Mitochondrial Aspartate Aminotransferase Expressed on the Surface of 3T3-L1 Adipocytes Mediates Saturable Fatty Acid Uptake (43854)

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Abstract. **Physicochemical studies have suggested that the 43-kDa plasma membrane fatty acid binding protein ($FABP_{pm}$) is closely related to the mitochondrial isoform of aspartate aminotransferase (mAspAT). In the present studies, mAspAT was not detected immunohistochemically or by immunoblotting in plasma membranes of proliferating 3T3-L1 fibroblasts. During controlled differentiation to an adipocyte phenotype, mAspAT became detectable by the second day of confluent growth, prior to accumulation of visible lipid droplets, and was strongly expressed in 8-day differentiated 3T3-L1 adipocytes. The pattern of expression paralleled the previously reported expression both of $FABP_{pm}$ and of the V_{max} for saturable uptake of long chain free fatty acids. As with anti-$FABP_{pm}$, antibodies to mAspAT selectively inhibited the uptake of [^{3}H]-oleate in 3T3-L1 adipocytes but not in fibroblasts, while having no effect on uptake of either 2-deoxyglucose or the medium chain fatty acid octanoate. Preabsorption of anti-$FABP_{pm}$ with mAspAT, or of anti-mAspAT with $FABP_{pm}$, abolished immunopositivity in immunohistochemical and immunoblotting studies, as well as the ability of either antibody to inhibit [^{3}H]-oleate uptake. These studies provide strong biologic evidence for the identity of $FABP_{pm}$ and mAspAT, and for the hypothesis that $FABP_{pm}$/mAspAT mediates the uptake of long chain free fatty acids.**
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The cellular uptake of long chain free fatty acids (FFA) has long been considered a passive process (1, 2). However, recent studies clearly demonstrate that a major component of FFA uptake in hepatocytes (3–6), adipocytes (7–9), cardiac myocytes (10, 11), and jejunal enterocytes (12), but not fibroblasts (13), exhibits the kinetic features of facilitated transport. Five distinct plasma membrane proteins of 22 (14), 40–43 (15, 16), 56 (17), 74 (18), and 88 kDa (19, 20) have been proposed as FFA transporters. The first to be identified, a 40- to 43-kDa plasma membrane fatty acid binding protein designated $FABP_{pm}$, was isolated initially from rat hepatocytes (15) and subsequently from several of the other cell types enumerated (9, 10, 12, 16). Extensive evidence in favor of the hypothesis that $FABP_{pm}$ is an FFA transporter includes the finding that, during differentiation of 3T3-L1 fibroblasts to an adipocyte phenotype, a progressive increase in the V_{max} for saturable FFA uptake is accompanied by a parallel increase in the expression of $FABP_{pm}$ in plasma membranes (13).

Surprisingly, $FABP_{pm}$ has been found to be related to the mitochondrial isoform of the well-studied enzyme aspartate aminotransferase (mAspAT) (21, 22). In the present study we demonstrate that adipocyte differentiation in 3T3-L1 cells is accompanied by *de novo* expression of mAspAT antigen in the plasma membrane and that antibodies to mAspAT selectively inhibit FFA uptake in the differentiated adipocytes. These data provide further evidence that $FABP_{pm}$ and mAspAT are very closely related if not identical.

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Materials and Methods

Materials. [^{3}H]-2-deoxyglucose (8.0 Ci/mmol), [^{3}H]-9-10-oleic acid (7.4 Ci/mmol) and [^{14}C]-octanoate (4.4 mCi/mmol) were obtained from New England Nuclear (Boston, MA); [^{125}I]-protein A (30 mCi/mg) from Amersham (Arlington Heights, IL); nonradioactive oleate, cytochrome-C, phloretin, dexamethasone, insulin, and methylisobutylxanthine from Sigma Chemical Co. (St. Louis, MO); FFA-free bovine serum albumin (BSA), phosphodiesterase, and Na-p-nitrophenyl 5′-thymidylate from Boehringer Mannheim (Indianapolis, IN); and all culture media and fetal bovine and calf serum from Gibco (Gaithersburg, MD). The ABC immunochemistry reagent kit was purchased from Vectasta (Boston, MA) and electrophoresis reagents from Bio-Rad (Richmond, CA).

Culture of 3T3-L1 Cells. 3T3-L1 cells were maintained in culture in exponential growth phase (23). As needed, aliquots of cells were allowed to grow to confluence, and after 2 days at confluence (Day 0), they were induced to differentiate by the method of Reed and Lane (24). By Day 7–9 after initiating differentiation, the monolayers consisted principally of adipocytes, which contained multiple, small, oil red O staining lipid droplets (25). Except as noted, fibroblasts 2 days post confluence and adipocytes 7–9 days after initiating differentiation were used in the studies described below. When required, single cell suspensions were prepared from the monolayer cultures (13). Single cell preparations were used only if >90% of cells excluded trypan blue.

Preparation and Characterization of Antibodies to FABPpm and mAspAT. Sinusoidally enriched plasma membranes were prepared from fresh, perfused rat liver as described (26). Membrane proteins were extracted, and $FABP_{pm}$ was purified by high performance liquid chromatography as reported elsewhere (21). mAspAT was purified to homogeneity from isolated rat liver mitochondria (27). Each of the purified antigens, which migrated as a single silver-stained 43-kDa band on SDS-PAGE, was used to prepare polyclonal monospecific antisera by immunizing outbred New Zealand white rabbits four to six times as described (15, 21). The titer and monospecificity of the resulting antisera were checked by double radial immunodiffusion on agar plates, immunoelectrophoresis, and immunoblot techniques (13). By agar gel diffusion each antiserum reacted at a titer of at least 1:32 with the purified antigen against which it was raised. In Western immunoblots employing homogenates of whole rat liver, each antiserum identified only a single band, at 43 kDa. Absorbed antibody reagents were prepared by preincubation of anti-$FABP_{pm}$ with an excess of mAspAT, or conversely, preincubation of anti-$FABP_{pm}$ with an excess of mAspAT (28).

Immunohistochemical Studies. Antisera were used in immunohistochemical studies at dilutions of 1:500–1:1000, as previously reported (13). Controls employed preimmune rabbit serum at the same dilutions. Detection was accomplished with the ABC reagent kit, using a goat anti-rabbit IgG, according to the manufacturer's instructions. For immunoblotting, detection was with [^{125}I]-protein A.

Isolation of Plasma Membranes from 3T3-L1 Cells. Plasma membranes from 3T3-L1 fibroblasts grown at confluence for 2 days (Day 0) and from 7–9 day adipocytes were prepared according to Thom (29) and Storch (30), and stored at −80°C until use. Protein assays were performed by the biuret reaction using the absorption at 562 nm of the bicinchoninic acid complex to quantitate the Cu^+ ion formed (31). As reported (13), the plasma membrane preparations were each enriched 5-fold in the plasma membrane marker phosphodiesterase (32); the activity ratios of the mitochondrial marker enzyme succinate dehydrogenase (33) in these same preparations were between 0.12 and 0.14 compared with cell homogenates.

Studies of FFA Uptake. [^{3}H]-oleate uptake studies were performed as described (13) in 2-day confluent fibroblasts and in adipocytes 7–9 days after initiation of differentiation. All experiments were performed in triplicate.

Antibody Inhibition of FFA Uptake. To study the effects of anti-$FABP_{pm}$ and anti-mAspAT on [^{3}H]-oleate uptake in 3T3-L1 cells, the IgG fractions of several monospecific anti-$FABP_{pm}$ and anti-mAspAT antisera were purified by ion exchange chromatography employing DE52 cellulose beads (34). Antibody inhibition studies were conducted as described (4, 9, 10, 13), in media containing 250 μM [^{3}H]oleate:500 μM BSA (unbound oleate concentration = 43 nM). To establish the specificity of the antibodies, the effects of anti-$FABP_{pm}$ and anti-mAspAT on the initial uptake velocities of [^{3}H]-2-deoxyglucose and [^{14}C]-octanoate were determined under conditions similar to those for [^{3}H]-oleate, as described (13).

Isolation of 3T3-L1 RNA. Total cellular RNA was isolated from fresh cell pellets obtained by trypsin treatment of culture dishes followed by two PBS washes. A maximum of 500 µl of cell pellet was disrupted with a polytron in 2.5 ml of extraction buffer containing guanidinium isothiocyanate. RNA extraction and isolation were accomplished through a CsCl gradient as described elsewhere (35). Samples were resuspended in RNAse-free distilled H_2O and stored at −70°C. RNA concentrations and integrity were estimated by spectrophotometry and ethidium bromide/agarose gel analysis. In particular, Northern blots employing several probes spanning different regions of the mAspAT RNA gave no evidence of degradation of the RNA samples.

Production of cRNA Probes. A full-length cDNA encoding rat liver mAspAT, including its mitochondrial leader sequence, was kindly provided by Dr. J. R. Mattingly (36). A 5′ *EcoRI-PstI* fragment of this cDNA (nucleotide positions 1 to 419) was cloned into the corresponding sites of the polylinker of the riboprobe capable vector pT3T7 (Boehringer Mannheim). The resulting plasmid was linearized with *NcoI* (position 89 in the cDNA). The linearized molecule was used to run a 356-bp riboprobe (including 26 bp from the plasmid vector) off the T3 promoter. Labeled antisense transcripts were produced with a riboprobe kit obtained from Promega and used according to manufacturer's instructions. The concentration of T3 RNA polymerase in the riboprobe reactions was adjusted down to a 10th of that recommended by the manufacturer to increase the yield of full-length transcripts. Probes were analyzed with 6% denaturing urea-polyacrylamide gel electrophoresis (PAGE). Only probes for which >95% of labeled material ran as full length was used.

RNAse Protection. Fifty micrograms of total cellular RNA were hybridized to cRNA probes in RNAse protection analyses as described previously (37, 38). A total of 2.5×10^5 cpm of [^{32}P]-labeled RNA probe was added to each sample in a final volume of 30 μl. The mixture was then hybridized overnight at 45°C. Following hybridization, heteroduplexes were digested with RNAse A (60 μg/ml) and RNAse T1 (3 μg/ml; Sigma) in a 37°C bath for 1 hr. Following proteinase K digestion, extraction, and precipitation, samples were analyzed under denaturing conditions in a 6% acrylamide gel. Gels were fixed in 7% TCA (Sigma) for 5 min and dried. Autoradiography was performed at −70°C for 12–72 hr.

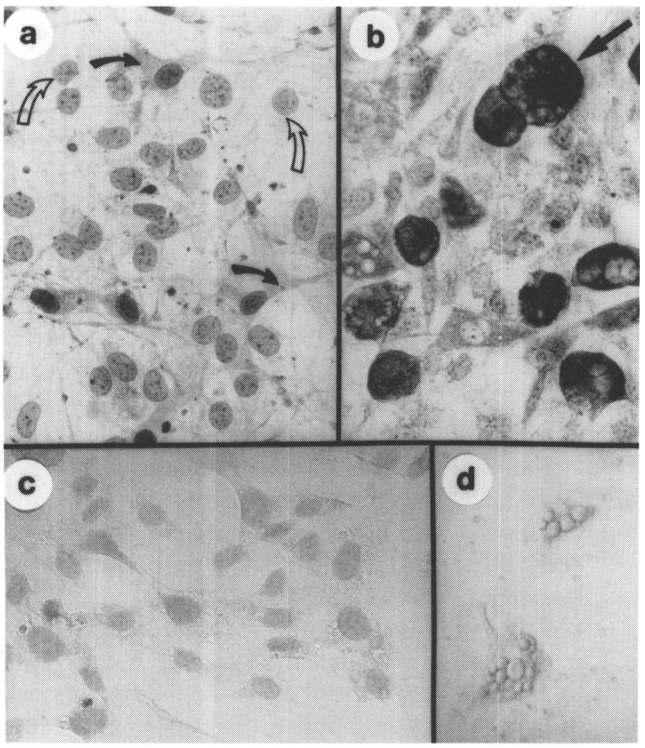

Figure 1. Immunohistochemical staining of 3T3-L1 cells with rabbit anti-rat mAspAT. Cells in Panel A and B were lightly counterstained with hematoxylin to highlight nuclei. (A) Fibroblasts after 2 days confluent growth. Some cells (solid arrows) are beginning to show positive immunohistochemical staining, but the majority (open arrows), like rapidly proliferating fibroblasts in exponential growth phase (not shown), are negative. (B) Day 8 adipocytes stain strongly for mAspAT. Plasma membrane distribution is evident at solid arrow. (C) Immunohistochemical staining of 7-day 3T3-L1 adipocytes using anti-mAspAT preabsorbed with $FABP_{pm}$. Note small lipid droplets within cells. The preabsorption process completely abolishes the immunohistochemical staining observed in Panel B. (D) Immunohistochemical staining of 8-day adipocytes using normal rabbit serum as primary antibody. No reaction is observed.

Results

Immunohistochemical Studies. Using the rabbit anti-rat mAspAT antiserum as the primary antibody, rapidly proliferating 3T3-L1 fibroblasts displayed no detectable mAspAT antigen either on the cell surface or intracellularly. Since these cells contain mitochondrial mAspAT, the lack of intracellular staining confirms that the cells were impermeable to the antibody under the conditions employed. By contrast, fibroblasts immunostained with the same antibody preparations after permeabilization of the plasma membrane exhibit extensive particulate intracellular mitochondrial staining (data not shown). Among 3T3-L1 cells grown to confluence for 1–2 days, which retained the spindle shape and contained no visible lipid droplets, a proportion expressed detectable surface mAspAT, although the majority remained negative (Fig. 1a). Once again, intracellular staining with the characteristic mitochondrial pattern was not observed, indicating that the positive reaction was on the cell surface. By contrast, all cells grown at confluence for 8 days or more with an obvious adipocyte morphology strongly expressed surface mAspAT antigen (Fig. 1b). The evolution of expression of mAspAT at different stages of 3T3-L1 cell differentiation from a fibroblast to an adipocyte morphology was identical to the evolution of expression of $FABP_{pm}$ antigen previously reported (13). 3T3-L1 adipocytes exhibited no immunohistochemical reaction if the anti-mAspAT was preabsorbed with purified $FABP_{pm}$ (Fig. 1c), with anti-$FABP_{pm}$ preabsorbed with mAspAT (not shown), or when the primary antibody was replaced with preimmune rabbit serum (Fig. 1d).

Identification of mAspAT in 3T3-L1 Cell Plasma Membranes. As detected by SDS-PAGE and immunoblotting, rabbit anti-rat mAspAT reacted with only a single 43-kDa band in homogenates of whole rat liver and in extracts of rat liver plasma membranes. An identical band was observed with a corresponding extract of mouse liver, indicating immunological cross-

reactivity between the mAspAT's of the two species (not shown). The antibody also reacted strongly with a 43-kDa band in extracts of 3T3-L1 adipocyte plasma membranes. By contrast, only a faint band was seen in immunoblots of plasma membrane extracts from 2-day confluent fibroblasts (Fig. 2). Membranes from exponentially growing fibroblasts were nonreactive. The evolution of immunoreactivity in plasma membrane extracts observed with anti-mAspAT was similar to that observed with anti-$FABP_{pm}$ (13). Neither absorbed antibody reagent exhibited any immunoreactivity against 3T3-L1 adipocyte plasma membrane extracts or against either purified antigen (Fig. 3), demonstrating that the epitopes detected by the two original antibody preparations are identical, irrespective of the immunogen employed to produce the antibody response.

Antibody Inhibition of Fatty Acid Uptake. As previously reported (13), at the [^{3}H]-oleate:BSA concentrations employed, oleate uptake velocity by 3T3-L1 cells increased progressively from the nonconfluent to the confluent fibroblast to the 8-day adipocyte stages (Fig. 4). As with anti-$FABP_{pm}$ (13), antibodies to rat mAspAT had little inhibitory effect on oleate uptake by 3T3-L1 fibroblasts. However, different anti-mAspAT preparations inhibited uptake of [^{3}H]-oleate by 3T3-L1 adipocytes by from 31% (Fig. 4) to 55%, a range of inhibition similar to that reported with anti-$FABP_{pm}$ in studies with a variety of cell types (4, 5, 9–13). In particular, the maximum inhibition observed in the present studies was similar to the maximum of 57% inhibition reported in 3T3-L1 adipocytes using anti-$FABP_{pm}$ (13). Lack of more complete inhibition in all of these studies presumably reflects the established presence of a separate, passive pathway for FFA uptake (6), as well as the possible existence of additional facilitated pathways mediated by other putative FFA transporters (14, 17–20). As with anti-$FABP_{pm}$ (13), the observed inhibition was selective for long chain FFA. The antibody had no effect on uptake of [^{3}H]-2-deoxyglucose or of [^{14}C]-octanoate, a medium chain fatty acid which enters cells by mechanisms different from those utilized by FFA of chain length $\geq C_{12}$ (8, 13) (Fig. 4). Neither of the absorbed antibody preparations exhibited any inhibitory effect on FFA uptake.

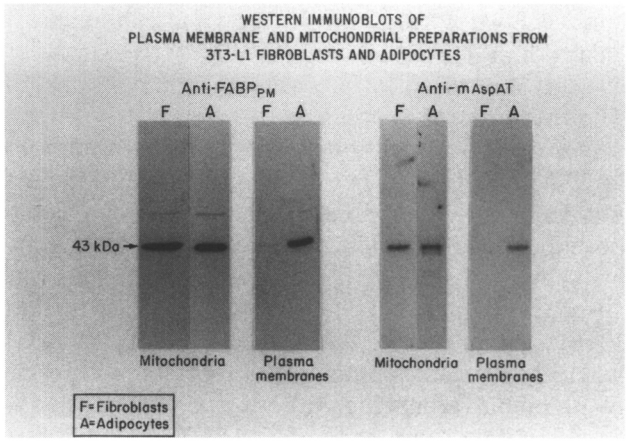

Figure 2. Immunoblots of mitochondrial and plasma membrane extracts from 3T3-L1 fibroblasts (F) and adipocytes (A), employing either anti-$FABP_{pm}$ (left) or anti-mAspAT (right). Both antisera detect a 43-kDa band in mitochondria isolated from either fibroblasts or adipocytes. Both antisera detect an identical, abundantly expressed 43-kDa band in adipocyte plasma membranes, which is either absent or only very weakly expressed in fibroblast plasma membranes.

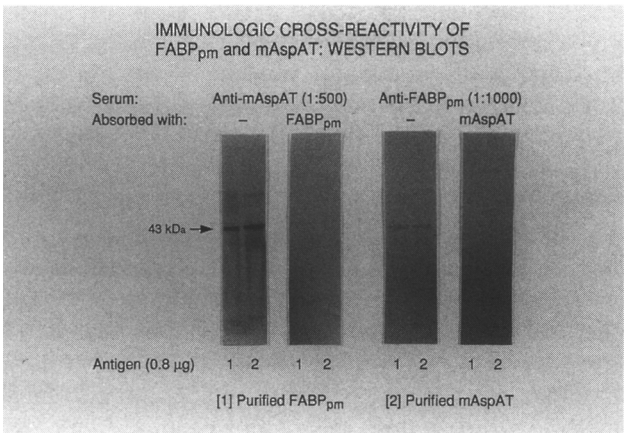

Figure 3. Immunoblots demonstrating that anti-mAspAT (left) and anti-$FABP_{pm}$ (right) are each immunoreactive against both purified $FABP_{pm}$ and mAspAT. For each antiserum, all immunoreactivity is abolished by preabsorption with the other antigen.

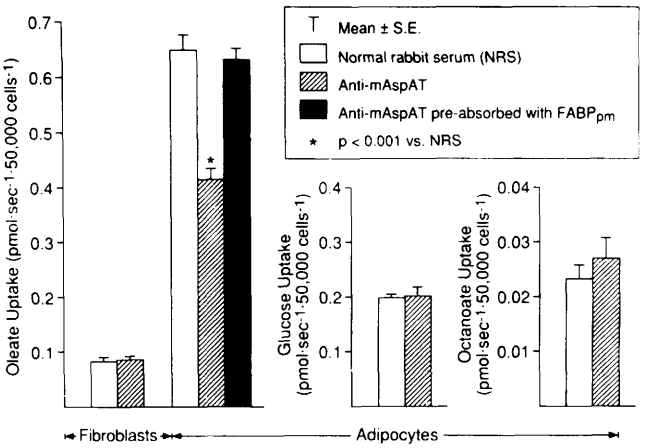

Figure 4. Effects of anti-mAspAT on uptake in 3T3-L1 cells. (Left) Effect of the antibody on [^{3}H]-oleate uptake compared to normal rabbit serum as control. The antibody is without effect in fibroblasts, in which uptake is predominantly nonsaturable, but significantly inhibits uptake in adipocytes, in which uptake is predominantly saturable. The inhibitory effect in adipocytes is completely abolished by pre-absorption of the antibody with $FABP_{pm}$. (Center and right) The antibody has no effect on the uptake of [^{3}H]-2-deoxyglucose and [^{14}C]-octanoate, respectively. Illustrated studies employed a 2.5 mg/ml concentration of IgG prepared from an antiserum positive by agar gel diffusion at a titer of 1:32, and are representative of results obtained with all available anti-mAspAT preparations. Because of the high degree of interspecies homology, high titer anti-mAspAT antisera are not readily available.

RNAse Protection Assays. A full-length mes-

sage corresponding to the size of the antisense probe minus the fragment of plasmid polylinker (26 bp) was visible in RNA specimens corresponding to both the fibroblast and adipocyte stages of 3T3-L1 cells (Fig. 5). A second band of lesser intensity and smaller size was observed only in the adipocyte stage. The appearance of this band is consistent with the postulated existence in adipocytes of a second, alternatively spliced message with high degree of homology to a portion of the antisense mAspAT riboprobe.

Discussion

$FABP_{pm}$ was first isolated and purified to homogeneity from rat hepatocyte plasma membranes in 1985 (15). By contrast, mAspAT is a long-known and well-studied enzyme readily purified from mitochondrial preparations (27), which has an important role in aminoacid metabolism but no known function in FFA metabolism or transport. For this reason, the finding that $FABP_{pm}$ and mAspAT were structurally related

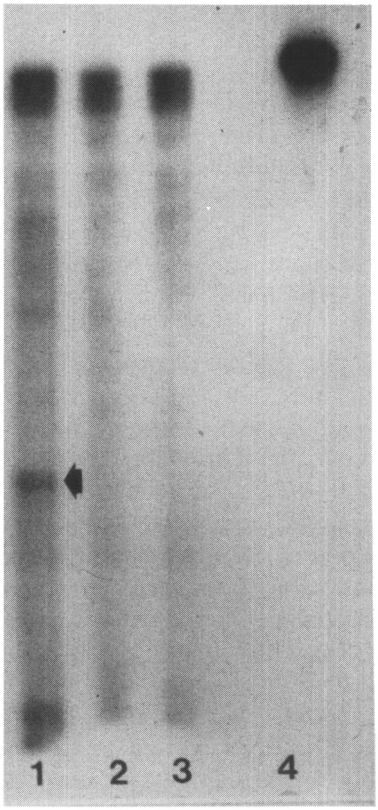

Figure 5. RNAse protection assay. Fifty micrograms each of total cellular RNA from 3T3-L1 Day 8 adipocytes (Lane 1) and 2-day confluent fibroblasts (Lane 2), or of a control yeast RNA (Lane 3) were hybridized to a probe derived from the 5′ end of an mAspAT cDNA. Samples were digested and processed as described in Materials Methods prior to electrophoresis and autoradiography. Lane 4 contains the unhybridized probe. Faint bands present in Lane 1 and 2 result from single base mismatches between the rat-derived probe and the mouse-derived samples. Arrow indicates a prominent band, lacking in the fibroblast sample, which consistently appears during differentiation to an adipocyte phenotype.

(21) was unexpected. Nevertheless, it has been established that the two proteins share an identical N-terminal aminoacid sequence of at least 35 residues, a similar aminoacid composition, identical mobility in SDS-PAGE and elution from four different HPLC columns, an unusually basic isoelectric point (pI = 9.1–9.3) with a similar pattern of multiple charge isomers, immunologic crossreactivity, similar mAspAT enzymatic activity and FFA binding properties, and an identical pattern of peptides after tryptic digestion (21, 22). The present study demonstrates that anti-mAspAT antibodies have the same ability to selectively block FFA uptake as do antibodies to $FABP_{pm}$. The data also establish that all of the $FABP_{pm}$ antigenic epitopes expressed on the cell surface during adipocyte differentiation in 3T3-L1 cells (13) are also detected by antibodies to mAspAT, and conversely. This is true because absorbtion of either antiserum with the other antigen (i) eliminates its reactivity against 3T3-L1 adipocytes in immunohistochemical studies; (ii) removes the ability of the antiserum to detect either $FABP_{pm}$ or mAspAT in Western blots; and (iii) eliminates the ability of the antiserum or its IgG fraction to inhibit FFA uptake. The accumulated data argue very strongly that $FABP_{pm}$ and mAspAT are identical.

Is $FABP_{pm}$/mAspAT a reasonable candidate to be a plasma membrane FFA transporter? Immunohistochemical, immunofluorescence, and immunoperoxidase labeling studies document that finding the protein on the cell surface reflects the biology of the situation and not artifact (21). Although mAspAT is generally considered a hydrophilic protein, its N-terminal domain specifically anchors it to membranes, and it exhibits a number of hydrophobic properties, reviewed elsewhere (22), which would not be anticipated from an examination of a conventional hydropathy plot. For example, it has been shown by freeze-fracture electron microscopy to intercalate into the hydrophobic core of membranes under certain circumstances. Moreover, four separate lines of evidence support the hypothesis that $FABP_{pm}$/mAspAT is an important mediator of cellular FFA uptake: (i) monospecific anti-$FABP_{pm}$ antibodies selectively inhibit FFA uptake in several cells and tissues that exhibit predominantly facilitated FFA uptake kinetics (4, 9, 10, 12, 13), including *Xenopus* laevis oocytes (39). These antibodies have no effect on uptake in fibroblasts (13), in which FFA uptake is predominantly passive. The inhibitory effect is highly specific; the antibodies do not influence the uptake of sulfobromophthalein (BSP), bilirubin, various bile acids, glucose, or the medium chain fatty acid, octanoate (3, 10, 13, 39); (ii) during differentiation of 3T3-L1 fibroblasts to an adipocyte morphology, a progressive increase in the V_{max} for saturable FFA uptake is accompanied by a parallel increase in the expression of

$FABP_{pm}$/mAspAT on the cell surface. Moreover, the saturable component of FFA uptake expressed during adipocyte differentiation is selectively inhibited by the same anti-$FABP_{pm}$ and anti-mAspAT antibodies (13); (iii) incorporation of $FABP_{pm}$ into synthetic liposomes reconstitutes FFA uptake (40); and (iv) in 3T3 fibroblasts, which do not express $FABP_{pm}$/mAspAT antigen on the cell surface, FFA uptake is predominantly nonsaturable. However, when such cells are transfected with a plasmid containing a full-length mAspAT cDNA downstream of a metallothionine promoter, they express abundant surface $FABP_{pm}$/mAspAT and exhibit a Zn^{++}-dependent increase of up to 6-fold in the V_{max} for FFA uptake (41). Thus, evidence supporting a role for $FABP_{pm}$/mAspAT in cellular FFA uptake is at least as great as has been marshalled in support of a transport function for any of the other putative FFA transporters (14, 17–20). Accordingly, although $FABP_{pm}$/mAspAT does not have the multiple membrane-spanning domains typical of many transporters, the possibility that it participates in the transmembrane transport of FFA requires serious consideration.

How $FABP_{pm}$/mAspAT is directed to two different sites in the cell to carry out two distinct functions poses an interesting question in cellular and molecular biology. The 80-fold increase in plasma membrane expression of $FABP_{pm}$ observed during adipocyte differentiation in 3T3-L1 cells greatly exceeds the contemporaneous increase in mAspAT within the mitochondria (13), suggesting that transfer of the protein to the plasma membrane is a carefully regulated process. How this regulation is effected has yet to be established. However, some speculations may be relevant.

$FABP_{pm}$ isolated from plasma membranes lacks the mitochondrial leader sequence with which mAspAT is initially synthesized as pre-mAspAT (21, 22). Indeed, it is most unlikely that a protein bearing this mitochondrial leader would be sorted to the plasma membrane. $FABP_{pm}$ could be synthesized as pre-mAspAT, and then directed to the plasma membrane by a post-translational modification involving cleavage of the mitochondrial leader. There is no known extramitochondrial mechanism to effect such a process. However, the mechanism by which mAspAT enters mitochondria has been studied with increasing intensity (e.g., 42–45), during the course of which the export of mature mAspAT from mitochondria after cleavage of the leader has been demonstrated. Heretofore, this has been considered a degradative pathway for the enzyme (43). However, preliminary studies have described increased plasma membrane expression of $FABP_{pm}$ following transfection of 3T3 cells with a full-length mAspAT cDNA (including the coding region for the leader sequence) (41, 46) but not after transfection with cDNAs encoding mutated forms with either a truncated or deleted leader sequence (46). These observations are consistent with mitochondrial uptake and subsequent cleavage of the leader sequence being part of the mechanism for sorting mature mAspAT to the plasma membrane. Pulse-chase studies to be conducted shortly may help to establish whether such a mechanism is operative.

An alternative mechanism is suggested by comparing the genomic structure of the mAspAT gene (47) with that of its cDNA (48). The mitochondrial leader sequence is encoded by Exon 1 and the N-terminal sequence of the mature protein by Exon 2 of the mAspAT gene, with more than 10 kb of relatively unexplored DNA constituting Intron I in between. The mAspAT mRNA is assembled by splicing out Intron I; the Exon 1/Exon 2 junction occurs precisely within the codon for the first aminoacid (serine) of the mature protein. An obvious possibility is that a distinct $FABP_{pm}$ mRNA arises by splicing an alternative first exon to Exon 2. Efforts to clone such an $FABP_{pm}$-specific mRNA from a rat liver cDNA library by conventional methods have been unsuccessful, as all screening reagents also react with the abundant mAspAT cDNA. Similarly, the possibility that an $FABP_{pm}$-specific mRNA could be cloned functionally in the *Xenopus* laevis oocyte system was rendered unlikely by the observation that an $FABP_{pm}$-mediated FFA transport system was strongly expressed in uninjected, control oocytes (39). Hence, this explanation remains hypothetical. It is, however, consistent with the results of the nuclease protection study reported above. This experiment suggests that 3T3-L1 fibroblasts contain only a single mRNA species highly homologous to the portion of mAspAT mRNA derived from Exon 1 and 2. In 3T3-L1 adipocytes, however, coincident with expression of $FABP_{pm}$/mAspAT on the cell surface, an additional mRNA species appears which is homologous to only a part of this region of the mAspAT mRNA. Precisely which part of the Exon 1/Exon 2 region of mAspAT mRNA gives rise to this band remains to be established.

Thus, preliminary observations have been made which are consistent with either of the obvious potential mechanisms for directing mature mAspAT to the plasma membrane. Although important molecular and cell biologic questions remain to be answered, the present study provides important support for the dual hypotheses (i) that $FABP_{pm}$ and mAspAT are identical, and (ii) that $FABP_{pm}$/mAspAT functions to facilitate saturable FFA uptake in specific cell types. Four other putative FFA transport proteins have been identified recently in various tissues (14, 17–20), for two of which cDNAs have been cloned (18, 20). None appears structurally related to $FABP_{pm}$/mAspAT. Further information clarifying the roles and possible interactions of all five putative FFA transporters is awaited with interest. Given the importance of FFA to multiple

aspects of cellular structure, function, metabolism, and gene regulation, it would not be surprising if there ultimately proved to be multiple facilitated mechanisms for their transport across plasma membranes.

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