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Acyl-CoA Metabolism and Partitioning

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Abstract

Long-chain fatty acyl-CoAs are critical regulatory molecules and metabolic intermediates. The initial step in their synthesis is the activation of fatty acids by one of 13 long-chain acyl-CoA synthetase isoforms. These isoforms are regulated independently and have different tissue expression patterns and subcellular locations. Their acyl-CoA products regulate metabolic enzymes and signaling pathways, become oxidized to provide cellular energy, and are incorporated into acylated proteins and complex lipids like triacylglycerol, phospholipids, and cholesterol esters. Their differing metabolic fates are determined by a network of proteins that channel the acyl-CoAs towards or away from specific metabolic pathways and serve as the basis for partitioning. This review evaluates the evidence for acyl-CoA partitioning by reviewing experimental data on proteins that are believed to contribute to acyl-CoA channeling, the metabolic consequences of loss of these proteins, and the potential role of maladaptive acyl-CoA partitioning in the pathogenesis of metabolic disease and carcinogenesis.

Keywords

Acyl-CoA binding protein; acyl-CoA synthetase; bubblegum; cancer; fatty acid binding protein; fatty acid transport protein; β -oxidation; metabolic syndrome; triacylglycerol

Partitioning of fatty acids and the formation of fatty acyl-CoAs

Long-chain fatty acids (FAs) derived from either *de novo* synthesis, dietary sources, or the turnover of triacylglycerol (TAG), phospholipids, and cholesterol esters have multiple metabolic fates. These fates include the entry of FAs into pathways of degradation, incorporation or reincorporation into complex lipids, esterification to proteins, and synthesis of eicosanoids. Long-chain FAs can also activate transcription factors, participate in intracellular signaling, and allosterically modulate enzyme reactions (Fig. 1). Apart from roles related to signaling and eicosanoid formation, each of these functions require the formation of a long-chain acyl-CoA by one of 13 acyl-CoA synthetases (ACS) that use long-chain (16–22 carbons) and very-long-chain (>22 carbons) FAs. The 13 ACS isoforms in three subfamilies (ACSL, ACSVL/FATP, ACSBg) are part of the 26-member ACS family, all

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of whose members contain related nucleotide (AMP/ATP) and FA binding motifs (185, 199). Most of the long-chain ACS proteins include a transmembrane domain anchor at the N-terminus, but several of the isoforms have 2 different start sites, one of which lacks an N-terminal transmembrane anchor, or have alternative internal exons that result from differential splicing (180). The nomenclature of the genes encoding the long-chain acyl-CoA synthetases was unified in 2004, and no ACSL2 exists (121).

Long-chain ACS isoforms activate FAs in an energetically costly two-step reaction that uses the equivalent of two high-energy bonds (198, 199):

As critical signaling molecules, acyl-CoAs are allosteric inhibitors of adenosine nucleotide translocase (ANT) (99, 175), liver glucokinase (192), acetyl-CoA carboxylase (ACC) (147, 150), HMG-CoA reductase (97), phosphofructokinase-1 (75), and hormone sensitive lipase (HSL) (75). Acyl-CoAs can also stimulate the release of transport vesicles (146, 158). Long-chain acyl-CoAs are excellent detergents and form micelles in aqueous solutions with the CoA groups exposed to the water phase (69). The measured critical micellar concentrations (CMC) for the most common long-chain acyl-CoAs, 18:1-CoA and 16:0-CoA, are about 32 μM and 42 μM , respectively (25, 179). However, within cells, acyl-CoAs are generally bound to proteins and membranes, so the concentration of acyl-CoAs would be too low to self-aggregate. Because of their amphipathic nature, acyl-CoAs can interfere with membrane integrity by acting as detergents; when high concentrations of acyl-CoAs are present, the permeability of membranes to small molecules like sucrose and citrate is altered (2). Myristoylation or palmitoylation of proteins requires acyl-CoAs, but to our knowledge, no changes in protein acylation have been found in mice or cells deficient in any ACS.

Because most mammalian cells contain several different long-chain ACS isoforms, we have hypothesized that each isoform may partition or channel its long-chain FA substrates into specific downstream pathways. Hypothesized differences in cell function include the use of the activated FAs for pathways that synthesize glycerolipids and cholesterol esters, for pathways of FA elongation or desaturation, for degradative pathways in the mitochondria, ER, and peroxisomes, for protein acylation, and for transcriptional regulation. In this review we will focus primarily on evidence that either supports or refutes the concept of specialized roles for the ACS isoforms that activate long-chain FAs.

Fatty acid use by acyl-CoA synthetases

FAs are carboxylic acids with long-chain hydrocarbon side groups. In animals, the predominant long-chain FAs are those of 16 and 18 carbons with varying degrees of saturation. FAs of 20 carbons, such as 20:4 ω 6 and 20:5 ω 3, form a small percentage of the total FA content in animals, but are precursors for multiple subfamilies of eicosanoids. The question of how FAs are transported into cells remains controversial, but whether FAs enter

via transport proteins or flip-flop across the plasma membrane, their thioesterification to coenzyme A prevents their exit. It has been variously speculated that FA entry might occur via junctions between the plasma membrane and the endoplasmic reticulum (ER). Alternatively, entry may be mediated by fatty acid binding protein (FABP) isoforms (47), or facilitated by the FA transport proteins (FATP/ACSVL) (52) that are themselves acyl-CoA synthetases. Several groups, however, have shown that the directionality of FA entry or “vectorial transport” is driven by the intracellular metabolism of the FAs (4, 47, 122).

Understanding the process that channels FAs into specific metabolic pathways requires consideration of the physical chemistry of hydrophobic FAs which must move in an aqueous environment. Further, in order to minimize futile cycles, synthetic and degradative pathways must be separated from one another both spatially and temporally. Cells overcome the problem of hydrophobicity by converting the FA to an amphipathic molecule by the thioesterification of coenzyme A to the carboxyl group. The ability of the cell to vectorially channel fatty acyl-CoAs towards or away from a metabolic pathway forms the basis of partitioning, and is likely to vary with cell type, intracellular location of carriers and enzymes, cellular energy status, and hormonal signals.

Acyl-CoA binding protein and fatty acid binding proteins

Selective partitioning of acyl-CoAs within cells requires methods to overcome hydrophobicity, because the amphipathic fatty acyl-CoAs can move freely both in the aqueous cytosol and in membrane monolayers. Two protein families, FA binding proteins (FABPs) and acyl-CoA binding protein (ACBP), may aid in FA and acyl-CoA movement within cells and are believed to protect cell membranes from the detergent effects of the acyl-CoAs.

FABPs are isoforms of a 10 member intracellular lipid-binding protein family which reversibly binds hydrophobic ligands and, in theory, traffics them throughout the cytosol to various organelles (178). A recent comprehensive review of FABP isoforms identifies metabolic alterations in knockout models, but definitive functions have not been established (187). FABP isoforms are ubiquitously expressed, but differ in stoichiometry, affinity and specificity toward related ligands that include FAs, acyl-CoAs, eicosanoids, and peroxisome proliferator-activated receptor ligands.

The amount of a specific FABP isoform in any tissue appears to reflect the tissue’s lipid-metabolizing capacity. For example, in hepatocytes, adipocytes and cardiomyocytes, which specialize in lipid metabolism, FABPs make up 1–5% of all cytosolic proteins (61). Evidence for the importance of FABPs in lipid metabolism comes from loss-of-function studies in mice. FABP1, which is strongly expressed in liver and intestine, is the only isoform that binds both FA and fatty acyl-CoA; the other FABP isoforms bind only FA (61, 165). Two independent *Fabp1*^{−/−} mouse models have been generated but, despite the importance of lipid metabolism in liver and intestine, neither model has an overt phenotype (119, 145). When mice are fed low fat chow, the liver of *Fabp1*^{−/−} mice appears histologically normal, and serum TAG and total free FA levels are unchanged, although alterations in individual FA species are observed (120). In one of the *Fabp1*^{−/−} models, the

hepatic content of phospholipid, cholesterol, and cholesterol ester is greater than in the controls (120). Although the loss of *Fabp1* reduces hepatic FA binding capacity, total liver lipid content, including TAG and free FA, remains unchanged. Only under extreme fasting conditions (48 hours) does the diminished FA binding capacity in the knockout mice reduce hepatic FA uptake, FA oxidation, and TAG levels (145). Although differences were observed in the effects of knockouts of the liver-type FABP1 and the intestinal FABP isoform, information related to acyl-CoAs was not provided (49). The adipose-type FABP4 (also known as aP2) is the major isoform in white and brown adipose tissue and macrophages (22, 113). Because disruption of the *Fabp4* gene in mice increases the cytosolic content of free FA, FABP4 is generally thought to facilitate FA transport between intracellular compartments for storage or export (23, 67), however this model provides no evidence for mistargeted intracellular FA. The heart-type FABP3, which is most abundantly expressed in heart, skeletal muscle, and brain, is induced in rat brown adipose by acute cold exposure (27, 206), or by a β_3 -adrenergic receptor agonist in mouse subcutaneous white adipose as cells became “beige” (141). Physiologically, the *Fabp3*^{-/-} perform each of these processes knockout model that most strongly supports a specific function for FABP in FA partitioning, because *Fabp3* deficient mice have defective FA oxidation and are more reliant on glucose as a substrate for energy production in both cardiomyocytes and muscle (39, 171). In addition, *Fabp3*^{-/-} mice are extremely cold intolerant (196).

In contrast to the multiple FABP isoforms, only a single ACBP has been identified. ACBP binds medium- and long-chain acyl-CoAs with high affinity, but does not bind free FA, acylcarnitine, or cholesterol (166). The affinity for acyl-CoAs is so much higher for ACBP than for liver-type FABP1 that it was suggested that ACBP is the major carrier of acyl-CoA in all cells including hepatocytes (163). ACBP expression and concentration are highest in liver, but ACBP is also present in high levels in the adrenal cortex, testis and epithelial cells. Because these tissues and cells specialize in secretion, they have high energy needs and may require ACBP to shuttle fatty acyl-CoAs towards energy producing oxidative pathways (7). Disruption of the ACBP homologue in yeast (ACB1), does not affect phospholipid synthesis or turnover, indicating that ACBP is not required for glycerolipid synthesis in yeast. However, yeast deficient in ACB1 have disordered plasma membrane structures as a result of aberrant and reduced sphingolipid synthesis (48). Highlighting the importance of ACBP in *in vivo* metabolism are studies from two separate *Acbp* deficient mouse models. In the first model, the authors concluded that ACBP is an essential protein required for embryonic development because an implantation defect results in embryonic lethality (94). The second knockout is viable, but does not indicate a role for ACBP in trafficking acyl-CoAs, although liver acyl-CoA levels are ~40% lower than in controls. Instead, the main effect of the knockout was an impaired skin barrier and the development of alopecia (5). In addition to their skin phenotype, *Acbp*^{-/-} mice undergo a crisis around the weaning period, exhibiting weakness and poor weight gain (143). At this time point, SREBP maturation is impaired and SREBP target genes involved in cholesterol biogenesis are not appropriately upregulated. It is unclear why two separately generated *Acbp* knockout models exhibit these two disparate phenotypes, but these models indicate that ACBP and the acyl-CoAs they bind are essential for normal growth and development.

Complex lipid synthesis and degradation

Exogenous FAs and those synthesized *de novo* primarily enter pathways of complex lipid synthesis to produce stored energy depots and phospholipid membranes, or they enter degradative pathways; degradation includes mitochondrial β -oxidation, peroxisomal β - or α -oxidation (40), and ER ω -oxidation (152, 176). Evolutionarily, cells have developed organelles that perform each of these processes, which is evidence of an additional level of fatty acyl-CoA partitioning. Further evidence for this type of partitioning may lie in the localization of synthetic and oxidative enzymes with specific organelles. Although it is generally thought that organelles have a single specialized function, in fact, both glycerolipid synthesis and ω -oxidation occur in the ER, whereas both alkyl lipid synthesis and β -oxidation take place in peroxisomes, and both FA synthesis and β -oxidation occur in mitochondria. It is not known how FAs and acyl-CoAs are independently directed into separate pathways within an organelle.

Glycerolipid Synthesis

The initial and committed step for the *de novo* synthesis of TAG and all glycerophospholipids is the acylation of *sn*-glycerol-3-phosphate with a fatty acyl-CoA to form 1-acyl-*sn*-glycerol-3-phosphate (lysophosphatidic acid) catalyzed by glycerol-3-phosphate acyltransferase (GPAT) (3). GPAT isoforms are present in the outer mitochondrial membrane (GPAT1 and -2) and in the ER (GPAT3 and -4) (201). Overexpression of GPAT1 in either isolated primary rat hepatocytes or *in vivo* in rat liver causes steatosis, confirming the important role of GPAT in initiating hepatic TAG synthesis (102, 140). Mouse knockout models of the GPAT isoforms have provided clues as to the partitioning of acyl-CoAs towards synthetic or oxidative pathways. In studies comparing *Gpat1*^{-/-} and *Gpat4*^{-/-} mice, for example, GPAT1, but not GPAT4, is required to incorporate *de novo* synthesized FA into TAG and to divert these FAs away from oxidation (200). The ER GPATs are likely to channel exogenously derived acyl-CoAs towards TAG synthesis. It is possible that the location of GPAT1 at the outer mitochondrial membrane serves to divert *de novo* synthesized fatty acyl-CoAs away from carnitine palmitoyltransferase-1 (CPT1)-mediated entrance into the mitochondria where they would be oxidized. This hypothesis makes sense from a cellular homeostatic standpoint in that newly synthesized FAs would not be oxidized but, instead, stored for later use when energy supplies are low. This example of acyl-CoA partitioning at the level of the mitochondria is at least partly controlled by the energy status of the cell and by the animal's hormonal status. With low cellular energy, activated AMP-activated kinase inhibits GPAT1 and favors mitochondrial β -oxidation, whereas dietary carbohydrate and insulin upregulate GPAT1 and promote TAG synthesis (139, 200).

Acyl-CoA degradation

The regulation of mitochondrial β -oxidation depends on cellular energy status. When ATP levels are low, acyl-CoAs are transported into the mitochondria by carnitine palmitoyltransferase-1 (CPT1). Mitochondrial β -oxidation of fatty acyl-CoAs is the major route of FA degradation, but very-long-chain FAs and branched-chain FAs are poorly oxidized in mitochondria, and, instead, are degraded in peroxisomes. The β -oxidation capability of peroxisomes terminates at medium-chain acyl-CoAs and produces chain-

shortened acyl-CoAs and acetyl- and propionyl-CoAs, which are transported out of the peroxisome as short- to medium-chain acyl-carnitines to be completely oxidized in the mitochondria (71). Depending on its chain length, the acyl-CoA is converted to the corresponding carnitine ester by one of two peroxisomal enzymes, carnitine acetyltransferase (CRAT) or carnitine octanoyltransferase (CROT) (42).

Despite the high-energy cost of acyl-CoA synthesis, numerous acyl-CoA thioesterases (ACOT) reverse this reaction. Because several ACOTs are upregulated by peroxisome proliferator-activated receptor (PPAR) - α under the same conditions that promote acyl-CoA synthesis and oxidation, their physiological function remains unclear. The requirement for free CoASH within mitochondria is very high, reflecting the importance of CoASH in both the citric acid cycle and β -oxidation, so it is possible that ACOT operates to ensure sufficient free CoASH to maintain optimal mitochondrial function.

Two distinct types of ACOT proteins (types I and II) are structurally dissimilar but functionally homologous, suggesting the proteins evolved through convergent evolution (9). Type I ACOTs (ACOTs 1–6) contain N-terminal β -sandwich and C-terminal α/β hydrolase domains, whereas type II ACOTs (ACOTs 7–13) use N-terminal hotdog-fold thioesterase domains (89). Of the type I ACOTs, ACOT1 is located in the cytosol, ACOT2 in mitochondria, and ACOT3–6 in peroxisomes. Of the type II ACOTs, ACOT8 is located in peroxisomes, ACOTs 7, -11, -12 and -13 are in the cytosol, and ACOT9, -10, and -13 are mitochondrial (89). Each of the ACOT isoforms has an acyl-chain length preference: recombinant ACOT3 prefers long-chain acyl-CoAs (12 carbons – 18 carbons), whereas ACOT5 prefers medium-chain acyl-CoAs like C10-CoA (202). ACOT8 uses acyl-CoA substrates ranging from 2 carbons to 20 carbons, both saturated and unsaturated (71, 72), and is strongly inhibited by CoASH (72). This broad substrate specificity and CoASH inhibition suggest that ACOT8 may sense CoASH content and regulate intra-peroxisomal acyl-CoA levels in order to ensure optimal flux through β -oxidation. Because few knockout models have been reported, it is difficult to understand the specific roles of the ACOTs. However, ACOT13 (Them2) deficient mice fed a high fat diet are protected from weight gain, hepatic steatosis and glucose intolerance, implying that ACOT13 is important for hepatic β -oxidation and gluconeogenesis (80). Additionally, ACOT13 deficient mice are more able to adapt to acute cold exposure, suggesting that ACOT13 in brown adipose (BAT) may diminish FA channeling into heat production or UCP1 activation (80). The studies involving both ACOT8 and ACOT13 suggest that ACOTs modulate acyl-CoA flux through oxidative pathways. Thus, there may be a reciprocal relationship between the ACSLs and ACOTs to regulate the metabolic fates of acyl-CoAs via either mitochondrial or peroxisomal oxidation.

The ω -hydroxylation of medium and long-chain saturated FAs is mediated by the family of Cyp450 4A fatty acid omega hydroxylases in hepatocytes. This represents an important secondary pathway for FA metabolism in liver under conditions in which hepatocellular fatty acid flux rates exceed the capacities of the dominant esterification and mitochondrial β -oxidation pathways (152). This alternative pathway, which synthesizes dicarboxylic fatty acids, diminishes acyl-CoA flux through both the mitochondrial and peroxisomal β -

oxidative pathways, perhaps preventing mitochondrial dysfunction. The ω -oxidation of 20:4 ω 6 initiates the synthesis of the eicosanoid family of signaling molecules (14).

Acyl-CoA synthetases and fatty acid transport proteins

The 26 enzymes that comprise the ACS family have significant sequence homology with highly conserved domains that correspond to an ATP/AMP binding site and a FA binding site (199). Crystallization studies of bacterial and yeast acyl-CoA synthetases (58, 64, 78) suggest that when the enzyme binds ATP, this induces a conformational change that opens a “gate” to the FA binding site (64). Once bound, the FA is converted to an FA-AMP intermediate. Coenzyme A (CoA) is then bound to the FA-AMP, and AMP is removed. Finally, the acyl-CoA and AMP are released, and the enzyme reverts to its original form.

Acyl-CoA synthetases are named for the FA chain length of the preferred substrate. Short-chain acyl-CoA synthetases (ACSS) activate acetate, propionate, and butyrate. Medium-chain acyl-CoA synthetases (ACSM) prefer FAs of 6-10 carbons, but can also activate longer-chain FAs. Long chain acyl-CoA synthetases (ACSL) activate FAs of 12-20 carbons. Very-long-chain acyl-CoA synthetases (ACSVL) can activate FAs longer than 20 carbons, but prefer 16 and 18 carbon FAs. Overlap in FA preference between the groups is common, and within each subfamily, some individual isoforms have preferences for a specific chain length or saturation. The FA saturation and chain length preference of each ACS enzyme has been hypothesized to relate to the size and shape of the FA-binding site (64). Site-directed mutagenesis of ACSL4 confirmed the FA-binding site and showed that specific amino acids in this site help to determine FA preference (186). In addition to FAs, certain ACSVL isoforms can use other molecules as substrates. ACSVL6 (FATP5) activates bile acids (70, 184), and ACSVL1 (FATP2) activates 3 α , 7 α , 12 α -trihydroxy-5 β -cholestanoate (132).

Although we initially hypothesized that the subcellular location of each acyl-CoA synthetase determines acyl-CoA partitioning, several of the ACS isoforms have been found on multiple membranes. For example, ACSL1 has been identified on the plasma membrane, ER, nucleus, mitochondria, peroxisomes, GLUT4 vesicles, and lipid droplets (6, 45, 51, 90, 100, 177). Several explanations are possible for the abundance of putative subcellular locations. The location of ACSL1 may actually differ in different cell types, perhaps related to the splice variants that are present (180). Alternatively, the localization studies may not have examined purified organelles. With overexpression studies, the protein may have been mislocalized. Finally, an ACS isoform may move from one location to another under different physiological conditions. For example, FATP1 may translocate from ER to the plasma membrane after insulin stimulation (205).

Supporting a relationship between location and function, endogenous ACSL1 in liver has been found on ER and mitochondria, corresponding to its effects on neutral lipid synthesis and FA oxidation (103), whereas cardiac ACSL1 has been identified on mitochondria, consistent with its large effect on FA oxidation (38). If one assumes that the identified location is accurate, one might suggest that ACSL3, which has been found on lipid droplets and ER, participates in FA uptake and glycerolipid biosynthesis (8, 159). In liver, FATP4 is located on the ER (133), ACSL4 is located on the ER, mitochondrial associated membrane,

and peroxisomes (100, 133), and ACSL5 has been found on the mitochondria (100, 101). Specific functions related to these sites have not been investigated.

Alternatively, studies of FA uptake may be an exception to the idea that location dictates function, because changing the expression level of intracellular ACSLs or FATPs alters cellular FA retention (47, 133, 207). In 3T3-L1 cells, for example, overexpressing FATP1 or FATP4 on the ER or overexpressing ACSL1 on the mitochondria increases FA uptake and retention by 40% (207). This increase in FA uptake may have occurred because of the altered FA or acyl-CoA concentration gradient as intracellular FAs are converted to acyl-CoAs, or because the addition of the CoA has trapped the FAs within the cell.

One mechanism by which substrates are sequentially channeled through a pathway is via multi-enzyme complexes (181). Thus, the location of ACSL1 may dictate where fatty acyl-CoAs are directed by allowing the ACSL to interact with proteins involved in downstream processing of fatty acyl-CoAs. For example, ACSL1 co-immunoprecipitates with CPT1a and voltage-dependent anionic channel (VDAC) on the outer mitochondrial membrane (95). CPT1a catalyzes the conversion of acyl-CoA to an acyl-carnitine, which is required to transport the FA into the mitochondrial matrix for oxidation (126). This complex of ACSL1, CPT1a, and VDAC could facilitate the transfer of the acyl-CoA product to CPT1. On the ER, entry into pathways of complex lipid synthesis might be similarly enhanced by protein interactions between ACSL1 and various acyltransferases. An alternative to a direct protein-to-protein transfer might be an ACS-mediated increase in the local concentration of its acyl-CoA product, thereby effecting a localized increase in the amount of substrate available for the downstream pathway.

Regulation of long-chain ACS isoforms

The expression of the ACS isoforms is highly regulated by both the nutrient status of the cell and by the developmental stage of the animal. Although changes in expression may suggest an association with lipid synthesis or FA oxidation, definitive roles have not been clarified. Total ACS activity in rat liver increases 7-fold from birth to adulthood (24), possibly related to the neonatal switch from maternally-supplied glucose in the fetus to the high fat content of milk delivered to the neonate after birth. In liver, *Acs11* and *Acs14* are upregulated with fasting and down-regulated with refeeding of a high sucrose diet. *Acs15* shows the opposite pattern, with higher expression during fasting and lower expression with refeeding (123), suggesting the potential for different functions or preference for endogenous vs. exogenous FA of the different isoforms. A high fat diet increases the expression of liver *Acs11* (123, 190). In liver, a 48-hour fast decreases the amount of ACSL1 on microsomes, whereas refeeding increases microsomal ACSL1 (100), changes that might suggest an association with TAG synthesis. On the other hand, changes in expression vary in different tissues. For example, a fasting/sucrose-refeeding protocol increases *Acs15* mRNA in liver, but not in intestine (151). In hamster liver, *Acs13* expression decreases with high fructose feeding (34), and increases with a high fat, high cholesterol diet (203).

Total long-chain ACS activity in adipose is decreased by exercise and noradrenaline (59, 174) and by fasting (123), stimuli which increase lipolysis, suggesting a role in re-

esterification. Norepinephrine or glucagon treatment rapidly decreases ACSL activity in adipocytes, and insulin quenches the effect of norepinephrine on ACSL activity within minutes (59). This rapid change in ACSL activity suggests that post-translational modifications occur to modulate ACSL activity in response to nutritional status and other stimuli. Using mass spectrometry, 25 phosphorylation and 15 acetylation sites were identified on ACSL1 in liver and brown adipocytes. When seven of these sites were mutated to mimic phosphorylation or acetylation, the activity of ACSL1 decreased, confirming the importance of post-translational modifications in regulating ACSL1 activity (43). The phosphorylation of ACSL1 and ACSL4 is also altered by fasting and *ob/ob* genotype in the liver (56), but how these changes in phosphorylation affect activity has not been studied.

PPAR γ agonists increase *AcsI* expression in adipocytes (118). Because PPAR γ is necessary for adipocyte differentiation and lipid accumulation, ACSL1 may play a role in early lipid accumulation, and *AcsI* gene transcription in adipocytes is increased by overeating, insulin, and triiodothyronine (82, 118, 174). Surprisingly, however, an adipose-specific knockout of *AcsI* resulted in larger adipose depots and diminished FA oxidation (37).

In the heart, PPAR α increases the transcription of *AcsI* (35). Incubation with either insulin or oleate also increases *AcsI* and *AcsI3* expression in rat cardiomyocytes (35). Between birth and adulthood, ACSL activity in mouse heart increases more than 10-fold, and *AcsI* mRNA increases 2.5-fold. During this same period, the heart switches from primarily glucose to FA as the preferred substrate for energy production, consistent with ACSL1-mediated activation of FAs destined for oxidation (38). *AcsI3* expression decreases more than 2-fold between embryonic day 16 and post-natal day 7, indicating its importance in fetal heart development (28).

AcsI3 mRNA is upregulated under disparate conditions, including induction by poliovirus protein 2A infection of HeLa cells (142). This requirement of ACSL3 for viral proliferation appears to be related to the incorporation of activated FAs into phosphatidylcholine. ER stress via activated GSK-3 β induces the expression of *AcsI3* in the hepatocarcinoma cell line HuH-7 and in mouse liver, and an shRNA-mediated knock down of *AcsI3*, but not *AcsI*, blocks ER stress-related lipid accumulation (16).

Channeling

Evidence that acyl-CoAs are channeled or partitioned into different pathways was first obtained in *Saccharomyces cerevisiae*, which expresses three well-studied long-chain ACS isoforms (termed Faa1-3p). Analysis of null alleles showed that the ability to use exogenous FA required Faa1p, whereas Faa2p and Faa3p activate only endogenous FA, and that none of these Faa proteins channel FA towards β -oxidation (79). Replacing yeast Faa null mutants with rodent ACSL or FATP isoforms indicated that complementation varies for FA uptake and incorporation (30, 194). Similarly, in ACS-deficient *Escherichia coli*, complementation studies showed that each of the 5 rat ACSL isoforms differs in its ability to channel FA into phospholipid synthesis and β -oxidation (15).

The differential effects of inhibiting FA incorporation into TAG and phospholipid in cultured rat hepatocytes and human fibroblasts also suggests the possibility of channeling in mammalian cells. Thus, the FA acid analog triacsin C decreases [^{14}C]oleic acid incorporation into TAG relative to phospholipid and oxidation products (73, 138). However, because triacsin C is a competitive inhibitor of ACSL1, ACSL3, and ACSL4 (193, 195), inhibition studies could not identify roles for the individual ACSL isoforms.

What can we learn from loss of function studies?

One way to learn about function is to observe the effect on animal or cell biochemistry in the absence of a particular gene. Knockouts have been made for several of the genes that encode proteins able to activate long-chain fatty acids. Multiple caveats impede the ability to come to firm conclusions based on knockout models. Problems include the fact that many of the ACSL isoforms have splice variants or different start sites, that the expression or activity of other ACSL isoforms may increase to compensate for the absent enzyme, that the long-term absence of a particular enzyme may induce changes in the cell or animal that modify or distort the effect of the missing protein, and that an ACSL isoform may not only be located on several subcellular membranes, but its location and function may also differ in different tissues. Thus, all interpretations of function derived from knockout animals remain tentative.

Long-chain acyl-CoA synthetases

ACSL1

ACSL1, the most extensively studied isoform, is highly expressed in liver, heart, white and brown adipose, and skeletal muscle (35). Multi-tissue and tissue-specific knockouts indicate that ACSL1 has different functions in different tissues. In liver, the knockout causes a 50% decrease in total ACSL activity, together with a 25-35% decrease in hepatic acyl-CoA content and a 20% decrease in the incorporation of [^{14}C]oleate into TAG (103). Although incorporation of oleate into phospholipid appeared to be unaffected, an analysis showing altered phospholipid species suggests that ACSL1 contributes specifically to the incorporation of 18:0-CoA (103). Because liver long-chain acyl-carnitines are 50% lower than in controls, it was concluded that lack of ACSL1 in liver impairs trafficking of acyl-CoAs into both TAG and oxidation pathways. These data could be interpreted as showing that ACSL1 either does not target its acyl-CoA product into a specific pathway or that, because of its dual location on both the mitochondria and the ER, ACSL1 partitions its product into both synthetic and degradative pathways.

In contrast to liver, tissue-specific knockouts of *Acs1* in highly oxidative tissues like heart (38) or white or brown adipose (37) strongly suggest that channeling towards β -oxidation is primary. In these tissues, the knockout causes an 80-90% decrease in total long-chain ACS activity together with profound decreases in FA oxidation, without altering the incorporation of [^{14}C]oleate into TAG or phospholipid. In *Acs1*^{-/-} heart, the uptake of the FA analog Br-[^{14}C]palmitate is lower than controls, whereas uptake of 2-deoxy-[^{14}C]glucose increases 8-fold, and in ACSL1-deficient brown adipose, the defect in FA oxidation impairs the ability of the mice to maintain a normal body temperature when placed at 4 °C (37, 38). Although *Acs13* mRNA is upregulated in both heart and brown adipose, this isoform is apparently

ineffective in supplying acyl-CoA for oxidation and thermogenesis. Similarly, in white adipose, the loss of ACSL1 activity causes a 50% decrease in [^{14}C]18:1 oxidation, but no alteration in FA incorporation into TAG or phospholipid; in fact, compared to controls, white adipose depots are 40% larger (37). Interestingly, an shRNA-mediated knockdown of *Acs11* in 3T3-L1 adipocytes supported a role for this isoform in FA re-esterification, suggesting that the function of ACSL1 in these cells may differ from that in mouse adipose tissue (112).

In macrophages from diabetic mice and humans, upregulated ACSL1 increases the metabolism of 20:4 ω 6 and enhances inflammation and atherosclerosis (84). Unlike the deficiency in liver, adipose, and heart, ACSL1 deficiency in macrophages did not impair either FA oxidation or the accumulation of neutral lipid (84). Surprisingly, the deficiency caused a reduction in the levels of 20:4 ω 6-CoA and blocked the increased production of PGE₂ that is usually observed in mice with type-1 diabetes. It was speculated that this finding was the result of either limited uptake and activation of 20:4 with depletion of the membrane phospholipid pool available as a substrate for phospholipase A2 (83) or was caused by lack of ACSL1-mediated activation of 18:2 as a substrate for the elongation and desaturation enzymes that convert 18:2-CoA to 20:4-CoA (85). In addition, when macrophages are activated by a variety of inflammatory signals, *Acs11* mRNA and protein increase markedly (167). In contrast, the absence of ACSL1 reduces lipopolysaccharide (LPS)-stimulated increase in 16:0-, 18:1-, and 20:4-CoA levels, diminishes multiple acyl- and alkyl-PC species, and decreases the turnover of 20:4 in several phospholipids, but does not affect the LPS-stimulated increase in ceramide species (167). Similar to macrophages, the lack of obvious partitioning in *Acs11* null endothelial cells results in a >50% decrease in total ACS activity but no change in 16:0 oxidation (106). These data show clearly that the function of ACSL1 in macrophages and endothelial cells differs fundamentally from its function in oxidative tissues and liver.

ACSL3

ACSL3 is expressed on the ER and on lipid droplets in most tissues (8, 159), but it is not known whether lipid accumulation is related to the presence of this isoform on one or both of these organelles. Mice deficient in ACSL3 have not been reported. Because FAs are ligands for the PPARs, enhanced conversion of FA to acyl-CoA could diminish PPAR activation; conversely, the absence of conversion might increase PPAR activation and the resulting transcription of PPAR target genes. To examine the role of ACSLs in regulating gene expression, small interference RNA (siRNA) was used to knock down several ACSL and FATP isoforms by at least 70% in rat primary hepatocytes (11). Only the knockdown of *Acs13* diminished the reporter activity of PPAR γ , ChREBP, SREBP1c, and liver X receptor (LXR) - α (but not PPAR α), the lipogenic target genes of these transcription factors, *de novo* FA synthesis from [^{14}C]acetate and, consequently, TAG and phospholipid synthesis. Because synthetic ligands did not overcome the inhibition, the authors concluded from this study that ACSL3 normally produces acyl-CoAs or downstream metabolites that regulate specific transcription factor corepressors or coactivators.

Knockdown of *Acs13* diminishes the incorporation of [^{14}C]acetate into lipid extracts, and *Acs13* expression increases in sucrose-fed mice and in *ob/ob* mice. These data suggest that

ACSL3 mediates transcriptional control of hepatic lipogenesis (11). Indirectly confirming this role, a fructose-induced knockdown of *AcsI3* in hamster liver reduces the expression of LXR- α , LXR β , and retinoid X receptor (RXR) β , although these changes could have resulted from the fructose itself rather than from a direct effect of ACSL3 (34).

In addition to its location on the ER, ACSL3 moves to lipid droplets when FAs are added to the medium of cultured cells, suggesting that it may provide acyl-CoAs for glycerolipid synthesis at the lipid droplet surface (45). It has been suggested that the N-terminus region that anchors the protein has a hairpin membrane topology on the cytosolic leaflet of the ER membrane and, presumably, on lipid droplets (159).

ACSL3 also interacts with the Src-family tyrosine kinase Lyn in the Golgi of HeLa and COS-1 cells and allows Lyn to be transported to the plasma membrane (149). Because a mutant of ACSL3 that lacks the AMP/ATP-binding motif can still function for Lyn transport, it was concluded that ACSL activity was not required for this role, however, enzyme activity was not measured (149). Although mammalian synthesis of 4 and 16-carbon acyl-CoAs for protein acylation have not been described, in *Xenopus*, triacsin C-mediated inhibition of ACSL activity blocks the palmitoylation of G α (197).

ACSL4

AcsI4 expression is prominent in the adrenal gland, ovary, testis, and brain, where it preferentially uses 20:4 ω 6 and 20:5 ω 3 (81). Because of this preference for polyunsaturated FA, ACSL4 has been implicated in eicosanoid metabolism. The importance of ACSL4 in neural development is demonstrated by human mutations that cause a form of X-linked mental retardation (130). In *Drosophila*, dAcsl is said to be “functionally homologous” to human ACSL4. dAcsl mutant flies have impaired synaptic vesicle transport and function, and accumulate axonal aggregates that can be rescued by human ACSL4. It was therefore suggested that dAcsl4 regulates the retrograde transport of synaptic vesicles, possibly by increasing specific lipid signaling molecules (111, 208). The signaling molecules might be related to eicosanoids, because siRNA treatment of several lines of cultured cells results in 3- to 7-fold increases in arachidonic acid metabolites like prostaglandins and hydroxyeicosatetraenoic acids (HETEs) when the cells are stimulated with IL1- β (93). Evidence that ACSL4 in the brain interacts with a signaling complex comes from a study of mice deficient in α -synuclein, a brain microsomal protein that promotes FA uptake and incorporation into phosphatidylcholine (55). In the absence of α -synuclein, the activity of total long-chain microsomal ACS decreases in brain, possibly due to its interaction with brain ACSL4. It was suggested that this complex may regulate the channeling of polyunsaturated FAs into selected phospholipids species (55), however other isoforms may also be responsible. ACSL6, too, activates FA that are incorporated into phospholipids in neuronal cells (117), and an siRNA knockdown of *AcsI1* reduces the levels of some phosphatidylcholine species that contain 20:4 ω 6 (93). In a related study in INS 832/13 cells, an siRNA knockdown of *AcsI4*, but not *AcsI5*, decreases basal and FA-stimulated glucose-stimulated insulin secretion by increasing the media content of epoxyeicosatrienoic acids (EETs) (91). In this study, the membrane content of EETs was reduced, suggesting that ACSL4 controls the intracellular content of unesterified EETs.

Additional evidence that ACSL4 regulates eicosanoid synthesis comes from the manipulation of ACSL4 splice-variant-1 in human arterial smooth muscle cells (54). Overexpression markedly increases the synthesis of 20:4 ω 6-CoA and its incorporation into phosphatidylethanolamine, phosphatidylinositol, and TAG. Because the amount of unesterified 20:4 ω 6 is reduced, compared with controls, prostaglandin E₂ (PGE₂) secretion is blunted. Conversely, inhibiting ACSL4 activity pharmacologically with either triacsin C or rosiglitazone, acutely increases PGE₂ release (54). Surprisingly however, long-term down regulation of *Acs4* by siRNA diminishes PGE₂ secretion (54). Because ACSL4 potentially controls both free arachidonate and arachidonate metabolites, it is an attractive target for treatment of inflammatory diseases, as well as for other disorders driven by dysfunctional eicosanoid metabolism.

ACSL5

The expression of *Acs15* is highest in intestinal mucosa, with lower amounts found in lung, liver, adrenal gland, adipose tissue, kidney, and brown adipose (129, 151). The hormonally-controlled increase of *Acs15* mRNA by insulin and by SREBP-1c, suggests that this isoform plays a role in TAG synthesis (1, 66). Supporting this interpretation, when *Acs15* was decreased by siRNA in primary rat hepatocytes, incorporation of radiolabeled 18:1 into TAG, phospholipids, and cholesterol esters was diminished (10). It is not known whether this apparent partitioning occurs via channeling to specific acyltransferases. On the other hand, cold exposure or treatment with the β 3-adrenergic receptor agonist CL316,243 causes white adipose to “beige” with increased expression of *Ucp1*, *Fabp3*, and FA oxidation genes, as well as *Acs15* (141). It may be that in adipose tissue, ACSL5 is linked to FA oxidation rather than to complex lipid synthesis.

The knockout of *Acs15* decreases total long-chain ACS activity 60% in jejunum with no alteration in the mRNA expression of the other major isoforms (129). Surprisingly, absorption of gavage oleate is normal in *Acs15* null mice, and when fed a high fat diet, weight gain with a high fat diet is similar to that of controls. These data do not support a critical function of ACSL5 in the absorption of dietary lipid.

ACSL6

Acs16 is most highly expressed in brain (46). Splice variants near the nucleotide binding site alter the enzyme properties so that the K_m value for ATP of ACSL6_v1 is 8-fold higher than that of ACSL6_v2 (195). An siRNA against *Acs16* in mouse neuroblastoma cells inhibits proliferation and neurite outgrowth, suggesting a block in phospholipid synthesis (86). This hypothesis is strengthened by studies in which overexpressed ACSL6 in differentiating PC12 cells enhances the internalization and accumulation of 22:6 ω 3 and the incorporation of [¹⁴C]-labeled 18:1, 20:4 ω 6, and 22:6 ω 3 into phospholipids and TAG. The authors concluded that ACSL6 does not channel specific FAs into TAG versus phospholipid, but that the enzyme functions primarily in docosahexanoic acid metabolism during neurite outgrowth (116, 117). Despite these intriguing studies, no additional information is available on the mammalian ACSL6.

The FATP/ACSVL/SCL27A Family

The first member of the FATP/ACSVL family was discovered by an expression cloning method that used a cDNA library from 3T3-L1 adipocytes to select proteins that facilitated the uptake of BODIPY 3823, a fluorescent fatty acid analog (168). Because the discovered protein was thought to transport long-chain fatty acids across the plasma membrane, it was named fatty acid transport protein (FATP), and the gene family was designated as SCL27A. Several of the FATP proteins were subsequently shown to be located on internal cellular membranes and, because they are members of the ACS family and most are long-chain and very-long chain acyl-CoA synthetases, they have been renamed ACSVL1-6. Whether these proteins have separate transport and activation functions remains controversial (52, 77). FATPs that are not located on the plasma membrane are thought to enhance FA uptake into cells because the FAs become trapped intracellularly as acyl-CoAs, whose subsequent metabolism ensures that uptake is unidirectional (207). The outlier in this family is FATP5, a bile acid-CoA synthetase that does not activate fatty acids (70, 184). Specificity of the functions for most of the ACSVL/FATP proteins remains inconclusive.

FATP1 (ACSVL4/SLC27A1)

Fatp1 mRNA is prominent in adipocytes, brain, heart, and skeletal muscle (168). Most studies of FATP1 have focused on its purported function as a FA transporter (52). In the mouse knockout fed a high fat diet, skeletal muscle contains less TAG, diacylglycerol and acyl-CoA than do wild type controls, and the animals are protected from fat-induced insulin resistance and intramuscular accumulation of fatty acyl-CoAs (88). FATP1 in brown adipose is upregulated in response to cold, and in mice null for *Fatp1*, body temperature falls below 30 °C after 10 hours at 4 °C (204). This apparent defect in the channeling of FA towards oxidation is supported by studies using adenovirus-overexpressed *Fatp1* in L6E9 myotubes, in which the protein physically interacts with CPT1, and the resulting increase in mitochondrial FA oxidation is blocked by etomoxir, an inhibitor of CPT1 (169). In related studies, transient *Fatp1* overexpression in rat soleus muscle increases the oxidation of 16:0 by 35% (65).

In the retina, FATP1 physically interacts with lecithin retinol-acyltransferase (LRAT) and retinal pigment epithelium isomerase (RPE65) to inhibit isomerase activity (17). LRAT uses an acyl-CoA (frequently 16:0) to esterify all-*trans* retinal to all-*trans* retinal ester. This substrate for RPE65 converts the retinal ester back to 11-*cis* retinal in a process that reforms rhodopsin in the visual cycle (17). An analysis of the neuroretina and retinal pigment epithelium from *Fatp1*^{-/-} and control mice shows no differences in FA composition, and it was speculated that FATP1 directs FA into the mitochondria (similar to its effects when overexpressed in myotubes and rat muscle) and that its absence diminishes energy production from FA, thereby accelerating ageing in retinas from aged mice (17).

FATP2 (ACSVL1/SLC27A2)

FATP2 is most highly expressed in liver, kidney, small intestine, and placenta (135). The protein is present on internal ER and peroxisomal membranes (62), but it increases FA uptake, consistent with the idea that intracellular metabolism drives FA uptake (92).

Although FATP2 activates trihydroxycholestanoic acid (THCA) (132), bile acids are normal in FATP2 null mice (62). A knockout of *Fatp2* in placenta does not alter neutral lipid accumulation (135). Compared to controls, the knockout mouse has 75-80% lower ACS activity (with 24:0) in liver and kidney, and 60% lower oxidation of 24:0, consistent with a peroxisomal location (62). Very-long-chain FAs do not accumulate, however, indicating that FATP2 is not essential for their degradation.

In experiments using adeno-associated virus-mediated shRNA to silence *Fatp2* in liver, the loss of FATP2 has no effect on total ACS activity with either 16:0 or 24:0, but activity in purified peroxisomes decreases by ~50% (41). In this knockdown, the accumulation of TAG after a high fat diet is ~20% lower than controls, and insulin sensitivity is greater. No differences in bile acids were noted.

FATP3 (ACSVL3/SLC27A3)

Fatp3 is most highly expressed in lung and in the steroidogenic tissues, adrenal cortex, ovary and testis (153). It is also present on the mitochondria of cultured neural cell lines and can activate both long-chain and very long-chain FAs (153). The expression of *Fatp3* is high in lung tumors (154) and in malignant glioma compared to normal glia (157). Knockdown by RNAi in human glioma and lung cell lines diminishes long-chain FA activation and inhibits anchorage-dependent and independent cell growth and the growth of xenografts via diminished Akt phosphorylation (154, 157). It is unknown how these effects are related to the enzymatic activity of FATP3.

FATP4 (ACSVL5/SLC27A4)

Fatp4 is expressed most prominently in heart, skeletal muscle and brown and white adipose (33). FATP4 protein is located on the ER (133), and like other acyl-CoA synthetases, it promotes insulin-stimulated FA uptake into C2C12 muscle cells, consistent with the idea that intracellular metabolism enhances cellular fatty acid uptake (29). An adipose-specific knockout of *Fatp4* causes adipocyte hypertrophy when the mice are fed a 40% fat diet, although the mice do not consume more food than controls (98). Total ACS activity with 18:1 is unchanged in knockout adipose, suggesting that FATP4 is a minor contributor to total ACS activity in fat cells, or that other ACS activities increase to compensate. Expression of mRNA from other FATP isoforms and of lipogenic genes is unchanged, and the cause of obesity was not determined. Because significant decreases were found in most phospholipid species and in cholesterol esters, the authors suggested that FATP4 might normally activate FA destined for phospholipid biosynthesis and that, in its absence, excess FAs in the ER would become available to other ACS isoforms that are coupled to TAG synthesis. Furthermore, because only subcutaneous adipose showed these lipid changes, whereas visceral adipose had decreases in lipolytic genes like *Hsl* and *Atgl* and increases in *Plin1*, the authors speculated that the function of FATP4 differs in each depot. Muscle FATP4 and lipid oxidation both increase in humans undergoing aerobic exercise training, suggesting that muscle FATP4 might channel FA towards oxidation (74). Although an antisense knockdown of FATP4 diminished 18:1 uptake by 50% in isolated enterocytes (182), the mouse knockout does not show any evidence for a role in dietary TAG or cholesterol absorption (173).

A human mutation in *Fatp4* causes the ichthyosis prematurity syndrome, which is characterized by premature birth, scaly erythroderma, and neonatal asphyxia. Similar to the human mutation, newborn *Fatp4*^{-/-} mice have a restrictive skin syndrome with a defective barrier that is incompatible with postnatal survival. Additionally, the mouse knockout shows abnormalities in the development of sebaceous and meibomian glands, with lower amounts of type II diester wax in sebum (108). Fibroblasts from *Fatp4*^{-/-} mice have lower ACS activity than wild type mice and the FA composition of phospholipids is altered (76). Replacement of FATP in suprabasal keratinocytes rescues these mice, indicating that a systemic metabolic defect is not responsible for the skin defect; rather, the ceramide content in the knockout containing 26 carbons or more is lower than in controls, and the content of ceramides with 24 or fewer carbons is higher (137). Newborn mouse skin also has a decreased proportion of ω -O-acylceramide (109). These data strongly suggest a role for FATP4 in ceramide biosynthesis in skin.

Mutations in RPE65, an enzyme in the retina that isomerizes all-*trans* retinyl esters to 11-*cis*-retinol, cause blindness (57, 115), and mice lacking RPE65 do not have functional rhodopsin (164). Like FATP1, FATP4 inhibits RPE65 either by competing with RPE65 for its substrate, all-*trans* retinyl palmitate, or by synthesizing 20:0-CoA, which may bind to the hydrophobic pocket of RPE65 (105). Confirming the physiological effect of FATP4, *Fatp4*^{-/-} mice have higher isomerase activity and the 11-*cis*-retinol product of RPE65 is regenerated more rapidly (105).

FATP5 (ACSVL6/SLC27A5)

FATP5 mRNA is prominent in liver, where it has been found on the plasma membrane and microvilli in the space of Disse (31). In addition to activating long-chain FAs, FATP5 activates C24 bile acids to their CoA forms. A knockout of FATP5 showed that, compared to controls, the *Fatp5*^{-/-} livers contain 48% less TAG and almost three times more phosphatidylserine. Both the secretion of TAG and fasting serum β -hydroxybutyrate concentrations are lower in the knockout (31). These changes in lipids were attributed to a defect in FA transport into the hepatocytes. Supporting this interpretation is the finding that the content of TAG is high in *Fatp5*^{-/-} heart and skeletal muscle, neither of which expresses FATP5, so because FAs were unable to enter the liver, they may have been diverted into other tissues (31). Similarly, AAV-mediated silencing of FATP5 reverses TAG accumulation in steatotic liver. Although this reversal was attributed to a decrease in FA uptake into hepatocytes, acyl-CoA synthetase activity was not measured (32).

In *Fatp5* null mice, concentrations of total bile acids are unchanged in liver, bile, urine, and feces, but most of the gall bladder bile acids remained unconjugated (70). It was suggested that re-conjugation of bile acids was defective because primary, but not secondary, bile acids were detected. *Fatp5* null mice also show decreased food intake and increased energy expenditure and do not gain weight on a high fat diet. These features are unexplained but may relate to recent findings that bile acids have systemic metabolic effects (96, 183).

FATP6 (ACSVL2/SLC27A6)

FATP6 is the most highly expressed FATP in heart, where the protein colocalizes with CD36 and caveolin on the sarcolemma (53). Apart from studies of FA uptake, no information is available on lipidomics or FATP6-dependent FA trafficking.

ACS Bubblegum 1 and 2

AcsBg was discovered in *Drosophila melanogaster*, where its absence causes neurodegeneration with dilated photoreceptor axons and elevations in very-long-chain FAs (134). Two mammalian homologs were subsequently identified, ACSBg1 in brain (185) and steroidogenic tissues (156) and ACSBg2 in testis and brainstem (155). Like the FATP/ACSVL proteins, the ACSBg1 isoform activates both long- and very long-chain saturated and unsaturated FAs (44); ACSBg2, however, activates only 18:1 ω 9 and 18:2 ω 6 (155). In neuron-derived Neuro2a cells, when RNA interference diminished ACSBg1 expression, the activation and β -oxidation of 16:0 decreased 30-35%, suggesting that ACSBg1 might direct FA towards oxidation (156). ACSBg1 mRNA is down-regulated by gonadotrophin in rat Leydig cells, and a mouse knockout model shows increases in the amounts of some long-chain fatty acids (16:0, 18:0, 18:1, and 18:2) in total lipids from testis, ovaries and brain, despite the presence of normal ACSL activity (172, 191). Nuclear inclusion vacuoles containing membrane debris that are normally observed in wild type Leydig cells at 15 months are found prematurely in the null mouse at 9 months, suggesting a role for ACSBg in eliminating aging organelles (172). No additional information is available on these null mice.

What can we learn from gain of function studies?

When a protein has been over-expressed, the interpretation of its physiological function is problematic. The transfected protein may be located in a membrane or organelle with which it is not normally associated. If adenovirus-mediated over-expression is used, virus toxicity may cause cells to function abnormally and even to lyse. With transgenic over-expression, the gene can insert into the DNA at a position that interrupts an unrelated function. The situations most likely to present problems in interpretation are those in which an over-expressed enzyme synthesizes large amounts of a product for which the downstream cellular machinery is unprepared to handle. Thus, the synthesis of a large amount of acyl-CoA may overwhelm downstream pathways that can neither use the acyl-CoAs for synthetic purposes nor degrade them quickly (106). The detergent properties of acyl-CoAs may then damage cell membranes and alter the functions of membrane-associated receptors, enzymes and transporters. One example of such acyl-CoA toxicity occurs when ACSL1 is over-expressed in heart (20, 21). As might be expected, the resulting lipotoxic cardiomyopathy is ameliorated by cardiac over-expression of diacylglycerol acyltransferase, a downstream enzyme that can use the accumulating acyl-CoAs to synthesize TAG and sequester the excess acyl-CoAs in cytoprotective lipid droplets (110).

In other studies in which ACSL and FATP/ACSVL isoforms are over-expressed in cultured cells, a common result has been to increase the incorporation of FA into glycerolipids. Thus, over-expression of FATP1 increases FA incorporation into TAG in HEK293 cells (60) and

skeletal muscle (50), and the overexpression of ACSL1 increases TAG reacylation (104). These overexpression studies led to a radically different interpretation of function than subsequent studies, which showed that the absence of either FATP1 or ACSL1 impairs FA oxidation. For a comprehensive review of ACSL over-expression studies, see (124).

Role of acyl-CoAs in disease

Obesity and type 2 diabetes mellitus

Obesity and type 2 diabetes mellitus are associated disorders that share the underlying features of insulin resistance and dyslipidemia. The dyslipidemia is characterized by hypertriglyceridemia, low HDL, and elevated free FA. Although the pathogenesis of the insulin resistance syndrome is controversial, three factors are present: 1) hypersecretion of insulin by pancreatic β -cells; 2) increases in intra-abdominal adiposity, with high circulating levels of free FA; and 3) insulin resistance in skeletal muscle. All three of these factors are associated with disordered FA metabolism and, secondarily, with disordered acyl-CoA metabolism.

In an attempt to mechanistically link the three commonly held factors, Prentki and Corkey hypothesized that elevated cytosolic long-chain acyl-CoAs cause insulin resistance. This hypothesis is based on the work of McGarry and Foster who showed that malonyl-CoA, the “signal of plenty,” inhibits CPT1, thereby blocking acyl-CoA transport into the mitochondria for β -oxidation (127, 128). Prentki and Corkey hypothesize that with nutrient surfeit, the rate of glucose metabolism increases in pancreatic β -cells, liver, and muscle, causing malonyl-CoA levels to rise and inhibit CPT1, which blocks mitochondrial β -oxidation of acyl-CoAs and allows acyl-CoAs to accumulate (160).

The accumulation of cytosolic long-chain acyl-CoAs in β -cells can modify the acylation state of key regulatory proteins involved in the regulation of ion channels and exocytosis of insulin (161). Indeed, in both cultured β -cells and rodent pancreatic islets, adding exogenous FA and glucose increases long-chain acyl-CoA content concomitantly with increased insulin secretion, basal hyperinsulinemia, and reduced prandial insulin release (19, 107, 162). It is unclear whether the resultant hyperinsulinemia results from insulin resistance or, conversely, is the driver of insulin resistance (170).

Insulin resistance in liver and muscle has been attributed to long-chain acyl-CoA activation of protein kinase C θ , which phosphorylates the insulin receptor and/or insulin receptor substrate-1 (IRS-1) and reduces the cells' ability to respond to insulin (87). Despite an associative study linking elevated hepatic long-chain-acyl-CoA content and plasma insulin levels (18), two knockout mouse models have not confirmed a direct connection between elevated hepatic acyl-CoA content and hepatic insulin resistance. In mice with liver-specific deficiency of *Acs11*, a 25-35% decrease in hepatic acyl-CoA content does not protect the mice from developing diet-induced insulin resistance (103), and in *Gpat1* deficient liver, which has a nearly 2-fold increase in acyl-CoA content, the mice are protected from diet-induced insulin resistance (144). Thus, at least in liver, acyl-CoA accumulation does not necessarily result in insulin resistance.

Evidence for muscle acyl-CoA accumulation as a cause of insulin resistance is better supported. In studies in both rats and humans, high fat feeding or direct lipid infusion increases intramuscular acyl-CoA levels and diminishes muscle uptake of glucose in response to insulin (18, 36). Conversely, when morbidly obese subjects lose weight, insulin sensitivity improves together with a reduction in intramuscular acyl-CoA levels (68). This indirect evidence associates the accumulation of cytosolic long-chain acyl-CoAs in muscle with the development of insulin resistance.

These studies do not support a direct role for long-chain acyl-CoA accumulation in the development of insulin resistance. While it is appealing to identify a single molecule as a unifying cause for the development of insulin resistance, it is more likely that long-chain acyl-CoAs are merely a marker for metabolically dysfunctional tissues.

Cancer

One hallmark of tumorigenesis is the upregulation of genes that encode enzymes that synthesize FAs and complex lipids (26, 131). Although lipids are required for enhanced membrane biosynthesis in rapidly proliferating cells, a role beyond that of simple cellular growth is suggested by the upregulation of isoforms that are specific for lipids with specialized properties. For example, upregulated *AcsI4* is particularly associated with hepatocellular carcinoma and aggressive cancers in breast, prostate, and colon (12, 114, 136, 188). ACSL4 prefers to activate 20:4 ω 6 (81) and promotes tumor cell survival by two separate mechanisms. In colon cancer, ACSL4 overexpression may prevent apoptosis by depleting pro-apoptotic unesterified 20:4 ω 6 (13, 189). In hepatocellular carcinoma, ACSL4 overexpression generates 20:4 ω 6-CoAs that might promote cell proliferation and growth by regulating signaling molecules like atypical protein kinase C (aPKC) or by binding a transcription factor like hepatic nuclear factor-4 α (HNF-4 α), antagonising its activity and enhancing tumor growth (40, 63, 148). In addition, chemical inhibition of ACS activity by triacsin C, which inhibits ACSL1, ACSL3, and ACSL4, but not ACSL5 or ACSL6, induces apoptosis in lung, colon and brain cancer cells (125). Although it appears that 20:4 ω 6-CoA is important for tumorigenesis, cell growth, and proliferation, more studies are needed to elucidate the mechanism by which 20:4 ω 6-CoA enhances growth.

Conclusion

The multiplicity of related ACS isoforms that can activate long-chain FAs has suggested that each may play a distinctive role in partitioning FAs towards or away from specific downstream pathways. Evidence from over-expression studies is suspect because of possible mislocalization of the overexpressed proteins, inability of downstream pathways to accept an excessive flux of acyl-CoA substrate, aberrant allosteric regulation of metabolic enzymes by acyl-CoAs, and damaging effects of acyl-CoAs on cellular membranes. Although loss of function studies are more likely to aid in elucidating differences in the various isoforms, only a handful of the 13 putative long-chain ACS isoforms has been investigated with a knockout or knockdown of expression model. Moreover, the interpretation of many studies is limited because a major focus has been on phenotypic abnormalities that are tissue-specific or related to high fat diets or FA transport. To elucidate specific acyl-CoA

trafficking, future studies should focus on the incorporation of specific fatty acids into the pathways outlined in Figures 1 and 2. Such studies may require radio- or isotopic labeling studies, lipidomic studies that identify minor lipid species and eicosanoids, and an analysis of acylated proteins. What we have learned so far, however, generally supports the idea of lipid channeling by individual long-chain ACS isoforms in specific organs or cells. ACSL1 and FATP1 activate FAs that are targeted to oxidation, whereas FATP4 deficiency alters the production of specific ceramides, ACSL3 modulates the production of transcription factor targets, and ACSL4 contributes to pathways of eicosanoid metabolism. In addition, the data also suggest that acyl-CoA partitioning may vary in different tissues and under different physiological conditions. How can this occur? The most likely answer is that the long-chain ACS isoforms physically partner with different specific downstream acyltransferases or degradation enzymes. The best example, so far, is that of liver ACSL1 and CPT1, which have been shown to interact (95), consistent with the finding that deficient ACSL1 diminishes FA oxidation.

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Abbreviations

ACBP	acyl-CoA binding protein
ACS	acyl-CoA synthetase
ACSBg	ACS bubblegum
ACSL	long-chain ACS
ACSVL	very-long-chain ACS
CPT1	carnitine palmitoyltransferase-1
ER	endoplasmic reticulum
FA	fatty acid
FABP	FA binding protein
FATP	fatty acid transport protein
TAG	triacylglycerol

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Summary Points

- Acyl-CoAs are critical metabolic intermediates and regulators of metabolism, but how they are partitioned into different metabolic pathways is poorly understood.
- Acyl-CoAs bind intracellularly to ACBP and to the liver-type FABP, but no evidence has been provided that these proteins traffic acyl-CoAs to selective locations within the cell.
- The multiple long-chain acyl-CoA synthetase isoforms are expressed in different cell types and are located in different organelles.
- Loss of function studies suggest that acyl-CoA synthetases contribute to acyl-CoA partitioning.
- Altered acyl-CoA partitioning may contribute to several disorders, including insulin resistance, type 2 diabetes mellitus, and cancer.

Figure 1.

Metabolic fates of long-chain fatty acids. Long-chain fatty acids from exogenous or endogenous sources are activated to acyl-CoAs by one of 13 acyl-CoA synthetase isoforms. The free fatty acids are ligands for nuclear transcription factors and 20-carbon fatty acids can be converted to a variety of signaling eicosanoids. The acyl-CoAs are transcriptional ligands, substrates for β - and ω -oxidation, and can be incorporated into complex lipids or used to modify proteins. ACS, acyl-CoA synthetase; EET, epoxyeicosatrienoic acids; ER, endoplasmic reticulum; FA, fatty acid; HETE, hydroxyeicosatetraenoic acid; PPAR, peroxisome proliferator-activated receptor.

Figure 2.

Acyl-CoA metabolism. The major initial enzymatic steps in the metabolism of long-chain acyl-CoAs include 13 independent thioesterases to hydrolyze acyl-CoAs, fatty acyl-CoA reductase, which converts the acyl-CoA to a fatty alcohol that will be incorporated into ether lipids, and ATs (acyl-CoA acyltransferases), which incorporate fatty acids into complex lipids and acylated proteins. In the major degradative pathways, CPT1 (carnitine palmitoyltransferase-1) converts acyl-CoAs to acylcarnitines that enter the mitochondria for β -oxidation, whereas very-long-chain acyl-CoAs begin to be oxidized in peroxisomes, releasing acetyl-CoAs, until they are chain-shortened to 8 carbons, which complete their oxidation in the mitochondria.

Table 1

Evidence for partitioning from loss of function studies

Gene	Tissue	FA partitioning information	Phenotype	References
<i>Acs11</i> KO	Liver-specific	Decreased FA incorporation into TAG & oxidation	None	(103)
	Adipose-specific	Decreased FA oxidation	Increased adipose mass; defective thermogenesis	(37)
	Multi-tissue- heart	Decreased FA oxidation	None	(38)
	Heart-specific	Decreased FA oxidation	None	(38)
	Macrophage-specific	Altered 20:4 metabolism and PGE ₂ production	Protects macrophages against diabetes-mediated inflammation	(84)
	Endothelial cell- specific	No information	None	(106)
<i>Acs13</i> KD	Rat hepatocytes	Glycerolipid synthesis on lipid droplets? Regulation of transcription factors	—	(11)
<i>Acs14</i> KD	Cultured cells, various	Altered eicosanoid metabolism	Human X-linked mental retardation	(91, 93)
<i>Acs15</i> KD	Primary hepatocytes	Decreased FA incorporation into glycerolipids and cholesterol esters	Decreased lipid droplet formation	(10)
<i>Acs15</i> KO	Total KO	No information	None	(129)
<i>Acs16</i> KD	Neuroblastoma cells	22:6 ω 3 metabolism?	Inhibited neurite outgrowth	(86)
<i>Fatp1/Acs14</i> KO	Skeletal muscle, BAT, L6E9 cells	Decreased FA oxidation	Defective thermogenesis	(204)
	Retina	? Decreased FA oxidation	Accelerated retinal ageing	(17)
<i>Fatp2/Acs11</i> KO	Total KO	? Decreased oxidation of 24:0	None	(135)
<i>Fatp3/Acs13</i> KD	Glioma	No information	Decreased anchorage-dependent growth	(157)
<i>Fatp4/Acs15</i> KO	Human mutation	Decreased type II diester wax in the sebum	Ichthyosis prematurity syndrome	(108)
	Keratinocytes	Decreased long-chain ceramides	Postnatal restrictive skin	(137)
<i>Fatp5/Acs16</i> KO	Gall bladder bile	Decreased conjugated bile acids	Low weight gain on a high fat diet	(70)
<i>Fatp6/Acs12</i>	---	No information	---	---
<i>AcsBg1</i> KD	Neuro2a cells	Decreased β -oxidation	---	(156)
<i>AcsBg1</i> KO	Various tissues	Increased amounts of some long-chain fatty acids	None	(172, 191)
<i>AcsBg2</i>	---	No information	---	---