Fatty acid binding proteins have the potential to channel dietary fatty acids into enterocyte nuclei

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Abstract Intracellular lipid binding proteins, including fatty acid binding proteins (FABPs) 1 and 2, are highly expressed in tissues involved in the active lipid metabolism. A zebrafish model was used to demonstrate differential expression levels of fabp1b.1, fabp1b.2, and fabp2 transcripts in liver, anterior intestine, and brain. Transcription levels of fabp1b.1 and fabp2 in the anterior intestine were upregulated after feeding and modulated according to diet formulation. Immunofluorescence and electron microscopy immunodetection with gold particles localized these FABPs in the microvilli, cytosol, and nuclei of most enterocytes in the anterior intestinal mucosa. Nuclear localization was mostly in the interchromatin space outside the condensed chromatin clusters. Native PAGE binding assay of BODIPY-FL-labeled FAs demonstrated binding of BODIPY-FLC12 but not BODIPY-FLC5 to recombinant Fabp1b.1 and Fabp2. The binding of BODIPY-FLC12 to Fabp1b.1 was fully displaced by oleic acid. In vivo experiments demonstrated, for the first time, that intestinal absorption of dietary BODIPY-FLC12 was followed by colocalization of the labeled FA with the first time, that intestinal absorption of dietary BODIPY-FLC12 was followed by colocalization of the labeled FA with the first time, that intestinal absorption of dietary BODIPY-FLC12 was followed by colocalization of the labeled FA with the first time, that intestinal absorption of dietary BODIPY-FLC12 was followed by colocalization of the labeled FA with the first time, that intestinal absorption of dietary BODIPY-FLC12 was followed by colocalization of the labeled FA with lipid absorbed by small enterocytes. Using BODIPY-FLC12 and proflavine as lipid tracers, it was demonstrated that dietary LCFA, and other lipophilic compounds marketed in a barrel capped by two short α-helices (18). The ligand binding site is within the large water-filled interior (19, 20), but binding sites and interaction modes with some other lipophilic compounds may vary (12).

Duplication of genes and entire genomes is a major evolutionary force driving the increasing complexity of organisms (21), and the history of the FABP family has been shaped by duplication events (8). The molecular diversity of FABPs, together with overlapping and tissue-specific expression patterns, may facilitate redundancy, neofunctionalization, and subfunctionalization of biochemical properties. The precise in vivo function of each FABP is not yet clearly understood. Distinct developmental and tissue-specific expression patterns support the hypothesis that cellular retinol and retinoic acid binding proteins, these abundant chaperone proteins are members of an ancient conserved multigene family of intracellular lipid binding proteins (4–6). The evolutionary relationships of vertebrate FABPs were clarified using phylogenetic and conserved synteny analyses (7, 8). They bind long-chain FAs (LCFAs) and other lipophilic compounds (9–12) and are believed to be implicated in FA intracellular uptake and transport, lipid metabolism regulation, protection from the harmful effects of nonesterified LCFAs, and modulation of cell growth and proliferation (13–17). Despite a low amino acid sequence identity among the various FABPs, these proteins fold in a highly conserved tertiary structure. A typical member of the family consists of 127–134 amino acid residues with 10 or 11 anti-parallel β-strands folded into a barrel capped by two short α-helices (18). The ligand binding site is within the large water-filled interior (19, 20), but binding sites and interaction modes with some other lipophilic compounds may vary (12).
of a subspecialization in ligand preferences and/or suggest diversity in uncharacterized functions.

It was demonstrated, using cell cultures, that FABPs may channel unesterified FAs and other lipophilic ligands into nuclei, potentially targeting them to transcription factors, and initiate nuclear receptor transcriptional activity (11, 22–33). To our knowledge, no in vivo data are currently available to support the hypothesis that exogenous FAs enter cell nuclei via their binding to FABPs. The dietary triacylglycerols are the major source of lipids in the intestinal lumen. Their hydrolysis releases large quantities of medium- to long-chain FAs absorbed by the enterocytes via complex mechanisms, involving both passive diffusion and protein-mediated transport (34–38). Once inside the cell, dietary FAs are reversibly bound to lipid binding proteins involved in the transport of FAs from the plasma membrane to cellular compartments (e.g., FABPs and acyl-CoA binding proteins). The intestinal mucosa and its enterocytes provide a very attractive system for evaluating the fate of exogenous FAs inside cells. FABP1 and FABP2 are the most strongly expressed FABP family members in the human small intestine (13, 14), and these proteins are found in abundance in absorptive cells (39, 40). In zebrafish, the anterior intestine is the major site of fat absorption (41–43), and different fabp genes were found expressed in the intestine including fabp1a, fabp1b.1, fabp1b.2, fabp2, fabp3, fabp6, fabp7a/fabp7b, fabp10a/fabp10b, and fabp11a (41, 44–47). The teleost ancestor experienced a whole-genome duplication event at the base of the teleost radiation (48) leading, in some cases, to the retention of pairs of duplicate genes (e.g., fabp1a/fabp1b). The resulting orthologs may have retained their initial subfunctions, as well as acquiring new functions (i.e., neofunctionalization), and/or duplicate genes may be preserved by partitioning ancestral subfunctions between them (i.e., subfunctionalization). In addition, tandem gene duplication may arise as demonstrated with fabp1b.1 and fabp1b.2 found adjacent in the zebrafish genome (44).

Zebrafish were used to study the expression pattern and dietary regulation of homologs to human FABP1 and FABP2, which are significantly expressed in the anterior intestine of this vertebrate model. The next step was cellular and subcellular immunolocalization of Fabp1b and Fabp2 along the intestinal villi. Third, an assessment of the fate of dietary fluorescent (BODIPY)-labeled analogs of FAs revealed that dietary BODIPY-FLC12 (medium chain) but not BODIPY-FLC6 (short chain) was colocalized with Fabp1b and Fabp2 in enterocyte nuclei.

MATERIALS AND METHODS

Animals

Wild-type zebrafish (Danio rerio) were produced in our facilities according to the rules approved by the Ministère de l’Agriculture, de l’Agroalimentaire et de la Forêt, France, under permit number A35-522-6. All experiments were conducted in conformity with the 2010/63/EU directive on the protection of animals used for scientific purposes. Larvae were obtained by natural mating and raised in embryo water [90 µg/ml Instant Ocean (Aquarium Systems, Sarrebourg, France), 0.58 mM CaSO4, 2H2O, dissolved in reverse-osmosis purified water] at 28.5°C with an 11:13 light/dark photoperiod.

Feeding experiments and tissue sampling

Zebrafish adults were fed ad libitum with TetraMin flakes (Tetra GmbH, Melle, Germany) containing 48% protein, 8% lipid, 11% ash, 2% fibers, and 6% moisture. Larvae were fed on ZF Biolabs formulated diet (FD) flakes (Tres Cantos, Spain), dried chicken egg yolk (CEY) powder, or olive oil (OO).

Zebrafish eleutheroembryos at 5 days postfertilization (dpf) and larvae at 15 dpf, as well as adult males, were sampled for real-time quantitative PCR (qRT-PCR). On the day preceding the nutritional trials, larvae and adults were divided into two groups and transferred into containers with embryo water but no food. After ~18 h (larvae) or 22 h (adults) food deprivation, one group of larvae or adults was fed for 2–2.5 h, while the second group continued to fast. After feeding, the eleutheroembryos/larvae were transferred into clean embryo water to eliminate any remaining food, and six pools of 25 eleutheroembryos (5 dpf) or 20 larvae (15 dpf) were immediately frozen in liquid nitrogen and stored at ~80°C until RNA extraction. Two separate batches were used as sources of larval experiments. The adult males were dissected, and six individual samples of liver and anterior intestine were isolated. The ventral white adipose tissue was carefully removed, as it was closely associated with the digestive tract on the ventral side and also with liver lobes at some points (49). Samples were immediately frozen in liquid nitrogen and stored at ~80°C until RNA extraction for the RT-PCR and qRT-PCR experiments. Larvae and adult animals from the fasted group were sampled and treated in the same way as the fed animals. In addition to the liver and anterior intestine, six separate brain samples were collected, one ovarian, one testicular, and one heart sample were collected from a pool of six animals, and one kidney sample was collected from a pool of four animals. All samples, except the heart, kidney, and ovarian tissues, were collected from male organisms.

For whole-mount in situ hybridization, 15 dpf zebrafish larvae (i.e., at a fully exotrophic nutritional stage) were fasted for 24 h and were then fed for 3 h with FD, dried CEY, or OO. Approximately 15 larvae per treatment or sampling point were fixed in PBS (137 mM NaCl, 2.7 mM KCl, 0.02 M PO4 ), and dehydrated by successive washes with methanol (25%, 50%, 75%, 95%, and 100%) and stored at ~20°C prior to analysis.

Dietary experiments with the green fluorescent BODIPY-FA analogs were performed using adult zebrafish. BODIPY [boron dipyrromethene (4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene)] and BODIPY-FL analogs (Life Technologies) were resuspended in methanol (1 µg/µl) and mixed well with a CEY emulsion (10 µl/ml), prepared from dried CEY mixed in water using an IKA Ultra-Turrax Homogenizer. The BODIPY-FLC12 (D8254), or BODIPY-FLC10 (D8292)-CEY emulsion was maintained at 37°C for 10 min for the solvent to evaporate. BODIPY-FLC10 (D8292) was used as a negative control. Animals previously starved for 5 days were fed ad libitum (labeled emulsion/embryo water = 1:600, v/v), and their food intake was checked visually. Samples were taken 3 h after feeding. Quantitative analysis of dietary BODIPY-FA analog fluorescence signal after intestinal absorption in zebrafish intestinal villi was determined using ImageJ free-processing software (National Institutes of Health, http://rsb.info.nih.gov/ij/) as previously described (50).

Extraction of total RNA, reverse transcription, and PCR analyses

Total zebrafish RNA was extracted using the NucleoSpin® RNA II extraction kit (Macherey-Nagel, Hoerdt, France) according
to the manufacturer’s instructions. Total RNA was checked for integrity by ethidium bromide staining in agarose borax gel and quantified using a NanoDrop ND-1000 spectrophotometer (Nxyor Biotech, Paris, France). Reverse transcription were performed with 1 µg total RNAs using the StrataScript™ qRT-PCR cDNA synthesis kit (Stratagene, Amsterdam, The Netherlands), with an oligo(dT) primer, according to the manufacturer’s instructions. The reaction mixture (20 µl) was incubated at 37°C for 30 min, 42°C for 1 h 30 min, and then 70°C for 10 min. Two reverse transcription and one reverse transcription negative (RTneg) control (i.e., without reverse transcriptase) were performed on each sample. Qualitative PCR was also carried out on 2 µl reverse transcription reaction product diluted 1:10 in a final volume of 20 µl containing 10 µl Brilliant® SYBR® Green qRT-PCR Master Mix and 0.25 µM of each primer. An aliquot of the PCR reactions was electrophoresed on 1.5% agarose gel containing ethidium bromide, and PCR products were visualized and photographed.

**qRT-PCR**

Transcripts were amplified using the MX 3000P qRT-PCR thermal cycler instrument (Stratagene). qRT-PCR amplification was carried out in a final volume of 20 µl, using 10 µl Brilliant® SYBR® Green qRT-PCR master mix (Stratagene), 0.25 µM oligodeoxynucleotide primers, and 3 µl diluted cDNA; 5 and 15 dpf zebrafish electrophoresed on 1.5% agarose gel contain volume of 20 µl containing 10 µl Brilliant® SYBR® Green qRT-PCR Master Mix and 0.25 µM of each primer. An aliquot of the PCR reactions was electrophoresed on 1.5% agarose gel containing ethidium bromide, and PCR products were visualized and photographed.

**Whole-mount in situ hybridization**

Whole-mount in situ hybridization was carried out on zebrafish larvae, sampled, and fixed in 4% PFA, as described previously. A clone purchased from the German Resource Center for Genome Research, Berlin, Germany (IMAGE: 7225625), corresponding to GenBank database dbEST zebrafish clone gb CO995259.1, was used to generate the fabp1b RNA probe. The sense (ZF-fabp1b F1) 5'-CAAGACTATTTGGAGAAGAGA-3' and antisense (ZF-fabp1b R1) 5'-TGAGATTTGGACACTTATTAAG-3' primers were designed from this clone and used for probe synthesis, as previously described (43), except that the primer annealing temperature (Tm) in the thermal profile was 55°C for fabp1b in the first PCR amplification. For the fabp2 RNA probe, the ZF-FB clone (41) was used, corresponding to a 203 bp PCR product of GenBank database dbEST zebrafish clone gbAJ132590 after amplification with the sense (oligo ZF1A) 5'-CCTTCGATCATGACCTTGACCCG-3' and antisense (oligo ZF 3A) 5'-CCGGCACTGGAATATTTACCTTAC-3' primers, subcloned using the pGEM-T Easy vector kit (Promega, France). The second PCR was performed with the two pGEM-T Easy vector cDNAs, using the T7 and SP6 universal primers, and then was described (43). As the fragments were sense oriented in the vector, the PCR template for the fabp1b.1 sense probe was produced using T7 universal primer, while the PCR template for the antisense probe was produced using SP6 universal primer and the inverse for the fabp2 probes. Both antisense and sense digoxigenin (DIG)-labeled RNA probes were synthesized using the DIG RNA labeling kit (SP6/ T7) (Roche Diagnostics, Meylan, France), following the manufacturer’s instructions. The 211 bp fabp1b probe was able to hybridize to fabp1b.1 and fabp1b.2 transcript variants. The fabp2 probe was 203 bp long. The protocol for in situ hybridization was as previously described (51, 52), except that prehybridization and hybridization were conducted at 60°C. The posthybridization stringent baths were at hybridization temperature, except for the last two baths in 0.2× SSC Tween at 57°C (30 min each). In contrast, the hybridization buffer contained 50% formamide, and the animals were incubated in preabsorbed sheep anti-DIG-AP Fab (Roche Diagnostics) fragments at 1:5,000 dilution at 4°C overnight. The antibody was rinsed in six PBS-Tween baths for 30 min each time.

**Amino acid sequence analyses**

Deduced protein sequences were extracted from the UniProt (53) database. Sequences were aligned (supplementary Fig. 1) using the ClustalW2 program (54).

**Recombinant Fabp1b.1 and Fabp2 production and purification**

Zebrafish fabp1b.1 and fabp2 full coding cDNA sequences (supplementary Table 1) were cloned in the pET5a vector (Promega). pET5a-Fabp1b.1 and pET5a-Fabp2 constructs were transformed into BL21 (DE3) Star Escherichia coli strain (Novagen). Bacterial cells containing the relevant expression plasmid were cultured in 2× yeast extract and tryptone media at 30°C for Fabp1b.1 and 37°C for Fabp2 for ~4 h before Fabp synthesis was induced by adding 0.4 mM isopropyl β-D-thiogalactopyranoside and incubating for a further 4 h. After centrifugation at 5,000 g for 10 min, the cells were collected and resuspended in cell lysis buffer (30 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, pH 8.3) and ruptured by sonication. Cell debris was removed by centrifugation at 15,000 g at 4°C for 30 min, and the supernatant was processed in two ammonium sulfate precipitation steps. (NH₄)₂SO₄ was first added to a final concentration of 30% with stirring at room temperature for 2 h, followed by centrifugation at 15,000 g for 15 min. The supernatant fraction was treated with (NH₄)₂SO₄ to a final concentration of 50% and centrifuged at 15,000 g for 15 min. The latter supernatant fraction (~40 ml) was dialyzed against 4 liters of 30 mM Tris-HCl, pH 8.3, at room temperature for 12 h, changing the buffer every 4 h, and finally concentrated by ultrafiltration. The concentrated fraction (~10 ml) was loaded onto a SephadexG50 fine gel filtration column (141 cm × 1.9 cm). Chromatography was performed at 4°C, using 30 mM Tris-HCl, pH 8.3, as the mobile phase at a flow rate of 15 ml/h. Fabp fractions were analyzed by SDS-PAGE, MALDI-TOF, and MALDI-TOF-TOF MS to determine protein purity.
Antibody production and characterization

Polyclonal antisera against recombinant zebrafish Fabp1b.1 and Fabp2 were obtained by injecting New Zealand white rabbits with 0.8 mg purified recombinant proteins dissolved in 0.4 ml 50 mM Tris-HCl, pH 8.3, together with an equal amount of Freund’s complete adjuvant. One month later, a booster injection containing 0.4 mg purified protein in Freund’s incomplete adjuvant. Bleed tests were carried out at days 0, 20, and 40, for antibody titration response measurement, and a final blood sample was collected at day 68. Blood was allowed to clot and retracted at 37°C for 1 h, followed by 16 h at 4°C, and the collected serum was stored at -20°C. Crude polyclonal antisera were purified by affinity column chromatography at room temperature after linking highly purified Fabp1b or Fabp2 to cyanogen bromide-activated Sepharose (Sigma-Aldrich), according to the manufacturer’s instructions. The eluate was washed by adding 0.1 M glycine and 0.15 M NaCl, pH 8.2, until its A280 nm reached zero. The antibodies were desorbed using 0.1 M glycine and 0.5 M NaCl, pH 2.6. The fractions collected were immediately spiked with 1:10 (v/v) Tris-HCl 1 M, pH 8.3, to neutralize the pH and stored at -20°C until use. Antiserum titration was performed by ELISA. Plates were coated with 100 ng highly purified Fabp1b.1 or Fabp2. All the following steps were conducted in PBS-Tween buffer. Plates were blocked with 5% BSA at room temperature for 1 h. Serial dilutions (1:200 to 1:102,400) of each antisera were incubated for 2 h at room temperature. Plates were extensively washed, incubated with Alexa-488 goat anti-rabbit IgG antibody (Molecular Probes), and, finally, extensively rewashed. The signal was measured in a Varioskan Flash spectrophotometer (Thermo Scientific). Antibody specificity was assessed by Western immunoblots. Following SDS-PAGE, total proteins extracted from 5 dpf zebrafish celereuberomys were transferred to Hybond-C membrane (GE Healthcare Amersham). Membranes were blocked with 0.1% BSA and 2% glycine in PBS-Tween, and then incubated in the presence of crude anti-Fabp1b.1 (1:10,000) or anti-Fabp2 (1:1,000) antisera or purified polyclonal antibodies at room temperature for 2 h. Membranes were washed and incubated with affinity purified anti-rabbit IgG alkaline phosphatase associated in goat (1:30,000; Sigma-Aldrich), at room temperature for 1 h and then washed again. Alkaline phosphatase activity was developed with 0.33 mg/ml nitro-blue tetrazolium and 0.165 mg/ml 5-bromo-4-chloro-3-indolylphosphate in developing buffer (100 mM Tris-HCl, pH 9.5, 0.1 M NaCl, and 5 mM MgCl2). Proteins extracted from 5 dpf zebrafish celereuberomys exhibited one immunoreactive band at the right predicted relative molecular mass of Fabp1b.1 and Fabp2, thus demonstrating the monospecificity of each antibody (supplementary Fig. 2).

Immunofluorescence labeling analyses

Immunofluorescence was measured on dissected zebrafish digestive cavity and anterior intestinal fragments. Tissues were fixed with 4% PFA in PBS at 4°C overnight and embedded in graded alcohol/paraffin and toluene/paraffin series, and then, finally, in paraplast. Sections 10 µm thick were then used for immunodetection and confocal microscopy. The sections were blocked in 5% normal goat serum, 0.1% BSA, and 0.05% Tween-20 in PBS (PBST) at room temperature for 20 min. Sections were incubated with rabbit preimmune serum, or purified polyclonal anti-Fabp1b (1:2,000), or anti-Fabp2 (1:200) primary antibodies in 1% normal goat serum and 0.1% BSA in PBST at 37°C for 2 h. After extensive washing, 4 × 5 min with PBS, the sections were incubated with secondary Alexa 488- or Alexa 594-conjugated goat anti-rabbit IgG (H+L) (Life Technology) at 1:1,000 dilution in PBS at room temperature for 1 h. The slides were then immersed in a solution of To-Pro 3 iodide (TP3) or 4',6-diamidino-2-phenylindole (DAPI) (1:4,000) to label the nuclei red or blue. Following several washes in PBS, samples were mounted with Gold ProLong anti-fade mounting medium (Life Technology), sealed with nail polish, and stored in a dark place at 4°C prior to analysis. Control sections were treated without the primary antibody. Sections were viewed using an Olympus FV-300 scanning laser confocal microscope.

Electron microscope immunocytochemistry

Fragments of anterior intestine were fixed in 1% glutaraldehyde and 4% formaldehyde in 0.1 M PBS, pH 7.4, for 12 h, changing the solution three times. After washing in the same buffer, the samples were embedded in LR white resin (Sigma-Aldrich). Ultrathin sections were treated successively with glycine 0.02 M and PBS, blocked with 1% BSA for 5 min, treated with primary antibody diluted in PBS containing 1% BSA for 1 h, washed with PBS, and then incubated with the 10 nm protein A-colloidal-gold complex (Sigma-Aldrich) in PBS containing 1% BSA for 1 h. All the incubation samples consisted of a 30 µl drop of solution at 25°C. Controls were prepared by floating sections on PBS to replace primary antibodies. Slices were contrasted with 5% uranyl acetate for 5 min and examined under a Zeiss EM902 electron microscope. Subnuclear immunogold particle density was determined using the area and particle tools in ImageJ free-processing software.

BODIPY-FL FAs binding to recombinant Fabps

Stock 2 mM solutions of BODIPY-FLC5 or BODIPY-FLC12 in ethanol were diluted in PBS prior to mixing and incubating with purified proteins for a few minutes. Prior to binding assays, the proteins were delipidated in batch using hydroxylalkoxypropyl-dextran (Sigma-Aldrich) chromatography, equilibrated with 50 mM Tris-HCl, pH 7.4, at 37°C for 45 min. The proteins were then diluted at the working concentration with 30 mM Tris-HCl, pH 8.3, sample buffer. Five microliters of 20 µM BODIPY-FLC5 or BODIPY-FLC12 were mixed with 20 µl 5.6 µM recombinant zebrafish Fabp1b.1, 12 µl 4.3 µM tropomyosin A (TPMA) from Echinococcus granulosus (GenBank: AAB65799.4), or 6.6 µl 7.5 µM BSA. A 12 µl sample of 20 µM BODIPY-FLC5 or BODIPY-FLC12 was mixed with 20 µl 30 µM recombinant zebrafish Fabp2. The binding of fluorescent carbon chain BODIPY FAs to recombinant Fabps was then evaluated by an in-gel fluorescence imaging method derived from the one previously described for visualizing BODIPY-arachidonic acid-tagged cellular proteins (55, 56). A dye-free 4+ loading sample buffer containing 200 mM Tris-HCl (pH 6.8), 20% glycerol, and 8% SDS was then added to each sample. Samples were then loaded on a 1.5 mm thick, 15% native polyacrylamide gel and electrophoresed using a running buffer containing 0.05 M Tris-HCl and 2.6 M glycerol. After in-gel fluorescence imaging (G:Box, Syngene), gels were stained with Coomassie blue R250 to check for protein loading and electrophoretic migration. All manipulations were done in the dark.

Fluorescence emission spectra of BODIPY-FLC5, or BODIPY-FLC12, in the presence and absence of Fabp1b.1 were determined at 25°C using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Santa Clara). BODIPY-FLC5 or BODIPY-FLC12 2 mM stock solutions in ethanol were diluted in PBS prior to mixing and incubating for 2 min with purified FABP1b.1. The protein sample was previously dissolved with 30 mM Tris HCl, pH 8.3, buffer. The emission spectrum was measured from 500 to 600 nm while the excitation wavelength was set at 488 nm.

Displacement experiments

Fabp1b.1 was dissolved in 30 mM Tris HCl, pH 8.3, sample buffer (5.6 µM, 22.5 µl) and incubated with BODIPY-FLC12 (20 µM, 7.5 µl) at room temperature for 10 min. The solution was then mixed with 3 µl unlabeled FA taken from a 5 mM stock solution.
in ethanol and supplemented with 10 µl loading sample buffer used for loading on the native polyacrylamide gel. Under these conditions, the unlabeled FAs were in ~100-fold molar excess (i.e., 0.55 mM) over the labeled FA and protein used. The following competitor ligands assayed were oleic acid (C18:1w9), linoleic acid (C18:2w6), and DHA (C22:6w3). The samples were mixed and allowed to equilibrate for 20 min at room temperature before loading 29 µl into the native polyacrylamide gel.

**Tandem MS**

Stained PAGE bands were faded, excised, and in-gel digested with trypsin in the appropriate buffer. The peptides resulting from the proteolytic digestion were identified by peptide mass fingerprinting and collision-induced dissociation using a 4800 MALDI-TOF-TOF mass spectrometer (Applied Biosystems). The MS data were analyzed using Mascot software (Matrix Science Ltd., London, United Kingdom) and searching the NCBInr and ZFIN zebrafish databases.

**Statistics**

Variations in transcription levels (normalized by reference genes) were analyzed for statistical significance using REST-384-version 2 software for multiple reference genes (http://rest.gene-genes) and/or a nonparametric Mann-Whitney test. The significance level was set at 0.05. Data are presented as mean ± SD. The same test was used to check for variations in normalized efla/ppiaa values.

**RESULTS**

**FABP1 and FABP2 homologs in zebrafish**

Tandem duplicated fabp1b.1 and fabp1b.2 on zebrafish chromosome 8 and fabp2 on chromosome 1 (http://zf.in.org/) generated proteins of the predicted molecular mass of 14,115 Da and 128 amino acids long, 14,461 Da and 128 amino acids long, and 15,090 Da and 132 amino acids long, respectively. Human FABP1 exhibited 54.33% and 41.73% identity with Fabp1b.1 and Fabp1b.2, respectively (supplementary Fig. 1). Zebrafish Fabp2 was identical in size and exhibited 66.67% identity with its human homolog (supplementary Fig. 1). Zebrafish Fabp1b.1 and Fabp1b.2 exhibited 21.09% and 15.62% identity with zebrafish Fabp2, while these two proteins showed 50.78% identity between them.

**Tissue-specific distribution of zebrafish fabp1b.1, fabp1b.2, and fabp2 transcripts**

Qualitative RT-PCR detected fabp1b.1 and fabp1b.2 transcripts in total RNA extracted from the anterior intestine in adult zebrafish, irrespective of the animal’s nutritional status (Fig. 1A). qRT-PCR indicated similarly high levels of transcripts for fabp1b.1 and fabp2 in the anterior intestine, by comparison with normalizing genes efla1 (Fig. 1B, D) and ppiaa (data not shown). The fabp1b.2 mRNA levels were around 3-fold lower (Fig. 1C, data not shown). The normalized level of fabp1b.2 transcripts was very high in brain (Fig. 1C, data not shown). Trace amounts of fabp2 transcripts were also expressed in adult brain while fabp1b.1 transcripts were not detected. By contrast, fabp1b.1 transcripts were detected in the liver, together with very small amounts of fabp2 mRNA, while fabp1b.2 transcripts were not detected (Fig. 1; data not shown). Zebrafish fabp1b.2 transcripts were also detected in ovaries, testicles, kidneys, and heart, and trace amounts of fabp2 transcripts were found in kidneys.

**Fig. 1.** The fabp1b.1, fabp1b.2, and fabp2 transcript expression in various adult zebrafish tissues. A: RT-PCR products of fabp1b.1 and fabp1b.2 transcripts resolved by electrophoresis on agarose ethidium bromide gels. The efla1 transcripts were analyzed to assess qualitative integrity of mRNAs. Lane 1: size standard. Lanes 2 to 7: RT-PCR products generated from total RNAs and extracted from dissected brain (b) (lanes 2 and 3), anterior intestine (i) (lanes 2 and 4), liver (l) (lanes 6 and 7) after a period of over 24 h fasting (lanes 2, 4, 6) or 22 h fasting followed by 2 h feeding (lanes 3, 5, 7). Lane 8: A negative control lacking reverse transcriptase generated no RT-PCR products. B–D: qRT-PCR analysis of fabp1b.1 (B), fabp1b.2 (C), and fabp2 (D) transcript expression was performed on fed animals after dissecting various tissues. Six independent liver or anterior intestine and four brain samples from individual adults were tested in triplicate in each condition. Zebrafish heart (h; pool from six animals), kidney (k; pool from four animals), ovary (o), and testicle (t) samples were subjected to a single determination in triplicate. The efla1 transcript levels were determined and used as a normalizing gene. The results shown are the number of copies of fabp1b.1, fabp1b.2, and fabp2 transcripts divided by the number of copies of efla1. nd, no amplification product detected.

**FABPs channel dietary fatty acids into enterocyte nuclei**

The reporter transcript expression was performed on fed animals after dissecting various tissues. Eight independent liver or anterior intestine and four brain samples from individual adults were tested in triplicate in each condition. Zebrafish heart (h; pool from six animals), kidney (k; pool from four animals), ovary (o), and testicle (t) samples were subjected to a single determination in triplicate. The efla1 transcript levels were determined and used as a normalizing gene. The results shown are the number of copies of fabp1b.1, fabp1b.2, and fabp2 transcripts divided by the number of copies of efla1. nd, no amplification product detected.
Regulating fabp1b.1 and fabp2 transcript levels by the nutritional status and lipid composition of the diet and cephalocaudal patterning of their expression in gut

The aim of the experimental work was to determine whether the fabp1b.1 and fabp2 transcript levels were regulated after feeding and/or according to the lipid composition of the diet. Suitable primer sets were designed on the basis of the cDNA sequences previously obtained to be used for qRT-PCR and primers for synthesizing specific probes for whole-mount, in situ hybridization. The modulation, if any, of transcript levels after feeding and by dietary lipid composition was evaluated by using commercially and in-house formulated synthetic larval diets and the techniques described previously.

The first step was to study whether regulation of fabp1b.1 and fabp2 transcript levels occurred in the zebrafish adult intestine and liver after feeding. As indicated in Fig. 2, transcript levels of fabp2 (P = 0.0087) were upregulated by 1.59-fold and fabp1b.1 by 1.36-fold (not significant) in the anterior intestine when the reference gene used was eef1a1. REST software, using both ppiaa and eef1a1 as reference transcripts, gave P = 0.021 for fabp2. This upregulation was not observed in the liver (Fig. 2). A similar study was then conducted at early developmental nutritional stages (i.e., first feeding at 5 dpf) and at the start of the exogenous nutritional phase of larval development (i.e., 15 dpf) (Fig. 3).

There were no significant differences in fabp1b.1 and fabp2 transcript levels between nourished and unnourished 5 dpf eleutheroembryos when normalized with the two reference genes, while a slight upregulation of fabp1b.1 was detected when using ppiaa only (×1.15-fold, P = 0.026). Transcripts levels of fabp1b.1 and fabp2 were also significantly upregulated after feeding in whole 15 dpf zebrafish larvae homogenates, irrespective of the reference gene used (Fig. 3). The fabp1b.1 transcript levels were 1.41 (P = 0.026) and 1.54 (P = 0.0022) higher, respectively, when eef1a1 and ppiaa were used as reference transcripts. REST software using the two reference transcripts at the same time gave a P value of 0.001. The fabp2 transcript levels were 1.59 (P = 0.026) and 1.71 (P = 0.0022) higher, respectively, when eef1a1 and ppiaa were used as reference transcripts. REST software using the two reference transcripts at the same time gave a P value of 0.004. The effect of diet composition on the level of fabp1b (fabp1b.1 + fabp1b.2) and fabp2 transcript levels was then studied by whole-mount in situ hybridization (Fig. 4). An upregulation of fabp1b and fabp2 hybridization signals between starved and fed conditions was detected, and the signal was apparently modulated according to the lipid composition of the diet. Larvae (15 dpf) nourished with FD experienced a high induction of the signal after feeding, while larvae fed with dried CEY or OO were in an intermediate state (Fig. 4).

Fig. 2. qRT-PCR analysis of fabp1b.1 and fabp2 transcript expression in the dissected anterior intestine (ai) and liver (l) of adult zebrafish. A: fabp1b.1. B: fabp2. After a period of over 24 h fasting (fasted group, fa) or 22 h fasting followed by 2 h feeding (fed group, fe), organs from 6 individuals were sampled and analyzed independently, in triplicate. The number of eef1a1 transcripts was also quantified and used to express the results as a number of copies of fabp1b.1 or fabp2 divided by the number of copies of the normalizing gene. Very similar results were obtained with ppiaa, used as another normalizing gene (data not shown). Data are means ± SD. ** P < 0.01, significant difference between fa and fe groups for the organ examined using the nonparametric Mann-Whitney test.

Fig. 3. qRT-PCR analysis of the expression of fabp1b.1 and fabp2 transcripts in whole, 5 dpf zebrafish eleutheroembryos and 15 dpf larvae homogenates. A: fabp1b.1. C: fabp2. Animals were sampled after a period of over 18 h fasting (fasted group, fa) or 18 h fasting followed by 2–2.5 h feeding with FD (fed group, fe). At each age, six independent pools of 25 (5 dpf) or 20 (15 dpf) animals were used to quantify fabp1b.1 and fabp2 transcript levels in triplicate. The eef1a1 and ppiaa were also quantified and used as normalizing genes. The results shown are the number of copies of the fabp1b.1 or fabp2 transcripts divided by the number of copies of eef1a1 (A, C) or ppiaa (B, D). Data are means ± SD. Means were compared in reference to the fasted group using the nonparametric Mann-Whitney test. Asterisks indicate significant differences (* P < 0.05; *** P < 0.005) between the fasted and fed groups, for the transcript and age concerned.
A differential expression pattern of \textit{fabp1b} genes along the anterior-posterior axis of the gut was demonstrated by whole-mount in situ hybridization (Fig. 4; supplementary Fig. 3). The \textit{fabp1b} (\textit{fabp1b.1} plus \textit{fabp1b.2}) and \textit{fabp2} transcripts were strongly expressed in the anterior intestine and its rostral expansion, and no mRNAs could be detected in the posterior intestine. A significant \textit{fabp2} transcript level was detected in the rectum, but no hybridization signal with \textit{fabp1b}.

Localization of Fabp1b and Fabp2 in adult zebrafish intestine

Histological sections of the digestive cavity were stained with monospecific polyclonal antibodies raised against recombinant zebrafish Fabp1b1.1 or Fabp2 (Fig. 5). It is important to notice that the antibody raised against Fabp1b1.1 may also detect an Fabp1b2 signal, due to a significant sequence identity between the two; from now on, we will refer to the signal generated by Fabp1b1. Fabp1b1 and Fabp2 were immunodetected at high levels in the anterior intestine. A lower-level Fabp1b signal was detected in the liver (Fig. 5A and data not shown). No Fabp1b (Fig. 5A) or Fabp2 (data not shown) labeling signal was found in the posterior intestine, testicles, white adipose tissue, or infiltrated pancreatic islets. Higher magnification revealed homogenous cytoplasmic Fabp1b and Fabp2 expression in the intestinal absorptive cells. Expression was detected through the cytoplasm, including the microvilli, and inside the nuclei of some enterocytes mostly outside condensed chromatin clusters.

Immunogold labeling electron microscopy was used for cellular and subcellular localization of Fabp1b (Fig. 6; supplementary Fig. 4) and Fabp2 (supplementary Fig. 5) in enterocytes. Gold particles were observed inside and on the surface of the microvilli, as well as in the terminal web. Immunogold particles were also abundant in the cytoplasm, outside the endoplasmic reticulum membrane, in close proximity to the plasma membrane, and inside the nucleus. No immunogold particles were detected inside oil globules, mitochondria, intercellular space, goblet cells, or lamina propria. Fabp1b nuclear immunogold particles were found in the interchromatin space with a labeling efficiency 4.34 times higher than in nuclear membrane-associated condensed chromatin and condensed chromatin clusters. The same was true of Fabp2, with 3.01-fold more labeling in the interchromatin space by comparison with the condensed chromatin areas.

Native PAGE binding assay of BODIPY-FL-labeled FAs to recombinant Fabp1b.1 and Fabp2

A native PAGE binding assay was used to check that recombinant Fabp1b.1 or Fabp2 were able to specifically bind BODIPY-labeled FAs (Fig. 7). TPMA, used as a negative control, was unable to bind the labeled FAs. We demonstrated that neither Fabp1b.1 nor Fabp2 were able to bind BODIPY-FLC\textsubscript{0} to any significant extent, but both of them avidly bound BODIPY-FLC\textsubscript{12}. While a very heavily labeled band was observed with BODIPY-FLC\textsubscript{12} (Fig. 7B, D), only a very faintly labeled band was observed with Fabp1b.1 and BODIPY-FLC\textsubscript{0} (Fig. 7B, lane 5). Adding SDS to a final concentration of 2% in the loaded sample improved the PAGE protein resolution without a drastic change in the quantity of BODIPY-FLC\textsubscript{12} fluorescence bound to FABPs (supplementary Fig. 6; data not shown). The inability of BODIPY-FLC\textsubscript{0} to bind to Fabp1b.1 was confirmed.
BODIPY-FLC₁₂ binding to Fabp₁b.₁ or Fabp₂. By increasing the amount of BSA loaded into the gel, our in-gel fluorescence imaging method confirmed that this protein was able to bind BODIPY-FLC₁₂ (supplementary Fig. 8).

We then demonstrated that oleic acid was able to fully abolish the binding of BODIPY-FLC₁₂ to Fabp₁b.₁ (Fig. 8). DHA had partial displacement activity under these experimental conditions, while linoleic acid was ineffective.

Feeding with BODIPY-labeled FAs
To investigate the involvement of Fabp₁b and Fabp₂ in the targeting of dietary FAs to nuclei, adult zebrafish were fed with BODIPY-FLC₁₂ (Fig. 9) or BODIPY-FLC₁₂ (Fig. 10; supplementary Fig. 9) mixed with dried CEY. Both fluorescent FAs were taken up by the enterocyte, and a wide cytoplasmic distribution was observed. These experiments also

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Fig. 5. Immunofluorescence localization of Fabp₁b and Fabp₂ in the digestive cavity of zebrafish. The histological sections were stained with monospecific polyclonal antibodies developed against recombinant zebrafish Fabp₁b₁ (A, B, E) or Fabp₂ (C, D, F) proteins, with Alexa 488-conjugated secondary antibody (green). The sections were counterstained with TP₃ to label the nuclei red. A: High levels of Fabp₁b₁ were immunodetected in the anterior intestine (ai) and lower levels in the liver (l) while no signal was detected in the posterior intestine (pi), testicles (t), adipose tissue (at), or pancreatic islets (p). B: In the intestinal mucosa, Fabp₁b₁ was detected inside the intestinal villi (iv) while the lamina propria (lp) was devoid of any signal. C, D: Immunofluorescent staining for Fabp₂ was similar to that of Fabp₁b₁, with high levels in iv. E, F: Higher magnification revealed Fabp₁b and Fabp₂ expression in the enterocytes (e), including the microvilli (mv), while goblet cell (gc) and infiltrated cells from lymph and blood vessels (lbv) were devoid of any immunofluorescent signal. Nuclear localization of Fabp₁b and Fabp₂ was highlighted by the yellow coloring in the nuclei of most enterocytes in the intestinal mucosa (B, D, E, F). TP₃ nuclear staining demonstrated that cells with fully condensed chromatin appeared homogeneously red and did not exhibit any nuclear Fabp signal. The nuclei located in the suprabasal position of a large number of enterocytes exhibited immunofluorescent labeling outside condensed chromatin clusters. White arrow: nucleus (n) with condensed chromatin clusters, immunolabeled with Fabp antibodies outside the clusters. White arrowhead: nucleus with condensed chromatin clusters, not immunolabeled with Fabp₁b antibody. Inserts in E, F are enlarged views of the region surrounded by dashed lines in the main pictures. il, intestinal lumen. Scale bar represents 75 µm in A, B, C, D; 20 µm in E, F.
showed that nuclear incorporation of FA analogs depended on the ligand carbon chain length. A strong BODIPY-FLC₅ signal was located in the basal region of the enterocyte and surrounding the nuclei, while a robust BODIPY-FLC₁₂ signal was also observed inside the nuclei. Fluorescence microscopy images demonstrated that the BODIPY-FLC₁₂ signal associated with the nuclear fraction was 18.05 ± 1.76% of the total fluorescence detected in the villi (7 villi analyzed which included 225 enterocyte nuclei). Some enterocyte nuclei were heavily stained while other ones with more condensed chromatin had low BODIPY-FLC₁₂ intranuclear labeling (Fig. 10; supplementary Fig. 9). The labeled enterocyte nuclei were 13.17 ± 2.04% of the total surface area analyzed demonstrating a significant nuclear fraction enrichment of BODIPY-FLC₁₂ label ($P < 0.0006$). Because both fluorescent FAs and Fabp1b and Fabp2 have a homogenous cytoplasmic distribution, it was difficult to assign a colocalization. However, an accumulation of both labels was observed in the basal region of the enterocyte, and colocalization of BODIPY-FLC₁₂ with Fabp1b and Fabp2 was observed inside the enterocyte nuclei, in the interchromatin space between the condensed chromatin areas (Fig. 10).

**DISCUSSION**

The primary role of all FABP family members is to bind FAs and other hydrophobic ligands, such as lipophilic...
Fig. 8. Displacement of BODIPY-FLC12 to recombinant Fabp1b.1. A: Representative native PAGE of purified recombinant Fabp1b.1 labeled with BODIPY-FLC12 and displaced by 100-fold molar excess of unlabeled FAs or not. Lane 1, Fabp1b; lane 2, Fabp1b.1 plus BODIPY-FLC12; lane 3, Fabp1b.1 plus BODIPY-FLC12 and oleic acid (C18:2w6); lane 4, Fabp1b.1 plus BODIPY-FLC12 and linoleic acid (C18:2w6); lane 5, Fabp1b.1 plus BODIPY-FLC12 and DHA (C22:6w3). B: Labeled Fabp1b.1 bands were detected for fluorescent, and full displacement of BODIPY-FLC12 to Fabp1b.1 was observed in the presence of oleic acid (lane 3). DHA exhibited partial displacement activity (lane 5), while linoleic acid was ineffective (lane 4). Lanes 3 to 5 come from the same gel with the lanes moved.

Fig. 9. The intestinal absorption of dietary BODIPY-FLC5, and simultaneous localization of Fabp1b and Fabp2 in zebrafish intestinal villi. Histological sections were counterstained with DAPI to label the nuclei blue (A–D). The sections were stained with monoclonal antibody (E–G) or Fabp2 (H) proteins with Alexa 594-conjugated secondary antibody (red). Adult animals were previously fed with BODIPY-FLC5 mixed with CEY powder and used as a negative control (see Materials and Methods for details) (I) or BODIPY-FLC5 mixed with CEY powder (J–L), and fluorescence was detected on histological sections in green fluorescent protein channel (green). (N–P) Merging between nuclei labeling, immunofluorescence signal of Fabps, and BODIPY-FLC5 or BODIPY-FLC5 signals are presented for each corresponding column of the figure. The apex of the villi is positioned at the bottom of each image. C, G, K, and O are enlargements of the indicated area on B, F, J, and M, respectively. No BODIPY-FLC5 signal was detected inside the nuclei and the intranuclear overlap between Fabp and DAPI labels is shown as pink spots (white arrows in N, O, P, e, enterocyte; lbv, lymph and blood vessels; mv, microvilli. Scale bar represents 20 µm, except 5 µm in C, G, K, O.
Intestinal absorption of dietary BODIPY-FLC₁₂ and its colocalization with Fabp1b and Fabp2 in zebrafish enterocytes. A–C: Histological sections were counterstained with DAPI to label the nuclei blue. D, E: The sections were stained with monospecific polyclonal antibodies developed against recombinant zebrafish Fabp1b.1 or Fabp2 proteins with Alexa 594-conjugated secondary antibody (red). F, H: Adult animals were previously fed with BODIPY-FLC₁₂ mixed with CEY powder (see Materials and Methods for details), and fluorescence was detected in the green fluorescent protein channel (green). L, J: Merged view of labeled nuclei, Fabp immunofluorescence signal, and BODIPY-FLC₁₂ signals are presented in the left and right columns of the figure. The apex of the villi is positioned at the bottom in each image. B, G are enlargements of the indicated large area from A, I, respectively. Inserts in A, F are enlargements of the indicated small area focused on an enterocyte nucleus with a strong BODIPY-FLC₁₂ signal. The selected nucleus has been surrounded by a yellow line after using the threshold and wand tool options of ImageJ software (see supplementary Fig. 9 for an enlarged view of these panels). Yellow intracytoplasmic granulation indicates an overlap between Fabp1b and BODIPY-FLC₁₂ (I, J, red arrow in G). Some nuclei with more condensed chromatin had low BODIPY-FLC₁₂ intranuclear labeling while the pink granules indicate intranuclear Fabp1b (white arrowhead in G). White and red asterisks indicate goblet cell and putative red blood cell nuclei, respectively, free of any Fabp1b and BODIPY-FLC₁₂ labeling (B, G). Intranuclear overlapping was observed between Fabp, BODIPY-FLC₁₂, and nucleus labels (white arrows in G, J). e, enterocyte; lbv, lymph and blood vessels; mv, microvilli. Scale bar represents 20 µm.

Fig. 10. Intestinal absorption of dietary BODIPY-FLC₁₂ and its colocalization with Fabp1b and Fabp2 in zebrafish enterocytes. A–C: Histological sections were counterstained with DAPI to label the nuclei blue. D, E: The sections were stained with monospecific polyclonal antibodies developed against recombinant zebrafish Fabp1b.1 or Fabp2 proteins with Alexa 594-conjugated secondary antibody (red). F, H: Adult animals were previously fed with BODIPY-FLC₁₂ mixed with CEY powder (see Materials and Methods for details), and fluorescence was detected in the green fluorescent protein channel (green). L, J: Merged view of labeled nuclei, Fabp immunofluorescence signal, and BODIPY-FLC₁₂ signals are presented in the left and right columns of the figure. The apex of the villi is positioned at the bottom in each image. B, G are enlargements of the indicated large area from A, I, respectively. Inserts in A, F are enlargements of the indicated small area focused on an enterocyte nucleus with a strong BODIPY-FLC₁₂ signal. The selected nucleus has been surrounded by a yellow line after using the threshold and wand tool options of ImageJ software (see supplementary Fig. 9 for an enlarged view of these panels). Yellow intracytoplasmic granulation indicates an overlap between Fabp1b and BODIPY-FLC₁₂ (I, J, red arrow in G). Some nuclei with more condensed chromatin had low BODIPY-FLC₁₂ intranuclear labeling while the pink granules indicate intranuclear Fabp1b (white arrowhead in G). White and red asterisks indicate goblet cell and putative red blood cell nuclei, respectively, free of any Fabp1b and BODIPY-FLC₁₂ labeling (B, G). Intranuclear overlapping was observed between Fabp, BODIPY-FLC₁₂, and nucleus labels (white arrows in G, J). e, enterocyte; lbv, lymph and blood vessels; mv, microvilli. Scale bar represents 20 µm.

As previously described (41), a significant level of fabp2 transcripts was detected in the rectum while no hybridization signal with fabp1b, thus highlighting the differential expression pattern of these genes. Levels of fabp1b.1 and fabp2 transcripts were upregulated in the anterior intestine after feeding, both at the larval stages and in adults. As previously reported (45, 47), these levels were modulated according to the lipid composition of the diet. Lipid-mediated regulation of gene transcription via the activation of nuclear receptors to modify the expression of genes coding for proteins involved in the lipid metabolism triggered the proliferation of intestinal cells and modulated the expression of molecular signals that may affect homeostasis (26, 36).

The second step of our study was to produce monospecific polyclonal antibodies against purified recombinant proteins from the two highly expressed fabps in the zebrafish anterior intestine (i.e., fabp1b.1 and fabp2). These tools were used for the cellular and subcellular immunolocalization of Fabp1b and Fabp2 in the digestive cavity and demonstrated a very high level of these FABPs in enterocytes in the anterior intestine. Given the very intense immune staining signal observed for both Fabps in the zebrafish intestinal villi, it is very likely that these proteins are present simultaneously in the same enterocytes. Immunofluorescence and immunocytochemical labeling methods revealed Fabps in the microvilli and cytosol, as previously demonstrated with human enterocytes from jejunal mucosa (39), as well as in the nuclei of most enterocytes in the anterior intestine. No staining signal was observed inside mitochondria or oil globules. Nuclei of cells present in the intestinal mucosa and with fully condensed chromatin, as well as cells from lymph and blood vessels, were devoid of any Fabp immunofluorescent signal. Electron microscopy immunodetection with gold particles demonstrated that enterocyte nuclear localization was mostly in the interchromatin space outside the condensed chromatin clusters. The nucleus is compartmentalized into chromosome territories and interchromatin spaces (68, 69). Zebrafish enterocyte nuclei revealed the conventional pattern with dense chromatin/heterochromatin enriched at the nuclear periphery and around the nucleoli, whereas less condensed/euchromatin regions extended toward the nuclear interior, together with dispersed interior clumps.
of condensed chromatin/heterochromatin. The spatial organization of eukaryotic genomes in the cell nucleus is linked to their transcriptional regulation (70, 71). However, it has been difficult to find general rules on the involvement of nuclear organization in transcriptional regulation. It remain to be determined whether Fabp1b and Fabp2 immunolabeling, found almost exclusively in less condensed/euchromatin regions, was located in transcriptionally permissive areas.

The third step was to evaluate whether fluorescent FA analogs were able to bind to recombinant zebrafish Fabp1b.1 and Fabp2. We demonstrated that neither recombinant Fabp1b.1 nor Fabp2 were able to bind significantly to BODIPY-FLC5 (short chain), but both of them bound avidly to BODIPY-FLC12 (medium chain). Our in-gel binding assay showed that Fabp2 in an oligomerization state was unable to bind to the labeled FA analog. Fluorescent short- to long-chain BODIPY-labeled FAs were used in cell culture to assess the cellular uptake and nuclear localization of unesterified FAs (72–75). These fluorescent FAs analogs, which have a fluorophore linked to the terminal (omega) carbon atom furthest from the carboxylate moiety, were also esterified into complex lipids after adding to cells/animals. Human FABP1 had high affinity for BODIPY-FLC12, in the same range as LCFA (74). FABP1 expression significantly enhanced the uptake of medium-chain BODIPY-FLC12 and differentially targeted BODIPY-FLC12 into nuclei, in marked contrast to the lack of effect of FABP1 expression on the intracellular distribution of short-chain BODIPY-FLC5 (73). The findings demonstrated that oleic acid was the only FA tested able to fully abolish the binding of BODIPY-FLC12 to zebrafish Fabp1b.1. This is interesting, as oleate binding causes a conformational change in rat FABP1 (76) and increases the nuclear localization of FABP1 and FABP2 (23, 33). Oleic acid may be involved in the transcriptional regulation of gene expression (77, 78), and the presence of either FABP1 or FABP2 magnifies the transcriptional activation mediated by PPARα (33).

The last step was to assess the fate of dietary fluorescent (BODIPY) FAs at the intestinal level, as the digestive physiology of zebrafish may be addressed using these labeled analogs (79). In vertebrates, the absorption and transport of short- to medium-chain FAs occurs differently in enterocytes, as LCFA are usually reesterified inside the cell. There is evidence indicating that both FABP1 and FABP2 participate in the cellular uptake and transport of unesterified FAs and the lipid metabolism (16, 80, 81). However, some authors report that they play a role in lipid sensing but not in direct dietary FA absorption (13, 62, 83). Cell culture models have proved very useful for mechanistic investigations into the role of FABPs in modulating nuclear receptors and gene transcription (26). For example, it was demonstrated that FABP1 interacted with PPARα (24, 27), and FABP4 with PPARγ (22), respectively, in cultured primary hepatocytes, HepG2 cells, and 3T3-L1 adipocytes. A physical contact between FABP1 and PPARα occurs during this activation, so it has been suggested that FABP1 is a coactivator in PPAR-mediated gene control (27). It has also been demonstrated that FABP5 served as coactivator in the nucleus of transfected cells for PPARβ/δ-mediated gene transcription control (25). FABP5 shuttles FAs directly to the nucleus where it may deliver the ligand to PPARβ/δ, thus enabling its activation (31, 32). In addition, DHA binding to FABP7 triggers the accumulation of FABP7 in the nucleus and modulates cell migration (28). In transfected COS-7 cells, nuclear accumulation of FABP1 and FABP2 is unlikely to be mediated by increased nuclear transport. FABP accumulation in the nucleus is due to a reduced rate of nuclear egress in the presence of the ligand and the dependent interaction of FABP with PPARα (33).

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