Mouse Very Long-chain Acyl-CoA Synthetase 3/Fatty Acid Transport Protein 3 Catalyzes Fatty Acid Activation but Not Fatty Acid Transport in MA-10 Cells*

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The family of proteins that includes very long-chain acyl-CoA synthetases (ACSVL) consists of six members. These enzymes have also been designated fatty acid transport proteins. We cloned full-length mouse Acsvl3 cDNA and characterized its protein product ACSVL3/acyl-CoA transport protein 3. The predicted amino acid sequence contains two highly conserved motifs characteristic of acyl-CoA synthetases. Northern blot analysis revealed that the mouse Acsvl3 mRNA is highly expressed in adrenal gland, testis, and ovary, with lower expression in the brain of adult mice. A developmental Northern blot revealed that Acsvl3 mRNA levels were significantly higher in embryonic mouse brain (embryonic days 12–14) than in newborn or adult mice, suggesting a possible role in nervous system development. Immunohistochemistry revealed high ACSVL3 expression in adrenal cortical cells, spermatocytes and interstitial cells of the testis, theca cells of the ovary, cerebral cortical neurons, and cerebellar Purkinje cells. Endogenous ACSVL3 was found primarily in mitochondria of MA-10 and Neuro2a cells by both Western blot analysis of subcellular fractions and immunofluorescence analysis. In MA-10 cells, loss-of-function studies using RNA interference confirmed that endogenous ACSVL3 is an acyl-CoA synthetase capable of activating both long-chain (C16:0) and very long-chain (C24:0) fatty acids. However, despite decreased acyl-CoA synthetase activity, initial rates of fatty acid uptake were unaffected by knockdown of Acsvl3 expression in MA-10 cells. These studies cast doubt on the designation of ACSVL3 as a fatty acid transport protein.

The transport of fatty acids into cells and their subsequent “activation” by thioesterification to CoA are fundamental processes required for entry of fatty acids into the metabolic stream (1). The mechanism of fatty acids entry into cells remains controversial. Some investigators argue that specific proteins are required to transport the fatty acid across the plasma membrane (2–6). Others have provided evidence that proteins are not necessary for translocation of fatty acids through the lipid bilayer (7, 8). One group of proteins proposed to mediate fatty acid entry into cells are the fatty acid transport proteins (FATPs)§ (4). The mammalian FATP family consists of six homologous proteins (FATP1-6) that share 35–58% amino acid identity.¶ Studies with cultured cells overexpressing FATP1-6 have demonstrated increased rates of accumulation of fluorescent or radiolabeled fatty acids (3, 9). However, interpretation of fatty acid transport studies is hampered by the fact that, once inside cells, fatty acids are rapidly metabolized. Metabolism will decrease the intracellular concentration of the unesterified fatty acid, shifting the concentration gradient across the plasma membrane to promote entry of additional fatty acids into the cell. The design of most transport studies does not distinguish between transport mechanisms that can occur in a protein-free phospholipid bilayer and transport plus metabolism.

Independent of studies on FATP and its potential role in fatty acid transport, Hashimoto and co-workers (10) used classical protein purification methods to isolate the first enzyme capable of activating very long-chain fatty acids (VLCFA; fatty acids containing more than 22 carbons). This enzyme with very long-chain acyl-CoA synthetase (ACSVL)³ activity was purified to homogeneity from rat liver peroxisomes, a known source of ACSVL activity. Sequencing of peptide fragments obtained from proteolytic digestion of this protein led to cloning of full-length Acsvl1 cDNA (11). Translation of the cDNA sequence revealed that the protein with the highest amino acid identity to ACSVL1 was not one of the five long-chain acyl-CoA synthetases but rather the protein now known as FATP1. ACSVL1 contains two highly conserved amino acid sequence motifs (12, 13). Motif 1 is an AMP-binding domain common to all known acyl-CoA synthetases and related enzymes whose reaction mechanism involves formation of an adenylated intermediate.

1 The abbreviations used are: FATP, fatty acid transport protein; ACSVL, very long-chain acyl-CoA synthetase; VLCFA, very long-chain fatty acid; siRNA, small interfering RNA; MAM, mitochondria-associated membrane fraction; X-ALD, X-linked adrenoleukodystrophy; EST, expressed sequence tag; contig, group of overlapping clones; PBS, phosphate-buffered saline; RT, reverse transcription.
2 P. A. Watkins, unpublished observations.
3 In 2004, the nomenclature for long-chain acyl-CoA synthetases was officially revised (43). The root designation “ACS” for acyl-CoA synthetase is followed by “L” for long-chain and a number. A similar convention for the very long-chain acyl-CoA synthetases, designated ACSVLx, is used in this paper. Genes are designated Acsxl, and proteins are designated ACSVL."
nated ACSB. The FATP nomenclature follows that of Stahl

activate bile acids to their CoA derivatives (28, 45) and is thus desig-

scribed as VLCS-H2/VLACSR/BACS has been shown to preferentially

sequences for two members of the

[1-14C]lignoceric acid (C24:0) were obtained from Moravek, Inc. (Invitrogen) in a 5% CO2 atmosphere. MA-10 cells (23) were kindly

supplemented with penicillin/streptomycin and 10% fetal bovine serum

method of Lowry

beled fatty acids were from Sigma. Protein was measured by the

ration, the expression pattern of this protein suggests that it may

ACSVL3 does not exhibit fatty acid transport activity. In addi-

tion, the expression pattern of this protein suggests that it may play an important role in brain development.

EXPERIMENTAL PROCEDURES

Materials and General Methods—[1,14C]Palmitic acid (C16:0) and [1,14C]lignoceric acid (C16:0) were obtained from Moravek, Inc. (9,10-

membrane, reactivity of the ACSVL3 antibody was assessed. Anti-actin

antibody for ACSVL3 was determined by Western blot analysis of

ers and two acyl-CoA synthetases belonging to two other families,

bers and two acyl-CoA synthetases, fatty acid transporters, or bifunctional proteins with both activities. Several laboratories have now demonstrated that at least four ACSVL/FATP proteins have acyl-CoA synthetase activity (18–21). We now report that a fifth member of the murine

protein family, ACSVL3 or FATP3, is also an acyl-CoA synthetase capable of activating both long- and very-long fatty acid substrates. We further report that endogenous ACSVL3 does not exhibit fatty acid transport activity. In addition, the expression pattern of this protein suggests that it may play an important role in brain development.

ACYSL/FATP nomenclature

The ACS nomenclature follows the convention established for mam-

malian long-chain acyl-CoA synthetases. The enzyme previously de-

scribed as VLCs-H2/VLACSR/BACS has been shown to preferentially

activate bile acids to their CoA derivatives (28, 45) and is thus desig-

scribed as VLCS-H2/VLACSR/BACS has been shown to preferentially

activated ACSB. The FATP nomenclature follows that of Stahl et al. (46).

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ACSL6 and BG1. Only mouse or human ACSVL3 was detected by the purified antibody (Fig. 1).

**Northern Analysis—** For tissue expression of *Acsvl3*, total RNA was prepared using the Trizol reagent (Invitrogen) from freshly harvested tissues from adult (~3-month-old) mice. Twenty micrograms of RNA was electrophoresed on a 1% agarose gel at 4 V/cm for 2.5 h and then transferred overnight to a Hybond-N+ membrane (Amersham Biosciences). A cDNA probe for detection of *Acsvl3* mRNA was prepared by excision of a 1196-bp fragment corresponding to bp 405–1601 using Eco47III and BspEI. For control, a 528-bp glyceraldehyde-3-phosphate dehydrogenase probe was prepared by PCR amplification using 5′-ACCACTATGGAGAAGCTGGTG-3′ and 5′-CTCATGTCAAGCCAGATGC-3′ as forward and reverse primers, respectively, and mouse brain cDNA as template. Conditions for probe labeling, hybridization, and detection were as previously described (20). For developmental analysis of *Acsvl3* expression, a Northern blot was prepared from 1–2 μg of poly(A)+ RNA as previously described (24). For this blot, the probe consisted of 1315 nucleotides corresponding to bp 807–2121 of *Acsvl3* cDNA. As a control for loading and transfer, the blot was probed with a mouse mouse cDNA of the ubiquitously expressed protein cyclophilin.

**RT-PCR Analysis—** Approximately 2 μg of total cellular RNA (extracted as described above) was reverse transcribed and PCR-amplified using the PerkinElmer Life Sciences RT-PCR system with forward primer 5′-AATGCGAACGGACACGTGACACACAT-3′ (within exon 6; see Table II) and reverse primer 5′-ATTCGAAAGTCCTCACAGAGGAGGA-3′ (within exon 10). The primer combination was selected such that a 824-bp *Acsvl3*-specific product can be amplified from cDNA but not from genomic DNA, potentially contaminating the RNA extracts. RT-PCR was performed under conditions not allowing quantitative analysis. As a positive control, RT-PCR of a 680-bp fragment of the ubiquitously expressed glyceraldehyde-3-phosphate dehydrogenase mRNA using forward primer 5′-ATCGCGTCTTCTCACACAT-3′ and reverse primer 5′-TCAACACCTGTTGCTGTA-3′ was performed on the same cDNA preparations.

**Animals and Their Care—** Wild type 129SvEv mice were obtained from Taconic, Inc. (Germantown, NY) and housed in facilities of The Johns Hopkins University School of Medicine. Animals were housed under controlled conditions, between 22 and 27 °C, on a 12 h-light/dark cycle, with food and water ad libitum. Procedures involving animals and their care were conducted in conformity with institutional guidelines that are in compliance with national and international laws and policies (47).

**Antibodies, Indirect Immunofluorescence, Direct Immunofluorescence, and Immunohistochemistry—** Polyclonal antiserum to the peroxisomal protein PMP70 was a gift from Dr. S. Gould. Monoclonal antibody to the endoplasmic reticulum marker, protein-disulfide isomerase, and polyclonal antibody to the mitochondrial marker, Mn-superoxide dismutase, were from Stressgen, and polyclonal antibody to a plasma membrane marker, E-cadherin, was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit antibody to BG1 was affinity-purified as previously described (25). For indirect immunofluorescence analysis, cells were fixed in 4% formaldehyde in PBS and permeabilized with 1.0% Triton X-100 prior to incubation with primary and secondary antibodies as described previously (27). In some experiments, cells were incubated following the manufacturer’s instructions with MitoTracker red (Molecular Probes, Inc., Eugene, OR) before fixation. Because affinity-purified polyclonal antibodies to ACSVL3 and BG1 were both raised in rabbits, double-labeling studies done by sequential application of antibodies resulted in an unacceptable level of nonspecific binding of fluorescence-labeled secondary antibodies. Therefore, these antibodies were also directly labeled using Zenon™ Rabbit IgG Labeling reagents (Molecular Probes). Alexa Fluor 555 and Alexa Fluor 488 reagents were used to fluorescently label ACSVL3 and BG1 antibodies, respectively, following the manufacturer’s protocol. For direct immunofluorescence, cells were incubated concurrently with both antibodies, washed extensively with PBS, fixed again with 4% formaldehyde, washed, and mounted. Control experiments containing labeling reagents prepared without either ACSVL3 or BG1 antibody resulted in no nonspecific labeling when used in conjunction with the opposite directly labeled antibody. For immunohistochemistry, tissues from 3-month-old mice were harvested, quickly frozen in liquid nitrogen, and stored at ~80 °C. Tissue sections were cut using a cryostat and fixed with 4% paraformaldehyde as described previously (28). Brain sections were 20 μm thick; sections of adrenal gland, testis, and ovary were 5–8 μm thick. After fixation, sections were incubated for 30 min with 0.6% H2O2 in methanol for 20 min with 5% normal goat serum. Sections were then incubated sequentially with Avidin D and biotin, 15 min each (Avidin/Biotin Blocking Kit; Vector Laboratories). Incubation with primary rabbit antibody (affinity-purified anti-hACSVL3 antibody; 1:100) was for 1 h at 37 °C or overnight at 4 °C. Peroxidase-based detection was done using a Vectastain ABC kit (Vector Laboratories). After counterstaining with hematoxylin-Harris stain for 30 s, sections were dehydrated and mounted with DPX mounting solution (Fluka Biochemika).

**RNA Interference—** Four pairs of cDNA oligonucleotides containing complementary sense and antisense *Acsvl3* sequences were obtained from Integrated DNA Technologies and used to synthesize small interfering RNA 21-mers (siRNA) using the Silencer kit (Ambion) as previously described (25). Targeted sequences of siRNA constructs 1–4 corresponded to bp 55–75, 695–715, 1243–1263, and 1855–1875, respectively, of the *Acsvl3* coding region. MA-10 cells were transfected with the individual siRNA constructs or a mixture of all four, essentially as described (25), except that cells were incubated with siRNA for only 12 h prior to the addition of the usual culture medium.

**Acyl-CoA Synthetase and Fatty Acid Uptake Assay—** Acyl-CoA synthetase assays utilizing radiolabeled palmitic acid (C16:0) and lignoceric acid (C24:0) were performed as previously described (29, 30). Fatty acid uptake assays were similar to those described by Trotter et al. (31).

**b** Length to the last nucleotide preceding the poly(A) sequence.
for protein determination, radioactivity was determined by liquid scintillation counting.

RESULTS

Cloning of Full-length Mouse Acsvl3 cDNA and Initial Characterization—Early evidence for the existence of ACSVL3 came from homology probing of the expressed sequence tag data base using the amino acid sequence of ACSVL1 as a probe. The highly conserved region now referred to as Motif 2 (12, 13) was found in numerous ESTs that could be placed into five groups of candidate ACSVL1 homologs, including ACSVL3. Full-length mouse Acsvl3 cDNA was cloned into the mammalian expression vector pcDNA3 as described under "Experimental Procedures." The nucleotide sequence shown in Fig. 2 contains 137 bp of 5′-untranslated region, a Kozak consensus sequence flanking the likely start codon (underlined), an open reading frame encoding 667 amino acids, and 117 bp of 3′-untranslated region containing a polyadenylation signal (boldface type). The Acsvl3 gene is found on mouse chromosome 3. The predicted amino acid sequence encodes a protein with a calculated molecular mass of 72,968 daltons and a pI of 7.59. Motif 1 (underlined italic type) and Motif 2 (underlined and boldface type) are also indicated in Fig. 2.

The predicted ACSVL3 amino acid sequence was found to share 35–47% identity and 54–78% overall similarity with the five other members of the murine ACSVL/FATP protein family. Examination of the amino acid sequence using both neural networks and hidden Markov models trained on eukaryotes revealed the presence of a signal peptide sequence with the most likely cleavage site between residues 19 and 20 (33). A Kyte-Doolittle hydropathy plot obtained using the TopPred II program (34) (available on the World Wide Web at bioweb.pasteur.fr/seqanal/interfaces/toppred.html) suggested that ACSVL3 contains one or two potential membrane-spanning regions. The genomic organization of Acsvl3 (Table II) was determined by genomic primer walking, in combination with in silico analysis. The intron/exon structure of the mouse gene is similar to that of its human ortholog.4

Tissue Expression of Acsvl3—Before examining the biological function of ACSVL3, it was first necessary to determine the tissues and the cell types within these tissues in which the protein was expressed. In addition, it was necessary to assess

4 P. Fraisel, Z. Pei, S. Fors-Petter, P. A. Watkins, and J. Berger, manuscript in preparation.
During mouse brain development, poly(A) control hybridization with a glyceraldehyde-3-phosphate dehydrogenase RNA. Blot analysis of determine which specific cell types expressed the protein. In immunohistochemical analysis of mouse tissues was used to determine the subcellular location of ACSVL3 in cell lines predicted to express the protein by the histochemical analyses. The mouse testis Leydig cell line MA-10 (23) and the mouse neuroblastoma cell line Neuro2a were found to express ACSVL3 both by RT-PCR and Western blot (data not shown). Subcellular fractionation by differential centrifugation revealed that ACSVL3, which migrates as an 84-kDa band on Western blots, was found primarily in the M fraction, which is enriched in mitochondria, of both MA-10 cells (Fig. 5A) and Neuro2a cells (data not shown). An additional band of ~58 kDa was occasionally seen in the M fraction and, to a lesser extent, in the peroxisome-enriched L fraction; based on RNA interference studies (below), this band appears to be nonspecific and unrelated to ACSVL3. No 84-kDa ACSVL3 band was seen in the N (nuclear), L (light mitochondrial), P (microsomal), or S (cytosolic) fractions. Indirect immunofluorescence studies were also done to examine the subcellular compartment(s) containing ACSVL3. Both COS-7 cells overexpressing the protein and MA-10 and Neuro2a cells that endogenously express ACSVL3 were examined. A fine, reticular immunostaining pattern was observed in COS-7 cells transfected with full-length Acsvl3 cDNA (Fig. 6A) that exhibited significant colocalization with the endoplasmic reticulum marker, protein-disulfide isomerase (Fig. 6, B and C).

**FIG. 3.** Tissue expression of *Acsvl3*. A, RT-PCR analysis of murine tissues. Mouse tissue RNA was obtained, reverse transcribed, and used as template for amplification of a fragment of Acsvl3 as described under “Experimental Procedures.” Amplification of a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe is also shown. C, Northern blot analysis of Acsvl3 mRNA during mouse brain development. Poly(A)* RNA (1–2 μg/lane) was extracted from dissected brain or whole head (lanes E12 and E14) at the indicated developmental stages and hybridized with 32P-labeled cDNA probes. A cyclolin (cyph) probe was used as loading control for this blot. The lanes during brain development are labeled as follows: E12–18, 12.5–18.5 days of gestation; P2–P30, days of postnatal development.

**FIG. 4.** Immunohistochemical localization of ACSVL3 in mouse tissues. Tissues were processed for immunohistochemical localization using affinity-purified anti-ACSVL3 antibody as described under “Experimental Procedures.” Cells containing ACSVL3 protein have a brownish appearance against the blue hematoxylin counterstain. In the brain sections (cerebral cortex, hippocampus, and cerebellum), neuronal expression is evident. In the adrenal cortex, cells of the outer zona glomerulosa (zg) and the inner zona reticularis (zr) showed greater expression than did the middle zona fasciculata (zf); the adrenal medulla (med) had no detectable ACSVL3. In testis, primary spermatocytes showed high expression, with lower levels observed in secondary spermatocytes and interstitial Leydig cells. Both internal and external theca cells of the ovary showed prominent ACSVL3 expression; some labeling of follicle cells was also evident.
be present in other fractions (44).

Membrane fragments are typically found in the P fraction, they can also be detected in the N, L, and P fractions, but not in the M fraction. Plasma membrane marker, E-cadherin, was de-enriched in microsomes (S), particulate (P), or cytosolic (I) fractions. The plasma membrane marker, E-cadherin, was detected only in the M fraction, the identical MAM isolation protocol; phosphatidylethanolamine was previously detected in purified mitochondrial fraction (Fig. 5A). Western blot analysis revealed that ACSVL3 was mainly in the purified mitochondrial fraction (Fig. 5B).  

Like ACSVL3, the acyl-CoA synthetase BG1 (also called Very Long-chain Acyl-CoA Synthetase 3) was found in the nuclear (N), light mitochondrial (L), enriched in peroxisomes, particulate (P), enriched in microsomes, or soluble (S; or cytosolic) fractions. The plasma membrane marker, E-cadherin, was detected in the N, L, and P fractions, but not in the M fraction. Plasma membrane sheets typically sediment in the N fraction; whereas plasma membrane fragments are typically found in the P fraction, they can also be present in other fractions (44). Western blot of MAM and purified mitochondria. A combined mitochondria/peroxisome-enriched (ML) fraction of MA-10 cells was subfractionated into purified mitochondria (Mito) and MAM fraction as described previously (25). MnSOD is found in purified mitochondrial fraction, an endoplasmic reticulum marker. D–O, endogenous ACSVL3 in MA-10 cells. Double labeling using anti-ACSVL3 (D) and MitoTracker red (E) showed partial colocalization of ACSVL3 with mitochondria in the merged image (F). Double-labeling with anti-ACSVL3 (G) and anti-FPMP70 (H) demonstrated that the punctate ACSVL3-containing vesicles were not peroxisomes when the images were merged (I). Double labeling with anti-ACSVL3 (J) and anti-protein-disulfide isomerase (K) revealed no colocalization in the merged image (L), unlike the observation of the overexpressed protein (A–C). The acyl-CoA synthetase BG1 (M) was also found in punctate structures associated with mitochondria (25), similar to ACSVL3 (N). However, the merged image (O) suggested that these vesicle populations were distinct.  

A combined mitochondria/peroxisome-enriched (ML) fraction of MA-10 cells was subfractionated into purified mitochondria (Mito) and MAM fraction as described previously (25). MnSOD is found in purified mitochondrial fraction, an endoplasmic reticulum marker. D–O, endogenous ACSVL3 in MA-10 cells. Double labeling using anti-ACSVL3 (D) and MitoTracker red (E) showed partial colocalization of ACSVL3 with mitochondria in the merged image (F). Double-labeling with anti-ACSVL3 (G) and anti-FPMP70 (H) demonstrated that the punctate ACSVL3-containing vesicles were not peroxisomes when the images were merged (I). Double labeling with anti-ACSVL3 (J) and anti-protein-disulfide isomerase (K) revealed no colocalization in the merged image (L), unlike the observation of the overexpressed protein (A–C). The acyl-CoA synthetase BG1 (M) was also found in punctate structures associated with mitochondria (25), similar to ACSVL3 (N). However, the merged image (O) suggested that these vesicle populations were distinct.
Acyl-CoA synthetases play a central role in fatty acid metabolism by converting a relatively inert fatty acid molecule into an activated form that can be utilized by numerous metabolic pathways (1). Fatty acyl-CoAs can be degraded for energy production or incorporated into triacylglycerol for storage. They are substrates for the synthesis of phospholipids, sphingolipids, and glycolipids and can acylate proteins or regulate gene expression. Based on this metabolic diversity, along with the wide range of fatty acid chain lengths in nature, it is not surprising that mammalian genomes encode about 25 distinct acyl-CoA synthetases.\(^5\)

The acyl-CoA synthetase reaction mechanism involves formation of an acyl-adenylate intermediate and the release of pyrophosphate, with subsequent displacement of AMP by CoA before SH. A similar reaction mechanism is employed by other enzymes, such as firefly luciferase and bacterial gramicidin-S synthetase, and all of these proteins contain a characteristic AMP-binding domain (14). This domain is present in the predicted amino acid sequence of ACSVL3 when analyzed by the Protein Families (PFAM) database (available on the World Wide Web at www.sanger.ac.uk/Software/Pfam/). Within the AMP-binding domain is a highly conserved sequence of 10 predicted amino acids referred to as Motif 1 (Fig. 2) that is nearly identical in all members of the ACSVL family, including ACSVL3: (FY)TSGTGLPK. Black et al. (15) described a “signature motif” that was conserved among members of the long-chain acyl-CoA synthetase family and found evidence that this region played a role in acyl chain-length substrate specificity of the enzymes. We described a highly conserved region in the ACSVL family that overlapped with the long-chain enzyme signature.

\(^5\) P. A. Watkins, manuscript in preparation.
motif, which we referred to as Motif 2 (12, 13). Within Motif 2 is a sequence of 11 amino acids that are identical in all six members of the ACSVL family: GDTRFWRKGENV. It should be noted that this family is designated “very long-chain” for two reasons: first, because at least five of the six members are capable of activating VLCFA, and second, to distinguish it from the long-chain (ACSL), medium-chain (ACSM), and other acyl-CoA synthetase families. Whereas both ACSL and ACSVL family enzymes can activate long-chain fatty acid substrates, only the latter are capable of VLCFA activation.

Several lines of evidence support the conclusion that ACSVL3 is indeed an acyl-CoA synthetase. First, the ACSVL3 amino acid sequence contains the two motifs thought to be critical for enzyme activity and also shares 35–42% overall amino acid identity with ACSVL1, ACSB, FATP1, and FATP4, proteins previously shown to have acyl-CoA synthetase activity (10, 18–21). Second, RNA interference studies indicated that disruption of ACSVL3 led to decreased cellular activation of both C16:0 and C24:0. Third, overexpression of the human ortholog of ACSVL3 in COS-7 cells led to an overall increase in VLCFA levels in this disease.

In addition to expression in neurons of the cerebral cortex and cerebellum, both ACSVL3 and BG1 are also expressed in the zona fasciculata of the adrenal cortex, ACSVL3 was most prominent in this zone. Expression was also observed in folicell cells. Whereas BG1 was found primarily in the zona glomerulosa and the zona reticularis. Whereas adrenal ACSVL3 could participate in the synthesis of stored cholesterol esters and/or triacylglycerol, the relationship between fatty acid activation and synthesis and secretion of steroid hormones is unclear. No obvious step in the conversion of cholesterol to steroid hormone is known to require activation of an acyl moiety. However, the presence of two distinct acyl-CoA synthetases, ACSVL3 and BG1, in steriodogenic tissues suggests that they perform a vital metabolic function.

Acknowledgments—We thank Dr. Mario Ascoli for the MA-10 cell line, Dr. Stephen Gould for antibody to PMP70, and Dr. Dennis Vance for antibody to phosphatidylethanolamine N-methyltransferase 2. We also thank Dr. James Hamilton for thoughtful discussions on fatty acid transport.

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