABP association with subcellular organelles arises by protein-protein interactions. For example, microscopic studies showed a partial localization of LFABP in the nuclear membrane of primary mouse hepatocytes (145). Further, transfection of the LFABP gene in fibroblasts also demonstrated localization in small part to the nucleus, and an increased level of unesterified FA in that compartment (55). Nuclear localization of LFABP may be related to its reported direct interactions with peroxisome proliferator–activated receptor (PPAR)-α and PPAR-γ (145). Interestingly, in HepG2 cells the transactivation of these nuclear transcription factors by FA depends on the cellular LFABP concentration, suggesting a function for LFABP in delivering PPAR ligands to the nucleus and thereby modulating target gene expression (145). Earlier studies showed that LFABP binds several carcinogens, and it was suggested that LFABP plays a role in both normal mitosis and carcinogen-induced cell proliferation; this evidence indirectly supports a role in cell growth that is as yet undefined, but that could conceivably include effects on gene transcription (15, 90). The *Lfabp* 5′ promoter region contains a peroxisome proliferator response element (112, 123); thus, LFABP may be involved in regulating its own expression. Different PPARs regulate transcription of the LFABP gene in liver and intestine. Although the PPAR-α/retinoid X receptor (RXR) heterodimer functions in the liver, PPAR-δ/RXR induces LFABP gene transcription in intestine (100, 112). The functional significance of this difference is not known.

Subcellular fractionation and immunoelectron microscopy have also revealed a small amount of LFABP in the peroxisomal matrix (8). This is of potential interest, as the phenotype of the LFABP-null mouse includes a defect in FA β oxidation in liver (79, 87) and intestine (W.S. Lagakos and J. Storch, unpublished observations). At present, it is thought that the mechanism by which LFABP reduces β oxidation is likely related to a ligand-transport function, as FA β-oxidative capacity was not decreased in the liver or intestinal homogenates of the *Lfabp*$^{-/-}$ mice.

It has been consistently found that no compensation with other FABPs occurs in LFABP-null mice; however, upregulation of acyl-CoA-binding protein and sterol carrier protein 2 has been reported by some but has not been found by others. Accumulation of cholesterol in liver of *Lfabp*$^{-/-}$ mice was likewise noted in some but not all reports (79, 80, 87, 88). A role for LFABP in cholesterol metabolism has been postulated but not definitively demonstrated. On a high-cholesterol diet, *Lfabp*$^{-/-}$ mice became obese and developed fatty livers (80); however, separate studies showed no such effect. In fact, the cholesterol-supplemented mice had reduced body weight and showed no evidence of hepatic triacylglycerol (TG) accumulation (89).

In addition to diminished FA oxidation, *Lfabp*$^{-/-}$ mice did not develop hepatic steatosis following a starvation or high-fat feeding challenge, in contrast to wild-type mice (79, 87, 88). Together, these results suggest that LFABP may function in partitioning of FA to different lipid metabolic pathways. Interestingly, *Lfabp*$^{-/-}$ mice were protected against obesity and hepatic steatosis (compared with wild-type mice) when they had been fed a diet high in saturated fat but not in polyunsaturated fat (89). Given that LFABP has increased specificity for binding unsaturated FA, the mechanism of this intriguing dietary effect remains to be understood. The transcription of the LFABP gene and that of the microsomal triglyceride transfer protein (MTP), which is known to be required for hepatic very-low-density lipoprotein (VLDL) production and intestinal chylomicron synthesis, were both recently shown to be coordinately regulated by PPAR-α/RXR-α and PPAR-γ coactivator-1-β (123). Involvement of LFABP in hepatic VLDL production is also suggested by the observation that LFABP knockout prevents the hepatic steatosis that is typically caused by treating mice with an MTP inhibitor.

Recently, strong evidence for an essential function of LFABP in lipoprotein biogenesis was reported. It was demonstrated that in intestine, LFABP is essential for budding of prechylomicron transport vesicles (PCTVs) from the endoplasmic reticulum (ER). Generation of PCTVs from the ER is rate limiting in the transit of absorbed dietary fat across the enterocyte (77). The protein responsible for budding this vesicle from ER membranes was identified as LFABP. IFABP had less than 25% of the activity of recombinant LFABP in PCTV generation. Cytosol from LFABP-null mice had 60% of the activity of wild-type mouse cytosol. These results indicate that LFABP is necessary for the budding of PCTV from intestinal ER membranes (86). Consistent with such a function, a reduction in intestinal lipid secretion following a bolus delivery of lipid to the duodenum was recently shown in *Lfabp*$^{-/-}$ mice (88).

INTESTINAL-TYPE FABP; FABP2

IFABP expression is confined to enterocytes of the small intestine (26), where it is coexpressed with LFABP. Like LFABP, IFABP is most abundant in the proximal intestine, although maximum levels of IFABP expression are shifted somewhat distally (17, 110). Expression of the IFABP gene is induced by the hormone peptide YY, which is secreted by ileal endocrine cells when dietary lipid reaches the distal part of the small intestine (43). As with LFABP, IFABP redistributes from a predominantly apical location to a more diffuse cytosolic location after fat feeding (6). Such characteristics underlie the long-held suggestion that IFABP, like LFABP, plays a role in dietary lipid absorption.

Distinct differences between the two proximal enterocytic FABPs, however, indicate that

each protein is likely to contribute at least some specific functional characteristics to the lipid absorption process. For example, IFABP binds saturated LCFA with higher affinity than unsaturated FA, like most of the FABPs but unlike LFABP, which has greater affinity for unsaturated FA than IFABP (103, 105). The far broader ligand specificity of LFABP is noted above. In vitro transfer studies have also demonstrated that IFABP and LFABP use fundamentally different mechanisms of FA transfer to and from membranes. Transfer from IFABP occurs by direct collisional interactions between the IFABP and membranes, as opposed to aqueous diffusion–mediated transfer for LFABP (54, 135). The helical portal region of the proteins has been shown to determine their transfer mechanism, and the helical region of IFABP is membrane interactive, with specific cationic residues participating in protein-membrane interactions (27, 28, 32, 146). Because the net surface charge of all cytosol-facing membranes is believed to be negative, charge-charge interactions may be a driving force for IFABP-mediated FA transfer within the cell. IFABP and LFABP genes are also regulated differently, as the IFABP promoter has not been reported to contain a peroxisome proliferator response element.

Although a large body of literature reports effects of IFABP transfection on lipid uptake and metabolism in cultured cells, these studies unfortunately show inconsistent results that do not lend themselves to straightforward interpretation; the same is true regarding LFABP transfection studies (126). It is not certain whether differences observed between transfected and nontransfected cells are due to secondary host cell differences, clonal variability, or actual FABP effects.

Insight into IFABP function has begun to emerge from studies of the *Ifabp*$^{-/-}$ mouse. Mice null for IFABP gained more weight and showed higher levels of serum TG than did wild-type mice (138), suggesting involvement of this protein in lipid absorption, metabolism, and/or secretion. Although results with the IFABP-knockout mouse clearly indicate that IFABP is not absolutely essential for dietary fat absorption (138), this may not be surprising in light of the extreme efficiency of lipid assimilation by the digestive tract, which is underscored by redundancy in digestive enzymes as well as the presence of several FABPs.

A role for IFABP in lipid assimilation and energy homeostasis is also suggested by the presence of a polymorphism in the human IFABP gene, which results in an Ala-to-Thr substitution at residue 54 and is associated with insulin resistance and dislipidemias in several (although not all) populations (36, 98, 101, 130). Plasma FA concentrations were higher following a test meal in Pima Indians homozygous for the Thr^{54} form relative to the more prevalent Ala^{54} form (101). Because the Thr^{54}-IFABP has a higher binding affinity for FA (11, 127), the apparently greater level of lipid assimilation in the Thr^{54} homozygotes implies that the effect is not simply due to greater binding of FA, as that would likely result in decreased, not increased, FA export from the enterocyte. Rather, this finding could support a more specific role for IFABP in cellular trafficking. Notably, increased fat absorption was reported in human fetal intestinal explants from IFABP-Thr^{54} heterozygotes relative to Ala^{54} homozygotes (63). On the other hand, as noted above, a complete absence of IFABP in the knockout mice was also correlated with higher levels of serum lipids, implying that IFABP has an inhibitory action on lipid assimilation.

A high frequency of the Thr^{54} allele has been detected in obese women and has been correlated with high tumor necrosis factor α (TNFα) levels, high fasting plasma insulin levels, and high leptin levels (4). TNFα is an inflammatory cytokine expressed in adipocytes that may lead to hypertriglyceridemia by decreasing hepatic lipoprotein lipase activity and increasing de novo FA synthesis (149); this suggests that IFABP-Thr^{54} is associated with obesity and insulin resistance, which may be mediated in part by inflammatory cytokines. A role for IFABP in the metabolic syndrome is also suggested by the results of high-fat feeding of the IFABP-null mice, which caused elevated body weight

in males and increased insulin insensitivity in both males and females (3, 138).

ADIPOCYTE-TYPE FABP; FABP4

Adipocyte FABP (AFABP) is also referred to as aP2, denoting its sequence similarity to the so-called myelin P2 protein, and as ALBP, adipocyte lipid-binding protein. It is expressed at high levels not only in white and brown adipose cells, but also in monocytes and macrophages (97). Its expression increases dramatically both during adipocyte differentiation and during the conversion of monocytes to activated macrophages.

Both adipocytes and macrophages express another FABP type, KFABP (FABP5). In wild-type C57BL/6J mice, KFABP is found at very low levels in fat cells, but is found at levels approximately equivalent to AFABP in macrophages. The regulation and function of AFABP and KFABP show certain similarities, which we discuss in this section; they also have several individual properties, and those are described in the subsequent section.

AFABP appears to bind only LCFAs with high affinity. This ligand specificity, coupled with the striking increase in AFABP expression (which is often used as a differentiation marker during adipocyte maturation), has led to the broad hypothesis that AFABP plays a role in TG storage and release in this cell type. The exact nature of its role in the adipocyte, as well as its function in the macrophage, is emerging from recent studies of animals null for AFABP, from tertiary structure and structure-function analyses, and from in vitro studies in cultured cells.

Although mice null for AFABP showed few phenotypic changes on a low-fat chow diet, high-fat feeding led to lower plasma insulin levels and body weight in the null animals compared with the wild-type animals (53). Other investigations, however, revealed that high-fat feeding of younger *Afabp*$^{-/-}$ mice did lead to hyperinsulinemia and obesity, although plasma glucose levels were somewhat lower than in similarly fed wild-type mice (115). In keeping with a possible alteration in glucose homeostasis, indirect calorimetry studies showed that in the fed state, the glycolysis activator fructose-2,6-diphosphate was higher in the AFABP-null mice relative to wild-type mice, although direct measurements of glucose oxidation did not significantly differ between the two genotypes (10). Some studies, but not all, have found modest decreases in β-adrenergic stimulated lipolysis (25, 113, 115) in AFABP-null mouse adipose tissue. The reason for the relatively minor adipose tissue phenotype is most likely related to the compensatory upregulation of adipocyte KFABP expression. As noted above, expression in wild-type mice is barely detectable; however, AFABP ablation leads to a dramatic increase in KFABP expression to levels approaching those of the wild-type AFABP (25, 113, 115). Such compensation might be expected to result in little alteration in adipose cell homeostasis, as it was found that in vitro AFABP and KFABP display similar ligand-binding properties, similar FA transfer rates, and a similar mechanism of LCFA transfer to membranes (115). Thus, the differences in reported effects of AFABP ablation noted above may be related to differential levels of KFABP upregulation found in separately bred mouse colonies. Indeed, comparative studies of AFABP-null and KFABP-transgenic mice indicated that functional effects on lipolysis appear to be related to the total level of FABP present in the adipose tissue, rather than the specific FABP type (48).

In contrast to the AFABP-null mice, mice null for KFABP did not exhibit a compensatory upregulation in adipocyte AFABP expression (73). This is likely because the levels of KFABP in adipose tissue are normally very low, obviating a need for compensation. The *Kfabp*$^{-/-}$ mice displayed modest effects on systemic glucose metabolism when fed a high-fat diet (73), which were similar to the effects of AFABP knockout (discussed above). However, studies of mice null for both AFABP and KFABP clearly showed that these proteins are involved in the development of the metabolic syndrome phenotype: The *AFABP*$^{-/-}$/*KFABP*$^{-/-}$ mice demonstrated strong protection against the development of hyperglycemia,

hyperinsulinemia, and hepatic steatosis, even in the face of marked obesity (23, 74). Small-molecule inhibitors of FA binding to AFABP were recently shown to ameliorate diabetic and atherosclerotic symptoms in mouse models of these diseases (35).

Unlike the relatively small effects of AFABP ablation in adipose tissue, dramatic effects of the gene knockout occur at the macrophage level, revealing an important role for AFABP in dietary lipid–induced atherosclerosis. The importance of inflammation in the development of atherosclerosis is increasingly appreciated, and it is thought that an aggressive inflammatory response combined with macrophage/foam cell lipid accumulation are critical events in the development of atherosclerotic heart disease (107). As AFABP is expressed in monocytes and macrophages, the *Afabp*$^{-/-}$ mice were crossed with the well-known model for dietary atherosclerosis, the *apoE*$^{-/-}$ mouse, to generate mice lacking both ApoE and AFABP. When fed a high-fat diet, the control apoE-null mice developed over 75% occlusion of coronary arteries in 12 weeks, as expected. In contrast, *ApoE*$^{-/-}$/*Afabp*$^{-/-}$ mice displayed only 10% occlusion, with smaller, less complex, and less macrophage-rich lesions (99). Importantly, bone marrow transplantation studies in mice, in which adipose AFABP expression was normal and only macrophage AFABP expression was targeted, showed that macrophage AFABP—rather than adipocyte AFABP—was responsible for the observed changes (62). Virtually identical results were reported in separate studies (21, 75). Thus, it is likely that macrophage AFABP is critical to the development of dietary atherosclerosis. The mechanism of this effect does not appear to be related to alterations in cellular FA transport properties (S. Kodukula and J. Storch, unpublished observations), and the mice remained hypercholesterolemic. Macrophage cholesterol esterification, although altered, is only modestly affected by AFABP ablation. In contrast, although the atherosclerosis-prone *ApoE*$^{-/-}$ mice showed increased expression of several inflammatory cytokines upon exposure to oxidized low-density lipoprotein (LDL), macrophages from the *ApoE*$^{-/-}$/*Afabp*$^{-/-}$ mice had much-reduced cytokine levels, in keeping with the lesser extent of atherosclerotic lesion formation (62). It is likely that the reduced levels of inflammatory cytokines underlie the decrease in atherosclerotic disease progression. The mechanisms by which AFABP regulates cytokine production are not known; however, both PPAR-γ– and NF-κB–dependent pathways appear to be involved (76). It is noteworthy that a polymorphism in the promoter region of the human AFABP gene that leads to decreased transcription and decreased AFABP protein levels is correlated with a modest but significant reduction in circulating TG levels, as well as with a decreased risk for coronary heart disease (136).

Interestingly, in contrast to the marked compensation in adipose tissue, KFABP levels in the macrophage did not change in the *Afabp*$^{-/-}$ mice, suggesting that in this tissue the proteins are more likely to have at least some distinct functions. Overall, it appears that for manifestation of clear effects on systemic glucose and lipid metabolism, deficiency of both AFABP and KFABP is required. In contrast, only AFABP deficiency is necessary to generate dramatic protection from dietary lipid–induced cardiovascular disease. Ablation of both genes on the apoE-deficient background confers both improved insulin sensitivity and glucose homeostasis as well as protection against atherosclerosis (22), although the contribution of adipose tissue versus macrophage FABPs is not certain in this model. Adenovirus-mediated overexpression of AFABP in cultured THP-1 macrophages was shown to increase cholesterol ester accumulation in macrophage foam cells (34), which suggests a primary role for AFABP in the process of atherosclerotic cardiovascular disease progression.

The effect of AFABP on inflammatory processes is not limited to dietary atherosclerosis, as allergic airway inflammation is also ameliorated by AFABP knockout (120), and mice null for AFABP and KFABP are protected from development of autoimmune encephalomyelitis (102). It is not yet clear whether the effects

of AFABP and KFABP that underlie their apparent proinflammatory properties are a result of changes at the cellular level or whether systemic effects that are secondary to cell-based changes are also involved. Although KFABP is well known to have a broad distribution, such that a knockout could have cellular effects in many tissues, the restricted tissue expression of AFABP may indicate a systemic effect. However, AFABP was shown to be expressed in dendritic and perhaps other cell types in the central nervous system under conditions of inflammatory or other stress (120). Moreover, not all models of atherosclerosis are dependent on AFABP; in transplant-associated atherosclerosis, which does not involve hypercholesterolemia, AFABP knockout did not prevent lesion formation and macrophage infiltration (99).

In addition to the abovementioned effects on transcriptional regulation and signal transduction pathways, the mechanism by which AFABP may affect intracellular lipid metabolism is thought to involve direct transport of its FA or FA metabolite substrate to and/or from different subcellular locations (126). Given reported observations that AFABP-null mice may have reduced adipose lipolytic activity, it is interesting that a screen for proteins that interact directly with hormone-sensitive lipase (HSL) identified AFABP (116). The interaction is supported by coimmunoprecipitation of HSL-AFABP complexes from rat adipose tissue, and it was also shown that incubation of purified HSL with AFABP causes an increase in HSL hydrolytic activity, presumably by decreasing the inhibition of the enzyme by FA (117). In support of the apparently overlapping functions of AFABP and KFABP in the adipocyte, KFABP, but not other FABP types, has also been shown to interact with HSL (57). Residues His-194 and Glu-199 on HSL were shown by alanine-scanning mutagenesis to be necessary for AFABP-HSL interaction and for the ability of AFABP to increase HSL activity (117).

The interaction with HSL requires that FA is bound to the AFABP (57). It is not clear, however, how the holo-AFABP would serve as an FA acceptor from HSL. In addition, other FABP types could also activate HSL, presumably by relieving the product inhibition on the enzyme, even though they showed no protein-protein interaction (57). These results complicate a straightforward interpretation of the functional role of direct FABP-HSL interactions.

Further investigation of the interaction has been performed in cultured C8PA lipocytes using fluorescent protein–tagged AFABP and HSL using FRET microscopy. The results showed that the HSL-AFABP complex exists (*a*) when cells are in the basal state, lipolytic activity is low, and HSL is localized in the cytoplasm, and (*b*) when cells are in the β-adrenergic stimulated state and HSL is found at the surface of the TG droplets and its activity is increased, indicating that the proteins may translocate to the lipid droplet surface as a complex (121). Recent FRET studies demonstrate that HSL phosphorylation is required for AFABP interaction and that lysine 21 on the α-I helix of AFABP is a necessary component of the protein-protein interaction site, as a K21-I mutant did not interact with HSL (122). Interestingly, this residue in the helical domain had been identified by in vitro structure-function analysis of AFABP as being required for protein-membrane interactions and FA transport to membranes (66). While HSL phosphorylation is required for its translocation to the lipid droplet (128) as well as for the AFABP interaction (122), cytoplasmic complexes were also found, when the HSL presumably is not phosphorylated. Thus, the functional significance of the AFABP-HSL interaction is potentially very interesting but is as yet uncertain.

The actions of AFABP may occur not only in the cytosolic compartment and on cytoplasmic organelles, but in the nucleus as well. Indeed, it seems likely that at least some of the systemic effects of the AFABP knockout, as well as its effects on expression of inflammatory cytokines and other immune modulators, reflect the role of AFABP as a regulator of nuclear transcription

factor activity. It is well appreciated that several LCFAs and their metabolites may act as ligands for the PPAR transcription factors (145), and it has been suggested that the FABPs are involved in delivery of ligand to the PPARs. Nuclear as well as cytoplasmic localization for AFABP has been observed in transfected CV-1 cells; however, expression resulted in an unanticipated decrease in PPAR transactivation, perhaps due to overexpression and ligand sequestration (46). A detailed analysis of PPAR-FABP interactions in transfected COS cells, however, revealed not only that ligand binding resulted in the translocation of AFABP or KFABP to the nucleus in a ligand-specific manner for each protein, but that nuclear translocation resulted in specific PPAR transactivation. AFABP was shown to transactivate as well as specifically interact with PPAR-γ, whereas KFABP specifically transactivated PPAR-β (131). A recent NMR study provided intriguing new evidence for the underlying structural basis of the AFABP-ligand interactions that effect nuclear translocation and PPAR-γ transactivation. FABP structures display multiple conformations in the solution state and are far more flexible in the absence of ligand, as discussed above (45, 49, 50). For the AFABP, binding of those ligands that result in nuclear translocation stabilizes the conformation, exposing a nuclear localization signal formed from nonadjacent residues (K21, R30, and K31) in the helical domain of the protein, whereas binding of nonactivating ligands does not stabilize that particular conformation (9, 37). The ligand-stabilized AFABP interacts with importins α or β, and the complex subsequently enters the nucleus. Similarly, three leucine residues located near the junction of the turn elements connecting the β sheets (L66, L86, and L 91) can form a so-called key conformation, which is the recognition signal for the export of the protein out of the nucleus (9, 37).

Thus, the mechanism by which the AFABP (and perhaps other members of the FABP family) effect different actions and interactions in various subcellular compartments may be due to subtle conformational changes on their surfaces caused by different ligands, which allow interaction with several different partners. A similar mechanism of action has been demonstrated for cellular retinol-binding protein-II binding of retinoic acid (RA) and delivery to the retinoic acid receptor (RAR) transcription factor (114).

KERATINOCYTE-TYPE FABP; FABP5; CUTANEOUS FABP; EPIDERMAL FABP

The skin-type FABP (KFABP) is rather widely expressed compared to other FABP types, with substantial levels found not only in skin but also, as noted above, in macrophages. KFABP is also expressed in liver, neuronal tissue, lung, and elsewhere. Its expression is dramatically upregulated in adipose tissue when the AFABP gene is ablated, and studies of mice null for both AFABP and KFABP demonstrate a role for KFABP in systemic glucose and lipid homeostasis (see the section on AFABP, above). In the macrophage, deletion of AFABP does not lead to increased KFABP expression, suggesting that there are at least some independent functions for the two proteins in that tissue. A marked increase in liver KFABP expression in atherosclerosis-prone LDL receptor–deficient mice fed a high-fat Western diet provides further evidence, albeit indirect, for a role in systemic lipid metabolism (51).

An understanding of the function of KFABP in normal and abnormal skin is emerging from studies of cultured keratinocytes and KFABP-null animals. In cultured keratinocytes, it was demonstrated that KFABP expression is essential for normal cell differentiation (131). In humans and rodents, KFABP is expressed in basal cell layers and, more strongly, in granular cell layers in normal skin (143). KFABP-knockout mice were found to have lower transepidermal water loss, as well as a delayed recovery in transepidermal water loss following disruption of the skin-lipid barrier (92, 93); however, they displayed no gross or histological changes in skin morphology. It is possible that the upregulation of HFABP expression in the skin of the KFABP-null animals led to the relatively

modest phenotype (93). KFABP/HFABP–double knockout mice have been generated (39), but a skin phenotype has not yet been reported.

KFABP is highly expressed in psoriatic skin; indeed, one of its earlier designations was psoriasis-associated FABP. This protein has been shown by cellular colocalization, in situ cross linking, and coimmunoprecipitation to interact with an S100 protein called psoriasin or S100A7 (42, 108). The S100 proteins are calcium-regulated signaling proteins, and both KFABP and S100A7 are overexpressed in psoriatic skin (41). The KFABP–S100A7 interaction is dependent on divalent cations (41), and the protein-protein complex redistributes in part from a cytosolic location to peripheral adhesion-like structures in response to calcium (108). The functional significance of the KFABP-S100A7 interaction is not yet clear.

The rat KFABP contains five cysteine residues, and biochemical analyses have shown that at least one major disulfide bond, that between Cys 120 and Cys 127, was present in the native protein despite the reducing conditions normally found in cytosol (91). The S-S bond is not required for FA binding (91); however, it has been reported that KFABP is covalently modified in vivo by the reactive acyl aldehyde 4-hydroxynonenal at Cys 120, in the process perhaps acting as an antioxidant protein by preventing the modification of other cellular proteins (16). Whether there exists a functional relationship between the Cys 120–Cys 127 disulfide bond formation and the modification of Cys 120 is not known.

A potential role for KFABP in cancer metastasis has also been suggested. Differential mRNA display and Northern analysis showed substantial upregulation of the KFABP gene in prostate and breast cancer cell lines relative to benign cell lines (58). In human prostate cancer, almost 75% of prostate carcinomas stained positively for KFABP, compared to fewer than 30% in benign prostatic hyperplasia (1). Other studies have shown that transfection of KFABP into a nonmetastatic epithelial cell line and inoculation of these transfected cells into syngenic rats resulted in significant tumor formation, whereas inoculation with vector-only transfected cells yielded no tumors (58). The tumors displayed KFABP expression, and tumor metastases also showed expression of the KFABP gene. Controls had no detectable expression, suggesting that KFABP may be involved in the induction of cancer metastases (58). Antisense KFABP transfection reduced the expression, the in vitro invasiveness, and the tumor size in nude mice (1). The tumorigenic KFABP-transfected cells were found to have elevated levels of vascular endothelial growth factor (VEGF), and conditioned media from these cells had robust angiogenic properties. Anti-VEGF antibodies could block the angiogenic potential of the media, suggesting that the KFABP-expressing cells induced metastasis via elevated expression of VEGF (59).

Several studies have explored a functional role for KFABP in the brain. Brain tissue expresses at least four different FABPs, including HFABP, brain-type FABP (BFABP), KFABP, and the myelin P2 protein (134, 140). KFABP is expressed at high levels in neurons during neurogenesis, particularly in the prenatal and postnatal periods (68). It is also dramatically upregulated by nerve growth factor in PC12 cells, and antisense knockdown of KFABP significantly decreases neurite outgrowth compared to mock-transfected cells (5). It has been proposed to function by transporting LCFAs or metabolites during neuronal differentiation; however, this has not been directly examined.

Other studies have suggested a role for KFABP in surfactant synthesis in alveolar type II cells (39) and in thymic immunity (94); however, these hypotheses require further examination. Judging from the evidence to date, it seems likely that KFABP plays cell-specific roles depending upon tissue function. Recent studies have suggested a potential mechanism by which KFABP might exert its effects, and it remains to be seen whether the various functions ascribed to KFABP thus far all involve this type of mechanism, which is related to nuclear hormone receptor activation. Specific KFABP

ligands have been shown to induce its translocation from the cytoplasmic to the nuclear compartment, and direct interaction of KFABP with PPAR-β has been demonstrated (131). RA is typically considered a ligand of the RA receptor, although it can also activate PPAR-β. Interestingly, although the RA-liganded RXR/RAR heterodimer leads to apoptosis and cell-growth inhibition, the RXR/PPAR-β heterodimer promotes cell survival. It was recently shown that delivery of RA to one or the other receptor subtype is determined by the ratio of KFABP to cellular retinoic acid–binding protein-II (119).

Overall, the two emerging mechanisms for KFABP function, like those of several other FABPs, relate to direct ligand binding and transport within the cytoplasmic compartment and to interactions with nuclear hormone receptors that regulate downstream processes, including signal transduction cascades and intermediary metabolic pathways.

HEART-TYPE FABP; FABP3

HFABP is highly expressed both in cardiac muscle and in skeletal muscle. It is also expressed to a lesser extent in many other tissues, including stomach, brain, lung, and mammary gland. The function of HFABP in muscle tissue appears largely related to a role in FA transport and metabolism, whereas its function in other tissues is less certain but may also include a lipid transport component. HFABP also affects cell proliferation and differentiation via mechanisms that are not yet fully understood.

Mice null for HFABP have provided important insight into its functional role. No substantial compensatory upregulation of any other FABP was found in the HFABP-null animals (18), making evaluation of their phenotype more straightforward. Thus, mice lacking HFABP showed dramatically decreased LCFA oxidation in muscle, which was offset by an increase in glucose utilization. Older animals were severely exercise intolerant and developed cardiac hypertrophy, further indicating defective lipid metabolism in muscle (18). Total heart FA uptake was also found to be markedly diminished in mice lacking HFABP (84). The alterations in uptake and metabolism were shown to occur at the cellular level, as cardiomyocytes isolated from HFABP-null mice showed marked reductions in LCFA uptake and oxidation and a concomitant increase in glucose oxidation (111). In giant vesicles prepared from *Hfabp*$^{-/-}$ hindlimb muscle, marked decreases in LCFA uptake were also found. Vesicles prepared from liver, which does not express HFABP, showed no effect of the HFABP deletion (70). Soleus muscle of HFABP-null animals also showed marked decreases in palmitate oxidation and increases in insulin-dependent glucose uptake (19, 31), although effects on glucose oxidation depended somewhat on dietary fat content and muscle TG content (2). There was no change in intramyocellular TG content in chow-fed mice, which was likely due to decreases in both TG hydrolysis and TG esterification in the HFABP-null soleus (19). Application of muscle contraction stimulus showed that HFABP-null muscles were still able to increase FA oxidation, albeit to a substantially lesser extent than were wild-type muscles (19). The mechanism underlying this tendency toward a normal response to contraction is not known, but the results imply that even in the face of complete absence of HFABP, normal muscle lipid metabolism can proceed to some extent.

Unlike muscle tissue, FAs are not a primary substrate for ATP production in the brain. Nevertheless, the brain utilizes FAs for phospholipid synthesis, and the abundance of polyunsaturated fatty acyl chains in brain phospholipids is well appreciated. Despite the expression of three other FABPs in brain, no compensatory changes in these or other FABPs were found in the HFABP-null brain (85). As the different FABPs show both developmental and cell-type specificities, this finding is not surprising; furthermore, it is consistent with the likely functional specificity for these proteins. HFABP-null brain showed a reduced phospholipid mass, and the incorporation of radiolabeled FAs showed that in the HFABP

knockouts, compared with wild-type mouse brain, less arachidonic acid was incorporated into total brain lipids. Interestingly, no such effect was found for palmitic acid, suggesting that HFABP may be specifically involved in n-6 FA transport/metabolism in the brain (85). Similar studies in heart tissue indicated a role for HFABP in 20:4n-6 incorporation into higher lipid classes as well, although no change in phospholipid mass was reported (84).

Although it has been clear for some time that an FABP-related protein termed mammary-derived growth inhibitor (MDGI) is actually HFABP (124), the biological properties attributed to MDGI, as well as subsequent studies of HFABP, raise the intriguing possibility that HFABP may act to modulate cell growth and differentiation. MDGI/HFABP has been found to inhibit the proliferation of mammary epithelial cells in culture, and MCF7 breast cancer cells transfected with the HFABP gene showed lower tumorogenicity when inoculated in nude mice (56). Given the apparent impact of HFABP on mammary epithelial cell growth, it is surprising that HFABP-null mice showed no discernable mammary phenotype (24). Nevertheless, indirect evidence supporting a role for HFABP in cell growth and differentiation also comes from proteomic analysis of differentiating mouse cardiomyocytes, which demonstrates a strong inverse correlation between HFABP/MDGI expression and that of proliferating cell nuclear antigen, a marker for proliferating cells (132). Interestingly, many of the effects of HFABP on mammary epithelia can be mimicked by an 11-residue C-terminal peptide. Expression of the peptide in human breast cancer cell lines led to diminished colony formation in soft agar as well as decreased tumor growth in nude mice inoculated with the peptide-producing cells (142). Although more work needs to be done to establish the generality of this finding, this result suggests that effects of HFABP on cancer cell proliferation may not, at least entirely, be related to its ligand binding properties.

Somewhat surprisingly, the effects of specific ligand binding on FABP functions are not well understood for many members of the protein family. For example, a variety of oxygenated metabolites of long-chain polyunsaturated FAs (PUFAs), in addition to the parent compounds, have been shown to bind to HFABP (144). As demonstrated earlier for a series of FAs binding to several of the FABPs (105), the affinities of the oxygenated compounds were found to be inversely related to their polarity, although some deviations were observed (144). The interaction of ligands with HFABP could lead to decreased free or effective concentrations of the ligands, thus moderating their activities, or to increased trafficking and delivery of the ligands to or from other proteins, specific membrane domains, or nuclear hormone receptors.

Interaction of HFABP with the putative membrane FA transporter CD36 in mammary tissue was shown by coimmunoprecipitation (125), and the expression of the two proteins was found to covary in several animal and muscle cell models, lending indirect support for coordinated action within the cell (137).

BRAIN-TYPE FABP; BLBP; FABP7

BFABP expression is associated with neuronal and glial cell differentiation (33, 61) and is more abundant in fetal than adult brain (118). Anti-BFABP antibodies were shown to block the extension of radial glial cell processes (33). BFABP's functions and mechanisms of action at the molecular level are not yet understood; however, a number of interesting findings are suggestive of its physiological role in the brain. Moreover, BFABP expression has been reported in other tissues, indicating extraneuronal functional effects as well.

BFABP expression was upregulated in an epidermal growth factor receptor–dependent manner in neurofibromatosis type 1 mutant mouse Schwann cells, which grow away from axons. In this system, anti-BFABP antibodies were shown to restore normal glial process growth and interaction with axons (82). Thus, BFABP appears to be involved in establishing the radial glial fibers that support normal neuronal migration. The expression of BFABP in

radial glia was substantially reduced in mice lacking Notch receptors, indicating a potential role for BFABP in Notch signaling (7). In BFABP-expressing malignant gliomal cells, relative to BFABP negative cells, nuclear factor 1 is in the dephosphorylated state; hypophosphorylation of this transcription factor is associated with the expression of glia-specific genes and increased glial differentiation (20). Moreover, it was recently shown that transfection of BFABP into a BFABP-negative malignant glioma cell line increases cell-migration properties and that knockdown of expression in a BFABP-positive cell line inhibited cell-migration properties, suggesting that BFABP may regulate the invasiveness of astrocytoma tumors (83). Furthermore, an analysis of human glial astrocytomas found that patient survival was inversely correlated with levels of BFABP expression (64).

Despite the role of BFABP in neuronal and glial cell differentiation, mice null for BFABP showed no gross morphological or histological alterations in the brain. There was no compensatory expression of other FABP types, either those normally expressed in brain or those not found in wild-type brain (95). Nevertheless, distinct behavioral differences were found in the *Bfabp*$^{-/-}$ mice, which displayed increased memory of fear and increased levels of anxiety. Interestingly, in neurons from the amygdala, the region of the brain associated with emotional memory, N-methyl-d-aspartate receptor activity in response to the n3 FA docosahexaenoic acid (DHA) was decreased in BFABP-null compared to wild-type mice, and decreased brain DHA content in the neonatal brain was found (95).

Examination of FA binding to BFABP has produced inconsistent results. It was reported that, in contrast to other FABPs, BFABP has a 40-fold greater affinity for the n3 FA DHA than for the n6 PUFA arachidonate (147). Such specificity was shown to a tenfold lesser degree or not at all in other reports (12, 105). Nevertheless, it is tempting to speculate upon a specific role for BFABP in DHA metabolism, given the high concentrations of DHA in brain.

A DHA-related role for BFABP is also indicated by studies in human mammary gland. A tumor growth inhibitor in mammary gland that induces mammary cell differentiation, initially called MRG for mammary-derived growth inhibitor related gene (142), was identified as BFABP (52). Overexpression of BFABP in a human breast cancer cell line caused increased differentiation, and treatment of the cells with DHA led to growth inhibition that was directly related to the level of BFABP expression, with BFABP-negative cells not responding to DHA treatment. No growth inhibitory effects were found with the n6 FA linoleate, irrespective of BFABP level (142).

Early pregnancy is known to reduce the risk of breast cancer, as pregnancy results in mammary cell differentiation. It has been suggested that a possible cause of this reduction in breast cancer risk is the reduction in n6/n3 PUFA ratio and increased n3 PUFA content found in the pregnant mammary gland (69). Further, in nonpregnant fat-1 transgenic mice, which can convert n6 to n3 PUFA, expression of BFABP resulted in a significantly greater reduction of the n6/n3 PUFA ratio, an increase in DHA levels in mammary tissue, and increased mammary cell differentiation (69). These results support a role for BFABP in n3 PUFA retention, possibly contributing to the effects of pregnancy on mammary gland differentiation.

OTHER FABPS

Two other members of the FABP family that bind LCFAs are the so-called myelin P2 protein (FABP8, also designated as MFABP) and testicular FABP (FABP9, also designated as TLBP or PERF15) (44). The functional properties of these FABPs remain largely unknown, although both exhibit fairly restricted expression patterns, suggestive of roles in peripheral nerve myelin and the testis, respectively. FABP9 is the sole FABP in testicular germ cells (60). Overexpression of FABP9 using a testicular germ cell–specific FABP transgene suggests that it is involved in determining germ cell fates and in the preservation of sperm quality (60).

CONCLUDING REMARKS AND FUTURE DIRECTIONS

In recent years an understanding of the functions of the FABPs and their potential mechanisms of action has begun to emerge (**Figure 2**). Evaluation of gene-ablated mice and in vitro studies indicate that different FABPs are likely to have overlapping as well as unique functions in specific tissues. FABPs appear to be involved in the extranuclear compartments of the cell, trafficking their ligands within the cytosol via interactions with organelle membranes and specific proteins. Several members of the FABP family have also been shown to function directly in the regulation of cognate nuclear transcription factor activity via ligand-dependent translocation to the nucleus.

It is interesting that the expression of many of the FABPs is regulated by binding of their ligands to PPAR transcription factors, and that these FABPs in turn appear to regulate specific PPAR transactivation. It remains to be understood how this feed-forward action of ligand channeling is homeostatically controlled.

Another issue remaining to be resolved concerns ligand binding by the FABPs and whether there are true functional roles for individual FABPs and specific ligands. For example, KFABP was shown to bind to and dramatically increase the half-life of the unstable lipoxygenase metabolite leukotriene A4 in rat basophilic leukemia cells, and it was suggested that KFABP functions in eicosanoid metabolism by allowing intermediary metabolites to be available for further metabolic conversion (30). Subsequent analysis, however, demonstrated that in addition to KFABP four other FABPs showed the same effect (148). Another unresolved issue concerns the putative role of LFABP in cholesterol binding and homeostasis.

Studies in rodent models have also shown that the FABPs are important in modulating systemic lipid and carbohydrate metabolism. The mechanisms underlying these changes likely include not only bulk changes in tissue lipid metabolism, but also modulation of signal transduction cascades and nuclear hormone receptor activation and, hence, downstream gene transcription. The intranuclear and extranuclear mechanisms of action of FABPs remain important avenues of investigation.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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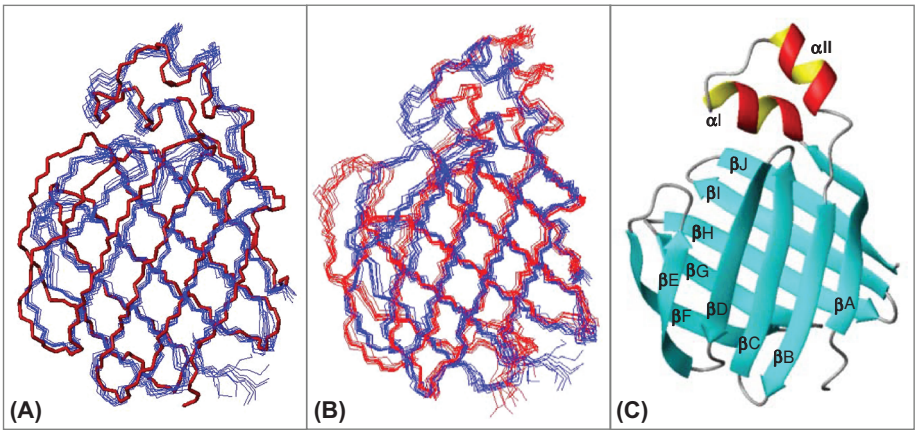

Figure 1

Nuclear magnetic resonance–derived solution structures for liver-type fatty acid–binding protein (LFABP), demonstrating specific conformational changes between unliganded and oleate-bound proteins. (*a*) Superposition of a family of holo-LFABP solution structures (*blue*) with the crystal structure (*thick red line*) (133). (*b*) Superposition of lowest-energy apo- (*red*) and holo-LFABP (*blue*) structures. The two molecules of bound oleate are not shown in the holo structure. (*c*) Ribbon diagram of the lowest-energy solution structure for apo-LFABP. Data from Reference 45.

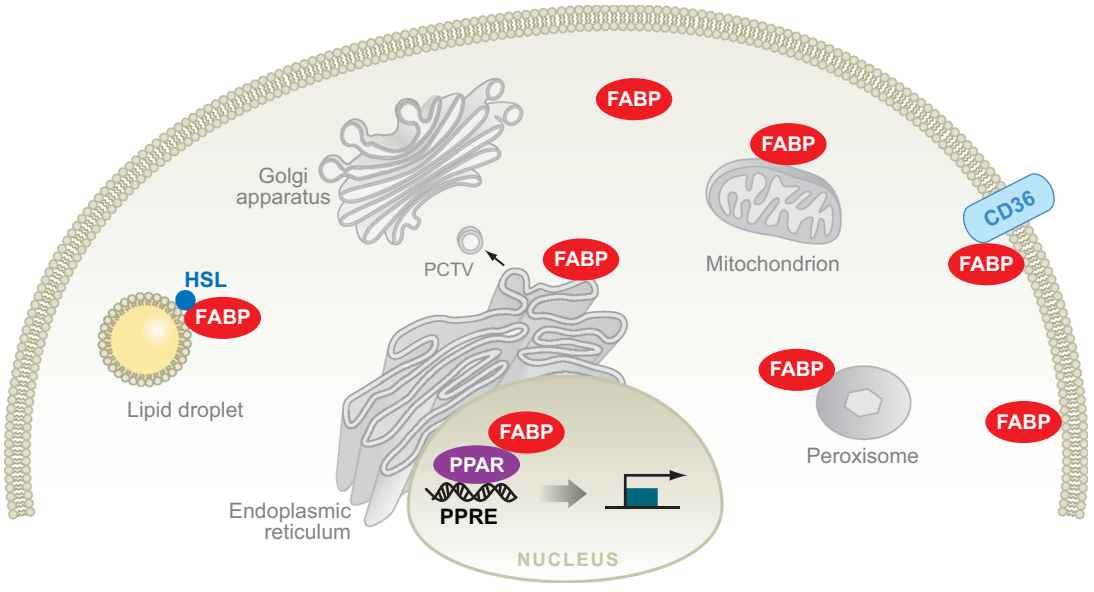

Figure 2

Potential functions of fatty acid–binding proteins (FABPs) in intracellular fatty acid disposition. FABPs are believed to act in the cytoplasmic compartment via specific interactions with subcellular organelles, including the endoplasmic reticulum, mitochondria, lipid droplets, and peroxisomes. Coordinated function with the putative transmembrane transporter CD36, the hormone-sensitive lipase (HSL), and a potential role in the formation of prechylomicron transport vesicles (PCTVs) are also depicted. FABPs are also likely to function in the nucleus by delivery of specific ligands to nuclear transcription factors such as the peroxisome proliferator–activated receptors (PPARs).

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Errata

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