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FATTY ACID TRANSPORTERS IN ANIMAL CELLS

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1. ABSTRACT

The mechanism by which fatty acids transverse the plasma membrane has been a controversial subject. Kinetic studies of fatty acid uptake suggested the presence of a protein carrier system in certain cells which exhibit rapid fatty acid influx and/or efflux such as hepatocytes, adipocytes and jejunal mucosal cells. Five plasma membrane proteins have been identified and proposed as candidates for fatty acid transporters thus far. These includes: Plasma Membrane Fatty Acid Binding Protein (FABPpm), Fatty Acid Translocase (FAT), caveolin, a 56-kDa renal fatty acid binding protein and Fatty Acid Transport Protein (FATP). The first four proteins were identified by classical biochemical techniques while FATP, the one most recently reported, was identified by expression cloning strategies. Each of these proteins has distinct primary amino acid sequence and tissue-specific pattern of expression. It remains to be determined whether the proteins identified to date function as individual polypeptides or as a single component of a larger complex. This review summarizes recent advances concerning the structure, function and regulation of these putative fatty acid transporters.

2. INTRODUCTION

Fatty acids serve as structural components for all organisms. Functioning as the biosynthetic precursors for glycero- and sphingolipids, membrane biogenesis requires *de novo* fatty acid production and/or active fatty acid uptake. In most membranes, the length and degree of unsaturation of the acyl chains affects lipid fluidity which can, in turn, have a profound modulatory role in regulating cellular functions such as endocytosis, exocytosis, ion channeling and transport (1). In addition, polyunsaturated fatty acids (PUFA) derived from essential fatty acids serve a regulatory role as precursors of biologically active signaling molecules such as leukotrienes, thromboxanes and prostaglandins (2). Metabolically, fatty acids are important energy substrates because of their high caloric content. In a typical diet of Western developed countries, approximately 30-40% of the dietary calories are derived from lipids, mainly in the form of di- and triglycerides. The linkage between excessive dietary lipid consumption and several common pathophysiologic disorders including heart disease, obesity and diabetes have been widely documented (3, 4).

3. FATTY ACID UPTAKE BY ANIMAL CELLS

Given the observations that fatty acids are central to a wide variety of cellular processes, it is somewhat surprising that the mechanisms by which dietary fatty acids enter the cell is still largely uncharacterized and has become a rather controversial subject (5). Due to their hydrophobicity, protonated fatty acids can partition into the lipid bilayer and diffuse across the membrane. The net movement of fatty acids down the concentration gradient would be driven by association with cytosolic lipid binding proteins and subsequent metabolism (6,7). Such biophysical experiments have fueled considerations of lipid transport across membranes as a chemical process, devoid of most

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Table 1. Putative fatty acid transporters

Year	Research Group	Name	Size[1] (kDa)	Source	Method	Ref
1985	Berk	FABPpm	43	Rat Hepatocytes	Oleate-Agarose Affinity Chromatography	14
1987	Fujii	-	56/60	Rat Renal Basolateral Membrane	Oleate-Sepharose Affinity Chromatography	16
1991	Trigatti	Caveolin	22	3T3-L1 Adipocytes	Photoaffinity Labeling	17
1993	Abumrad	FAT	88/53	Rat Adipose Tissue cDNA Library	Covalent Labeling	18
1994	Lodish	FATP	63/70	3T3-L1 Adipocyte cDNA Library	Expression Cloning	19

[1] When two numbers are present, the first represents the apparent mass determined via SDS-PAGE while the second corresponds to predicted mass from cDNA clones.

biological considerations. In contrast, kinetic studies of fatty acid uptake in cells such as hepatocytes, adipocytes and jeujunal mucosal cells which exhibit rapid fatty acid influx and/or efflux revealed the presence of a saturable, temperature-sensitive, and phloretin-inhibitable uptake system. These findings suggest the presence of a protein-mediated transport system for fatty acids (8,9,10). These two views need not be mutually exclusive for most cellular fatty acid transport studies suggest a mixed kinetic effect; a low capacity, high affinity proteinaceous component that functions at low substrate concentrations coupled with a high capacity, low affinity diffusional process that functions at elevated fatty acid levels. The purpose of this mini-review is not to argue the relative merits of the diffusion vs. transport schools, this has been done very effectively in the recent literature (11, 7, 10), but to summarize what is known about the proteins that have been identified as candidates as fatty acid transporters.

4. PUTATIVE FATTY ACID TRANSPORTERS

Five plasma membrane proteins have been reported and proposed to function in fatty acid movement across the plasma membrane (Table 1). These proteins have been referred to as either translocases, permease and transporters, depending upon the group that has identified them and the rigor to which functional definitions of transport have been employed. It should be stressed that hypotheses which involve more than one transport protein functioning in a particular cell type (Table 2), analogous to multiple hexose transporters (12,13) are still quite valid and bear investigation. Of the candidate transporters, four have been identified as lipid-binding proteins by virtue of fatty acid affinity chromatography or by association with reactive fatty acids analogues (14, 15, 16, 17, 18). The fifth protein, named fatty acid transport protein (FATP), was identified by expression cloning strategies but has not been shown to associate with lipids (19).

In order to establish the function of a protein as a fatty acid transporter, several criteria must be met. These include: 1) the protein should be localized to the plasma membrane; 2) the protein should have high affinity for fatty acids and that the binding should be selective and reversible; 3) treatment of cells with antibodies directed to the protein should inhibit fatty acid uptake; and 4) saturable fatty acid uptake should be linked to the expression of this protein. The available information on these five proteins are reviewed in the following sections.

4.1 Plasma Membrane Fatty Acid Binding Protein (FABPpm)

A characteristic of a fatty acid transporter is its ability to bind free fatty acids. Following this rationale, Berk *et al.* (14) employed oleate-agarose affinity chromatography to identify proteins from the plasma membrane of hepatocytes with high fatty acid affinity. Using the criteria of specific oleate binding, they identified a 40-kDa protein from rat hepatocyte (14) and jejunal microvillous membranes (15) with such characteristics. Binding studies revealed that the protein binds a variety of unesterified long-chain fatty acids such as oleate, exhibiting an apparent dissociation constant (K_d) of 78 nM. By immunohistochemical analysis, it was subsequently shown that the protein is expressed on the plasma membrane. To distinguish the plasma membrane fatty acid binding protein from cytoplasmic fatty acid binding proteins, this 40-kDa protein was named plasma membrane fatty acid binding protein (FABPpm) for its high affinity for long-chain fatty

Table 2. Tissue distribution of the putative fatty acid transporters.

Protein	Homology[1]	Tissue Distribution[2]						
		L	M	H	A	I	K	T
FABPpm	mAspAT	+	+	+	+	+	?	?
56kD	?	?	?	+	?	?	+	?
Caveolin	-	?	?	?	+	?	?	?
FAT	CD36	-	+	+	+	+	-	+
FATP	VLC-FACS	+	+	+	+	-	+	+

[1] mAspAT: mitochondrial aspartate aminotransferase;
VLC-FACS:very long-chain fatty acyl-CoA synthetase
[2] L:liver; M:skeletal muscle; H:heart; A:adipose; I:intestine;
K:kidney; T:testis; +:present; -:absent; ?:unknown.

acids and its cellular location. FABPpm was subsequently isolated from myocardium and adipose tissue (20,21,22) and its functional role in fatty acid transport was further characterized by antibody inhibition studies (21, 22, 23, 24). Incubation of cultured hepatocytes with anti-FABPpm antibodies markedly reduced the rate and extent of saturable fatty acid uptake, however large amounts of antibody were necessary to achieve such inhibition. Evidence for the involvement of FABPpm in fatty acid transport was further supported by the reconstitution of transport activity with liposomes containing FABPpm (25).

Biochemical analysis of FABPpm revealed the striking finding that FABPpm is identical in primary sequence and immunologically indistinguishable from the mitochondrial aspartate aminotransferase (mAspAT) (26,27). Two isoforms of aspartate aminotransferase (E.C. 2.6.1.1) are expressed in most animal tissues -- one located in the cytoplasm and the other in the mitochondrial matrix (28). The two isoenzymes catalyze transamination reactions linking the urea and Krebs cycle. In addition, mAspAT is central to gluconeogenesis and the malate-aspartate shuttle essential for transporting reducing equivalents from the cytosol to the mitochondria. However, until the surprising finding by Berk and colleagues of the protein being a plasma membrane fatty acid binding protein, neither isoenzyme had any previously documented role in fatty acid transport. FABPpm and mAspAT have essentially identical electrophoretic mobilities, isoelectric points, absorption spectra, amino acid compositions and chromatographic characteristics. Monoclonal antibodies specific for mAspAT react with both proteins, and conversely, monoclonal antibodies specific for FABPpm react with mAspAT. Both antibodies are capable of specifically inhibiting in a dose-dependent manner [^{3}H]-oleate uptake by hepatocytes. mAspAT has the same affinity for oleate as does FABPpm while purified FABPpm also possesses the aminotransferase activity of mAspAT. Transfection studies have shown that expression of mAspAT cDNA in 3T3 fibroblasts leads to cell surface expression of FABPpm and an increase in saturable fatty acid uptake (29), suggesting that mAspAT is identical to FABPpm and that mAspAT/FABPpm expression is linked to fatty acid uptake.

FABPpm is expressed on the plasma membrane; however, it is unclear how the protein is sorted to this site. It is unlikely that the same mitochondrial leader will serve as the plasma membrane targeting signal. To examine the question of enzyme targeting, the mAspAT gene and its cDNA have been cloned (30,31). Transfection of 3T3 fibroblasts with the full-length mAspAT cDNA resulted in an increase in FABPpm expression on the plasma membrane whereas transfection with cDNAs with a mutated leader sequence did not increase plasma membrane FABPpm expression (32,33), indicating that the mitochondrial leader sequence is important in FABPpm expression. It is speculated that mitochondrial uptake of mAspAT and subsequent cleavage of the leader sequence may lead to the sorting of mature mAspAT to the plasma membrane (34); however, there are no known examples of a similar extra-mitochondrial sorting mechanism. Examination of the genomic organization of the mAspAT gene leads to an alternative proposal that mRNA species with different leader sequences may be produced by alternative splicing of exon 1. Mature mAspAT mRNA is formed by joining the coding region (exon 2) with the 5' untranslated region and the mitochondrial leader sequence located in exon 1. The size of intron 1 is over 10 kb and has not been fully characterized (30). It is possible that an alternative exon 1 may be present in the long intronic sequence. Studies on 3T3-L1 RNA suggested that the 5'translated region of FABPpm RNA is different

from that of mAspAT. Expression of FABPpm on the plasma membrane is induced during 3T3-L1 preadipocyte differentiation (34). Ribonuclease protection assays with 3T3-L1 adipocyte RNA region of mAspAT RNA. These results suggest that alternative splicing of exon 1 may be the mechanism of directing FABPpm localization to different cellular compartments (34). However, convincing evidence supporting this hypothesis remains to be established.

FABPpm is expressed in a wide variety of tissues and its expression is up-regulated during preadipocyte differentiation. FABPpm expression is elevated in white adipose tissue of Zucker fatty (*fa/fa*) rats relative to wildtype animals. Consistent with insulin being a negative regulator or FABPpm expression, Zucker diabetic fatty (ZDF/Gmi^{TM}-*fa/fa*) animals express markedly elevated levels of FABPpm (35). The effects of insulin appears to be tissue-specific since the expression levels of FABPpm in adult +/+, *fa/fa* and ZDF livers are similar (35).

4.2 Fatty Acid Translocase (FAT)

FAT was identified by Abumrad and colleagues using protein crosslinking agents that block fatty acid uptake in adipocytes. In 1984, Abumrad *et al*. demonstrated that fatty acid uptake is irreversibly inhibited by pretreatment of cells with stibene compounds such as DIDS (4,4'-diisothiocyanostilbene-2,2'disulfonate) and STIS (4-acetamido -4' isothiocyanostilbene -2, 2' -disulfonic acid) (9). Incubation of cells with tritiated dihydro DIDS resulted in labeling of a membrane component with molecular mass between 80 and 90 kDa. Subsequently, a protein of a similar size in adipocyte plasma membranes was also labeled using tritiated sulfo-N-succinimidyl oleate (SSO), a fatty acid derivative which crosslinks covalently with nucleophiles in proteins (36). The 88-kDa radioactive band, which was later identified and named fatty acid translocase (FAT), was eluted from the gel and further purified. N-terminal amino acid analysis of FAT revealed that the sequence is essentially identical to that of human CD36, a ubiquitous membrane protein expressed in a variety of tissues (37). The cDNA of FAT was isolated from a rat adipose library using a synthetic oligo deduced from the N-terminal sequence (18). The nucleotide sequence of FAT is 79% similar and 75% identical to that of human CD36, whereas their amino acid sequences have 85% similarity and 79% identity, suggesting that FAT may be the rat adipose homologue of human CD36. Consistent with this view, antibodies directed against bovine CD36 react with both human CD36 and rat FAT (38). The FAT cDNA sequence contains an open reading frame for a 472-amino acid protein with a predicted molecular mass of 53 kDa. The discrepancy in molecular mass of FAT from 88 kDa as observed in SDS-PAGE using the lipophilic crosslinking reagents is probably due to extensive glycosylation. FAT contains 10 potential N-linked glycosylation sites, of which 8 are also conserved in human CD36. Hydropathy analysis of the deduced FAT amino acid sequence predicts two transmembrane domains, one at each end resulting in a hairpin-like configuration with 95% of the protein exposed to the extracellular environment. The C-terminal sequence $CXCX_5K$ of CD36, which has been postulated to interact with tyrosine kinases (36), is also found in FAT. Amino acid sequence comparison with muscle fatty acid-binding protein (M-FABP) also revealed a significant homology between amino acids 127-279 of FAT and M-FABP. The predicted secondary structure of this region of FAT consists of a single beta strand followed by two alpha helices in tandem and then by at least seven beta strands -- a structure similar to M-FABP. In addition, two out of the three conserved amino acid residues (R126 and Y128) in M-FABP, which are required for efficient binding to fatty acids, are also conserved in FAT as R272 and Y275 (39).

Northern blot analysis of FAT mRNA expression revealed an extra protected band which is not present in the preadipocyte RNA control. The sequence of this protected fragment is only partly identical to the 5' untranslated

FAT mRNA expression is tissue-specific and under metabolic control (18). Its levels of expression is high in cardiac muscle, skeletal muscle, intestine, spleen and adipose tissue, low in the testis and is undetectable in liver and kidney. FAT mRNA is expressed in a differentiation-dependent manner; strong induction was observed during 3T3-F442A and Ob1771 preadipocyte differentiations. Treatment of cultured adipocytes with dexamethasone, a synthetic glucocorticoid, resulted in a significant increase in FAT mRNA accumulation in parallel with an elevated fatty acid uptake. In addition, it has been shown that expression of FAT mRNA in fibroblasts is induced by fatty acids and other lipophilic molecules such as peroxisome proliferators (40) suggesting that the FAT gene may be controlled by members of the peroxisome proliferator activated receptor class of transcription factors.

The binding characteristics of a variety of long-chain fatty acids (stearic, oleate, arachidonate, palmitate and linoleate) by FAT are very similar (39). The binding capacity of FAT is estimated to be 3 moles of fatty acids per mole of FAT. As demonstrated by the Lipidex assay, the binding of fatty acid to FAT is reversible and hence is distinct from covalent lipidation found in other proteins. Transfection studies also demonstrated that expression of FAT in Ob17PY fibroblasts parallels the increase in fatty acid uptake (41).

4.3 Caveolin

During the studies on induction of cellular fatty acid binding proteins during 3T3-L1 preadipocyte differentiation, Trigatti *et al.* (17) utilized the photoreactive fatty acid analogue 11-m-

diazirinophenoxy[11-^{3}H]undecanoate (11-DAP-[^{3}H]undecanoate) to label cellular proteins with high affinity for lipophiles. In addition to the 15-kDa cytoplasmic fatty acid binding protein, ALBP, a 22-kDa plasma membrane was also labeled. Labeling of this 22-kDa protein with 11-DAP-[^{3}H]-undecanoate was specific and saturable, the protein has a high affinity to the fatty acid analogue with a K_d of 216 nM (42), similar to the characteristics of long-chain fatty acid uptake by adipocytes (8,9). Subsequently, the identity of this protein was found to be caveolin by immunological techniques (11). Caveolin is a 22-kDa integral membrane protein originally described as a substrate for tyrosine kinase in rous sarcoma virus-transformed fibroblasts (43). It is a structural component of the caveolae (44) , which is involved in receptor-mediated solute uptake (45,46). Caveolae are flask-shaped pits on the plasma membrane implicated in vesicle trafficking which cycle between two conformations, open and closed. Gerber *et al.* proposed that free fatty acids diffuse into the open caveolae and are bound by caveolin. The pH of the cavity decreases when the caveolae cavity is closed resulting in a protonation of the lipid, release from the bound caveolin and subsequent partitioning of the lipid into the plasma membrane and hence favor diffusion across the bilayer.

4.4 56-kDa Renal FABP

In 1987, a 56-kDa protein from rat renal basolateral membrane (16) and cardiac myocyte sarcolemmal membrane (47) was purified which exhibited high affinity binding of fatty acids. The purification procedures include nonionic detergent solubilization, followed by gel filtration, ion-exchange chromatography and oleate-Sepharose 4B affinity chromatography. The protein appeared to be amphiphilic in nature as it formed aggregates of various sizes in the absence of the detergent. Its electrophoretic mobility on gels was not altered by the omission of 2-mercaptoethanol, suggesting the protein consists of a single polypeptide chain and does not contains any disulfide bonds. It exhibits high affinity binding to fatty acids, the K_d for palmitate is 0.79 μM with similar affinities observed for other long-chain fatty acids. It has been proposed to be a fatty acid transporter due to its high affinity to fatty acids; however, no further characterization or functional data on this protein is available to support this claim.

4.5 Fatty Acid Transport Protein (FATP)

A variety of molecular biological techniques offer a new approach for the identification of fatty acid transporters. By using expression cloning strategies, Schaffer and Lodish successfully identified a novel protein from 3T3-L1 adipocytes (19) that has the characteristics of a fatty acid transporter. Experimentally, a cDNA library from 3T3-L1 adipocytes was transfected into COS7 fibroblasts and the resultant cells assayed for the uptake of BODIPY 3823 (4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-3-indacene-3-dodecanoic acid), a fluorescence fatty acid analogue, by fluorescence-activated cell sorting. Plasmid DNA from the most fluorescent 0.03% of cells was recovered and used for further screening. After three such rounds of screening, two classes of cDNA were identified -- one has 94% identity to rat liver fatty acyl-CoA synthetase, the other does not share any extended homology with known mammalian sequences (19). This novel cDNA contains a single open reading frame for a 646 amino acid protein with a predicted molecular mass of 71 kDa. The protein was named fatty acid transport protein (FATP).

3T3 fibroblasts stably transfected with FATP showed a marked increase in the uptake of oleate and the fluorescent fatty acid analogue BODIPY 3832. Uptake is saturable and specific for long-chain fatty acids. The Km for oleate was estimated to be 200 nM, comparable to the values obtained from 3T3-L1 adipocytes and BFC-1 adipocytes (48). 70% of [^{14}C]-oleate taken up by the transfected cells was found to be unmetabolized, suggesting that FATP functions as a transporter instead of a fatty acid-metabolizing enzyme. The activity was specific for fatty acids, other metabolites such as sugars and amino acids were not transported.

In vitro translated FATP and western blot analysis of transfected cell extracts revealed that FATP has an apparent molecular mass of 63 kDa. Hydropathy analysis on FATP amino acid sequence predicts four to six potential transmembrane domains, depending upon the parameters used for analysis. Immunohistochemical studies using FATP specific antibodies demonstrated that the protein is expressed on the cell surface.

Expression of FATP mRNA is tissue specific. High levels of expression are found in heart, skeletal muscle and adipose tissue, lower levels of expression are observed in brain, lung, kidney and liver whereas spleen and intestine do not have detectable FATP mRNA. FATP mRNA is present at low levels in 3T3-L1 preadipocytes; however, its levels are 5- to 7-fold higher in fully differentiated cells (49). Although the net expression level of FATP increases markedly during adipose conversion, expression of FATP mRNA transiently decreases 50-70% during the early phase of differentiation. This led to the finding that the decrease in FATP message is due to the repression by insulin present in the differentiation-inducing medium (49). Insulin exerts its inhibitory effects on FATP expression inconsistent with a negative regulatory role of insulin on FATP gene expression (49). This is further supported by the finding that FATP expression is upregulated in the adipose tissue of the insulin-resistant Zucker diabetic fa/fa rats (35).

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                                                                  1
                                                 70
cfatp1     MREMPDSPKF ALVTFVVYAV VLYNVN.SVF WKFVFIGYVV FRLLRTDFGR RALATLPRDF AGLKLLISVK
cfatp2     ~~~~MKLEEL VTVMLLTVAV IAQNLPIGVI LAGVLILYIT ..VVHGDFIY RSYLTLNRDL TGLALIIEVK
mfatp      ~~~~MRAPGA GTASVASLA. LLWFLGLPWT WSAAAAFCVY VGGGGWRFLR IVCKTARRDL FGLSVLIRVR
yfatp      ~~~~~MSPIQ VVVFALSRIF LLLFRLIKLI ITPIQKSLGY LFGNYFDELD RKYRYKEDWY IIPYFLKSVF
Consensus  -------P-- --V-----A- -L-------- ---------- ------DF-- R---T--RD- -GL--LI-V-
                                                                              *

           71                                                                 140
cfatp1     STIRGLFKKD RPIHEIFLNQ VKQHPNKVAI I......... EIESGRQLTY QELNALANQY ANLYVSEGYK
cfatp2     IDLWWRLHQN KGIHELFLDI VKKNPNKPAM I......... DIETNTTETY AEFNAHCNRY ANYFQGLGYR
mfatp      LELRRHRRAG DTIPCIFQAV ARRQPERLAL V......... DASSGICWTF AQLDTYSNAV ANLFRQLGFA
yfatp      CYIIDVRRHR FQNWYLFIKQ VQQNGDHLAI SYTRPMAEKG EFQLETFTYI ETYNIVLRLS HILHFDYNVQ
Consensus  ---------- --I---F--- V---P---A- ---------- --------T- ---N---N-- ANL----G--
                        *           *

           141                                                                210
cfatp1     MGDVVALFME NSIDFFAIWL GLSKIGVVSA FINSNLKLEP LAHSINVSKC KSCITNINLL PMFKAAREKN
cfatp2     SGDVVALYME NSVEFVAAWM GLAKIGVVTA WINSNLKREQ LVHCITASKT KAIITSVTLQ NIMLDAIDQK
mfatp      PGDVVAVFLE GRPEFVGLWL GLAKAGVVAA LLNVNLRREP LAFCLGTSAA KALIYGGEMA AAVAEVSEQL
yfatp      AGDYVAIDCT NKPLFVFLWL SLWNIGAIPA FLNYNTKGTP LVHSLKISNI TQVFIDPDAS NPIRESEEEI
Consensus  -GDVVA---E N---FV--WL GL-KIGVV-A --N-NLK-EP L-H----S-- K--I------ -------E--
            ** **         *   *   *   *   *   * *      *      *

           211                                                                280
cfatp1     LISDE.IHVF LAG.TQVDGR HRSLQQDLHL FSEDEPPVID GLNFRSVLCY IYTSGTTGNP KPAVIKHFRY
cfatp2     LFDVEGIEVY SVGEPKKNSG FKNLKKKLDA QITTEPKTLD IVDFKSILCF IYTSGTTGMP KAAVMKHFRY
mfatp      GKSLLKFCSG DLGPESILPD TQLLDPMLAE APTTPLAQAP GKGMDDRLFY IYTSGTTGLP KAAIVVHSRY
yfatp      KNALPDVKLN YLEEQDLMHE LLNSQSPEFL QQDNVRTPLG LTDFKPSML. IYTSGTTGLP KSAIMSWRKS
Consensus  ---------- --G------- ---L---L-- ---------- ---F---L-- IYTSGTTG-P K-A---H-RY
                                                                  ******** * * *

           281                                                                350
cfatp1     FWIAMGAGKA FGINKSDVVY ITMPMYHSAA GIMGIGSLIA FGSTAVIRKK FSASNFWKDC VKYNVTATQY
cfatp2     YSIAVGAAKS FGIRPSDRMY VSMPIYHTAA GILGVGQALL GGSSCVIRKK FSASNFWRDC VKYDCTVSQY
mfatp      YRIAAFGHHS YSMRAADVLY DCLPLYHSAG NIMGVGQCVI YGLTVVLRKK FSASRFWDDC VKYNCTVVQY
yfatp      SVGCQVFGHV LHMTNESTVF TAMPLFHSTA ALLGACAILS HGGCLALSHK FSASTFWKQV YLTGATHIQY
Consensus  --IA------ ------D--Y --MP-YHSAA -I-G-G---- -G---V-RKK FSAS-FW-DC VKY--T--QY
                           *  *       *        *       * **** **         *  **

           351                                                                420
cfatp1     IGEICRYLLA ANPCPEEKQH NVRLMWGNGL RGQIWKEFVG RFGIKKIGEL YGSTE...GN SNIVNVDNHV
cfatp2     IGEICRYLLA QPVVEEESRH RMRLLVGNGL RAEIWQPFVD RFRV.RIGEL YGSTE...GT SSLVNIDGHV
mfatp      IGEICRYLLR QPVRDVEQRH RVRLAVGNGL RPAIWEEFTQ RFGVPQIGEF YGATE...CN CSIANMDGKV
yfatp      VGEVCRYLLH TPISKYEKMH KVKVAYGNGL RPDIWQDFRK RFNIEVIGEF YAATEAPFAT TTFQKGDFGI
Consensus  IGEICRYLL- -P----E--H -VRL--GNGL R--IW--F-- RF----IGE- YG-TE----- ----N-D--V
            ** *****        *  *       **** *  **  *   **    ***  *  **           *

           421                                                                490
cfatp1     GACGFMPIYP HIGSLYPVRL IKVDRATGEL E.RDKNGLCV PCVPGETGEM VGVI.KEKDI LLKFEGYV.S
cfatp2     GACGFLPISP LTKKMHPVRL IKVDDVTGEA I.RTSDGLCI ACNPGESGAM VSTI.RKNNP LLQFEGYL.N
mfatp      GSCGFN..SR ILTHVYPIRL VKVNEDTMEP L.RDSEGLCI PCQPGEPGLL VGQI.NQQDP LRRFDGYV.S
yfatp      GACRNYGTII QWFLSFQQTL VRMDPNDDSV IYRNSKGFCE VAPVGEPGEM LMRIFFPKKP ETSFQGYLGN
Consensus  GACGF----- ------P-RL -KVD--T-E- --R-S-GLC- -C-PGE-G-M V--I-----P L--F-GY---
           * *               *               *   * *      ** *      *           **

           491                                                                560
cfatp1     EGDTAKKIYR DVFKHGDKVF ASGDILHWDD LGYLYFVDRC GDTFRWKGEN VSTTEVEGIL ..QPVMDVED
cfatp2     KKETNKKIIR DVFAKGDSCF LTGDLLHWDR LGYVYFKDRT GDTFRWKGEN VSTTEVEAIL ..HPITGLSD
mfatp      DSATNKKIAH SVFRKGDSAY LSGDVLVMDE LGYMYFRDRS GDTFRWRGEN VSTTEVEAVL ..SRLLGQTD
yfatp      AKETKSKVVR DVFRRGDAWY RCGDLLKADE YGLWYFLDRM GDTFRWKSEN VSTTEVEDQL TASNKEQYAQ
Consensus  ---T-KKI-R DVF--GD--- --GD-L--D- LGY-YF-DR- GDTFRWKGEN VSTTEVE--L ---------D
                 *       **   **         **  *   *     *   **  **   ******   **  *******   *
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           561                                                                  630
cfatp1     ATVYGVTVGK MEGRAGMAGI ..VVKDGTDV EKFIADITSR LTENLASYAI PVFIRLCKEV DRTGTFKLKK
cfatp2     ATVYGVEVPQ REGRVGMASV VRVVSHEEDE TQFVHRVGAR LASSLTSYAI PQFMRICQDV EKTGTFKLVK
mfatp      VAVYGVAVPG VEGKAGMAAI ADPHSQLDPN SMY.....QE LQKVLASYAR PIFLRLLPQV DTTGTFKIQK
yfatp      VLVVGIKVPK YEGRAGFAVI KLTDNSLDIT AKTKLLNDSL SRLNLPSYAM PLFVKFVDEI KMTDNLIKF~
Consensus  --VYGV-VP- -EGRAGMA-I ---------- ---------- L---L-SYA- P-F-R----V --TGTFK--K
             * *  *    **  * *                          * ***  * *          *

           631                                          677
cfatp1     TDLQKQGYDL VACKGDPIYY WSAAEKSYKP LTDKMQQDID TGVYDRI
cfatp2     TNLQRLG..I MDAPSDSIYI YNSENRNFVP FDNDLRCKVS LGSYPF~
mfatp      TRLQREGFDP RQT.SDRLFF LDLKQGRYVP LDERVHARIC AGDFSL~
yfatp      ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~
Consensus  T-LQ--G--- -----D---- ---------P ---------- -G-----
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Figure 1. Comparison of amino acid sequences of FATP homologues.

The primary amino acid sequences of the murine FATP (mfatp), the yeast homologue (yfatp), and the two *C. elegans* homologues (cfatp1 & cfatp2) were aligned by using the GCG sequence analysis package. The consensus sequence represents identical residues found in at least three proteins. Positions with perfect identity among all four proteins are marked by asterisks.

Three putative homologues of murine FATP (mFATP) have been predicted from the genomic sequences of other organisms based on their primary amino acid homologies -- one from *Saccharomyces cerevisiae* (yFATP) (50) and two from *Caenorhabditis elegans* (cFATP1 and cFATP2) (51). The amino acid sequence of yFATP is 40.5% similar and 31.8% identical to mFATP. Both *C. elegans* homologues are about 63% similar and 42% identical to mFATP, while they are 68.4 % similar and 50.7 % identical to each other. Alignment of the primary amino acid sequences of all four proteins revealed striking conservation in several regions (Fig. 1), suggesting that these peptide sequences are central to the function of FATP. The N-terminal sequences are diverse while the C-terminal moieties are more similar. A potential acyl-adenylate binding site (position 261-273) is well-conserved in all four proteins; such sequence is also found in the *E. coli* fatty acid transporter FadL and other enzymes catalyzing through the formation of acyl-AMP intermediates. Based on the fact that this sequence is present in all four proteins, it is likely that fatty acids transport by FATP may follow a similar mechanism. A stretch of about 20 amino acids at position 525 to 547 in Fig. 1 is well-conserved among the four members. This peptide sequence is also found in the rat very-long-chain fatty acyl-CoA synthetase, suggesting that these two proteins may share some common steps in their actions . Functional studies on protein with these sequences mutated may provide important information on how fatty acids are transported by FATP. The amino acid sequence of FATP has been well-conserved throughout the eukaryotic evolution, implicating that FATP is an important protein carrying out vital functions in all eukaryotic cells. The identification by biochemical techniques of a lipid binding site on FATP has yet to be reported.

Disruption of the yeast FATP gene resulted in profound effects on the uptake and utilization of extracellular fatty acids in the mutant strain (*fat1*Δ) (52). Yeasts are unable to grow in media containing cerulenin, a fatty acid synthesis inhibitor; however, growth can be restored by the addition of long-chain fatty acids to the media. Comparing to the wild-type strain, *fat1*Δ has a much slower growth rate in cerulenin-containing medium supplemented with fatty acids, suggesting that yeast FATP is essential for efficient utilization of extracellular long-chain fatty acids. Despite having higher acyl-CoA synethetase activities, *fat1*Δ strain showed marked decreases in uptake of BODIPY-3823 and oleate. These data strongly support the notion that FATP functions as a long-chain fatty acid transporter.

5. CONCLUSIONS

While controversy continues on the mechanism by which fatty acids traverse the plasma membranes of cells, a number of putative fatty acid transporters have been identified. For many cell types, it is not clear what the physiological significance of multiple transporters might imply. For example, while the substrate specificity of several transporters has been reported, by no means has an exhaustive analysis of such activities been carried out. Moreover, the metabolic relationships of one protein to another have insofar as relative activities and specificities need to be determined. What is clear is that for the first time, systems are available for the analysis of specific proteins in order to utilize a combination of genetic and biochemical methods to address fatty acid transport. Because of the relatively recent nature of the field, it is not clear at this time if the proteins identified to date function as individual polypeptides or as a single component of a larger complex. This point is worth noting for the transport activity of transfected cells is often not as robust as the activity in the parental homotropic environment

suggesting the possibility of transporter associated proteins. This is particularly important in considering the mechanism of lipid transport, either being energy-linked or simply facilitates the diffusion of lipids. Lastly, it can be predicted that if lipid transport does occur via a protein mediated factor(s), it is likely that certain pathophysiologies will be linked to its dysfunction. Whatever the outcome, it is likely that our view of hydrophobic ligand transfer across membranes will significantly change in the next decade. The proteins identified so far will be intense targets of examination in the years to come.

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