Ileal Bile Acid-binding Protein, Functionally Associated with the Farnesoid X Receptor or the Ileal Bile Acid Transporter, Regulates Bile Acid Activity in the Small Intestine*

Received for publication, July 11, 2005, and in revised form, August 31, 2005 Published, JBC Papers in Press, October 17, 2005, DOI 10.1074/jbc.M507454200

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Bile acids secreted in the small intestine are reabsorbed in the ileum where they activate the nuclear farnesoid X receptor (FXR), which in turn stimulates expression of the ileal bile acid-binding protein (I-BABP). We first hypothesized that I-BABP may negatively regulate the FXR activity by competing for the ligands, bile acids. Reporter assays using stable HEK293 cell lines expressing I-BABP revealed that I-BABP enhances rather than attenuates FXR activity. In these cells I-BABP localizes predominantly in the cytosol and partially in the nucleus, a distribution that does not shift in response to FXR expression. In vitro binding assays reveal that recombinant I-BABP is able to bind 35S-labeled FXR and that chenodeoxycholic acid (CDCA) stimulates this interaction modestly. When FLAG-tagged FXR was expressed in stable cells, the FXR-I-BABP complex in the nuclear extracts was more efficiently immunoprecipitable with anti-FLAG antibodies in the presence of CDCA. These results indicate that I-BABP stimulates FXR activity through a mutual interaction augmented by bile acids. When stable cells were transfected with an expression plasmid of the ileal bile acid transporter (IBAT) essential for the reabsorption of conjugated bile acids, the 14C-labeled conjugated bile acid, glycocholic acid, was more efficiently imported via IBAT in the presence than absence of I-BABP, whereas no change was observed in 14C-labeled CDCA uptake, which is independent of IBAT. Immunofluorescent staining analysis revealed that these two proteins co-localize in the vicinity of the plasma membrane in stable cells. Taken together, the current data provide the first evidence that I-BABP is functionally associated with FXR and IBAT in the nucleus and on the membrane, respectively, stimulating FXR transcriptional activity and the conjugated bile acid uptake mediated by IBAT in the ileum.

Bile acids are synthesized from cholesterol in the liver and secreted into the small intestine, where they facilitate absorption of fat and fat-soluble vitamins by solubilizing dietary lipids. Most are reabsorbed from the lower small intestine and returned to the liver. The bile acid synthesis rate in the liver is tightly regulated by the activity of a key enzyme of this pathway, cholesterol 7α-hydroxylase (CYP7A1).3 Bile acids returned to the liver induce FXR activity and suppress transcription of the CYP7A1 gene by the inhibitory effect of SHP (small heterodimer partner), one of the FXR target genes, on the activity of LRH-1 (liver receptor homologue-1), which positively regulates the gene expression of this enzyme (1, 2). This negative feedback pathway for bile acid synthesis is critical for maintenance of whole body cholesterol homeostasis.

The primary bile acids produced in the liver are usually converted into either glycine- or taurine-conjugated bile acids prior to excretion into the bile. The active uptake of conjugated bile acids from the intestine is mediated by the ileal bile acid transporter (IBAT) located on the apical membrane of the ileal enterocyte (3). Generation of IBAT-null mice revealed that this transporter is essential for efficient intestinal absorption of bile acids and that alternative absorptive mechanisms are unable to compensate for the loss of IBAT function (4). Once bile acids are taken up in the ileocytes, the ileal bile acid-binding protein (I-BABP), an abundant 14-kDa cytosolic protein, binds them. I-BABP is one of the target genes for FXR in the small intestine and therefore becomes more abundant in the presence of bile acids (5). Indeed, both FXR and I-BABP are co-expressed along the small intestine. I-BABP is thought to be involved in facilitating the uptake of bile acids and their intracellular trafficking in the small intestine, but its exact functions remain to be specifically elucidated.

I-BABP belongs to the fatty acid-binding protein (FABP) family, the members of which share a remarkably similar structure along with a small molecular mass (14–15 kDa) (6, 7). Unlike the other family members, I-BABP is unique in that its ligands, bile acids, directly govern its genetic expression by the activity of FXR. Because these two proteins bind bile acids, we first speculated that there is feedback regulation between FXR and I-BABP. The present study was undertaken to explore this possibility. We then focused on a possible functional interaction between I-BABP and IBAT when conjugated bile acids are imported through the plasma membrane. We show herein that I-BABP is functionally associated with FXR and IBAT in the nucleus and on the membrane, respectively, and stimulates the FXR transcriptional activity and the conjugated bile acid uptake mediated by IBAT in the ileum.

* This work was supported by research grants from the Ministry of Education, Science, Sports, and Culture of Japan and the program for promotion of Basic Research Activities for Innovative Biosciences. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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3 The abbreviations used are: CYP7A1, cholesterol 7α-hydroxylase; FXR, farnesoid X receptor; RXR, retinoid X receptor; IBAT, ileal bile acid transporter; I-BABP, ileal bile acid-binding protein; FITC, fluorescein isothiocyanate; FABP, fatty acid-binding protein; CDCA, chenodeoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; FBS, fetal bovine serum; PPAR, peroxisome proliferator-activated receptor; LBD, ligand-binding domain.
**EXPERIMENTAL PROCEDURES**

**Materials**—CDCA, GCA, and glycochenodeoxycholic acid (GCDDCA) were purchased from Sigma. Deoxycholic acid, lithocholic acid, and urodeoxycholic acid were from Wako (Osaka, Japan). [Carboxyl-14C]CDCA was from American Radiolabeled Chemicals Inc. and [1-14C]GCA (sodium salt) was from Amersham Biosciences.

**Cell Culture**—HEK293 cells were maintained in medium A (Dulbecco’s modified Eagle’s medium (Sigma), 100 units/ml penicillin, and 100 μg/ml streptomycin) supplemented with 10% fetal bovine serum (FBS) at 37 °C under 5% CO2 atmosphere. Caco-2 cells were maintained in medium A supplemented with 10% FBS and 1% non-essential amino acids at 37 °C under 5% CO2 atmosphere. The cells cultured for 10 days after total confluency were considered differentiated.

**Plasmid Constructs**—Expression plasmids for human FXR and human retinoid X receptor α (RXRα) were described previously (8). To generate pl-BABP900, a BglII-HindIII PCR fragment coding the 5’ promoter region (−862/+30) of the human I-BABP was inserted into a pGL3 basic vector (Promega). The reporter mutant construct, pl-BABP900mut, which contains mutations in the FXR-responsive element (5’-GGGCCCCGCTCT-3’, mutations underlined), was synthesized by a PCR-assisted method using a site-directed mutagenesis kit (Stratagene) following the manufacturer’s instructions. The expression plasmid pGal4-FXR was constructed by inserting the fragment encoding human FXR into a Gal4 DNA-binding domain expression vector, pM (Clontech). The expression plasmid of human I-BABP, pME-hIB-ABP, was constructed by fragment encoding human I-BABP into a pME18S vector (9). The expression vector pCMV-hIBAT and anti-human IBAT antibodies were kind gifts from Dr. Paul A. Dawson. The expression plasmids pFLAG-IBAT and pFLAG-FXR were generated by inserting the fragment encoding human IBAT and FXR, respectively, into p3XFLAG-CMV-7.1 (Sigma).

**Stable Cell Lines**—HEK293 cells were transfected with an expression plasmid, either pME-hIBABP or pME18S, together with a bacterial deaminase expression plasmid, pPAM2-BSD (Funakoshi, Japan). The cells were cultured with a selection medium containing 8 μg/ml blastidcin (Invitrogen), and the surviving clonal cells were collected. The I-BABP level in each cell line (HEK-IBABP-1, -2, and -3) was determined by Western blot analysis.

**Northern Blot Analysis**—Total RNA was isolated using an RNA preparation kit (Isogen, Nippon Gene Corp.). The RNA was fractionated by electrophoresis in a 1% formaldehyde-agarose gel and transferred to nylon membranes (Hybond-N, Amersham Biosciences). Probes for human I-BABP and 36b4 (8) were labeled with [α-32P]dCTP (3000 Ci/mmol; Amersham Biosciences) using a random-primed DNA labeling kit (Megaprime DNA labeling system, Amersham Biosciences). The membrane was hybridized with radioactive cDNA probes, and the signals on the membrane were quantified using an image-analyzing system (FLA-3000, FujiFilm Inc.).

**Western Blot Analysis**—HEK293 cells stably expressing I-BABP were set up in medium A supplemented with 10% FBS on day 0. On day 2, the cells were harvested, and total cellular proteins were fractionated by SDS-15% PAGE. Western blot analysis was carried out using rabbit polyclonal antibodies against human I-BABP (6) or human IBAT (10) with chemiluminescent substrate (ECL, Amersham Biosciences). The signals were quantified with a LuminolMager (LAS-3000, FujiFilm).

**Reporter Assays**—Reporter assays were performed as described previously (11). HEK293 cells (35-mm dishes) were transfected by the calcium phosphate method with 0.2 μg of a reporter plasmid, 0.01 μg of phRL-TK, an expression plasmid encoding Renilla luciferase (Promega), and expression plasmids (0.1 μg each) for human FXR and human RXRα. Forty-eight hours later, both the firefly and Renilla luciferase activities were quantified using a Dual-Luciferase™ reporter system (Promega) according to the manufacturer’s instructions (12, 13).

**In Vitro Pull-down Assays**—[35S]Metionine-labeled human FXR was prepared by in vitro translation with the TNT T7 coupled reticulocyte lysate system (Promega). I-BABP-Sepharose was prepared by covalently binding recombinant human I-BABP to N-hydroxysuccinimide-activated Sepharose (Amersham Biosciences). IgG-Sepharose was also prepared using unrelated mouse IgG. For in vitro pull-down assays, [35S]Metionine-labeled FXR was incubated with Sepharose beads for 2 h in buffer A (20 mM HEPES, pH 7.9, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 0.1% Nonidet P-40, 5% glycerol, protease inhibitors). To assess whether I-BABP-FXR interaction depends on ligands, the assay was carried out with or without 50 μM CDCA in buffer A. After the beads were washed several times, the proteins were eluted with an SDS-loading buffer and separated by SDS-PAGE. The signals on the membrane were quantified using an image-analyzing system (FLA-3000, FujiFilm).

**Immunocytochemistry for I-BABP and IBAT Localization**—HEK293 cells stably expressing I-BABP (HEK-IBABP-3) were grown on glass chamber slides and, at 80% confluency, were transiently transfected with the expression plasmid pFLAG-IBAT and then fixed with 4% paraformaldehyde 24 h after transfection. To detect localization of I-BABP and FLAG-IBAT, cells were stained with anti-I-BABP antibodies (1:100) and anti-FLAG antibodies (1:100; M2, Sigma) followed by Cy3-conjugated donkey anti-rabbit IgG (1:400; Jackson ImmunoResearch Laboratories) and FITC-conjugated donkey anti-mouse IgG antibodies (1:400; Jackson ImmunoResearch Laboratories), respectively. Fluorescence staining was visualized using an Olympus FV500 confocal microscope.

**RESULTS**

**Bile Acids Induce I-BABP Gene Expression in Differentiated Caco-2 Cells**

Through a Positive Bile Acid-responsive Element in Its Promoter—I-BABP is one of the target genes of FXR in the small intestine. A previous in vivo study showed a significant expression of I-BABP mRNA in the mouse ileum, where FXR gene expression was exclusively found to be elevated (5). The present experiment demonstrates that in differentiated Caco-2 cells the expression level of I-BABP mRNA is quite low under normal culture conditions and is tremendously augmented by various bile acids, which are known as FXR ligands, but not by the bile acid urodeoxycholic acid, a non-ligand (Fig. 1A, UDCA). To confirm the FXR-dependent induction of I-BABP gene expression, reporter assays using the human I-BABP promoter were performed. Fig. 1B shows that HEK293 cells lacking endogenous FXR require forced expression of both FXR and RXRα to a more than 10-fold induction of luciferase activity, which is specifically dependent on a positive bile acid-responsive element previously identified in the I-BABP promoter (5, 16). These results clearly show that I-BABP expression is regulated exclusively by the activation of FXR via ligand binding. A previous study also demonstrated a dramatic drop in I-BABP mRNA levels in bile
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Acid-depleted mice treated with a bile acid-binding resin, in contrast to a marked increase after chronic bile acid feeding (5). These findings prompted us to hypothesize the possibility of negative feedback regulation by the robust induction of I-BABP, which competes with FXR for the available ligands and inactivates the transcriptional activities of FXR.

I-BABP Stimulates FXR Transcriptional Activities—To investigate the effect of I-BABP on FXR transcriptional activities, reporter assays were conducted in HEK293 cells expressing I-BABP transiently or stably. First we employed a heterologous Gal4 system using a fusion protein, including a Gal4 DNA-binding domain and full-length FXR, to analyze the functional correlation between I-BABP and FXR in transient transfection assays (Fig. 2A). In contrast to our working hypothesis, forced expression of I-BABP indeed stimulated the Gal4-FXR activities in the presence of 30 or 100 μM CDCA. When a native I-BABP promoter was used, a slight, but not significant, enhancing effect of I-BABP was observed (data not shown). To further confirm the I-BABP effect, we established three stable HEK293 cell lines (HEK-IBABP) with different levels of I-BABP and carried out reporter assays using these cell lines. Fig. 2B shows that HEK-IBABP-3 cells were the most abundantly expressed I-BABP, whereas no I-BABP was detected in parental and mock-transfected HEK293 (HEK-mock) cells. In both types of luciferase assays using the native I-BABP promoter and a heterologous Gal4 system with these stable cell lines, the FXR transcriptional activities were augmented in the presence of CDCA (30 or 100 μM), almost in an I-BABP expression level-dependent manner (Fig. 2, C and D). These results clearly indicate that I-BABP stimulates FXR activities in the presence of bile acid rather than competing for bile acid ligands to FXR.

I-BABP Localizes Predominantly in the Cytosol but Also in the Nucleus in HEK-IBABP-3 Cells—To examine whether I-BABP co-localizes with FXR in the nucleus and whether CDCA affects localization, both HEK-mock and -IBABP-3 cells were transfected with pFLAG-FXR, and their intracellular localization was analyzed by Western blotting. In Fig. 3, an 0.05 volume of either the nuclear or the cytosol fraction was subjected to SDS-PAGE and Western blotting using an anti-I-BABP antibody. C, HEK293 cells were transfected with pFLAG-FXR and pG5Luc together with phRL-TK and then cultured with a medium containing 10% charcoal-stripped FBS at the various concentrations of CDCA for 24 h. The luciferase activities without CDCA are considered as 1. D, HEK293 cells were transfected with pFLAG-FXR and pG5Luc together with phRL-TK and then were cultured with a medium containing 10% charcoal-stripped FBS at the various concentration of CDCA for 24 h. The luciferase activities without CDCA are considered as 1. D. These results clearly indicate that I-BABP stimulates FXR activities in the presence of bile acid rather than competing for bile acid ligands to FXR.
I-BABP Interacts with FXR, and Bile Acid Strengthens the Interaction

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To reveal the molecular mechanism by which I-BABP stimulates FXR transcriptional activity, we explored the protein-protein interaction in vitro. Fig. 4A shows that resins conjugated with recombinant I-BABP, but not with unrelated IgG, trapped 35S-labeled FXR even in the absence of bile acid and more FXR in the presence of CDCA (on average 1.6-fold more in three separate experiments). To further confirm the interaction, we determined the I-BABP-FXR complex in the nucleus of HEK-IBABP-3 cells transfected with the expression plasmid pFLAG-FXR. The nuclear extracts were prepared and incubated with a cross-linker, dithio-bis(succinimidyl propionate), to strengthen the protein-protein interaction, and then FXR was immunoprecipitated with anti-FLAG antibodies. Although CDCA did not affect the nuclear localization of these two proteins, in the presence of CDCA I-BABP was more efficiently co-immunoprecipitated with FXR (Fig. 4B, lanes 3 and 4), consistent with the results shown in Fig. 4A. In HEK-mock cells, CDCA did not have any effect on FLAG-FXR (as shown in Fig. 3), and I-BABP was not immunoprecipitable (lane 1). These results suggest that I-BABP facilitates the FXR activities through protein-protein interaction.

Co-expression of both I-BABP and IBAT Stimulates the FXR Activity of Conjugated Bile Acids—Most bile acids are thought to be absorbed as a conjugated form from the ileum, where both IBAT and I-BABP are exclusively expressed. To see the biological relationship between I-BABP and IBAT, HEK-IBABP-3 cells transfected with an expression vector of IBAT were incubated with conjugated bile acids, and the FXR activity in these cells was examined. Although none of the glycine-conjugated bile acids enhanced the luciferase activities without the expression of IBAT (data not shown), the FXR activity was enhanced by both conjugated bile acids (GCA and GCDCA) in HEK-mock and HEK-IBABP-3 cells expressing IBAT (Fig. 5A). A greater increase in luciferase activity was observed in HEK-IBABP-3 cells than in HEK-mock cells in the presence of GCA or GCDCA. Fig. 5B shows that the IBAT expression levels in the two cell lines were almost equal with or without bile

FIGURE 3. Intracellular distribution of I-BABP in HEK-IBABP-3 cells. HEK-mock and HEK-IBABP-3 cells transfected with either pFLAG-FXR or an empty vector were incubated with or without 100 μM CDCA for 4 h. Nuclear or cytosolic fractions (0.05 volume) prepared from cells in a 60-mm dish were subjected to SDS-PAGE and Western blotting with anti-I-BABP or anti-FLAG antibodies. The signals were quantified with a phosphorimaging device (LAS-3000, Fujifilm).

FIGURE 4. In vitro and in vivo analyses of the interaction between FXR and I-BABP. A, [35S]methionine-labeled human FXR prepared by in vitro translation was incubated with either I-BABP- or IgG-Sepharose beads in the presence or absence of 50 μM CDCA. After the beads were washed several times, the proteins were eluted with an SDS-loading buffer and separated by SDS-PAGE. The signals on the membrane were quantified using an image-analyzing system (FLA-3000, Fujifilm). B, HEK-mock and HEK-IBABP-3 cells transfected with pFLAG-FXR were incubated with or without 100 μM CDCA for 4 h. Whole cell lysate was subjected to SDS-PAGE and Western blotting. Nucleus fractions prepared from cells cultured in five 100-mm dishes were subjected to the cross-linking reaction using dithio-bis(succinimidyl propionate) following immunoprecipitation (IP) with anti-FLAG antibodies. The immunoprecipitates were analyzed by Western blotting with anti-I-BABP or anti-FLAG antibodies.

FIGURE 5. Co-expression of both I-BABP and IBAT stimulates the FXR activity by conjugated bile acids. A, HEK-mock and HEK-IBABP-3 cells were transfected with an IBAT expression plasmid, pCMV-hIBAT, pGAL4-FXR, and pGL3Luc, together with phRL-TK, and then cultured with a medium containing 10% charcoal-stripped FBS at the various concentrations of conjugated (GCA and GCDCA) or unconjugated bile acids for 24 h. Luciferase assays were performed as described under “Experimental Procedures.” Luciferase activities without any bile acids are considered as 1. The values given are the average of data from more than three experiments performed in triplicate. B, whole cell lysate was subjected to SDS-PAGE and Western blotting with anti-I-BABP or anti-IBAT antibodies.
I-BABP Is Associated with FXR and IBAT

I-BABP is a 14-kDa cytoplasmic protein that belongs to the FABP family, the members of which share a remarkably similar structure. Like other FABPs, the biological functions of I-BABP have remained only incompletely understood. On the basis of the findings that I-BABP gene expression is exclusively regulated by the function of FXR and that these two proteins are capable of binding the same ligands, bile acids, we first hypothesized negative feedback regulation of FXR activity by I-BABP via a competition for bile acids. In contrast, we provide evidence here that I-BABP is able to interact with FXR in the nucleus and stimulate its transcriptional activity. Although I-BABP enhances FXR transcriptional activity quite modestly, this positive effect is nevertheless biologically relevant considering the abundance of I-BABP in the ileum. It has been reported that other members of the FABP family are also able to interact with various nuclear receptors and augment their transcriptional activity. L-FABP interacts with PPARα and PPARγ, but not with PPARδ, in a ligand-independent manner and plays the role of a positive regulator of activity for these nuclear receptors (18). It has also been demonstrated that adipocyte and keratinocyte FABPs selectively enhance the activities of PPARγ and PPARδ, respectively, and that these FABPs relocate to the nucleus in response to selective ligands (19). Furthermore, these FABPs interact directly with the PPAR subtype and enhance the transcriptional activities of these receptors (20). Taken together, the evidence indicates these family members containing I-BABP exert their effects by interacting directly with the respective receptors, and the resulting complexes are likely to mediate the delivery of ligands to the receptors. However, we did not observe any I-BABP translocation to the nucleus in response to bile acids. It has been established that CRABP-II, adipocyte FABP, and keratinocyte FABP share the same nuclear localization signal motif in their receptors (20).

We next examined the intracellular co-localization of I-BABP and IBAT on the membrane, using HEK293 cells expressing both proteins to elucidate the molecular mechanism of the increased uptake of conjugated bile acids mediated by the combination of IBAT and I-BABP. In HEK-IBABP-3 cells, I-BABP localized mainly in the cytoplasm but also in the nucleus, consistent with the result shown in Fig. 3. Transiently expressed IBAT was stained on the plasma membrane and the cytoplasmic organelles. The overlaid image shows the co-localization of these proteins in the vicinity of the plasma membrane (Fig. 7, yellow staining).

DISCUSSION

No change in the co-localization image was found with conjugated or unconjugated bile acid treatment (data not shown). This co-localization might explain the functional relationship of I-BABP and IBAT in accelerating the influx of conjugated bile acids.

FIGURE 6. Effects of I-BABP on the uptake of bile acids. HEK293 cells were set up on day 0 and transfected with pCMV-hIBAT and/or pME-I-BABP on day 1. After a 16-h incubation with radiolabeled bile acids (3 μM [3H]CDCA (A) and 3 μM [3H]GCA (B)) on day 3, the cells were harvested, and incorporated radioactivity levels were determined. The values given are the average of data from more than three experiments performed in triplicate. C, whole cell lysate was subjected to SDS-PAGE and Western blotting with anti-I-BABP or anti-IBAT antibodies.

FIGURE 7. I-BABP and IBAT co-localize in the vicinity of the plasma membrane. HEK-IBABP-3 cells grown on glass chamber slides were transfected with an expression plasmid, pFLAG-IBAT, and fixed with 4% paraformaldehyde 24 h after transfection. To detect localization of I-BABP and FLAG-IBAT, the cells were stained with anti-I-BABP and anti-FLAG antibodies followed by Cy3-conjugated donkey anti-mouse IgG and FITC-conjugated donkey anti-rabbit IgG, respectively. Fluorescence staining was visualized using an Olympus FV500 confocal microscope. Left panel, Cy3 anti-I-BABP immunostaining; center panel, FITC anti-FLAG immunostaining to detect FLAG-IBAT; right panel, overlay of Cy3 and FITC images. The yellow color represents co-localization of I-BABP and FLAG-IBAT.

Acids. We further examined whether I-BABP might stimulate the uptake of conjugated bile acids through IBAT. The uptake of [3H]-labeled conjugated bile acid (GCA) was notably augmented in the presence of both I-BABP and IBAT, whereas [3H]-labeled CDCA did not change in the presence or absence of I-BABP/IBAT (Fig. 6, A and B). Similar results were obtained when cells were incubated for a shorter time (4 h, data not shown). Fig. 6C shows that IBAT expression was constant in the absence or presence of I-BABP. These results suggest that the transport of conjugated bile acids mediated by IBAT through the apical membrane is facilitated by intracellular I-BABP.

We next examined the intracellular co-localization of I-BABP and IBAT on the membrane, using HEK293 cells expressing both proteins to elucidate the molecular mechanism of the increased uptake of conjugated bile acids mediated by the combination of IBAT and I-BABP. In HEK-IBABP-3 cells, I-BABP localized mainly in the cytoplasm but also in the nucleus, consistent with the result shown in Fig. 3. Transiently expressed IBAT was stained on the plasma membrane and the cytoplasmic organelles. The overlaid image shows the co-localization of these proteins in the vicinity of the plasma membrane (Fig. 7, yellow staining).
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molecules, but I-BABP does not contain this motif (20). These findings suggest that different types of nuclear localization systems exist, some of which are ligand-inducible or ligand-independent, for the different FABP family members.

The exact mechanism by which I-BABP enhances the FXR transcriptional activity without any increase in the translocation of these proteins to the nucleus in response to bile acids remains uncertain. There are several conceivable but still speculative possibilities in terms of I-BABP functions. The interaction between FXR and I-BABP in the nucleus may contribute to local elevation of bile acid concentration around the ligand-binding domain (LBD) of FXR, thereby facilitating the delivery of ligands to the LBD. The LBD is occupied with co-repressor proteins in the absence of bile acids, whereas binding of ligands to FXR induces a conformational change within the LBD, resulting in the release of existing co-repressor proteins and permitting the association of co-activator proteins. Thus, it is possible that I-BABP interacting with FXR may accelerate the dissociation of co-repressor proteins and/or the association of a co-activator protein, PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1α), which mediates a series of events leading to transcriptional activation of the target genes (21). Alternatively, a slight chance that I-BABP makes PGC-1α extend its association with FXR is unable to be disregarded. The molecular mechanism underlying the stimulation of the FXR transcriptional activity by I-BABP is now under investigation.

I-BABP has been postulated to be involved in intracellular bile acid trafficking and to facilitate the uptake of bile acid in the small intestine (22, 23). Indeed, the current data show that I-BABP is functionally associated with IBAT in enhancing bile acid influx efficiency when both are expressed in HEK293 cells. A previous study using a photoaffinity labeling technique revealed that I-BABP might interact with IBAT (24). We hypothesized that the C-terminal cytoplasmic tail of IBAT, which consists of ~40 amino acids, might interact with I-BABP because the last transmembrane domain adjacent to the C-terminal tail can bind bile acids (25). To obtain direct evidence for such mutual interaction, we examined whether I-BABP forms a complex with a soluble glutathione S-transferase fusion protein, including the IBAT C-terminal tail, when expressed in HEK293 cells, but failed to detect such a complex. Immunostaining analysis revealed only a co-localization of these two proteins along the membrane. These results suggest that I-BABP might be coupled functionally with IBAT without a tight protein-protein interaction. In contrast with these findings, a previous work demonstrated that daily administration of IBAT in the form of plasmid and anti-IBAT antibodies. We also thank Dr. Kevin Boru of Pacific Edit for review of the manuscript.

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I-BABP Is Associated with FXR and IBAT

In conclusion, we have presented findings that reveal that I-BABP interacts with FXR, which also can bind bile acids, and enhances FXR transcriptional activity. Moreover, I-BABP co-localizes with IBAT along the plasma membrane and facilitates the IBAT-mediated influx of conjugated bile acids. Through these effects I-BABP promotes the biological functions of bile acids by attenuating their deterrent effects in the ileum, where all three of these proteins are exclusively expressed.

Acknowledgments—We thank Dr. Paul A. Dawson for the IBAT expression plasmid and anti-IBAT antibodies. We also thank Dr. Kevin Boru of Pacific Edit for review of the manuscript.
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