POINT/COUNTERPOINT

Introduction
How Do Long-Chain Free Fatty Acids Cross Cell Membranes? (43985)

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Long-chain, non-esterified free fatty acids (FFA), the building blocks from which triglycerides are synthesized, are important energy substrates in various tissues, essential building blocks for the lipid components of cell membranes, and precursors for the synthesis of important biological mediators such as the prostaglandins. They are also increasingly being recognized as important intracellular mediators of gene expression. These multiple roles suggest that careful regulation of all aspects of FFA disposition, including cellular uptake, would be advantageous. However, although the intracellular metabolism of FFA and their transport in plasma as complexes with albumin have been extensively studied, little attention was paid until recently to the mechanisms by which FFA enter and leave cells. This neglect occurred both for methodologic reasons and because the cellular uptake of FFA was long considered to be an entirely unregulated process in which FFA partitioned passively into the lipid bilayer of the plasma membrane.

Since 1981, a series of studies from several groups clarified the influence of albumin binding on the uptake kinetics of FFA and other bound ligands, making more detailed studies of the uptake process feasible (1–6). Once it became clear that FFA uptake occurred principally from the very small unbound ligand pool in plasma and not from the albumin-bound compartment, it was quickly reported that a major component of FFA uptake in certain cell types, including hepatocytes, adipocytes, and cardiac and skeletal myocytes, in fact exhibited all the kinetic properties of a facilitated transport process: saturation, *trans*-stimulation, *cis*-inhibition, and counter transport (7–18). These observations, which—if valid—cannot be explained by purely passive diffusion, have suggested to many investigators the existence of a facilitated uptake process for FFA, which appears to exist in parallel with a nonsaturable, presumable diffusive pathway.

The kinetic evidence favoring facilitated FFA transport was sufficiently persuasive to some investigators to initiate a search for FFA transporters. Indeed, no fewer than five putative FFA transporters have by now been identified by various techniques in the plasma membranes of several tissues (Table I) (19–23). cDNA clones have been identified for three of them (19, 22, 23). A complex facilitated FFA transport system, involving both a peripheral membrane protein which serves as an "acceptor" and a distinct transmembrane transporter, also has been well characterized in *Escherichia coli* (24).

Despite the identification of putative transporters, the evidence for facilitated transport has been unconvincing to some investigators, particularly those with a strong focus on the physicochemical aspects of membrane:solute interactions. These investigators have documented that the purely passive, physicochemical flux of FFA across certain synthetic lipid membranes occurs at rates which far exceed those observed *in vivo*, and would be more than adequate to meet cellular needs. They argue that, in these circumstances, there would be no need for cells to have additional, specialized FFA transport mechanisms. The converse, of course is also possible: namely, that these purely synthetic membranes are poor models for biological

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Table I. Identification and Isolation of Plasma Membrane Fatty Acid–Binding Proteins

Lab (reference)	Year	Size (kDa)	Name	Technique[a]	Sites[b]	TMSD[c]	Homologies	Function[d]
Berk[e] (19)	1985	43	FABPpm	AC, PhA-L	L, A, My, I, E	4(?)	mAspAT	Ab-I, LR, GenExpr
Fujii (20)	1987	56/60	—	AC	K, My	?	?	N.R.
Trigatti (21)	1991	22	—	PhA-L	A	?	?	N.R.
Abumrad[e] (22)	1993	88/53	FAT	Coval-L	A, My, M, I, T	2	CD36	UT-I
Lodish[e] (23)	1994	63/71	FATP	Exp-Clon	A, My, M (K,B,L,Lu)	6	FACS/FACL[f]	GenExpr

[a] AC, affinity chromatography; PhA-L, photoaffinity labeling; Coval-L, covalent labeling; Exp-Clon, expression cloning.
[b] A, adipose tissue; B, brain; E, endothelium; I, intestine; K, kidney; L, liver; Lu, lung; M, skeletal muscle; My, cardiac muscle; T, testis.
[c] TMSD, trans-membrane spanning domains.
[d] Ab-I, Selective inhibition of uptake by antibodies to protein; LR, reconstitution of transport by insertion of protein into synthetic liposomes; GenExpr, expression of protein and transport function follows expression of cloned genetic message (cDNA or cRNA); UT-I, Selective inhibition of uptake by covalent labeling of protein; N.R., not reported.
[e] cDNA cloned.
[f] FACS/FACL, fatty acid co-A synthase/ligase.

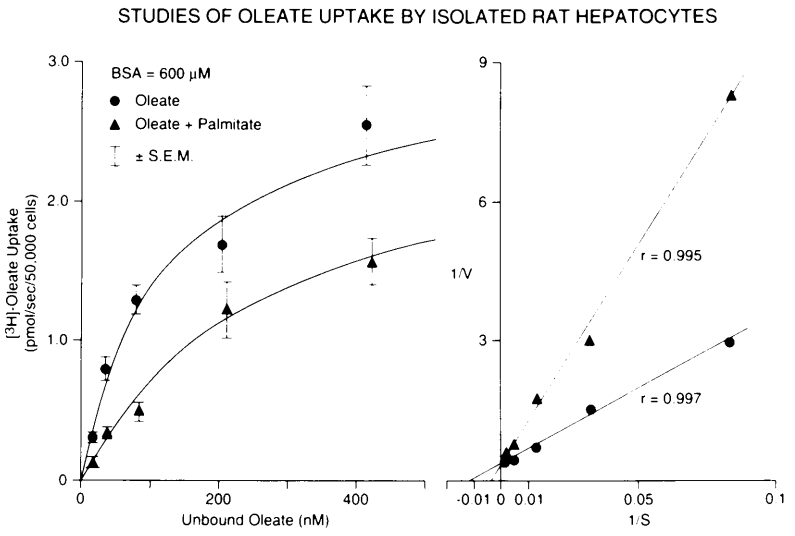

Figure 1. Initial rate of oleate uptake by isolated hepatocytes, expressed as a function of the unbound oleate concentration (O_u), in the presence and absence of 55 n*M* unbound palmitate. The albumin concentration in all studies was 600 μ*M*. Employing a wide range of oleate:BSA molar ratios to vary O_u, uptake was saturable when plotted against O_u both in the absence and in the presence of palmitate. Analysis of these data in terms of Michaelis-Menten kinetics indicates competition for transport between these two fatty acids. (left panel) Linear plot. (right panel) Double reciprocal plot. (Reproduction from Ref. 16.)

plasma membranes, and that FFA transfer rates in, for one, synthetic liposomes *in vitro* are not reflective of the passive transfer rates of FFA across plasma membranes *in vivo*.

The debate over the processes underlying the transmembrane movement of amphipathic molecules is not restricted to FFA. Similar questions have been raised, often by the same research groups, about the transport of such molecules as bilirubin, bile acids, and phospholipids.

In the following two articles, a leading proponent of each of the two major schools of thought about FFA transport summarizes his point of view. Professor David Zakim of the New York Hospital—Cornell Medical Center offers an elegant and highly lucid summary of the reasons why some investigators continue to believe that cellular FFA uptake is a purely passive, physicochemical process. Professor Wolfgang Stremmel of Heidelberg argues equally strongly for the existence of protein-mediated transport processes for FFA and other amphipaths.

My personal view favors the existence of facilitated transport system for FFA. Indeed, Professor Stremmel and I collaborated in the past on studies in this area and were the first to identify a putative FFA transporter, which we designated plasma membrane fatty acid–binding protein (FABPpm) (19). Nevertheless, Dr. Zakim argues this case here, as he has elsewhere (25), with great persuasiveness, and raises a number of difficult and pointed questions which keep us from becoming complacent about our point of view.

Table II. Evidence for the Identity of FABPpm and mAspAT

The two proteins have similar or identical:
- Molecular masses: 43 kDa by SDS-PAGE, 44.5 kDa by MS
- pI: 9.1 with multiple, characteristic charge isomers
- Chromatographic behavior in multiple systems
- Amino acid composition
- N-terminal amino acid sequence (35 residues)
- Tryptic digests
- mAspAT enzyme specific activity: 205–210 U/mg
- K_d for oleate binding: 7–8 × 10^{-8} *M*
- Photoaffinity labeling
- Immunologic epitopes

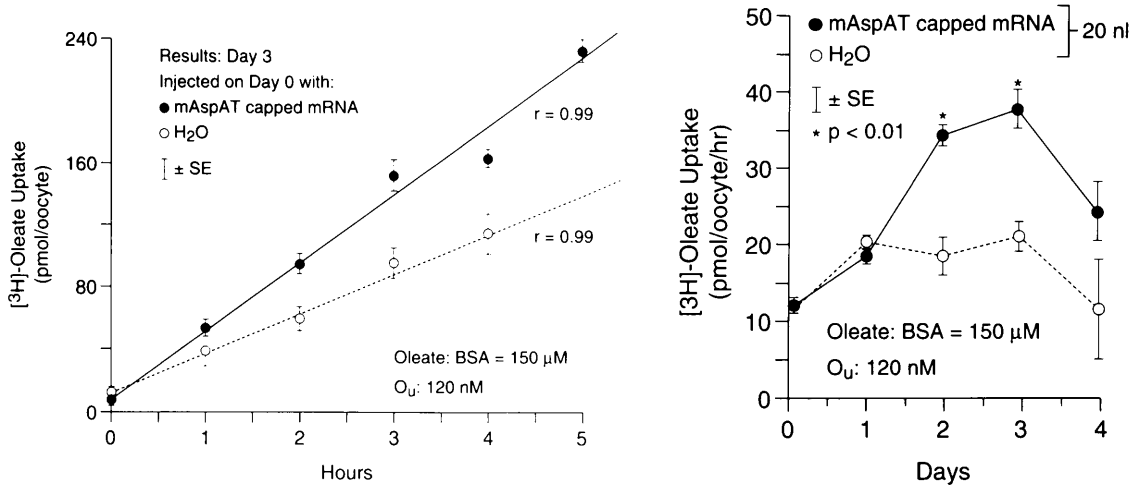

Figure 2. [^{3}H]-oleate uptake by *Xenopus laevis* oocytes from solution containing 150 μ*M* BSA. (left panel) Typical uptake curves measured over 4–5 h on Day 3 after injection with either capped mAspAT mRNA (●—●) or H_2O (○---○). Uptake rate was determined by linear regression. Correlation coefficients are invariably >0.95 when healthy oocytes are employed. (right panel) Oleate uptake rates in Xenopus oocytes at various times after injection with a capped mAspAT mRNA or with H_2O, as described above. Highly significant increases in uptake were observed on Day 2 and 3 after injection. Uptake declined appreciably by Day 4. (From Ref. 33.)

Ultimately, however, even if I cannot address all of Dr. Zakim's questions at the present time, the following evidence convinces me that cellular FFA uptake is a protein-mediated transport process.

First, FFA uptake does exhibit kinetic properties strongly suggestive of facilitated transport. The high-affinity binding of FFA to albumin extracellularly and to cytosolic FABPs intracellularly complicates the design and interpretation of the necessary experiments, but with meticulous attention to detail, such defining properties as competitive inhibition are readily demonstrable both in the perfused rat liver and in isolated hepatocyte systems (Fig. 1) (16). Second, although antibodies to FABPpm do not enter viable cells (26) and do not, therefore, alter intracellular metabolism, FFA uptake is highly selectively inhibited by such antibodies in certain cell types. Specifically, they inhibit FFA uptake in cells exhibiting FABPpm on the plasma membrane (10–12, 14, 15, 26, 27, 31), but not in fibroblasts (27), which do not display appreciable amounts of this protein. The antibody effects are highly specific. Uptake of glucose, or of medium chain fatty acids (which do not enter cells by diffusion) are not affected. And third, incorporation of FABPpm into synthetic liposomes appears to reconstitute FFA uptake (28). Similar effects are not seen with other, control proteins.

Defining the role of FABPpm in mediating FFA uptake was enormously complicated by the discovery that it is identical to the mitochondrial isoform of aspartate aminotransferase (29–31). The physicochemical and immunologic evidence favoring their identity is summarized in Table II.

Since pre-mAspAT had been cloned (32), the dual hypotheses that FABPpm was identical to mAspAT, and that this protein-mediated FFA uptake was testable in now standard transfection studies. In a mammalian system, the 3T3 fibroblast, transfection with plasmid pMAAT2, containing a full-length pre-mAspAT cDNA under the control of a Zn^{++}-inducible metallothionein promoter resulted in the *de novo* Zn^{++}-sensitive appearance of FABPpm on the plasma membrane, which was highly correlated with an up to a 10-fold, Zn^{++}-sensitive increase in saturable FFA uptake (26). Similarly, microinjection of *Xenopus laevis* oocytes with a capped mAspAT cRNA led to a selective increase in FFA uptake by the oocytes (Fig. 2) (33).

Selectively antibody inhibition, liposome reconstitution, and genetic expression have become the widely accepted techniques by which the function of a candidate transport protein is confirmed (33). If FFA transport is mediated by a specific protein, as the data just summarized strongly suggest, then the process is not one of simple, passive diffusion. I find the data in this regard quiet compelling. But I would sleep better if I could answer all of Dr. Zakim's questions.

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