

# Properties and physiological significance of fatty acid binding proteins

Norbert H. Haunerland<sup>a</sup> and Friedrich Spener<sup>b,\*</sup>

<sup>a</sup>*Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6*

<sup>b</sup>*Department of Biochemistry, University of Münster, Wilhelm-Klemm-Str. 2, 48149 Münster, Germany*

*\*Correspondence address: Tel.: +49-251-83-33100; fax: +49-251-83-32132*

*E-mail: spener@uni-muenster.de(F.S.)*

## Abbreviations

CRABP: cellular retinoic acid binding protein; CRBP: cellular retinol binding protein; FABP: fatty acid binding protein; I-BABP: intestinal bile acid binding protein; iLBP: intracellular lipid binding protein; ITC: isothermal titration calorimetry; PPAR: peroxisome proliferator activated receptor; PPRE: peroxisome proliferator responsive element; RAR: retinoic acid receptor; RARE: retinoic acid responsive element; RXR: retinoid X receptor.

## 1. Introduction

In 1978, Ockner et al. [1] discovered a small protein in the cytosol of certain rat tissues that bound fatty acids and consequently named it “fatty acid binding protein” (FABP). Since then, such FABPs have been found in many tissues of many different organisms which include mammals, fish, birds, and insects. Some of these proteins were originally characterized in a different context (organic anion binding protein, Z-protein) and only later were found to be FABPs. All FABPs are members of a large multigene family now called “intracellular lipid binding proteins” (iLBPs) with various functions in the transport and metabolism of their ligand fatty acids and other lipophilic ligands. Many excellent reviews have been published on different aspects of these proteins (for a recent review see Ref. [2]), which are remarkably conserved throughout the animal kingdom. While their roles in different cells, tissues, and organisms may vary, common features become apparent in the context of metabolic tasks and conditions. The purpose of this review is to summarize current knowledge about these proteins, and to provide insight into their roles in different organisms.

## 2. FABPs as members of the iLBP family

FABPs as members of the iLBP family have traditionally been named after the tissue from which they were first isolated. Liver-type, heart-type, and intestinal-type FABP (L-FABP, H-FABP, I-FABP) have been the first to be discovered [1], and later the aP2 protein was recognized as adipocyte-type (A-) FABP [3]. With the increasing availability of ESTs and gene array data, it has become clear that most iLBPs are not confined to a single tissue. This, however, does not necessarily mean that they are un-specifically expressed, as tissues always contain different cell types. For example, heart tissue contains not only cardiomyocytes, but also significant amounts of epithelial and smooth muscle cells as well as some adipocytes. Moreover, even defined cells such as adipocytes express more than one FABP-type [4]. This is even more apparent when FABPs expressed in non-mammalian animals are considered: for example, the most prominent FABP-type expressed in shark liver [5] clearly belongs to the same subfamily (see below) as H-FABP, while the FABPs found in the livers of other fish species of chicken and are basic proteins, yet distantly related to the mammalian L-FABP [6].

In this review, the widely accepted nomenclature for FABP that is based on the tissue occurrence will be used. The numerical classification used by Genbank may be more accurate, but less intuitive. In Table 1 the classical names, alternative designations found in the literature and the GenBank designations are summarized, as is the occurrence of the proteins in tissues of mature animals.

FABPs are expressed in vertebrate (mainly mammals, fish, birds) and invertebrate species. Pertaining to the latter, two FABPs are expressed in the midgut of the tobacco hornworm (*Manduca sexta*) [7] and believed to be involved in lipid digestion. The FABP from the flight muscle of locusts has been especially well characterized [8,9]. It is present in high concentration and shares many characteristics with its mammalian H-FABP counterparts. They have a high sequence homology to other insect proteins that have been identified only at cDNA levels, namely from the fruit fly (*Drosophila melanogaster*) [10] and the mosquito *Anopheles gambiae* [11]. A protein found in the brain of the tobacco hornworm, initially identified as a cellular retinoic acid binding protein (CRABP) [12], belongs to the same subfamily as H-FABP as well (see below). Surprisingly, FABPs have also been found to be prominent arthropod allergens, e.g. in the dust mites *Blomia tropicalis* [13] and *Acarus siro* [14]. In the fluke *Schistosoma mansoni* [15] and various other parasitic worms [16], FABPs are considered essential for lipid absorption, since these animals are unable to synthesize complex lipids de novo [17].

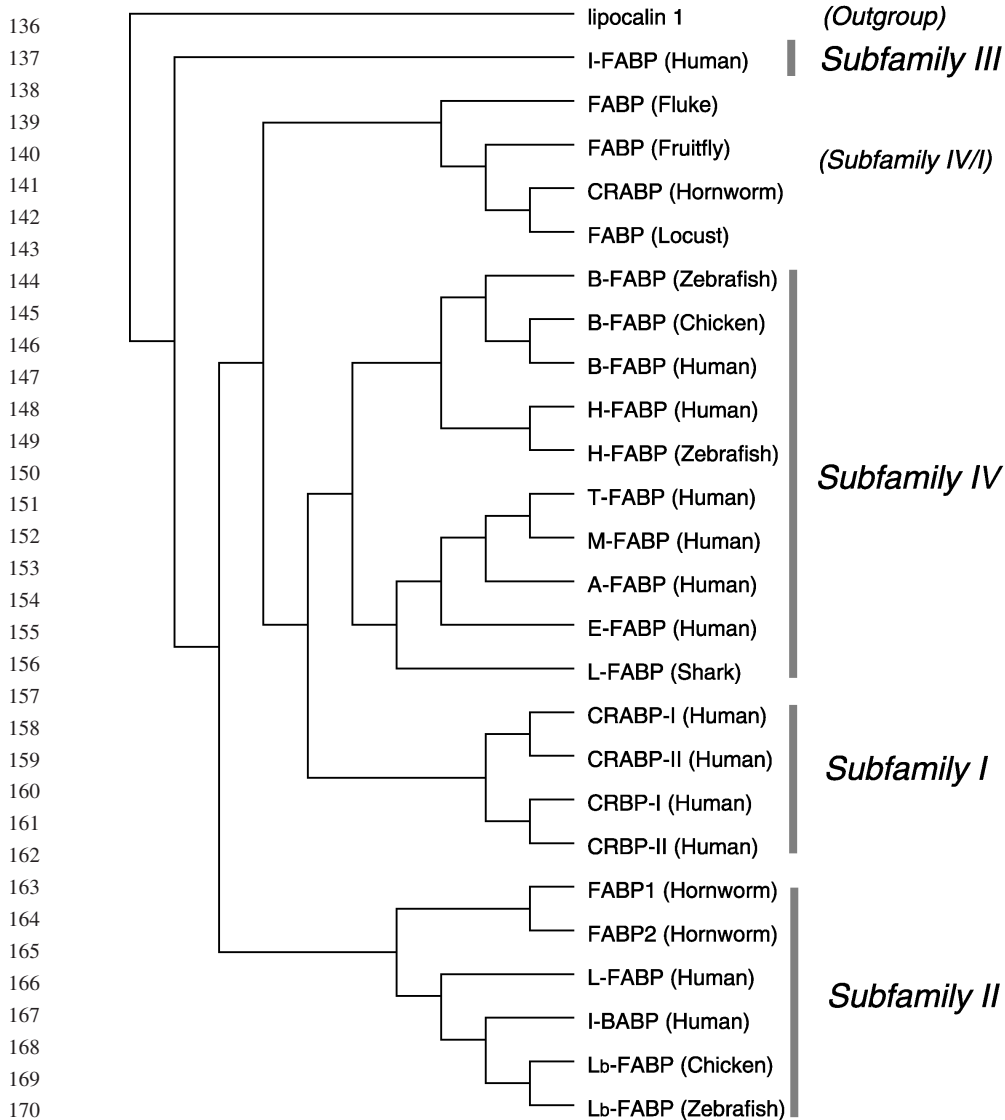
Given the wide distribution of iLBPs throughout the animal kingdom, it is apparent that they belong to an ancient gene family. Major gene duplications gave rise to the separate subfamilies. Multiple alignments of iLBP sequences and construction of phylogenetic trees by the Clustal W algorithm illustrate this relationship as shown in Fig. 1. Four major subfamilies for the mammalian proteins have been categorized based on this sequence homology and, in addition, on ligand binding characteristics [18] (see Table 1 and Fig. 1):

- (I) The intracellular retinoid binding proteins [19] can be further subdivided into the cellular retinoic acid binding proteins (CRABP I and II) and the cellular retinol binding proteins (CRBP I and II).

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Table 1  
Nomenclature and expression pattern for intracellular FABPs

iLBP-type	Alternatives names	Gene name (human)	Mammalian expression	Non-mammalian expression
L-FABP (liver)		FABP1	Liver, intestine, kidney, lung, pancreas	
I-FABP (intestinal)		FABP2	Intestine	
H-FABP (heart)	M-FABP (muscle) MDGI	FABP3	Heart, skeletal muscle, kidney, lung, mammary, placenta, testis, stomach, ovary	Fish muscle, bird muscle, insect muscle, fish ovary
A-FABP (adipocyte)	ALBP aP2	FABP4	Adipose tissue	Fish muscle (?)
E-FABP (epidermal)	E-FABP KLBP mal1	FABP5	Skin, adipose tissue, lung, brain, heart, skeletal muscle, testis, retina, kidney	
I-BABP (intestinal)	ILBP Gastrotropin	FABP6	Ileum	
Brain FABP	B-FABP R-FABP	FABP7	Brain, neurons	Bird brain, retina
M-FABP (myelin)	mP2 Myelin P2	FABP8	Schwann cells	
T-FABP (testis)	T-FABP	FABP9	Testis	
Lb-FABP (liver basic)	L-FABP	FABP10		Fish, chicken, iguana liver
Midgut FABP				Insect midgut



171 **Q6** Fig. 1. Phylogenetic tree for the iLBP family. Sequences for the vertebrate and invertebrate members of the iLBP  
 172 gene family were aligned with Clustal W. The tree was constructed with the neighbor joining method, using lens  
 173 lipocalin as an outgroup. For mammalian iLBPs only the human paralogs are shown. For the subfamily concept  
 174 see Sections 2 and 3 in the text.

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176 (II) L-FABP and I-BABP (intestinal bile acid binding protein) are closely related based  
 177 on sequence homology and both stand out because of their unusual ligand binding  
 178 specificities. L-FABP, which binds a broad range of ligand molecules (acyl-CoAs,  
 179 heme, squalene, bilirubin and certain eicosanoids), is the only FABP that forms a  
 180 complex with two fatty acid molecules at the same time [20–22].

181 (III) I-FABP is rather singular in sequence characteristics and binds one fatty acid  
182 molecule.

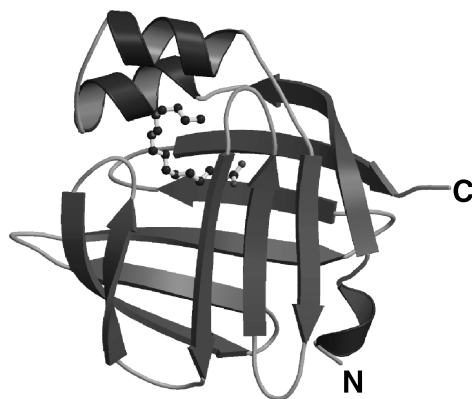
183 (IV) This iLBP subfamily comprises the largest number of different types of FABPs,  
184 i.e. H-, A-, E- (epidermal-type), M- (myelin-type), T- (testis-type), and B- (brain-  
185 type) FABP. They all bind only a single fatty acid molecule.

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187 Generally, the non-mammalian FABPs fall into one of the subfamilies as defined above  
188 and shown in [Table 1](#) and [Fig. 1](#), attesting to the considerable evolutionary conservation of  
189 this protein family. Various papers have discussed the phylogenetic relationship between  
190 the different members of the FABP family [3,23,24]. From phylogenetic analysis it is  
191 likely that a common ancestor gene branched out into two major families more than 900  
192 million years ago, long before the vertebrate–invertebrate divergence. Thus, subfamily II  
193 includes not only L-FABP and I-BABP, but also the insect midgut FABPs. The FABP  
194 from insect muscle is assembled not only with the H-FABP expressed in mammalian heart  
195 and skeletal muscle cells, but also with the cellular retinoid binding proteins, since  
196 subfamilies I and IV are believed to have split after the vertebrate–invertebrate  
197 divergence [25].  
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### 200 3. Structure and conformation of FABPs and their ligands

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202 The iLBPs are small proteins of 127–134 amino acids, whose expression in *E. coli*  
203 made available substantial quantities of recombinant protein for biophysicists and  
204 structural biologists to gain deeper insights into structure and binding properties of these  
205 proteins. Thus, three-dimensional structures have been determined by X-ray crystal-  
206 lography [22,26–30] and/or NMR [31–35] for all types of the mammalian iLBPs, with the  
207 exception of T-FABP. In addition, the crystal [36] and solution structure [37] of the  
208 chicken basic liver-type (Lb-) FABP are known. Of the invertebrate FABPs, the three-  
209 dimensional structures of a midgut FABP from tobacco hornworm [38] and of the  
210 H-FABP from desert locust [9] have been solved. From this wealth of data it has become  
211 clear that the tertiary structure of all iLBPs is highly conserved, despite the considerable  
212 differences in their primary structure. Sequence identities in this protein family range from  
213 25% for some paralogous members to over 90% for some orthologs. The common  
214 structural feature is a 10-stranded  $\beta$ -barrel, made of two orthogonal antiparallel 5-stranded  
215 sheets that form the “clam”-shaped binding cavity [39]. The opening of this clam,  
216 considered the portal domain, is framed on one side with the N-terminal helix-turn-helix  
217 domain, a further common structural motif of all iLBPs ([Fig. 2](#)). The 10 antiparallel  
218 strands that form the barrel is the salient feature of iLBPs within the “calycin” superfamily  
219 of lipid binding proteins, whose other families, the avidins and lipocalins, are  
220 characterized by 8-stranded antiparallel barrels forming the binding cavity [40].

221 In the binding pocket of iLBPs the deprotonated carboxyl group of the bound ligand is  
222 generally buried inside the cavity for electrostatic interaction with one or two arginine  
223 residues, in addition to be hydrogen bonded by a tyrosine- or serine-OH and an ordered  
224 water molecule [27]. Nonetheless, important differences between individual iLBP-types  
225 exist, which influence binding kinetics and affinity as well as the mechanism of ligand



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239 **Q6** Fig. 2. Three-dimensional structure of *holo* E-FABP (with palmitic acid) [29]. All iLBPs have the characteristic  $\beta$ -barrel structure, in which 10 antiparallel  $\beta$ -strands form the “clam”-shaped ligand binding site, framed by the helix-turn-helix domain as part of the portal. In E-FABP, fatty acid is bound in a U-shaped conformation, characteristic for subfamily IV iLBPs.

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transfer [18,41]. FABP-type specific affinities for fatty acids are due to different volumes of the binding cavities and to the amino acid side chains facing one side of the fatty acid’s hydrocarbon chain directly, and indirectly the other side via ordered water molecules. This view is not uncontested, however (see Section 4).

A close-up inspection of protein structure and ligand conformation by crystallographic techniques fosters the above-mentioned subfamily concept for iLBPs:

- (I) The conformation of the characteristic isoprenoid tail of the retinoid ligands is extended and the  $\alpha$ -ionone ring located close to the helix-turn-helix domain, whereas the functional group is always deeply immersed into the binding cavity. Here Arg111 and 132 and Tyr134 directly bind all-*trans* retinoic acid in the case of CRABPI and II (cellular retinoic acid binding proteins) [42] which is a scenario similar to that of straight-chain fatty acid binding in proteins of subfamily IV. In CRBP I and II (cellular retinol binding proteins), which bind either all-*trans* retinol or retinal, Gln108 interacts with the functional group of the ligand [43,44] and in CRBP III and IV, variants binding only retinol, Gln108 is replaced by His [45,46].
- (II) Of the two fatty acids bound by L-FABP, one is coordinated in a bent conformation electrostatically via Arg122 and an extensive hydrogen-bonding network involving Ser124 and 39 located at the bottom of the protein cavity, which again is reminiscent of fatty acid binding in subfamily IV. The second fatty acid in L-FABP adopts a rather linear shape, with the acyl chain in the cavity extending down towards the center of the other fatty acid molecule and the carboxylate sticking out of the fatty acid portal, thus being solvent exposed and pH sensitive [22]. Interestingly, although I-BABP contains the respective residues (Arg121, Ser123 and 38), it binds fatty acid only weakly, instead of a bile acid molecule with high affinity. Again, the bulk steroid molecule is inside the cavity and the carboxylate group at the protein–solvent interface [47].

- 271 (III) The fatty acid bound by I-FABP adopts a slightly bent conformation, reverse in  
272 direction to the second fatty acid in L-FABP, thus the carboxylate group is located  
273 deep inside the protein cavity directly coordinated to the side-chain of Arg106 similar  
274 to the ligands' carboxylate bound by proteins belonging to subfamilies I and IV [26].
- 275 (IV) The FABP-types of this subfamily all bind only a single fatty acid molecule in a  
276 U-shaped conformation. While the carboxylate group is bound electrostatically and  
277 hydrogen bonded via Arg106 and 126 as well as Tyr128 (H-FABP numbering), the  
278 hydrocarbon chain is located close to Phe57 (Leu60 in E-FABP) at the fatty acid  
279 portal [27]. Several unique features in this iLBP subfamily have been reported only  
280 recently. First, human E-FABP contains six cysteine residues, of which C120 and  
281 C127 form a disulfide bridge inside the protein cavity [29]. Secondly, human  
282 B-FABP binds oleic acid in the common U-form conformation, but very long-chain  
283 docosahexaenoic acid (DHA) in a helical conformation [30]. It remains to be seen  
284 whether the latter is a consequence of chain-length, or not a specific feature for  
285 binding  $n - 3$  fatty acids. The three-dimensional structure of insect muscle FABP  
286 has been solved for the *apo*-protein only [9]. It is remarkably similar to mammalian  
287 H-FABP, although steric limitations seem to predict a somewhat different shape of  
288 the ligand in the binding pocket.  
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#### 291 4. The binding and transfer of fatty acids by FABPs

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293 As far as we know, the obvious task of FABPs is to bind fatty acids. A total of eight  
294 FABP-types are expressed in various mammalian tissues each carrying out distinct  
295 metabolic tasks. Is fatty acid binding to these FABPs a mere variation of a common  
296 structural "leitmotiv", with little consequence for binding affinities? Or do the small  
297 structural differences in the binding sites lead to binding selectivities for distinct fatty acid  
298 structures? It is not easy to decide which view is correct, and literature data on this aspect  
299 are somewhat controversial.

300 The ADIFAB reagent is a covalently modified I-FABP, with a fluorescent label that  
301 changes its emission maximum upon the binding of fatty acids [48,49]. On the one hand,  
302 data elaborated with this ADIFAB assay have been interpreted in terms of the "solubility  
303 hypothesis", which states in a first approximation that the solubility of a given fatty acid in  
304 the bulk aqueous phase drives its affinity for any FABP. The binding site of I-FABP is  
305 considered to act similar to a non-polar solvent, and hence its affinity for different fatty  
306 acids is mainly determined by the entropic contribution of the hydrophobic effect.  
307 Recently, however, thermodynamic parameters for ligand double bonds were incorporated  
308 into the calculation of dissociation constants to reflect physico-chemical properties of a  
309 given FABP binding site, in fact, the enthalpic contribution to binding. For all FABP-types  
310 and their ligand fatty acids tested so far, the values for  $K_d$ s found with the ADIFAB  
311 method are between 2 and 200 nM.

312 On the other hand, far greater variations in binding constants were found with other  
313 methods. The earliest assays used charcoal to remove unbound fatty acid from the solution  
314 and calculated binding constants from the ratio of charcoal- and protein-bound  
315 radioactivity [50]. Soon charcoal was replaced by a lipophilic dextrane derivative,

Lipidex 1000 [51]. This material has strong affinity to fatty acids at 37 °C, and can be used to delipidate FABP. At 0 °C, however, protein-bound fatty acids were shown to remain bound to FABP, while unbound fatty acids were adsorbed to Lipidex. Determination by this method afforded dissociation constants between 0.2 and 0.4  $\mu\text{M}$  which are now considered too high, because of the low temperature and the time required to separate Lipidex from FABP [18]. More reliable values can be obtained by measuring dissociation constants without physically separating free from bound ligands, such as fluorescence-based methods like the ADIFAB assay. Another popular approach is isothermal titration calorimetry (ITC), which measures the heat absorbed or released upon binding of the ligand to the protein [18]. For mono- and polyunsaturated fatty acids, dissociation constants in the 10–300 nM range have been determined, whereas remarkably larger values were found for saturated fatty acids, for which the ADIFAB method suggests very strong affinity. The reasons for these discrepancies are not clear, but could be related to solubility problems. A comparison is shown in Table 2, taking the example of B-FABP.

It follows from this short discussion (for more details, see Ref. [18]) that absolute values of dissociation constants depend on the method used for their determination. Their relative values, however, are comparable from method to method, in particular for Lipidex and ITC data. Some of the latter can be explained on the basis of crystallographic studies [52]. Moreover, further insights into binding can be gained by inspecting the dynamic properties of FABPs through various NMR techniques, Fourier transform infrared spectroscopy and recent molecular dynamics calculations [18]. These studies lead to the following conclusions: (i) Differences in the backbone dynamics of various FABPs can be correlated to preferences for specific fatty acids and their relative binding affinities. (ii)

Table 2  
Dissociation constants for human B-FABP/ligand complexes determined by the ADIFAB and ITC method

Ligand fatty acid class	$K_d$ (nM)	
	ADIFAB, 37°C <sup>a</sup>	ITC, 30°C <sup>b</sup>
Saturated		
Palmitic acid	7	7100 <sup>c</sup>
Stearic acid	2.3	13,500 <sup>c</sup>
Monounsaturated		
Oleic acid	7	46.7 $\pm$ 1.4
Polyunsaturated $n - 6$		
Linoleic acid	11	115 $\pm$ 19
Arachidonic acid	18	207 $\pm$ 19
Polyunsaturated $n - 3$		
Docosahexaenoic acid	13	53.4 $\pm$ 4.1
$\alpha$ -Linolenic acid	21	27.5 $\pm$ 1.3

<sup>a</sup>Ref. [49].

<sup>b</sup>Ref. [30].

<sup>c</sup>By Lipidex assay and referenced to  $K_d = 47$  nM for oleic acid as obtained by ITC.

361 The *apo*-conformation of the protein can adapt to a particular ligand fatty acid and is thus  
362 stabilized by reduced backbone flexibility in some *holo*-FABPs [53], even “structured”  
363 water molecules as part of the tertiary structure may add to this stability. (iii) In the portal  
364 region, the backbone structures generally display an increased conformational variability.

365 Finding the correct answer to the questions raised at the start of this section is not easy.  
366 Certainly, preferences for interactions of certain FABP-types with structurally defined  
367 fatty acid classes can be recognized, such as E-FABP with saturated fatty acids, I-FABP  
368 with saturated and monounsaturated fatty acids, H-FABP with  $n - 6$  polyunsaturated fatty  
369 acids, L-FABP with mono- and  $n - 3$  polyunsaturated fatty acids, and B-FABP with  $n - 3$   
370 polyunsaturated fatty acids. This would have functional implications. A tenet to this  
371 statement is that all binding data published originate from *in vitro* assays that may not  
372 reflect the complexity seen within a cell *in vivo*. According to Weisiger [52], “free”  
373 unbound fatty acids in the aqueous cellular compartments originate from their spontaneous  
374 membrane-to-membrane transfer that is very slow and depends on the mean diffusional  
375 excursion ( $d_m$ ) of a fatty acid from the membrane. The bulk of the “free” fatty acid  
376 molecules in the cell, however, is bound to membranes and to intracellular binding  
377 proteins, particularly FABPs. When intracellular transfer of fatty acids beyond  $d_m$  is  
378 needed, certain FABPs act as “membrane-inactive” binding proteins, and catalyze  
379 the diffusional transfer step by increasing fatty acid concentration in the soluble  
380 (= diffusible) pool; others act as “membrane-active” binding proteins that catalyze fatty  
381 acid dissociation from donor membranes and rebinding to acceptor membranes through  
382 FABP-membrane collisions. This intriguing concept received convincing support by  
383 elegant studies at the molecular level, which demonstrated that L-FABP and CRABP II  
384 belong to the membrane-inactive, non-collisional group, while all other FABP-types  
385 investigated are membrane active and catalyze collisional transfer [54].

386 This collisional transfer of fatty acids from the FABP to zwitterionic and anionic  
387 membranes relies on interactions with positively charged amino acid residues in the helix-  
388 turn-helix motif and in turns belonging to the portal domain of respective FABPs [55–57].  
389 Thus, modulation of fatty acid transfer rates in either direction depends on electrostatic  
390 interactions of the protein with membrane lipid or protein; additional hydrophobic  
391 interactions appear to be at work as well. If this concept is true, why does a cell need  
392 membrane-inactive FABP, such as L-FABP at all? It has been proposed that membrane-  
393 active FABPs would lose diffusional mobility and thus ability to catalyze efficient fatty  
394 acid transfer in cells densely packed with membranes that require efficient fatty acid  
395 transfer between membranes over some distance. Hepatocytes and enterocytes are such  
396 cell types, and both express L-FABP [58].

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## 399 5. Metabolic actions of FABPs

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401 In contrast to the very detailed knowledge of the structure and binding characteristics of  
402 FABPs, much less is known about their biological functions. The fact that they bind fatty  
403 acids suggests that these proteins participate in various aspects of lipid transport and  
404 metabolism. Many studies have demonstrated that FABPs modulate metabolic reactions *in*  
405 *vitro*, but this does not imply that similar effects occur in living cells. Given the poor

solubility of fatty acids in water, one can expect, for example, that the presence of FABP in a buffer increases the availability of fatty acid to enzymes, thus leading to increased metabolic rates in vitro. FABPs are believed to serve the following cellular tasks:

- uptake of fatty acids into the cell;
- formation of cytosolic pool for fatty acids to be rapidly utilized and, concomitantly, to avoid detergent effects on cellular proteins and structures;
- targeting of fatty acids to specific metabolic pathways and modulation of enzymatic activities;
- involvement in fatty acid signaling and gene regulation;
- affecting cellular growth and differentiation;

For the first three tasks indirect evidences are available and will be generally addressed first in this section, followed by a detailed account of the specific FABP-types. The other two tasks will be dealt with in Sections 7 and 8.

*Uptake of fatty acids into the cell.* The various mechanisms and accompanying phenomena of fatty acid uptake are being dealt with in more detail in Chapters 2, 4, 5, and 6 of this book. In these processes FABPs would be at the receiving end in the cytosol. But the need for such cellular proteins in mediating fatty acid uptake, however, remains controversial [59]. General experimental approaches have been transfection of immortalized cultured cells with a certain FABP and determination of fatty acid uptake either by radioactivity or fluorescence. Thus, L-FABP enhanced initial uptake of oleic acid into L-cell fibroblasts [60] as did A-FABP in transfected CHO-cells, but not a non-binding mutant [61]. When endogenous L-FABP concentrations were decreased by transfecting HepG2 cells with antisense L-FABP cDNA, fatty acid uptake decreased accordingly [62]. On the other hand, expression of L-FABP mRNA in oocytes of *Xenopus laevis* had no effect on fatty acid uptake [63] as had the transfection of L6 myoblast with A- and H-FABP [64]. By the same token transfection with I-FABP cDNA of rat hBRIE 380 cells, murine L-cell fibroblasts, and human Caco-2 cells did not change the uptake kinetics of fatty acids [65–68]. The effect of FABP on fatty acid uptake obviously differs with respect to FABP-type and/or cell-type. Reasons can be the unknown coupling of the uptake process to cellular utilization of the fatty acid incorporated and, of course, the unknown proportions of the mechanisms contributing to the translocation of the fatty acid through the membrane.

*Cytosolic pool for fatty acids.* Due to the amphipathic nature of fatty acids, their accumulation in large quantities would result in the formation of micelles in the cytosol and damage to cellular membrane structures. FABP may protect against such damage, especially in cells that encounter large fatty acid fluxes. The protein may also modulate the regulatory effects of fatty acids on enzymes or on nuclear transcription factors.

*Cytosolic fatty acid transport and targeting.* Given the poor solubility of fatty acids in aqueous media, protein-mediated transport of fatty acids may be necessary to achieve high fluxes of fatty acids within cells. Indeed, tissues that metabolize large amounts of fatty acids, such as muscle of adipose tissue, have a high FABP content. FABP increases the total concentration of fatty acids in the cytosol, and it may transport fatty acids more rapidly through the aqueous phase (see Section 4). The proteins may also deliver fatty

451 acids to specific intracellular compartments or enzymes, for example, to mitochondria  
452 for  $\beta$ -oxidation, or to acyl-CoA synthetases for esterification and subsequent storage  
453 as triglycerides.

454 It is difficult to conclusively determine how a particular FABP functions in a living cell,  
455 especially since many cells express more than one member of the FABP gene family.  
456 However, functional conclusions can be drawn from metabolic differences in cells, tissues,  
457 and animals with different FABP content. At the cellular level, such differences can be  
458 induced through the transfection of cell lines with various FABPs. FABP levels can also be  
459 modified through experimental conditions, such as diet, hormones, or exercise. More  
460 recently, dramatic progress with respect to functional aspects has come from gene  
461 disruption studies. Knock-out mice for L-FABP, H-FABP, I-FABP, A-FABP, and E-  
462 FABP have shed light at the different functions of these proteins, but also revealed that  
463 other members of the gene family may compensate at least partly for the loss of one  
464 particular FABP. Other cues were obtained from comparing FABP orthologs in different  
465 animals. This approach is especially useful for animals that have adapted to extreme rates  
466 of lipid metabolism. In assessing the potential functions of FABPs, it is important to  
467 distinguish between the individual members of this gene family, and to consider the  
468 metabolic functions of the tissues in which they are expressed. Depending on the tissue,  
469 fatty acids need to be directed to different compartments, or to different pathways. Data  
470 from experimentally modified animals or different, specially adapted species support  
471 functions of FABP in intracellular fatty acid trafficking, but the details of underlying  
472 mechanisms have yet to be determined.

473 *L-FABP*: Liver is a major place of biosynthesis and detoxification, and L-FABP has  
474 long been speculated to function in directing fatty acids or related metabolites to the  
475 appropriate sub-cellular compartments. It may increase fatty acid acylation rates by  
476 making fatty acid more accessible to acyl-CoA synthetase [69]. Circumstantial evidence  
477 for a transport function was obtained from comparative studies between hepatocytes from  
478 male and female rats. In female cells, where FABP expression is 20% higher than in males,  
479 the fatty acid diffusion rate was markedly increased [70]. Other studies have also  
480 demonstrated that L-FABP modulates the uptake of fatty acids. In L-FABP knock-out  
481 mice, hepatic uptake of fatty acids from the blood was reduced by 50%. This is most likely  
482 a direct consequence of the markedly reduced fatty acid binding capacity ( $-80\%$ ) in the  
483 cytosol of liver cells, which do not express any other FABP. The cells, however,  
484 maintained normal levels of non-esterified fatty acids, triglycerides, and total lipids [71].  
485 Due to its wide range of ligands that includes xenobiotics, it has been suggested that  
486 L-FABP may also play a role in mitogenesis [72] (see Section 8).

487 *I-FABP*: Three different members of the FABP gene family are strongly expressed  
488 in the small intestine, albeit in different regions: cells of the proximal area of the  
489 small intestine express mostly L-FABP, while I-FABP is found in the medial region. The  
490 distal region expresses the intestinal bile acid binding protein (I-BABP). Since the small  
491 intestine is involved in dietary lipid absorption, it is plausible that these proteins mediate  
492 the uptake of lipids and their subsequent release into the bloodstream. The link between  
493 fatty acid uptake and I-FABP content is supported by various observations in cultured  
494 cells: Fatty acid uptake into undifferentiated stem cells was increased 1.7-fold following  
495 transfection with I-FABP, while the reduction of I-FABP levels in cultured enterocytes by

496 epidermal growth factor treatment resulted in reduced fatty acid uptake [73,74]. Other  
497 evidence supports a pivotal role of I-FABP in lipid absorption in vivo: A common  
498 mutation in this FABP gene doubles the affinity of I-FABP for fatty acids and results in  
499 increased fatty acid uptake, a finding that may explain why Pima Indians, a high incidence  
500 population group, are predisposed to type 2 diabetes [75,76]. However, targeted gene  
501 disruption of the I-FABP gene in knock-out mice did not impair their intestinal lipid  
502 absorption [77]. This, however, may be due to the overexpression of L-FABP in the  
503 intestine of these animals [78]. Like in other FABP knock-out models, an alternative  
504 FABP seems to compensate for the loss of I-FABP in the intestine of I-FABP null mice.

505 *A-FABP*: In adipocytes, free fatty acids are mostly incorporated into triacylglycerol for  
506 subsequent storage. A-FABP is therefore thought to direct fatty acids towards  
507 esterification at intracellular membranes where the long-chain acyl-CoA synthetases are  
508 located. Supporting data have been produced in experiments with primary and cultured  
509 adipocytes (reviewed in Ref. [79]). Alternatively, a role for A-FABP may arise during  
510 lipolysis, when free fatty acids are released from lipid droplets catalyzed by hormone  
511 sensitive lipase. As this enzyme is subject to feedback inhibition by fatty acid, it seems  
512 logical that rapid removal of fatty acids is required for efficient lipid mobilization. Indeed,  
513 A-FABP interacts directly with hormone sensitive lipase, making it possible to sustain  
514 rapid transport of fatty acids to the plasma membrane for export, or towards  
515 re-esterification at other organelles [80].

516 In order to study A-FABP function in vivo, a targeted disruption of its gene was  
517 generated in mice [81]. The mice appeared to be of normal phenotype, developed normally  
518 and were fertile. The morphology of adipocytes, and their fatty acid composition and  
519 uptake rates were unaltered. These findings, however, cannot be taken as indication that  
520 this FABP is not essential, as its loss greatly increased the expression of E-FABP in  
521 adipocytes, which normally makes up only 1% of total FABP in these cells [82]. While no  
522 changes in lipid metabolism were apparent in these animals when reared normally,  
523 differences were seen after diet-induced obesity. In contrast to wild-type mice, A-FABP  
524 null mice showed no increase in serum triglyceride levels, and remained sensitive to  
525 insulin. The concentrations of free fatty acid in the adipocytes were elevated, while  
526 lipolysis was reduced by 40% [83].

527 A-FABP is also expressed in macrophages which take up oxidized LDL and contribute  
528 to the development of atherosclerosis. Atherosclerotic lesions from hypercholesterolemic,  
529 ApoE-deficient mice contained high levels of A-FABP, and it has been demonstrated that  
530 oxidized LDL induces A-FABP expression. Double knock-out mice lacking both the  
531 ApoE and the A-FABP gene developed smaller lesions with fewer macrophages,  
532 indicating that macrophage A-FABP plays an important role in the formation of  
533 atherosclerotic lesions [84–86].

534 *E-FABP*: Epidermal FABP is the most universally expressed member of this gene  
535 family. It is the most abundant FABP in the skin. It may play a role in the maintenance of  
536 the water-permeability barrier of the epidermis, as suggested by recent studies with knock-  
537 out mice [87]. E-FABP null mice were of normal phenotype, and no differences were  
538 visible in histological examinations. No differences were seen in the epidermal fatty acid  
539 composition, but the basal *trans*-epidermal water loss was lower than that in wild-type  
540 animals. When the lipid barrier was damaged by acetone treatment, the recovery period

541 required to reach the basal level was much longer than in wild-type animals [88]. A  
542 significant increase in H-FABP expression was observed in the liver of neonatal mice,  
543 where E-FABP is normally strongly expressed [87]. Adipocytes of E-FABP knock-out  
544 mice showed a higher capacity for insulin-stimulated glucose transport; higher systemic  
545 insulin sensitivity was also observed [89]. In contrast, transgenic mice overexpressing  
546 E-FABP were less sensitive to insulin. The expression of E-FABP and A-FABP in  
547 adipocytes is interdependent: When E-FABP is overexpressed, the levels of A-FABP are  
548 reduced [90], while A-FABP knock-out mice reveal highly elevated levels of E-FABP  
549 expression [82].

550 *B-FABP*: This protein is found at its highest levels in developing brain [91]. The protein  
551 is expressed in glia cells, and its expression is regulated in response to interactions with  
552 neurons [92,93]. Unlike most other FABPs, B-FABP does not bind palmitic acid, but  
553 requires a longer hydrocarbon chain and a higher degrees of desaturation [94]. Its natural  
554 ligand appears to be DHA, the very long-chain fatty acid that is essential for the  
555 development of the nervous system. The expression of B-FABP in the brain coincides with  
556 its requirement for DHA, and therefore B-FABP is believed to be involved in the signaling  
557 pathways between developing neurons and glia cells [95]. B-FABP is also prominent in  
558 neural development of avian species, for example, in the neurogenesis of glial cells in  
559 chicken retina [96]. In contrast to the mammalian central nervous system, which is fully  
560 developed at maturity, the brain of birds shows significant levels of neurogenesis in the  
561 adult stage. The presence of B-FABP in adult bird brain, and its anatomical distribution  
562 lends credence to its role in neural migration and synaptic reorganization [97].

563 *H-FABP*: Perhaps, the clearest link between FABP and fatty acid metabolism is seen up  
564 to date for H-FABP. This protein is the only FABP expressed in various muscle tissues, in  
565 both vertebrates and invertebrate species [98,99]. The protein is highly conserved, even  
566 between insects and mammals, and is found in all muscles that metabolize fatty acids. A  
567 strong correlation exists between the fatty acid oxidation capacity of a muscle and its H-  
568 **Q2** FABP content, as illustrated in Fig. 3. Smooth muscle that depends largely on  
569 carbohydrates possesses very low levels of this FABP, while the content in red muscles  
570 increased. With higher  $\beta$ -oxidation rates typical for various red muscles, equally increased  
571 levels of H-FABP can be found [100]. Cardiac tissue, which depends mostly on lipid for  
572 energy supply and encounters the highest  $\beta$ -oxidation rates of all mammalian muscles,  
573 also has the highest FABP content (up to 5% of all cytosolic proteins). This observation  
574 applies also to non-mammalian muscles, which need to sustain high metabolic rates for  
575 long periods: Approximately, 9% of all cytosolic proteins are H-FABP in flight muscles of  
576 the Western sandpiper, a migratory shorebird found along the Pacific coast of North and  
577 South America; this high FABP content again reflects the fatty acid oxidation rates  
578 sustained in these muscles [101]. Higher metabolic demands exist for migratory insects as  
579 well, which retrieve energy during endurance flights exclusively through  $\beta$ -oxidation [8].  
580 A classical example is the flight muscle of desert locust, which oxidizes almost 1  $\mu$ M  
581 of fatty acid per minute and gram tissue, as H-FABP makes up almost one-fifth of all  
582 soluble proteins.

583 In all these muscles, elevated levels of H-FABP expression have been observed as a  
584 consequence of endurance training or otherwise increased fatty acid utilization. For  
585 example, chronic electrical stimulation in rat soleus muscle led to a 30% increase in

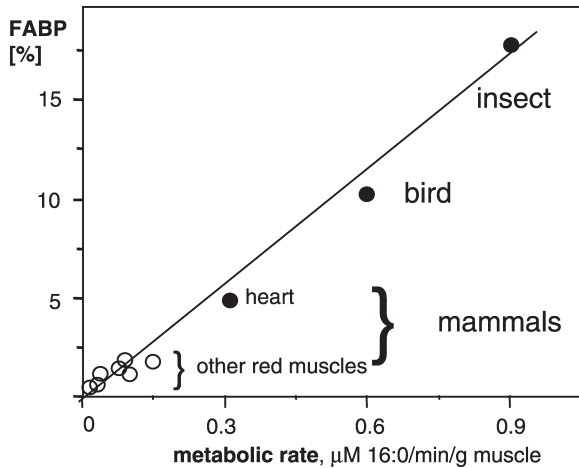


Fig. 3. Correlation between fatty acid oxidation capacity and FABP content in different muscles. Metabolic rates, expressed as the oxidation of  $\mu\text{g}$  of palmitate per minute and gram tissue, for mammalian muscles were taken from Ref. [100], for other muscles from Ref. [159]. FABP values for mammalian muscles were obtained from Refs. [100,160], for locust flight muscle from Ref. [8] and for sandpiper flight muscle from Ref. [161].

H-FABP expression [100], and in vivo experiments confirmed this finding: after 8 weeks of swimming, the concentration of H-FABP in rat skeletal muscle increases by 30%, though not in the heart [102]. Diets enriched with polyunsaturated fatty acids led to similar effects in skeletal muscle. In spite of the already extreme H-FABP content of locust flight muscles, its further expression still can be induced, both in response to exercise and to increased fatty acid supply alone [103]. As discussed in more detail below, H-FABP may act as a fatty acid sensor and modulates the expression of its own gene. This would assure that H-FABP levels are appropriate for the fatty acid transfer rates required to fuel muscle activity.

Studies in H-FABP knock-out mice confirm the importance of H-FABP for fatty acid transport and metabolism. The absence of H-FABP did not result in phenotypical differences, and the histology of skeletal and cardiac muscle appeared normal [104]. However, fatty acid uptake was reduced markedly in cardiac tissue ( $-80\%$ ) and isolated cardiomyocytes ( $-45\%$ ). Because of the impaired fatty acid uptake, cardiac muscle contraction in these animals relied on glucose oxidation, which can provide sufficient energy to resting animals [105]. Higher metabolic rates, however, could not be sustained. When exercised, H-FABP null mice fatigued quickly, a finding that lends support to the essential role of H-FABP in cardiac metabolism. Since no other FABPs are expressed in cardiac cells, a compensation mechanism as observed in other knock-out models may not be possible.

In contrast to vertebrates, fish appear to express both H-FABP and a protein more similar to A-FABP in their heart and skeletal muscle [106]. This is noteworthy because fish muscles also serve as the major lipid storage organ. The presence of A-FABP and

631 H-FABP would be consistent with distinct roles of these proteins in lipid metabolism: A-  
632 FABP could direct fatty acids towards storage, for example, during the early stages of  
633 migration when food intake exceeds the energy demand. H-FABP should be more  
634 prominent during spawning when vast quantities of energy are needed.

635

636

## 637 **6. Regulation of FABP gene expression**

638

639 From the functional data discussed above, it is not surprising that cells in tissues with  
640 prominent roles in fatty acid metabolism are especially rich in FABP. Moreover, FABP  
641 levels often increase as a consequence of increased fatty acid exposure. How is this  
642 achieved at the molecular level?

643

644 All FABPs share an identical gene structure of four conserved exons and three introns  
645 of variable size [4,107]. This overall gene structure is of ancient origin, as it is even found  
646 in non-mammalian species. The exon/intron boundaries are in identical positions in all  
647 FABPs, with the only exception that the second intron has been lost in several, but not all  
648 insect FABPs [108]. All FABP promoters contain a classical TATA box. The elements  
649 that control the tissue-specific expression of FABP are currently only poorly understood,  
650 but potential enhancer sequences have been characterized for several genes. These include  
651 two HNF1 $\alpha$  regulatory elements in the L-FABP promoter [109], a fat-specific enhancer  
652 required for A-FABP expression in adipocytes [110], and several binding sites for  
653 members of the POU transcription factor family that control B-FABP expression [111]. A  
654 concise promoter region that contained an atypical MEF2 binding site was shown to be  
655 responsible for the muscle-specific expression of H-FABP [112].

656

657 Better understood is the up-regulation of various FABP genes by fatty acids. It has long  
658 been known that the induction of FABP expression in response to lipid-rich diet [113] or  
659 endurance training [114] is the result of increased intracellular concentrations of fatty  
660 acids, which in turn activate nuclear transcription factors [115,116]. The best known of  
661 such transcription factors are the subtypes of the peroxisome proliferators activated  
662 receptor (PPAR  $\alpha$ ,  $\beta$ ,  $\gamma$ ), so called because of their activation by xenobiotic peroxisome  
663 proliferators in rodents [117]. Long-chain fatty acids and certain eicosanoids are  
664 considered as their natural ligands. PPARs bind as heterodimers with the subtypes  $\alpha$ ,  $\beta$ ,  $\gamma$   
665 of the retinoid receptor RXR to direct-repeat elements (peroxisome proliferators response  
666 elements, PPREs) in the promoter region of the genes that they regulate.

667

668 While circumstantial evidence suggests that PPARs are involved in the regulation of  
669 various FABP genes, proof has been provided for A-FABP [118] and L-FABP [119] only.  
670 In reporter-gene and transactivation assays Tontonoz et al. [118] have shown that the  
671 murine A-FABP gene is regulated by the binding of PPAR $\gamma$ 2 and RXR $\alpha$  to a direct-repeat  
672 element 5.2 kb upstream of the FABP gene. The expression of the rodent L-FABP gene in  
673 the liver is under the control of PPAR $\alpha$  bound to a PPRE around 110 bp upstream of the  
674 transcriptional start site; interestingly, its expression in intestinal cells is controlled by  
675 PPAR $\beta$ , which binds to the same response element as PPAR $\alpha$  in the liver [120].

676

677 Several studies have demonstrated that treatment of muscle cells with the PPAR $\alpha$   
678 agonist Wy14,643 resulted in elevated FABP mRNA levels, and concluded that the H-  
679 FABP gene is also under the control of PPAR $\alpha$  [121]. Although a direct-repeat sequence

676 reminiscent of a PPRE can be found in the distal promoter of rodent H-FABP genes, the  
677 involvement of this element could not be demonstrated. The absence of a functional PPRE  
678 in the human H-FABP promoter raises the possibility that PPARs may act indirectly  
679 through cross-talk with other nuclear receptors. Alternatively, the observed induction of  
680 gene expression by PPAR agonists could instead be a consequence of increased fatty acid  
681 uptake into the myocyte, caused by the induction of the membrane fatty acid transporter  
682 FAT/CD36 that is known to be controlled by PPAR $\alpha$  [121]. While it has been proposed  
683 that transcription factors other than PPARs may be involved in fatty acid mediated gene  
684 control [122], such factors have not been extensively studied. To this end, insights can be  
685 obtained from invertebrates, which do not express PPARs [123], but the ortholog of H-  
686 FABP, which can be induced by fatty acids [103]. It is interesting to note that a different  
687 fatty acid response element (FARE) has been identified in the promoter of the H-FABP  
688 gene from locust muscle [108,124]. Unlike PPRE, the locust FARE is an IR-3 element, a  
689 palindromic sequence containing two hexanucleotide half-sites (AGTGGT, ATGGGA)  
690 separated by three nucleotides reminiscent of a steroid hormone response element.  
691 Reporter gene constructs containing the locust FABP promoter were expressed in rat  
692 myoblasts cells, and treatment with fatty acids resulted in a twofold increase in expression.  
693 Deletion of the element did not affect the basal expression rate, but completely eliminated  
694 induction by fatty acid. Nuclear proteins from rat myoblasts bound to the element in gel-  
695 shift experiments, but additional fatty acid was required to achieve the same effect with  
696 nuclear proteins from locust muscle [124]. Perhaps, higher concentrations of fatty acids  
697 are required in the latter tissue, because its large FABP content may prevent full access of  
698 a signaling fatty acid to the nuclear receptor.

699 The locust FARE appears to be conserved in evolution: similar elements can be found  
700 not only within the proximity of putative FABP genes from other insects (*D. melanogaster*  
701 and *A. gambiae*), but also in the promoters of all mammalian H-FABP genes. In the latter  
702 case, however, the hexanucleotide half-sites (consensus sequence AGAAGA and  
703 AGGTGA) are pointing outwards, forming an everted repeat sequence [125]. It remains  
704 to be seen whether these elements alone are responsible for the regulation of the H-FABP  
705 gene by a fatty acid, and which transcription factors are involved. In any case, it appears  
706 that indeed there is more than one way by which fatty acids can control gene expression.

## 707 **7. The role of FABPs in fatty acid signaling and gene transcription**

710 The induction of A- and L-FABP mRNA expression by fatty acids and retinoids,  
711 involving heterodimers of PPAR and RXR subtypes, is a paradigm for all genes having a  
712 PPRE. It follows the general scheme for gene activation by lipophilic ligands that bind to  
713 nuclear receptors of the steroid hormone receptor superfamily [126]. In A- and L-FABP  
714 expressing cells, fatty acids thus induce their own intracellular binding proteins, a finding  
715 that insinuates that these proteins may be the vehicles for targeted transfer of the  
716 hydrophobic activators into the nucleus, where they become agonists of transcription  
717 factors [126,127]. Other examples from the iLBP family include CRABP (subfamily I)  
718 and I-BABP (subfamily II). CRABPs transport retinoic acid to the nucleus, and their genes  
719 are under the control of retinoic acid response elements (RARE), which in turn are  
720

721 activated by the complex of retinoic acid with RAR and RXR [128]. I-BABP is up-  
722 regulated by its ligand as well, via the farnesoid X receptor FXR, a nuclear receptor that is  
723 activated by bile acid [129].

724 The members of the iLBP family are well suited to deliver ligands into the nucleus: as  
725 small cytosolic proteins of ~ 15 kDa, FABPs may readily pass nuclear pores or enter by  
726 **Q3** a specific recognition signal the nuclear compartment. Indeed, immunolabeling  
727 techniques allowed to detect nuclear localization of L-FABP in hepatocytes already in  
728 1989 [130], of B-FABP in astrocytes [131], of A-FABP in 3T3-L1 adipocytes [132],  
729 and of H-FABP in mammalian [133] and insect myocytes [8]. In locust muscle, the  
730 cytosolic levels of FABP increase rapidly after adult ecdysis, and the nuclear levels were  
731 shown to increase proportionally. Thus, it is conceivable that FABPs transfer fatty acids  
732 to PPARs or other nuclear receptors, which in turn are activated to enhance transcription.  
733 While the ligand exchange could be simply a matter of fatty acid affinities between  
734 binding protein and nuclear receptor, recent studies point towards direct interactions  
735 between FABP and PPARs [134]. L-FABP and PPAR $\alpha$  co-localize in the nucleus of  
736 mouse hepatocytes and, as shown in vitro, the binding protein interacts via protein-  
737 protein contacts with PPAR $\alpha$  and  $\gamma$ . These contacts are required for the activation of  
738 gene expression in response to treatment of HepG2 cells with PPAR ligands, including  
739 long-chain fatty acids. Tan et al. [135] obtained similar results using the COS cell  
740 model: A-FABP and E-FABP interact directly with PPAR $\gamma$  and  $\beta$ , respectively, and  
741 co-expression of the binding protein and respective PPAR subtypes enhance gene  
742 activation. Moreover, it appears translocation of the FABP into the nucleus itself is a  
743 regulated process, with a massive import in response to ligand binding. The primary  
744 structures of FABPs do not carry nuclear import signals; therefore, other mechanisms  
745 must be operative. In the case of L-FABP, the negatively charged carboxylate group of  
746 the second fatty acid molecule at the surface of the *holo*-protein has been considered  
747 such a recognition signal [136,137].  
748

749 While complete mechanistic details are not yet understood, it seems that FABPs act  
750 as fatty acid sensors and mediators in the regulation of gene expression, as illustrated  
751 in Fig. 4. This does not mean that the mechanism by protein-protein contacts is  
752 exclusive for the ligand to become agonist. Moreover, for reasons not yet known,  
753 conflicting data have been reported for the ligand dependence of these protein-protein  
754 contacts. On the one hand, the interaction of L-FABP with PPAR $\alpha$  or  $\gamma$  has been  
755 shown to be independent of the presence of ligand [134]; on the other hand, A-FABP  
756 interacted with PPAR $\gamma$  and E-FABP with PPAR $\beta$  only in the presence of ligand [138].  
757 It is interesting to note the parallels between these FABPs and other iLBPs. It was  
758 found that CRABP II, but not CRABP I interacts with the retinoic acid receptor  
759 (RAR $\alpha$ ); this collisional contact leads to the transfer of all-*trans* retinoic acid from the  
760 binding protein to the nuclear receptor [139]. Although the affinity of 9-*cis* retinoic  
761 acid to CRABP II is much lower affinity than that of the *trans* isomer, it can be  
762 transferred by the same collisional mechanism to RXR $\alpha$  [140]. Therefore, L-, A-, E-  
763 FABP, and CRABP II appear to play complementary roles in gene regulation;  
764 protein-protein contacts are necessary between nuclear receptors and these binding  
765 proteins and thus can be addressed as co-activators of nuclear receptors [140].

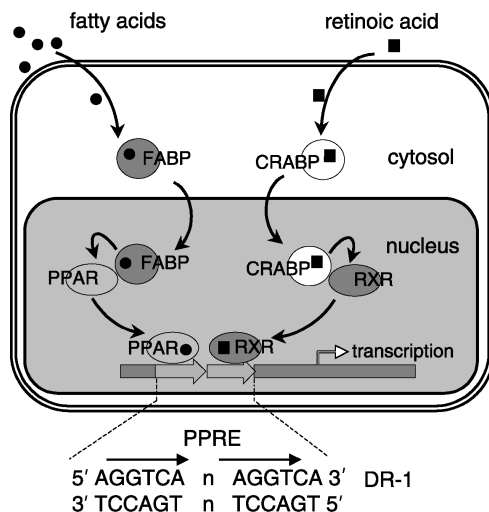


Fig. 4. The path of signaling fatty acids to the nucleus (bold arrows). Protein–protein contacts between iLBP (L-, A-, E-FABP, CRBP II) and the nuclear receptors are shown. The binding proteins deliver fatty acids and retinoic acid to the nucleus, where they are transferred by collision to their respective transcription factors (specific subtypes of PPAR and RXR). Nuclear receptor heterodimers then bind to PPRE for gene transcription.

## 8. Role of FABPs in cell growth and differentiation

Siding with the notion that FABPs target their lipophilic ligands, e.g. fatty acids or xenobiotics, to the nucleus to affect the cell cycle, we would expect either mitogenesis or growth arrest, the latter with or without differentiation. This modulation brought upon by the binding protein can be seen in the light of its cytosolic sensor function in signaling (Section 7), which may be operative only at low concentrations of the ligand [135]. However, if directed nuclear transport does not take place, the effect will be adverse in either direction, as FABP in a concentration-dependent manner would buffer the lipophilic ligands and prevent them from interacting with their nuclear targets.

L-FABP of subfamily II increased proliferation affected by mitogens and carcinogens in transfected liver and hepatoma cells [72,141]. Carcinogenic peroxisome proliferators became more potent in cells co-transfected by L-FABP, leading to higher cell proliferation rates due to targeting [142].

In contrast, FABPs of subfamily IV reveal growth inhibitory action, for which only a few other peptides are known such as interferons and transforming growth factor  $\beta$ . Thus, loss of A-FABP was correlated with progression of human bladder transitional cell carcinoma [143] and E-FABP, upon application to skin, reduced proliferation of melanoma cells, while normal skin fibroblasts were unaffected [144]. The gene product of a “mammary derived growth inhibitor-related inhibitor gene” (MRG), later identified as B-FABP, suppressed tumor growth in a nude mouse model and breast cancer cell proliferation after transfection with MRG [145,146]. Finally, transfection of MCF-7 cells, a human breast cancer line, with cDNA encoding bovine H-FABP reduced cell growth, in addition, the H-FABP producing transfectants reduced in vivo tumorigenicity [147]. At

811 present it is not clear whether or not growth inhibition is due to the FABP itself or to its  
812 putative ligand. But it is also tempting to speculate in the case of B-FABP that the high  
813 affinity-ligand DHA (Table 2) would exert the inhibitory effect.

814 The background of these observations during the last 15 years was the discovery of  
815 bovine “mammary derived growth inhibitor” (MDGI) in 1987 [Böhmer et al., JBC, 262].  
816 It was soon recognized as a variant of H-FABP [148] and finally identified as a preparation  
817 of H-FABP contaminated with small amounts of closely related A-FABP [149]. MDGI was  
818 a potent inhibitor of epithelial proliferation in various mammalian organ and cell cultures  
819 [150]. MDGI, and H-FABP alone also showed anti-proliferation activity in breast cancer  
820 cells and H-FABP expression seemed to be reduced in malignant breast tumors [151]. When  
821 administered extracellularly, however, the anti-tumor activity of H-FABP was not due to a  
822 bound ligand, but could be mapped to a C-terminal fragment of the protein [152]. More  
823 details on MDGI-activities of FABPs can be found in a review published in 1998 [153].

824 In mammary gland organ culture, growth inhibition was associated with functional  
825 differentiation in the presence of MDGI or H-FABP; in fact, this differentiation is  
826 preceded by heavy expression of H-FABP in the mammary epithelial cells, which then  
827 promotes milk protein synthesis in the differentiated cells [154]. Based on this  
828 observation, it was argued that H-FABP acts as a differentiation factor. A-FABP as  
829 well was assumed originally to be such a factor as it was expressed in the course of  
830 differentiation from preadipocytes to adipocytes of both primary cells and the 3T3-L1 cell  
831 model. Yet it was soon recognized that the fatty acids themselves (transported by E-FABP  
832 in the preadipocyte?) are the trigger of differentiation and, as a result A-FABP and PPAR $\gamma$   
833 among others are expressed. In fully differentiated adipocyte culture, removal of fatty  
834 acids from the medium and re-supplementation decreased and replenished A-FABP  
835 mRNA levels, respectively [155]. From today’s perspective we can ascribe to A-FABP a  
836 carrier function in fatty acid signaling to the nucleus to interact with PPAR $\gamma$  and a  
837 transport function needed during the time of heavy triacylglycerol accumulation. Indeed,  
838 tissue-specific enhancer and proximal promoter regions of the A-FABP gene interact  
839 with adipogenic transcription factors in a time-dependent manner [156]. In line with this,  
840 H-FABP in C2C12 cells was induced upon differentiation from the myoblast to the  
841 myotube stage [157]. A careful follow-up study demonstrated later that E-FABP in  
842 myoblasts is down-regulated during differentiation, while H-FABP was induced at later  
843 stages of differentiation when energy retrieval in the cells shifts from glycolysis to  
844  $\beta$ -oxidation, indicative of a metabolic transport function of the binding protein [158].  
845

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847

## 848 9. Outlook

849

850 Much progress has been made in the last decade in the study of the structure and  
851 binding behavior of the FABPs. Much of the current research activity is directed to  
852 understand the control of their gene expression, and the interactions of FABPs with other  
853 proteins in the cell. Undoubtedly, these studies will help to more fully understand the  
854 pleiotropic roles of these intracellular transport proteins, especially with respect to signal  
855 transduction, both at the molecular and the cellular level. It is the belief of the authors that

856 analysis of this conserved gene family in various organisms will continue to provide new  
857 insights into their regulatory functions.

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