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Phosphorylation and Acetylation of Acyl-CoA Synthetase- I

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Abstract

Long chain acyl-CoA synthetase 1 (ACSL1) contributes 50 to 90% of total ACSL activity in liver, adipose tissue, and heart and appears to direct the use of long chain fatty acids for energy. Although the functional importance of ACSL1 is becoming clear, little is understood about its post-translational regulation. In order to investigate the post-translational modifications of ACSL1 under different physiological conditions, we overexpressed ACSL1 in hepatocytes, brown adipocytes, and 3T3-L1 differentiated adipocytes, treated these cells with different hormones, and analyzed the resulting phosphorylated and acetylated amino acids by mass spectrometry. We then compared these results to the post-translational modifications observed *in vivo* in liver and brown adipose tissue after mice were fasted or exposed to a cold environment. We identified universal N-terminal acetylation, 15 acetylated lysines, and 25 phosphorylation sites on ACSL1. Several unique acetylation and phosphorylation sites occurred under conditions in which fatty acid β-oxidation is normally enhanced. Thirteen of the acetylated lysines had not previously been identified, and none of the phosphorylation sites had been previously identified. Site-directed mutagenesis was used to introduce mutations at three potential acetylation and phosphorylation sites believed to be important for ACSL1 function. At the ATP/AMP binding site and at a highly conserved site near the C terminus, modifications of Ser278 or Lys676, respectively, totally inhibited ACSL1 activity. In contrast, mutations of Lys285 that mimicked acetylation (Lys285Ala and Lys285Gln) reduced ACSL activity, whereas full activity was retained by Lys285Arg, suggesting that acetylation of Lys285 would be likely to decrease ACSL1 activity. These results indicate that ACSL1 is highly modified post-translationally. Several of these modifications would be expected to alter enzymatic function, but others may affect protein stability or protein-protein interactions.

Keywords: Fatty acid metabolism; Beta-oxidation; Post-translational modification; Triacylglycerol synthesis, phosphorylation; Acetylation

Abbreviations: ACCS: Acetyl-CoA Synthetase; ACSL: Long-chain acyl-CoA Synthetase; AICAR: 5-aminoimidazole-4-carboxamide riboside; BAT: Brown Adipose Tissue; GFP: Green Fluorescent Protein; MOI: Multiplicity of Infection; SIRT: Sirtuin Deacetylase

Introduction

Acyl-CoA synthetases (ACS) catalyze the ATP-dependent ligation of fatty acids with coenzyme A and comprise a 26 member mammalian family whose members are named for fatty acid chain length preference and identified by homologies in two major amino acid regions [1]. The five isoforms of long chain acyl-CoA synthetase (ACSL) are each encoded by a separate gene and catalyze the activation of saturated and unsaturated fatty acids of 8-22 carbon chains [2]. Most of their acyl-CoA products are directed towards either oxidation pathways or towards the synthetic pathways for triacylglycerol, phospholipids and cholesterol esters. Long chain acyl-CoAs are also important signaling molecules in lipid metabolism, insulin secretion, and glucose uptake, storage, and metabolism [3,4].

ACSL1, the major mRNA isoform in liver, adipose tissue, and heart [5], contributes 50% of total ACSL activity in liver and 80 to 90% in adipose tissue and heart [6-8]. ACSL1 is regulated transcriptionally by PPARα [9] and by nutrient and hormonal signals in a tissue specific manner [10]. For example, obesity increases ACSL1 mRNA in adipose, but changes in ACSL1 mRNA expression in the liver vary, depending on the obesity model examined [10]. Exercise decreases ACSL1 mRNA and activity in adipose, while increasing both ACSL1 mRNA and activity in gastrocnemius muscle [10].

Indirect evidence suggests that ACSL1 is also regulated post-translationally. For example, mice that have been either fasted or fasted then refed show disparate changes in ACSL1 mRNA, protein expression, and activity [5]. Thus after fasting, *Acsl1* mRNA expression increases in gastrocnemius, inguinal adipose, and liver, while ACSL1 protein levels increase in gastrocnemius, but decrease in inguinal adipose and liver. mRNA expression is unchanged in gonadal adipose tissue after a 48 h fast, but adipose protein levels and ACSL specific activity decrease [5]. Acute regulation of ACSL1 is also suggested by decreased enzyme activity in rat adipocytes after they are incubated with the membrane-permeable cAMP analog, dibutyryl-cAMP [11].

In order to investigate the post-translational modifications of ACSL1 under different physiological conditions, we used mass spectrometry to analyze overexpressed ACSL1 in hepatocytes, brown adipocytes, and differentiated 3T3-L1 adipocytes after treatment with different hormones and drug activators of protein kinase A or AMP-activated kinase (AMPK). Because *Acsl1* mRNA is regulated by PPARα [9] and is required for fatty acid β-oxidation [6-8], we also analyzed endogenous ACSL1 in liver and brown adipose tissue from mice under conditions in which fatty acid oxidation would be upregulated, i.e. fasting or exposure to a cold environment. We found universal N-terminal acetylation, and identified 15 acetylated lysines and 25 phosphorylation sites on ACSL1. Thirteen of the acetylated lysines had

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not previously been identified, and none of the phosphorylation sites had been previously identified. Several modifications were unique to a specific tissue or physiological condition and many modified critical functional regions of the protein. This study indicates a remarkable array of post-translational modifications that may control the activity of ACSL1.

Experimental Procedures

Animals and tissue fractionation

Animal protocols were approved by the UNC Institutional Animal Care and Use Committee. C57Bl/6 mice were maintained on a 12-h light/dark cycle with free access to water and low fat diet (Prolab 5P76 Isopro 3000; 5.4% fat by weight). Six to 11 week old mice were euthanized by cervical dislocation after each of four treatments: fed, fasted for 20 h, fasted for 9 h at room temperature, or fasted for 4 h and then exposed to 4°C for 5 h while continuing to fast. Liver and brown adipose tissues (BAT) were immediately removed and all subsequent steps were performed at 4°C. Unless stated otherwise, buffers contained proteinase inhibitor (Roche) and phosphatase inhibitor cocktail I and II (Sigma, St. Louis MO). For crude mitochondria collection, liver was rinsed with ice cold phosphate buffered saline and homogenized with 10 up-and-down strokes in a Teflon-glass motorized homogenizing vessel (setting 6) in cold buffer H (10 mM HEPES-KOH, pH 7.4, 0.25 M sucrose, 1 mM EDTA and 1 mM dithiothreitol). Crude mitochondria were collected as previously described [12], except that the pellet was resuspended in gentle lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40,5 mM EDTA), and homogenized in a Dounce tissue grinder with a glass pestle. For lysate collection, liver or BAT was homogenized in gentle lysis buffer and the lysate was collected after centrifuging at 600 x g.

Cell culture

Cells were maintained at 37°C in 5% CO_2/air. Lysates were collected in cold RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) or gentle lysis buffer supplemented with proteinase inhibitor (Roche) and phosphatase inhibitor cocktail I and II (Sigma, St. Louis MO).

Hepatocyte isolation and adenovirus infection

Primary hepatocytes were isolated from male Wistar rats (250-300 g) by the UNC Cellular Metabolism and Transport Core. Hepatocytes were seeded at a density of 4.5×10^6 cells per 100-mm collagen-coated dish in Minimal Essential Media supplemented with 10% Fetal Bovine Serum (v/v) and 50 units/ml penicillin/streptomycin. For adenovirus infection, recombinant adenoviruses Ad-*Acsl1* that carries a C-terminal Flag tag or Ad-GFP (control) [13] were added at an MOI of 20 for 24 h. Cells were either untreated or treated for 20-30 min with one of the following hormones: 200 nM insulin, 1 mM 5-aminoimidazole-4-carboxamide riboside (AICAR), 2 mM dibutyryl-cAMP, or 10 μM isoproterenol.

Brown adipocyte differentiation and adenovirus infection

Interscapular BAT from wild-type mice (postnatal day 5) was removed, minced, and digested for 30 min with collagenase (CSL1, 0.2%, Worthington) in isolation buffer (123 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 5 mM glucose, 100 mM HEPES, 4% bovine serum albumin) [14]. Precursor cells were collected and maintained in growth medium (Dulbecco's Modified Eagle's Medium with 10 mM HEPES, 25 mM glucose, 10% fetal bovine serum, 100 units/ml penicillin/streptomycin, 17 μM D-pantothenic acid, 33 μM D-biotin, 100 μM ascorbic acid, 8.3 mM L-glutamine and 4 nM insulin) [15]. Confluent cells (post-confluence day 2) were differentiated with growth medium supplemented with 50 nM insulin, 50 nM T3 and 1 μM rosiglitazone. Four days later, differentiated brown adipocytes were infected with adenovirus (Ad-GFP or Ad-*Acsl1*) using Lipofectamine (Invitrogen, Carlsbad, CA) at an MOI of 100 [16]. Four days after infection, adipocytes were preincubated in serum-free Dulbecco's Modified Eagle's Medium with 0.5% fatty acid-free bovine serum albumin for 4 h before hormone treatment for 30 min. Cells were treated with either 10 μM of the β3-adrenergic receptor agonist CL-316243, 200 nM insulin, or 20 μM forskolin.

3T3-L1CARΔ1 Adipocyte Differentiation and Adenovirus Infection

A 3T3-L1 adipocyte cell line stably expressing the truncated receptor for coxsackievirus and adenovirus receptor (3T3-L1CARΔ1) was a gift from Dr. D.J. Orlicky (University of Colorado). 3T3-L1CARΔ1 preadipocytes were cultured in Dulbecco's Modified Eagle's Medium containing 25 mM glucose, 10% fetal bovine serum and 100 U/ml penicillin/streptomycin [17]. Differentiation was induced in 2-d post-confluent cells with 0.25 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 100 nM insulin for 48 h [18]. Cells were then grown supplemented with 100 nM insulin, which was replaced every 2 d thereafter until analysis. After 6 days, the adipocytes were infected with adenovirus (Ad-GFP or Ad-*Acsl1*) at an MOI 200 for 16 h. Adipocytes were serum starved for 3 h before hormone treatment with 500 nM insulin for 20 min, 2 mM dibutyryl-cAMP for 30 min, or 200 μM 1,2-dioctanoyl-*sn*-glycerol for 30 min.

Immunopurification

Immunoprecipitation was performed in RIPA buffer or gentle lysis buffer at 4°C. ACSL1-Flag was immunoprecipitated overnight from Ad-*Acsl1* infected cells using anti-Flag M2 affinity agarose gel (Sigma, St. Louis MO). Ad-GFP infected cells were used as a negative control. Endogenous ACSL1 was immunoprecipitated overnight with rabbit anti-ACSL1 (21st Century Biochemicals, Marlborough, MA); target sequence Ac-EVHELFRYFRMPELIDIC-amide) from crude liver mitochondria, liver lysate, or BAT lysate. Crude mitochondria from liver-specific $Acsl1^{L-/-}$ liver or lysates from adipose-specific $Acsl1^{A-/-}$ BAT were used as negative controls. The immune complex was incubated with protein A/G agarose beads for 1 h (Santa Cruz Biotechnology, Santa Cruz, CA). The immune complex beads were treated with 4X LDS buffer (Invitrogen, Carlsbad, CA) supplemented with 1 mM dithiothreitol at 70°C for 10 min before alkylation by 2 mM iodoacetamide [DOI: 10.1038/nprot.2008.123]. The supernatant was subjected to electrophoresis in a NuPAGE 4-12% Bis-Tris gel, and the gel was stained for total protein using GelCode Blue Stain (Invitrogen, Carlsbad, CA).

LC-MS/MS

The 78 kDa bands were excised, cut into 1 mm^3 cubes, destained, and incubated with trypsin, and peptides were extracted [DOI: 10.1038/nprot.2008.123]. Reversed-phase nanoflow liquid chromatography-tandem mass spectrometry (nLC-MS/MS) was performed on an ABI TEMPO capillary LC coupled to an Applied Biosystems 4000QTrap mass spectrometer. Peptides were reconstituted in 15 μl of 0.5% formic acid and incubated for 10 min at 25°C with shaking. The pH of the solution was neutralized with 7.5 μl of 0.1 M NH_4HCO_3 and incubated for 10 min at 25°C with shaking. A 10 μl aliquot was loaded and desalted on a C18 trap at 20 μl/min with 95% A (95% water/5% acetonitrile with 0.1% formic acid) and 5% B (5% water/95% acetonitrile with 0.1%

formic acid) and separated on a 75 μm inner diameter × 15 cm Acclaim PepMap 100 C_{18} nanoLC column (LC Packings). The gradient flowed at 300 nl/min and started at 95% A and 5% B, increased to 40% B from 15 to 140 min, and was again increased to 90% B from 140 to 150 min. The gradient was held at 90% B for 10 min before the original conditions were resumed. An acetonitrile blank was run after each sample; the gradient started at 95% A and 5% B, increasing to 90% B at 5 min, and held for 40 min before returning to baseline conditions. Mass spectra were acquired for the samples and the blanks with Analyst Software v1.4.1 (AB SCIEX, Framingham, MA) scanning the mass range of 400-1800 m/z and the 3 most abundant multiply-charged peaks were selected for MS/MS. MS/MS centroid peak lists were created from precursors of all charge states using Mascot.dll script. Proteome Discoverer v1.3 (Thermo Fisher Scientific) was used for data analysis. MS/MS spectra were first searched against the Swiss-Prot (November 8, 2010 release) and reversed databases using a Mus or Rattus taxonomy filter with MASCOT v2.2.1 and a *Mus musculus* or *Rattus norvegicus* database subset for SEQUEST v1.2 with up to 1 missed cleavage allowed and carbidomethyl (C) static modification with a precursor tolerance of ± 1.8 Da and a MS/MS tolerance of ± 0.8 Da. The search results filtered for medium and high confidence peptides (defined according to the Percolator algorithm [19] with a q-value threshold of 0.05) and for proteins with more than 2 peptides (only rank 1 peptides were counted, and if a peptide belonged to more than one protein, it was only counted in the top scored protein) with protein grouping enabled are shown in Supplementary Table 1. All of the search results files belonging to each species, rat and mouse, respectively, were opened into a multi-consensus report. We filtered for high-confidence peptides (defined according to the Percolator algorithm [19] with a q-value threshold of 0.01) and for proteins with more than 5 peptides (only rank 1 peptides were counted and if a peptide belonged to more than one protein it was only counted in the top scored protein). The remaining proteins, including all proteins in all protein groups, were exported as a FASTA file to use in a second search for modifications. The second search was performed in Proteome Discoverer v1.3 (Thermo Fisher Scientific) using SEQUEST v1.2. MS/MS spectra were searched against the rat or mouse subset and corresponding decoy database created in the first pass search with up to 2 missed cleavages allowed and acetylation (K, N-terminal), carbamidomethyl (C), deamidation (NQ), oxidation (M), and phosphorylation (S,T, Y) variable modifications with a peptide tolerance of ± 1.8 Da and MS/MS tolerance of ± 0.8 Da. The smaller subset database was used to search for modifications in order to reduce the false discovery rate. The search results were filtered for high confidence peptides (defined according to the Percolator algorithm [19] with a q-value threshold of 0.01) and for proteins with more than 2 peptides (only rank 1 peptides were counted and if a peptide belonged to more than one protein it was only counted in the top scored protein). The modification search results for ACSL1 are shown in Supplementary Table 3. Replicates are biological replicates.

Site-directed mutagenesis

Full-length rat ACSL1 cDNA with a C-terminal Flag epitope was subcloned from a previously constructed plasmid to mammalian expression vector pcDNA3.1. PCR-based site-directed mutagenesis was performed to mutate single amino acids according to Stratagene's Quickchange Site-Directed Mutagenesis Manual. The primers (Supplemental Table 2) were synthesized by the UNC Nucleic Acids Core Facility. The full length ACSL1 with mutations was confirmed by sequencing by the UNC Genome Analysis Facility.

Transient transfection

CHO cells were routinely cultured in Modified Eagle's Medium containing 10% fetal bovine serum and 100 U/ml penicillin/streptomycin. CHO cells were plated a cell density of 2.0 x 10^5 per well in 6-well dishes and transfected 18 h after plating with 2 μg of plasmid carrying rat ACSL1 or mutants (Fugene 6, Roche, Florence, SC). Cell homogenates were collected 24 h post-transfection in ice-cold Medium A (10 mM Tris, pH 7.4, 250 mM sucrose, 1 mM EDTA, 1 mM dithiothreitol, and Protease Inhibitor Mixture (Sigma, St. Louis MO)). Aliquots were stored at -80°C until use.

ACSL assay

ACSL initial rates were measured with 50 μM [1-^{14}C] palmitic acid (Perkin Elmer, Waltham, MA), 10 mM ATP, and 0.25 mM CoA in total membrane fractions (0.5-4.0 μg) [20].

Sequence												Rat hepatocytes					Mouse Brown Adipocytes				3T3-L1			
Site	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5	None [4]	Ins [3]	AICAR [3]	cAMP [3]	Iso [1]	None [1]	Ins [2]	Fors [1]	CL [1]	None [1]	Ins [1]	cAMP [1]	di8 [1]
M1						**M**	E	V	H	E	L	xxxx	xxx	xxx	xxx	x	xx	x	x	x	x	x	x	x
K49	Y	A	T	R	P	**K**	A	L	K	P	P	x												
K52	R	P	K	A	L	**K**	P	P	C	D	L	x	x	x	xx	x		x	x		x			x
K217	E	G	V	E	N	**K**	L	T	P	C	L	xxxx	xx	xxx	xxx		xx	x	x	x	x	x		
K223	L	T	P	C	L	**K**	I	I	V	I	M	xxxx	xx	xxx	xxx	x	xx	x	x	x	x	x		
K341	L	C	H	G	A	**K**	I	G	F	F	Q					x								
K387	A	N	T	S	V	**K**	R	W	L	L	D			x	x									
K504	N	Y	Q	A	A	**K**	G	E	G	E	V			x										
K533	A	E	A	L	D	**K**	D	G	W	L	H		x											
K544	T	G	D	I	G	**K**	W	L	P	N	G		x											
K562	K	K	H	I	F	**K**	L	A	Q	G	E		xxx	xx	x	x	x			x	x			x

x, Identified acetylated lysines. Each "x" indicates that the modification was identified and multiple "x's" indicate that the modification was identified by the corresponding number of replicates. For example, "xx" indicates that the modification was identified in 2 replicates.
[ ] Number of replicates.
Abbreviations: Ins, insulin; AICAR, 5-aminoimidazole-4-carboxamide riboside; cAMP, dibutyryl-cyclic adenosine monophosphate; Iso, isoproterenol; Fors, forskolin; CL, CL-316243; di8,1,2-dioctanoyl-*sn*-glycerol
Note: ACSL1 was immunopurified from samples using RIPA buffer. Some of the untreated liver and brown adipocyte immunopurifications were done with gentle lysis buffer

Table 1: Acetylation sites found in overexpressed ACSL1. Ad-Acsl1-Flag was overexpressed in rat hepatocytes, mouse brown adipocytes or 3T3-L1 adipocytes, and treated as indicated.

Western blot

Homogenates from transfected CHO cells (30 μg) were separated by electrophoresis on a 10% polyacrylamide gel and transferred to nitrocellulose membranes (Bio-Rad). Protein expression of ACSL1 with and without mutations was detected by anti-ACSL1 polyclonal antibody (Cell Signaling 4047). GAPDH was used as the loading control (Abcam, Cambridge, MA; ab36840).

Results and Discussion

Acetylated lysines in overexpressed and endogenous ACSL1

To investigate the post-translational regulation of ACSL1 in different cell types and after different hormonal treatments, we overexpressed ACSL1 in rat hepatocytes, mouse brown adipocytes, and differentiated 3T3-L1 adipocytes. As has been reported for 80% of mammalian proteins, including in rat liver mitochondrial ACSL1 [21], the N-terminal methionine was acetylated under all conditions (Tables 1, 2). Although the function of N-terminal acetylation is unknown [22], in yeast an N-terminal acetylation appears to act as a degradation signal [23].

Eleven lysines were acetylated within the over-expressed protein (Table 1). Nine of these acetylated lysines had not been previously reported. Lys217 and Lys223 were acetylated in all rat hepatocytes and mouse brown adipocytes and in untreated and insulin-treated 3T3-L1 cells. In hepatocytes, Lys562 was also acetylated in all treatment groups, except in untreated hepatocytes. In contrast, Lys562 was acetylated in both untreated mouse brown adipocytes and untreated 3T3-L1 cells. Treatment of mouse brown adipocytes and 3T3-L1 cells with CL-316243, a β3-adrenergic agonist that increases fatty acid oxidation and thermogenesis [24], and 1,2-dioctanoyl-*sn*-glycerol (di8), respectively, also resulted in Lys562 acetylation. Five acetylated lysines (K49, K341, K504, K533, and K544) were unique to cell types or hormone treatment (Table 1). Acetylated Lys544 was previously identified in mouse liver after a calorie-restricted diet and in the livers of both fasted and fed mice [25,26].

Because post-translational modifications could be an artifact of overexpression, we also analyzed endogenous ACSL1 from mouse liver and brown adipose tissue (Table 2). None of the lysine acetylations, except Lys387, identified in the endogenous ACSL1, had been previously identified. Acetylated Lys387 was identified in liver after mice were fed a calorie restricted diet [25]. Although fewer total modifications were present in the endogenous ACSL1 compared to the overexpressed protein, three of the nine acetylated lysines identified in the endogenous samples were at the same sites. Thus, the overexpression and endogenous data complemented each other. Also congruent with the data from overexpressed ACSL1, Lys562 was the most prevalent acetylation in the endogenous samples in all treatment groups. Six of the acetylated lysines found endogenously (K52, K241, K250, K387, K641, and K649) were unique to a particular treatment or tissue.

Phosphorylated Residues in Overexpressed and Endogenous ACSL1

In overexpressed ACSL1 in rat hepatocytes, mouse brown adipocytes, and differentiated 3T3-L1 adipocytes that were either untreated or treated with different hormones, we identified 20 different phosphorylated residues (Table 3). None of these phosphorylation sites had been previously identified. Unlike the identified acetylated lysines, no specific residues were phosphorylated in all cell types or under all hormone treatments. However, Ser58 was consistently phosphorylated in hepatocytes in all treatment groups, and thirteen phosphorylation sites were unique either to a specific cell type or to a specific hormonal treatment (T46, T65, Y119, S123, T281, S299, T384, S385, T434, S440, T442, T445, and T527).

Seven residues were phosphorylated in endogenous ACSL1 from mouse liver and brown adipose tissue (Table 4). Two of these phosphorylated sites were identified in overexpressed ACSL1. Three of the endogenous phosphorylated sites were unique to treatment and tissue. Interestingly, Thr219 was phosphorylated in the fed, but not in the fasted condition in both liver and brown adipose tissue (Table 4). Thr219 was also phosphorylated when ACSL1 was overexpressed in hepatocytes treated with insulin, AICAR, or cAMP.

Critical functional regions

The 26 ACS family members have considerable homology [1]. Analysis of amino acid motifs in mammalian ACS isoforms and crystallization studies of an ACS from *Thermus thermophilus* HB8 have led to the designation of functional motifs (Figures 1, 2) [27].

Sequence												Mouse Liver					Mouse Brown Adipose					
Site	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5	Fed [3]	4 h fast [2]	20 h fast [1]	4°C [1]	RT [1]	None * [1]	Fed [2]	4 h fast [0]	20 h fast [3]	4°C [2]	RT [1]
M1						**M**	E	V	H	E	L	xxx	xx	x	x	x	x	xx		x	x	x
K52	R	P	K	A	L	**K**	P	P	C	D	L							x				
K223	L	T	P	C	L	**K**	I	I	V	I	M	x						x				x
K241	V	E	R	G	K	**K**	C	G	V	E	I									x		
K250	E	I	I	S	L	**K**	A	L	E	D	L									x		
K387	A	N	T	S	L	**K**	R	W	L	L	D											x
K428	S	S	L	G	G	**K**	V	R	L	M	I							x		xx		x
K562	K	K	H	I	F	**K**	L	A	Q	G	E	xx	x	x	x	x		x		x		x
K641	L	D	D	L	L	**K**	G	K	N	A	G							x				
K649	K	N	A	G	L	**K**	P	F	E	Q	V	x										

x, Identified acetylated lysines. Each "x" indicates that the modification was identified and multiple "x's" indicate that the modification was identified by the corresponding number of replicates. For example, "xx" indicates that the modification was identified in 2 replicates.
[ ] Number of replicates.
* Endogenous ACSL1 isolated from differentiated brown adipocytes
Abbreviations: RT, room temperature
Note: ACSL1 was immunopurified, using gentle lysis buffer, from liver crude mitochondria, except for one of the 4 hr fasting liver samples (which was a lysate), and from brown adipose tissue lysates.

Table 2: Acetylation sites found in endogenous ACSL1 from mouse liver or brown adipose tissue.

Sequence													Rat hepatocytes					Mouse Brown Adipocytes				3T3-L1			
Site	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5		None [4]	Ins [3]	AICAR [3]	cAMP [3]	Iso [1]	None [2]	Ins [1]	Fors [1]	CL [1]	None [1]	Ins [1]	cAMP [1]	di8 [1]
T46	T	F	W	Y	A	**T**	R	P	K	A	L		x												
S58	P	P	C	D	L	**S**	M	Q	S	V	E		x	xx	x	x(x)	(x)								
S61	D	L	S	M	Q	**S**	V	E	V	T	G		xx			(x)	(x)								
T65	Q	S	V	E	V	**T**	G	T	T	E	G						(x)								
Y119	K	P	N	Q	P	**Y**	E	W	I	S	Y													x	
S123	P	Y	E	W	I	**S**	Y	K	Q	V	A				x										
T219	V	E	N	K	L	**T**	P	C	L	K	I			x	x	x						x			
S230	I	V	I	M	D	**S**	Y	D	N	D	L		x		x	x						x			
Y231	V	I	M	D	S	**Y**	D	N	D	L	V		x		x							x			
T261	G	R	V	N	R	**T**	K	P	K	P	P		x											x	
T281	F	T	S	G	T	**T**	G	N	P	K	G		x												
T290	K	G	A	M	V	**T**	H	Q	N	I	M		x			x									
S299	I	M	N	D	C	**S**	G	F	I	K	A		x												
T384	F	G	Q	A	N	**T**	S	V	K	R	W					(x)									
S385	G	Q	A	N	T	**S**	V	K	R	W	L					(x)									
T434	V	R	L	M	I	**T**	G	A	A	P	V				xx										
S440	G	A	A	P	V	**S**	A	T	V	L	T				x										
T442	A	P	V	S	A	**T**	V	L	T	F	L				x										
T445	S	A	T	V	L	**T**	F	L	R	A	A				x										
T527	K	D	P	A	R	**T**	A	E	A	L	D			x											

x, Identified phosphorylations. Each "x" indicates that the modification was identified and multiple "x's" indicate that the modification was identified by the corresponding number of replicates. For example, "xx" indicates that the modification was identified in 2 replicates.
[ ] Number of replicates.
( ) Ambiguous site (i.e. S58 or S61).
Abbreviations: Ins, insulin; AICAR, 5-aminoimidazole-4-carboxamide riboside; cAMP, dibutyryl-cyclic adenosine monophosphate; Iso, isoproterenol; Fors, forskolin; CL, CL-316243; di8,1,2-dioctanoyl-*sn*-glycerol

Note: ACSL1 was immunopurified from samples using RIPA buffer. Some of the untreated liver and brown adipocyte immunopurifications were done with gentle lysis buffer.

Table 3: Phosphorylation sites found in overexpressed ACSL1. Ad-Acsl1-Flag was overexpressed in rat hepatocytes, mouse brown adipocytes, or 3T3-L1 adipocytes, and treated as indicated.

Sequence													Mouse Liver					Mouse Brown Adipose					
Site	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5		Fed [3]	4 h fast [2]	20 h fast [1]	4°C [1]	RT [1]	None* [1]	Fed [2]	4 h fast [0]	20 h fast [3]	4°C [2]	RT [1]
T67	V	E	V	T	G	**T**	T	E	G	V	R		x									x	
S123	P	Y	E	W	I	**S**	Y	K	Q	V	A		x										
Y124	Y	E	W	I	S	**Y**	K	Q	V	A	E		x										
T219	V	E	N	K	L	**T**	P	K	C	L	K		x						x				x
S423	F	H	K	I	Q	**S**	S	L	G	G	K										x		(x)
S424	H	K	I	Q	S	**S**	L	G	G	K	V										x		(x)
Y500	V	E	E	M	N	**Y**	L	A	S	K	G		x										
S503	M	N	Y	L	A	**S**	K	G	E	G	E		x		x								

x, Identified phosphorylations. Each "x" indicates that the modification was identified and multiple "x's" indicate that the modification was identified by the corresponding number of replicates. For example, "xx" indicates that the modification was identified in 2 replicates.
[ ] Number of replicates.
( ) Ambiguous site (e.g. S423 or S424).
* Endogenous ACSL1 isolated from differentiated brown adipocytes
Abbreviations: RT, room temperature
Note: ACSL1 was immunopurified using gentle lysis buffer from liver crude mitiochondria, except for one of the 4 hr fasting liver samples (which was a lysate), and the brown adipose tissue lysates.

Table 4: Phosphorylation sites found in endogenous ACSL1from mouse liver lysate, liver crude mitochondria, or brown adipose tissue lysate.

These include the ATP/AMP binding site (Motif I), Gate motif (Motif IV), Adenine binding site (Motif III), Fatty acid binding (Motif II), Linker region (continued Motif II), and Motif V. Lysine acetylation or phosphorylation at these sites is likely to regulate ACSL1 activity and, thus, long-chain fatty acid metabolism.

Modifications in the fatty acid binding region (Motif II)

Lys533 and Lys544 were acetylated in the fatty acid binding region of ACSL1 (Figure 1). Both Lys533 and Lys544 were acetylated in one replicate of overexpressed ACSL1 in hepatocytes incubated with insulin. A previous study found that Lys544 of ACSL1 is acetylated in mouse liver mitochondria before and after a 12 h fast [26], and that after a 2-wk calorie-restricted diet, the amount of acetylation on Lys544 decreased 2.7-fold while protein expression of SIRT3 and lysine acetylation of acyl-CoA synthetase medium chain-1 (ACSM1) and ACSS2 increased [25]. SIRT3 also deacetylates and activates ACCS2 [28]; although the

modified ACCS2 lysine is not structurally comparable, the data suggest a role for lysine deacetylation and up-regulation of fatty acid activation to acyl-CoA during caloric restriction. Thus, the relative amount of acetylation may be important at this residue. Changes in the relative amounts of lysine acetylation of ACS family members have also been associated with ethanol exposure. In rats, ethanol exposure increases total lysine acetylation of ACSS2 more than 4-fold, and it was suggested that hyperacetylation and inactivation of ACSS2 might play a role in hepatotoxicity [29]. Because ethanol-induced hyperacetylation is independent of SIRT3 expression levels, the sirtuin deacetylases may respond to different physiological stimuli.

Because ACSL1 channels fatty acids towards oxidation in heart and adipose tissue [8,30], activation of ACSL1 is likely to occur during fasting or caloric restriction when cellular energy is low. Because these conditions increase the expression and activity of the sirtuin deacetylases [31] and activate AMPK, it seems likely that deacetylation would increase β-oxidation.

Modifications in the linker region (Motif II)

Lys562, which located just after the Linker region, a six amino acid sequence that connects the N- and C-terminal regions (Figures 1, 2) [32], was acetylated in all treatment groups of ACSL1 overexpressed in hepatocytes, in untreated and di8-treated 3T3-L1 adipocytes, and in ACSL1 overexpressed in untreated and CL-316243-treated brown adipocytes. Lys562 was also acetylated under most conditions in endogenous ACSL1 from crude liver mitochondria. The Linker region is structurally important for catalysis; after the adenylate is formed, the C-terminal domain rotates ~140° to expose the active site to the opposite side of the C-terminal domain [27]. Lys562 is equivalent to Lys609 in *Salmonella enterica* ACSS. Acetylation at *S. enterica* Lys609 prevents adenylate formation without affecting the formation of the thioester [33]. Lys609 is part of the $PX_4GK\theta X(R/K)$ consensus sequence within the Fatty acid binding site/ Linker motif (where θ is the amino acid alanine, valine, leucine, isoleucine, or methionine) within the active site [32]. Further, mutating the homologous lysine in *Arabidopsis thaliana* 4-coumarate-CoA ligase abolishes its catalytic function [34].

Other modified sites

Similar to the decreased amount of Lys544 found after caloric restriction (see above), the amount of acetylation on Lys387 decreased 2.4-fold [25]. As predicted for possible AMPK-mediated modifications, a decrease in Lys387 acetylation with caloric restriction may enhance β-oxidation. Lys387 was uniquely acetylated in overexpressed ACSL1 in untreated brown adipocytes.

Unique modified sites

Several additional acetylated and phosphorylated residues were unique to a particular treatment or cell type. Lys641 was uniquely acetylated in brown adipose tissue from fed mice (Figure 2). This acetylation site lies near a fragment of Motif V, which is conserved in ACS family members and contains a lysine that is required for activity [1].

Several of the acetylation and phosphorylation sites identified in the overexpressed ACSL1 samples were not observed in the endogenous samples. These types of discrepancies could result if the amount of modified peptide were below the detection limit, if sample loss occurred during preparation, if the modification was lost during sample handling or detection, or if actual biological differences were present. Because few acetylated lysines and phosphorylated residues were unique to a cell type or hormone treatment, we cannot exclude the

Figure 1: Summary of acetylation and phosphorylation sites identified by nLC-MS/MS from overexpressed rat ACSL1 in hepatocytes and endogenous ACSL1 in mouse liver for all treatment groups. The sequence shown is rat ACSL1 (P18163). However, the amino acid residues shown in parenthesis indicate sequence variations between rat and mouse that vary at the post-translational modification site. The numbers above the amino acids indicate the amino acid position of the modification; forward slashes are present to separate the numbers. The AMP binding site, Gate motif, Adenine binding motif, Fatty acid binding site, Linker region, and the consensus fragment of Motif V are underlined; Roman numerals designate the motifs, as described in reference [27].

1 52
MEVHELFRYFRMPELIDIRQYVRTLPTNTLMGFGAFAALTTFWYATRPKALKPPCDLSMQSVEVTGTTEGVRRSAVLEDDKLL

LYYYDDVRTMYDGFQRGIQVSNDGPCLGSRKPNQPYEWISYKQVAEMAECIGSALIQKGFKPCSEQFIGIFSQNRPEWVTIEQ

217/219 223 241
GCFTYSMVVVPLYDTLGTDAITYIVNKAELSVIFADKPEKAKLLLEGVENKLTPCLKIIVIMDSYDNDLVERGQKCGVEIIGL

250
KALEDLGRVNRTKPKPPEPEDLAIICFTSGTTGNPKGAMVTHQNIMNDCSGFIKATESAFIASPEDVLISFLPLAHMFETVVE
AMP binding/ I Gate motif/ IV

387
CVMLCHGAKIGFFQGDIRLLMDDLKVLQPTIFPVVPRLLNRMFDRIFGQANTSVKRWLLDFASKRKEAELRSGIVRNNSLWDK

423/424 428
LIFHKIQSSLGGKVRLMITGAAPVSATVLTFLRAALGCQFYEGYGQTECTAGCCLSLPGDWTAGHVGAPMPCNYIKLVDVEDM
Adenine binding/ III

562
NYQAAKGEGEVCVKGANVFKGYLKDPARTAEALDKDGWLHTGDIGKWLPNGTLKIIDRKKHIFKLAQGEYIAPEKIENIYLRS
Fatty Acid binding/ II Linker/ II

641
EAVAQVFVHGESLQAFLIAIVVPDVEILPSWAQKRGFQGSFEELCRNKDINKAILEDMVKLGKNAGLKPFEQVKGIAVHPELF

SIDNGLLTPTLKAKRPELRNYFRSQIDELYSTIKI
Motif V

Figure 2: Summary of acetylation and phosphorylation sites identified by nLC-MS/MS in overexpressed rat ACSL1 in brown adipocytes in cell culture and in endogenous ACSL1 in mouse brown adipose tissue for all treatment groups. The sequence shown is rat ACSL1 (P18163). However, the amino acid residues in parenthesis indicate the sequence variations between rat and mouse that vary at the post-translational modification site. The numbers above the amino acids indicate the amino acid position of the modification; forward slashes are present to separate the numbers. The AMP binding site, Gate motif, Adenine binding motif, Fatty acid binding site, Linker region, and the consensus fragment of Motif V are underlined; Roman numerals designate the motifs, as described in reference [27].

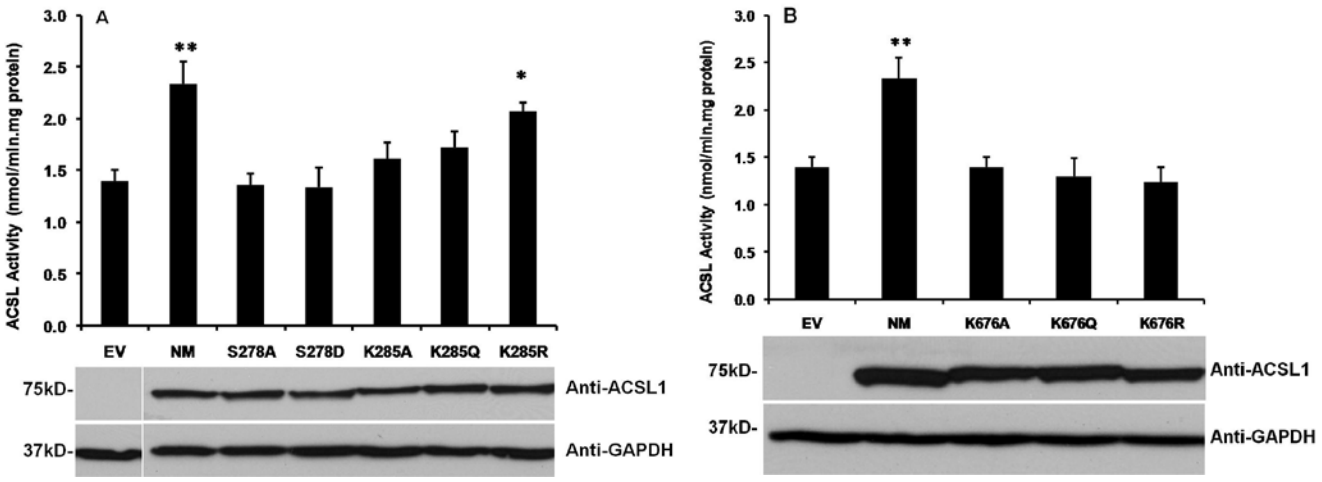

Figure 3: ACSL activity change by ACSL1 mutants in CHO cells. CHO cells were transfected with plasmids carrying empty vector (EV), or a full-length rat ACSL1 cDNA either without mutation (NM), or with mutations to replace single amino acids A) at Ser278 with alanine (S278A) or aspartic acid (S278D), at Lys285 with alanine (K285A), glutamine (K285Q), or arginine (K285R), or B) at Lys676 with alanine (K676A), glutamine (K676Q), or arginine (K676R). Cell homogenates were collected 24h post-transfection. Total ACSL activity was measured using [1-^{14}C]palmitate, data are means ± S.E. (n=3-6). * p<0.05, ** p<0.01. Western blotting with anti-ACSL1 antibody was performed with GAPDH as loading control, which has been repeated three times.

possibility that ACSL1 is regulated quantitatively by some of these post-translational modifications. Additionally, more biological replicates will be necessary to discern the reproducibility of the modifications under specific physiological conditions.

Mutations of Lys285 Ser278, and Lys676

To determine whether specific post-translational modifications alter ACSL1 activity, CHO cells were transfected with wild-type or mutated rat ACSL1. We elected to examine Lys285, Ser278, and Lys676. Lys285 and Ser278 are highly conserved residues that are located within the ATP/AMP binding site and were uniquely modified in hepatocytes by AICAR although the modifications had medium or

low confidence peptide identifications (falling below the q value = 0.1). We also modified Lys676 because it is a highly conserved residue in the motif KXXK/R that is present in all ACSs [1]. Ser278 was mutated to mimic the phosphorylated (S278D) and non-phosphorylated (S278A) states, whereas Lys285 and Lys676 were mutated to mimic the acetylated (K285A, K285Q, K676A, K676Q) or non-acetylated (K285R, K676R) states [35]. Transfected wild-type rat ACSL1 doubled ACSL total activity in CHO cells, but mutations of Ser278 diminished the total ACSL activity to the level observed with the empty vector, indicating total inhibition of ACSL1 activity (Figure 3A) and showing that Ser278 is critical. Lys172 in FadD13 from *Mycobacterium tuberculosis* is equivalent to Lys285, and has been proposed to bind to the phosphate group of ATP via electrostatic interactions [36]. Both K285A and K285Q, which should mimic acetylated Lys285, decreased the overexpressed ACSL1 activity by 60-70%, whereas K285R, which mimics deacetylated Lys285, maintained the activity increase caused by overexpressed ACSL1. These results suggest that acetylation of Lys285 would decrease ACSL1 activity. The fact that Ser278 and Lys285 were modified in concert in hepatocytes that had been treated with AICAR, strongly suggests that ACSL1 might be activated in liver under conditions when the cellular energy supply is deficient. Further investigation of these sites is necessary. Because these peptides were low confidence, a multi-enzyme approach or high mass accuracy instrument may help improve the identification of these modifications.

According to modeling of *M. tuberculosis* FadD13 [35], Lys676 would lie very near the phosphate group of ATP. Acetylation of the comparable Lys residue on the mitochondrial ACSS2 completely inactivates this enzyme, and deacetylation of the site by SIRT3, reactivates it [28]. Although our MS coverage of ACSL1 included this site, Lys676 was not acetylated under any condition in any of the tissues examined. However, all three mutations, Lys676Ala, Lys676Gln, and Lys676Arg, totally inhibited overexpressed ACSL activity (Figure 3B). Thus, similar to the comparable Lys487 residue in FadD13 [35], Lys676 appears to be essential for ACSL1 activity.

Discussion

Recent studies of global lysine acetylation modifications found in the HeLa and mouse liver cell proteomes reveal the complexity and ubiquity of this modification (reviewed in [37]). Lysine acetylation is an important regulator of metabolism [38,39] and is prevalent in mitochondria where more than 20% of proteins are estimated to contain acetyllysine modifications [26]. Several acetylated lysine residues from members of the acyl-CoA synthetase superfamily have been identified in these global acetylome studies (Supplemental Table 4). Similarly, although phosphorylation is a well-known regulator of cellular function and several phosphorylation sites in ACSL1 and other ACS family members were previously identified (Supplemental Table 5), little is known about the effect of phosphorylation on ACSL1 activity.

Functional effects of posttranslational modifications have been investigated in only a handful of ACS family members. Acetylation within the Linker region on Lys661 of mouse ACSS1 or on Lys635 in ACSS2, a mitochondrial isoform, deactivates these enzymes [40] and ACSS1 and ACSS2 can be reactivated by the sirtuin deacetylases SIRT1 and SIRT3, respectively [40]. Human ACSS2 is regulated similarly by SIRT3 on Lys642 [41]. In mice, when SIRT1 deacetylates and activates ACSS1, fatty acid synthesis increases [40]. During fasting, deacetylation and activation of ACSS would produce acetyl-CoA, a substrate used for both ketone production and the citric acid cycle. Other increases in fatty acid oxidation are also linked to SIRT-mediated deacetylation [42]. In our study, the comparable highly conserved lysine residue, Lys676, was not acetylated under any condition. However, mutating this lysine totally abrogated ACSL1 activity. Thus, if Lys676 were acetylated under some physiological condition, it is highly likely that ACSL1 would be inactivated, as this region seems to be critical for ACSL1 activity.

Although we identified a large number of lysine acetylation and phosphorylation sites of ACSL1, further studies will be required to understand the functional consequences of the majority of these modifications. Because few studies have found physiologically induced changes in ACSL1 specific activity, we suspect that many of the identified phosphorylations and acetylations do not simply alter enzyme activity but, instead, may act in concert under specific conditions. Further, because ACSL1 directs its acyl-CoA product towards both triacylglycerol synthesis and β-oxidation in liver [6], but only towards β-oxidation in adipocytes [7] and heart [8], it is likely that some of the modifications, rather than altering enzyme activity, might affect ACSL1 protein stability or protein-protein interactions.

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References

1. Watkins PA, Maiguel D, Jia Z, Pevsner J (2007) Evidence for 26 distinct acyl-coenzyme A synthetase genes in the human genome. J Lipid Res 48: 2736-2750.
2. Coleman RA, Lewin TM, Van Horn CG, Gonzalez-Baró MR (2002) Do acyl-CoA synthetases regulate fatty acid entry into synthetic versus degradative pathways? J Nutr 132: 2123-2126.
3. Faergeman NJ, Knudsen J (1997) Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling. Biochem J 323: 1-12.
4. Zhang L, Keung W, Samokhvalov V, Wang W, Lopaschuk GD (2010) Role of fatty acid uptake and fatty acid beta-oxidation in mediating insulin resistance in heart and skeletal muscle. Biochim Biophys Acta 1801: 1-22.
5. Mashek DG, Li LO, Coleman RA (2006) Rat long chain acyl-CoA synthetase mRNA, protein and activity vary in tissue distribution and in response to diet. J Lipid Res 47: 2004-2010.
6. Li LO, Ellis JM, Paich HA, Wang S, Gong N, et al. (2009) Liver-specific loss of long chain acyl-CoA synthetase-1 decreases triacylglycerol synthesis and beta-oxidation and alters phospholipid fatty acid composition. J Biol Chem 284: 27816-27826.
7. Ellis JM, Li LO, Wu P-C, Koves TR, Ilkayeva O, et al. (2010) Adipose acyl-CoA synthetase-1 (ACSL1) directs fatty acids towards β-oxidation and is required for cold thermogenesis. Cell Metab 12: 53-64.
8. Ellis JM, Mentock SM, Depetrillo MA, Koves TR, Sen S, et al. (2011) Mouse cardiac acyl coenzyme a synthetase 1 deficiency impairs fatty acid oxidation and induces cardiac hypertrophy. Mol Cell Biol 31: 1252-1262.
9. Schoonjans K, Watanabe M, Suzuki H, Mahfoudi A, Krey G, et al. (1995) Induction of the acyl-Coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. J Biol Chem 270: 19269-19276.
10. Coleman RA, Lewin TM, Muoio DM (2000) Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. Ann Rev Nutr 20: 77-103.
11. Hall M, Saggerson ED (1985) Reversible inactivation by noradrenaline of long-chain fatty acyl-CoA synthetase in rat adipocytes. Biochem J 226: 275-282.
12. Pellon-Maison M, Montanaro MA, Coleman RA, Gonzalez-Baro MR

(2007) Mitochondrial glycerol-3-P acyltransferase 1 is most active in outer mitochondrial membrane but not in mitochondrial associated vesicles (MAV). Biochim Biophys Acta 1771: 830-838.

13. Li LO, Mashek DG, An J, Doughman SD, Newgard CB, et al. (2006) Overexpression of rat long chain acyl-CoA synthetase 1 alters fatty acid metabolism in rat primary hepatocytes. J Biol Chem 281: 37246-37255.
14. Klein J, Fasshauer M, Ito M, Lowell BB, Benito M, et al. (1999) Beta(3)-adrenergic stimulation differentially inhibits insulin signaling and decreases insulin-induced glucose uptake in brown adipocytes. J Biol Chem 274: 34795-34802.
15. Ailhaud G, editor (2001) Adipose Tissue Protocols. Totowa, NJ: Humana Press. 197-224.
16. Miyoshi H, Perfield JW 2nd, Obin MS, Greenberg AS (2008) Adipose triglyceride lipase regulates basal lipolysis and lipid droplet size in adipocytes. J Cell Biochem 105: 1430-1436.
17. Orlicky DJ, DeGregori J, Schaack J (2001) Construction of stable coxsackievirus and adenovirus receptor-expressing 3T3-L1 cells. J Lipid Res 42: 910-915.
18. Ross SA, Song X, Burney MW, Kasai Y, Orlicky DJ (2003) Efficient adenovirus transduction of 3T3-L1 adipocytes stably expressing coxsackie-adenovirus receptor. Biochem Biophys Res Commun 302: 354-358.
19. Kall L, Canterbury JD, Weston J, Noble WS, MacCoss MJ (2007) Semi-supervised learning for peptide identification from shotgun proteomics datasets. Nat Methods 4: 923-925.
20. Polokoff MA, Bell RM (1978) Limited palmitoyl-CoA penetration into microsomal vesicles as evidenced by a highly latent ethanol acyltransferase activity. J Biol Chem 253: 7173-7178.
21. Distler AM, Kerner J, Hoppel CL (2007) Post-translational modifications of rat liver mitochondrial outer membrane proteins identified by mass spectrometry. Biochim Biophys Acta 1774: 628-636.
22. Polevoda B, Sherman F (2000) Nalpha -terminal acetylation of eukaryotic proteins. J Biol Chem 275: 36479-36482.
23. Hwang CS, Shemorry A, Varshavsky A (2010) N-terminal acetylation of cellular proteins creates specific degradation signals. Science 327: 973-977.
24. Inokuma K, Okamatsu-Ogura Y, Omachi A, Matsushita Y, Kimura K, et al. (2006) Indispensable role of mitochondrial UCP1 for antiobesity effect of beta3-adrenergic stimulation. Am J Physiol Endocrinol Metab 290: 1014-1021.
25. Schwer B, Eckersdorff M, Li Y, Silva JC, Fermin D, et al. (2009) Calorie restriction alters mitochondrial protein acetylation. Aging Cell 8: 604-606.
26. Kim SC, Sprung R, Chen Y, Xu Y, Ball H, et al. (2006) Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mol Cell 23: 607-618.
27. Hisanaga Y, Ago H, Nakagawa N, Hamada K, Ida K, et al. (2004) Structural basis of the substrate-specific two-step catalysis of long chain fatty acyl-CoA synthetase dimer. J Biol Chem 279: 31717-31726.
28. Hallows WC, Lee S, Denu JM (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. Proc Natl Acad Sci USA 103: 10230-10235.
29. Picklo MJ, Sr. (2008) Ethanol intoxication increases hepatic N-lysyl protein acetylation. Biochem Biophys Res Commun 376: 615-619.
30. Ellis JM, Li LO, Wu PC, Koves TR, Ilkayeva O, et al. (2010) Adipose acyl-CoA synthetase-1 directs fatty acids toward beta-oxidation and is required for cold thermogenesis. Cell Metab 12: 53-64.
31. Wohrl BM, Volkmann S, Moelling K (1991) Mutations of a conserved residue within HIV-1 ribonuclease H affect its exo- and endonuclease activities. J Mol Biol 220: 801-818.
32. Gulick AM (2009) Conformational dynamics in the Acyl-CoA synthetases, adenylation domains of non-ribosomal peptide synthetases, and firefly luciferase. ACS Chem Biol 4: 811-827.
33. Starai VJ, Celic I, Cole RN, Boeke JD, Escalante-Semerena JC (2002) Sir2-dependent activation of acetyl-CoA synthetase by deacetylation of active lysine. Science 298: 2390-2392.
34. Stuible H, Buttner D, Ehlting J, Hahlbrock K, Kombrink E (2000) Mutational analysis of 4-coumarate:CoA ligase identifies functionally important amino acids and verifies its close relationship to other adenylate-forming enzymes. FEBS Lett 467: 117-122.
35. Wang YH, Tsay YG, Tan BC, Lo WY, Lee SC (2003) Identification and characterization of a novel p300-mediated p53 acetylation site, lysine 305. J Biol Chem 278: 25568-25576.
36. Khare G, Gupta V, Gupta RK, Gupta R, Bhat R, et al. (2009) Dissecting the role of critical residues and substrate preference of a Fatty Acyl-CoA Synthetase (FadD13) of Mycobacterium tuberculosis. PLoS One 4: e8387.
37. Norris KL, Lee JY, Yao TP (2009) Acetylation goes global: the emergence of acetylation biology. Sci Signal 2: pe76.
38. Close P, Creppe C, Gillard M, Ladang A, Chapelle JP, et al. (2010) The emerging role of lysine acetylation of non-nuclear proteins. Cell Mol Life Sci 67: 1255-1264.
39. Zhao S, Xu W, Jiang W, Yu W, Lin Y, et al. (2010) Regulation of cellular metabolism by protein lysine acetylation. Science 327: 1000-1004.
40. Hallows WC, Lee S, Denu JM (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. Proc Natl Acad Sci U S A 103: 10230-10235.
41. Schwer B, Bunkenborg J, Verdin RO, Andersen JS, Verdin E (2006) Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. Proc Natl Acad Sci U S A 103: 10224-10229.
42. Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, et al. (2010) SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 464: 121-125.