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Acyl-coenzyme A synthetases in metabolic control

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Abstract

Purpose of review—The 11 long-chain (ACSL) and very long chain acyl-coenzyme A (acyl-CoA) synthetases [(ACSVL)/fatty acid transport protein] are receiving considerable attention because it has become apparent that their individual functions are not redundant.

Recent findings—Recent studies have focused on the structure of the acyl-CoA synthetases, their post-translational modification, their ability to activate fatty acids of varying chain lengths, and their role in directing fatty acids into different metabolic pathways. An unsettled controversy focuses on the ACSVL isoforms and whether these have both enzymatic and transport functions. Another issue is whether conversion of a fatty acid to an acyl-CoA produces an increase in the AMP/ATP ratio that is sufficient to activate AMP-activated kinase.

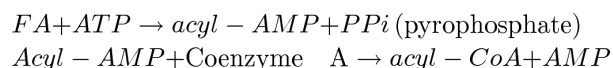
Summary—Future studies are required to determine the subcellular location of each ACSL and ACSVL isoform and the functional importance of phosphorylation and acetylation. Purification and crystallization of mammalian ACSL and ACSVL isoforms is needed to confirm the mechanism of action and discover how these enzymes differ in their affinity for fatty acids of different chain lengths. Functionally, it will be important to learn how the ACSL isoforms can direct their acyl-CoA products toward independent downstream pathways.

Keywords

β -oxidation; acyl-CoA synthetase; AMP-activated kinase; fatty acid; fatty acid transport protein; glycerolipid synthesis

Introduction

Of the 26 mammalian acyl-coenzyme A (acyl-CoA) synthetases (ACS), at least 11 can activate the major long-chain fatty acids (FAs) of 16–22 carbons [1,2*]. These include members of the long-chain [long-chain acyl-CoA synthetases (ACSL)] and very long chain [very long chain acyl-CoA synthetases (ACSVL)/fatty acid transport protein (FATP)] subfamilies that catalyze a two-step reaction:



This review will focus primarily on very recent advances in the study of these long-chain ACSs in metabolic regulation, highlighting new studies on structure, function, and regulation.

Structure and sequence of acyl-coenzyme A synthetase

Acyl-CoA synthetases are homologous in their sequences. The ACS catalytic domain shares homology with the ANL (A_{cy}l-CoA synthetases, N_{on}ribosomal peptide synthetase adenylation domains, and L_{uciferase} enzymes) super-family, a family consisting of ACS, nonribosomal peptide synthetase, and luciferase [3**]. The homology between ACS and luciferase is so similar that mutation of a single residue (L345S) allows the *Drosophila* *melanogaster* ACS to gain luciferase activity [4**]. For all ANL superfamily enzymes, the C-terminal domain is rotated ~140° after adenylation-formation; in ACS this rotation results in the conformation that forms thioesters. Analysis of the only reported mammalian ACS crystal structure revealed the following: (1) in the adenylation-forming conformation a conserved lysine, Mg²⁺, and the P-loop holds the pyro-phosphate in the active site; (2) in the thioester conformation the Arg472-Glu365 salt bridge sterically blocks ATP binding; and (3) the switch of the adenylation-forming conformation to the thioesterase conformation requires the presence of pyrophosphate [5*].

Acyl-coenzyme A synthetase and fatty acid uptake

FA may enter cells via both concentration-based flipping and protein-mediated transport [6]. Cellular retention may depend on 'vectorial acylation', the trapping of exogenous FA as an acyl-CoA within the cell and its subsequent use in downstream metabolism [7]. Although FATP1 was discovered in a functional FA uptake screen [8], several FATP family members are not present on the plasma membrane [7], and so they are also called ACSVL to distinguish their ability to activate FA with chain lengths as long as 26 carbons [1]. Although FA uptake increases in NIH fibroblasts [9] and COS (C_V-1 (simian) in O_{ri}gin, and carrying the S_V40 genetic material) cells overexpressing ACSL1 [10], it is unaffected by over-expressing [11] or knocking out ACSL1 in primary hepatocytes [12**] or 3T3-L1 adipocytes [13**]. The conflicting data concerning results of FA uptake by the same ACSL isoforms likely reflect differences in techniques for measuring uptake [7], the artificial effect of overexpression, tissue-specific subcellular location, and tissue-specific interacting proteins.

Fatty acid channeling

Although each ACSL and ACSVL isoform activates a broad range of long-chain FA, their respective physiological roles remain unknown. We hypothesized that different ACS isoforms channel FA into specific downstream pathways that could vary in a tissue-specific manner [7]. For example, despite a 50% decrease in total ACSL activity, primary hepatocytes from ACSL1 liver-specific knockout mice had small reductions in FA incorporation into both triacylglycerol (TAG) and β -oxidation products [12**]. The ACSL1 knockout livers had reduced long-chain acyl-carnitine content and reduced rates of FA oxidation. The minimal phenotype suggests that alternative pathways for FA oxidation and TAG synthesis are active in the *Acs1*^{L-/-} liver.

In contrast, primary epididymal adipocytes from ACSL1 adipose-specific knockout mice have unchanged FA incorporation into TAG, diacylglycerol (DAG), or phospholipid (PL), but reduced β -oxidation products (J.M. Ellis, R.A. Coleman, unpublished data). However, siRNA knockdown of ACSL1 in 3T3-L1 adipocytes does not affect de-novo lipogenesis or FA oxidation, but increases FA efflux [13**], leading the authors to conclude that ACSL1 activates FA that is re-esterified after lipolysis. Pulse-chase labeling experiments could verify reduced rates of re-esterification and confirm this interpretation.

Knocking down ACSL3 in rat primary hepatocytes decreases labeled acetate incorporation into TAG and PL [14**]. In human hepatoma Huh7 cells, siRNA knockdown of ACSL3 decreases oleate incorporation into phosphatidylcholine (PC) and secreted VLDL and increases cellular-free oleate, whereas knockdown of ACSL1 and ACSL4 does not alter oleate incorporation into cholesterol ester (CE), TAG, phospholipids, or free FA [15**]. Differential effects of individual ACSL iso-forms support the notion that ACSL isoforms channel FA to distinct metabolic fates.

Several overexpression studies are inconsistent with the knockdown studies and provide strikingly contradictory interpretations of the role of ACSL1. For example, when ACSL1 is overexpressed in rat primary hepatocytes, FAs are incorporated into DAG and phospholipids but not into CE or secreted TAG [11]. Overexpressed ACSL5 increases FA incorporation into DAG and TAG but does not affect FA used for β -oxidation [16]. In contrast, however, over-expressing either human ACSL3 or rat ACSL5 in HepG2 cells increases palmitate oxidation [17]. Further, TAG content increases in NIH-3T3 fibroblasts when ACSL1/ FATP1 is overexpressed [18] and in insulin-stimulated 3T3-L1 adipocytes when ACSL1 is knocked down [13**].

We see two potential problems. The first is that over-expression of an enzyme may overwhelm downstream pathways that cannot readily accommodate excess substrate; the second problem is that studies in cell lines are not always translatable to primary cells. Thus, proteomic analysis of mitochondria isolated from white adipose tissue, brown adipose tissue, and 3T3-L1 adipocytes reveals substantial differences [19**]. Compared with mitochondria from adipose tissue, many proteins are downregulated in 3T3-L1 mitochondria. Further, medium-chain ACS (ACSM) isoforms are more abundant than ACSL isoforms in white adipose tissue mitochondria, suggesting greater medium-chain FA oxidative metabolism. ACSL1 and ACSL6 are particularly enriched in white adipose mitochondria, whereas ACSL5 is more abundant in mitochondria from brown adipose. However, despite the high abundance of ACSL5 in brown adipose mitochondria, ACSL1 is required for adaptive thermo-genesis and normal FA oxidation in brown adipose (J.M. Ellis, R.A. Coleman, unpublished data). Thus, the function of ACSL5 in BAT remains unknown.

FA channeling could occur via protein–protein interactions. Immunoprecipitation of endogenous FATP1 from 3T3-L1 adipocytes followed by mass spectrometry identified mitochondrial 2-oxoglutarate dehydrogenase (OGDH), a key enzyme in the tricarboxylic acid cycle [20**]. FATP1 enhances OGDH activity in proteoliposomes, whereas FATP1 knockdown in 3T3-L1 adipocytes showed decreased OGDH and TCA cycle activity. Over-expressed FATP1 and carnitine palmitoyltransferase-1 (CPT1) also co-immunoprecipitate

from L6E9 myotubes and rat skeletal muscle *in vivo* [21*]. In L6E9 myotubes, FATP1 overexpression enhances FA oxidation, suggesting that protein–protein interactions of FATP1 facilitate activities of FA oxidation proteins. Overexpressed ACSL1 and FATP1 co-immunoprecipitate in 3T3-L1 adipocytes [22], but it is not known whether endogenous ACSL1 and FATP1 proteins interact.

The subcellular location of ACS enzymes, which was recently reviewed [23*], may influence the downstream metabolism of the acyl-CoA product. However, sub-cellular location studies have yielded conflicting interpretations. Most have examined subcellular fractions that are limited in purity. Data from overexpression studies are also problematic because the location of over-expressed proteins may not mimic the endogenous condition. In one such example, the subcellular locations of overexpressed and endogenous FATP4/ACSVL5 differed [24]. Confocal microscopy with tested isoformspecific antibodies will be required to determine precise locations of the acyl-CoA synthetases.

Acyl-coenzyme A synthetase enzyme regulation

Broad mass spectrometry surveys reveal a number of post-translational modifications to ACS enzymes, although their functional significance is generally unknown. An exception is the regulation on Lys-609 located on the C-terminus of the *Salmonella enterica*, ACS, for which lysine acetylation inhibits activity by preventing formation of the thioester [25]. Similar regulation occurs by the sirtuin (SIRT) acetylases on Lys-661 and Lys-635 of acetyl-CoA synthetase-1 and acetyl-CoA synthetase-2, respectively, and human acetyl-CoA synthetase 2 is regulated on Lys-642 [26]. In response to a calorie-restricted diet, acetylation of Lys-534 of ACSM1 and Lys-510 of acyl-CoA synthetase family member-2 (Q8VCW8) increased 2.75-fold and 2.3-fold, respectively [27**].

Role of acyl-coenzyme A synthetase in transcriptional regulation

ACSL can theoretically regulate transcription by modulating the intracellular content of FA and acyl-CoA, which are ligands for transcription factors. FA, for example, is a ligand for peroxisome proliferator-activated receptor (PPAR) α , β , or δ and may suppress SREBP1c target genes, and acyl-CoA is a possible ligand for HNF4 α [28]. The role of ACS in regulating transcription was addressed by a study that used siRNA to knock down the long-chain ACS isoforms that are expressed in liver, ACSL1, 3, 4, and 5, and FATP2, 4, and 5 [14**]. In primary rat hepatocytes, only an siRNA that targeted ACSL3 had effects on transcription. This siRNA elevated intracellular FA 82% and diminished the expression of PPAR γ , as well as reporter gene activity for PPAR γ , ChREBP, SREBP1c, and liver X receptor α (LXR α), together with the expression of target genes for these transcription factors. Decreased use of [1-¹⁴C]acetate for de-novo lipogenesis and complex lipid synthesis confirmed the effect of diminishing the expression of genes involved in lipid synthesis. Although one might conclude that the knockdown of ACSL3 decreased these transcriptional activities via an increase in a specific pool of FA ligand, Bu *et al.* argue that ACSL3 must control transcription in a ligand-independent manner because synthetic ligands did not normalize transcriptional activities of LXR and PPAR γ .

Long-chain acyl-coenzyme A synthetase's role in disease

ACSL is required for complex lipid synthesis in rapidly growing cells and the fungal metabolite triacsin C was initially reported to inhibit ACSL, diminish the synthesis of phospholipids, and decrease cell proliferation [29]. Triacsin C caused apoptosis in glioma cells, and, although it inhibits ACSL1, 3, and 4, but not ACSL5, overexpression of ACSL5 rescued the glioma cells [30, 31]. In this case, ACSL5 appeared to compensate for the inhibited isoforms. In a related publication, ACSVL3 (FATP3) was elevated in human gliomas [32]. ACSVL3 siRNA inhibited glioma cell growth and the cells became less tumorigenic. Because these features were reversed when active myr-Akt was expressed, ACSVL3 was thought to maintain oncogenesis by regulating Akt function.

Mutations in ACS genes have been identified in large screening studies. An *Acs15* single nucleotide polymorphism (SNP) (rs2419621; C>T) increased *Acs15* mRNA abundance in skeletal muscle and was associated with improved weight loss. This SNP increases the transcriptional rate of *Acs15* by enhancing gene activation by MyoD [33]. Because this ACSL5 SNP increases the differentiation of skeletal myoblast cells, persons with this mutation may have an advantageous ability to increase muscle mass and caloric expenditure with diet and exercise.

High levels of *Acs13* gene methylation in umbilical cord white blood cells were strongly correlated with levels of maternal polycyclic aromatic hydrocarbons and with the mRNA abundance of *Acs13* [34]. In a separate cohort, *Acl3* methylation was associated with polycyclic aromatic hydrocarbon exposure and childhood asthma [34]. These data suggest that maternal environmental exposures may affect childhood asthma through the methylation of the *Acl3* gene.

The knockdown of *Acs14* in neurites reduces dendritic spine formation, suggesting a role for ACSL4 in the maturation and remodeling of neurons and providing a link to the X-linked mental retardation associated with human mutations in ACSL4 [35]. In another study, a SNP (rs1324805) in the *Acs14* gene was more prevalent in patients with the metabolic syndrome [36]. A third study found an *Acs14* SNP (rs7887981) associated with increased liver fat or with increased circulating insulin and TAG in overweight participants from two different cohorts [37]. It is unknown how these SNPs affect the function of ACSL4, nor is it clear how the preference of ACSL4 for long-chain polyunsaturated FA may be involved.

A mutation within the *Fatp4* gene is associated with the ichthyosis prematurity syndrome (IPS) [38]. Fibroblasts from an IPS patient had reduced ACSVL activity and reduced incorporation rates of very long chain fatty acids into complex lipids. The skin findings are like those of *Fatp4* null mice, which die in early life with hyper-proliferative hyperkeratosis [39], and suggest that FATP4 plays a role in early epidermal development and that other ACS enzymes cannot compensate for a deficit of this enzyme.

Effect of fatty acid activation on AMP-activated kinase

Because the synthesis of each acyl-CoA uses the energy equivalent of two ATP molecules and produces one AMP, it has been suggested that FA activation might increase cellular

AMP/ATP ratios and activate AMP-activated kinase (AMPK) (Fig. 1). AMPK would then increase FA oxidation while blocking TAG synthesis. It would be surprising, however, if the conversion of FA to acyl-CoA triggered the activity of an energy-deficit indicator in settings of energy surplus when TAG synthesis is prominent.

Studies in animals have not been performed to compare ACSL-mediated activation of AMPK under different physiological conditions, but metabolic adaptations to fasting and chronic caloric restriction in heart, muscle, and liver do not include changes in AMPK activity [40], and when mice are placed in a cold environment, AMPK is not activated for several days despite increased ACSL-mediated FA activation [41].

Some experiments, however, do support ACSL-mediated activation of AMPK. In 3T3-L1 adipocytes, stimulation of lipolysis resulted in activated AMPK [42**]. The authors suggested that activation of AMPK during lipolysis would 'restrain the energy depletion and oxidative stress'. It might also permit use of released FA for β -oxidation within the adipocyte and conserve glucose during a 'fast'. In a situation more akin to 'feeding', AMPK activation increases in 3T3-L1 adipocytes during insulin-stimulated palmitate uptake, although the AMP/ATP ratio changes minimally [43**].

Studies in heart [44] and skeletal muscle [45*,46,47] suggest that AMPK may be sensitive to the 'lipid status' of the cell and that activation may be influenced by intracellular FA availability, independent of cellular AMP levels, with FAs, themselves, activating AMPK [48*]. FA added to L6 myotubes increases AMPK activity by 40–50%, together with phosphorylation of AMPK Thr¹⁷² and acetyl-CoA carboxylase- β Ser²¹⁸, and the rate of palmitate oxidation is almost doubled, without an increase in AMP content or LKB1/AMPKK activation [49]. Dominant-negative AMPK adenovirus reduced basal FA oxidation and inhibited the stimulatory effects of FA pretreatment on FA oxidation, suggesting that FA might improve AMPK as a substrate for LKB1. However, in assays with purified components, 10–50 μ mol/l long-chain acyl-CoAs directly inhibited LKB1, suggesting that the rise in long-chain acyl-CoA content that occurs when FA synthesis is enhanced would negate the effect of increased AMP to prevent simultaneous synthesis and FA oxidation [50]. In addition, strong evidence supports the idea that protein kinase A phosphorylates and inactivates AMPK [51], thereby allowing hormone-sensitive lipase activation to diminish and slowing the rate of lipolysis. Although the cellular concentration of long-chain acyl-CoAs has been estimated at between 5 and 160 μ mol/l [52], acyl-CoAs are amphipathic and are probably bound either to cell membranes or acyl-CoA-binding protein; neither their free concentration nor their availability as substrates or inhibitors can be known with certainty.

Conclusion

Although much has been accomplished, many important questions remain. Future studies are required to determine the subcellular location of each ACSL and ACSVL isoform. Purification and crystallization of mammalian ACSL and ACSVL isoforms is needed to confirm the mechanism of action and discover how these enzymes differ in their affinity for FAs of different chain lengths. Functionally, it will be important to learn the conditions

under which ACS activity activates AMPK and how the ACSL isoforms can direct their acyl-CoA products toward independent downstream pathways.

Acknowledgments

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 260).

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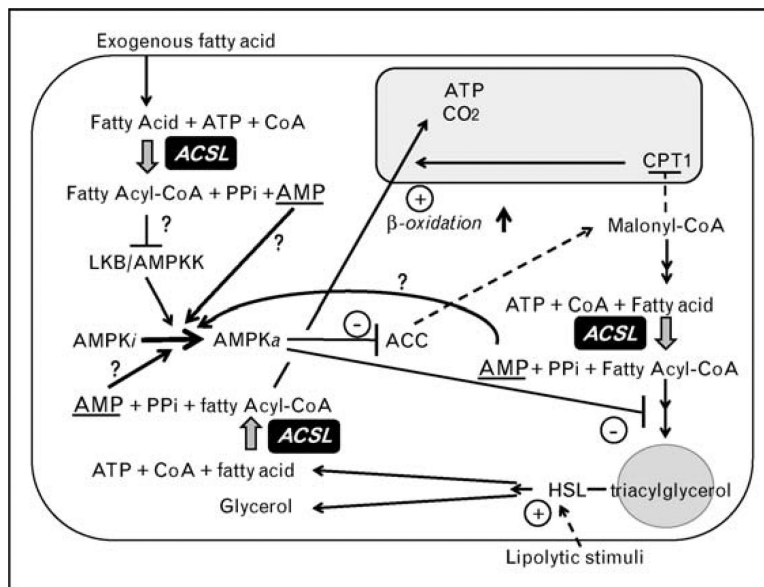


Figure 1. Activation of fatty acids by long-chain acyl-coenzyme A synthetase may activate AMP-activated kinase

When exogenous fatty acids enter the cell, are synthesized *de novo*, or are hydrolyzed from intracellular triacylglycerol stores, they must first be activated by a long chain acyl-CoA synthetase (ACSL) to form fatty acyl-CoAs. They can then enter downstream metabolic pathways. Because ATP is a substrate for ACSL and AMP is a product, the increased AMP/ATP ratio, a signal of low cellular energy stores, could theoretically activate AMP-activated kinase (AMPK). Activated AMPK inhibits triacylglycerol synthesis and acetyl-CoA carboxylase (ACC). The resulting decrease in malonyl-CoA levels diminishes fatty acid synthesis and also relieves the inhibition of carnitine palmitoyltransferase-1 (CPT1) by malonyl-CoA, allowing fatty acyl-CoA to be converted to acyl-carnitine and enter the mitochondria for β -oxidation. Thus, activated AMPK would generate an increase in ATP production. A prolonged lipogenic stimulus [which activates hormone-sensitive lipase (HSL)], may also inactivate AMPK to modulate these effects. It seems unlikely, however, that ACSL would activate AMPK under physiological conditions that stimulate triacylglycerol synthesis.