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2 Review

6 Long-chain acyl-CoA esters in metabolism and signaling: Role 7 of acyl-CoA binding proteins

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Long-chain fatty acyl-CoA esters are key intermediates in numerous lipid metabolic pathways, and recognized as important cellular signaling molecules. The intracellular flux and regulatory properties of acyl-CoA esters have been proposed to be coordinated by acyl-CoA-binding domain containing proteins (ACBDs). The ACBDs, which comprise a highly conserved multigene family of intracellular lipid-binding proteins, are found in all eukaryotes and ubiquitously expressed in all metazoan tissues, with distinct expression patterns for individual ACBDs. The ACBDs are involved in numerous intracellular processes including fatty acid-, glycerolipid and glycerophospholipid biosynthesis, β-oxidation, cellular differentiation and proliferation as well as in the regulation of numerous enzyme activities. Little is known about the specific roles of the ACBDs in the regulation of these processes, however, recent studies have gained further insights into their *in vivo* functions and provided further evidence for ACBD-specific functions in cellular signaling and lipid metabolic pathways. This review summarizes the structural and functional properties of the various ACBDs, with special emphasis on the function of ACBD1, commonly known as ACBP.

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1. Introduction

Long-chain fatty acyl-CoA esters (LCACoA) not only serve as essential intermediates in lipid metabolism, but are also recognized as regulators of a wide range of cellular functions [1]. This entails that the intracellular concentration of LCACoA is carefully controlled in order to allow continuous flux to either lipid biosynthetic processes or degradation while still serving as an intracellular regulator of signaling, ion channels and metabolic enzymes. The intracellular concentration of LCACoA is primarily controlled by *de novo* fatty acid synthesis, the activity of acyl-CoA synthetases (ACSL) and acyl-CoA thioesterase (ACOT), rate of β-oxidation and finally the concentration of cellular LCACoA binding proteins. To this end liver type fatty acid binding protein (FABP1), sterol carrier protein 2 (SCP2) and acyl-coenzyme A binding protein (ACBP) [2] have all been shown to bind LCACoA esters,

however, in contrast to FABPs and SCP2, ACBP binds only LCACoA esters. ACBP was discovered independently by five different groups as (1) a brain peptide, diazepam binding inhibitor (DBI) capable of inhibiting diazepam binding to the GABA receptor [2], (2) a bovine adrenal peptide stimulating cholesterol transport into Leydig cell mitochondria [3], (3) an intestinal peptide regulating insulin release in pigs [4], (4) a CCK-releasing peptide in the intestine [5] and finally (5) a polypeptide able to induce termination of fatty acid synthesis by binding acyl-CoA esters with high affinity [6].

Until a few years ago ACBP was thought to be the only high-affinity binding protein that exclusively bound acyl-CoA esters. However, during the past decade, numerous proteins have been identified, which comprise ACBP as a domain. Thus, these proteins, termed acyl-CoA binding domain proteins (ACBD), now comprise a large multigene family of intracellular lipid-binding proteins, to which ACBP, synonymous with DBI, belongs (Fig. 1). However,

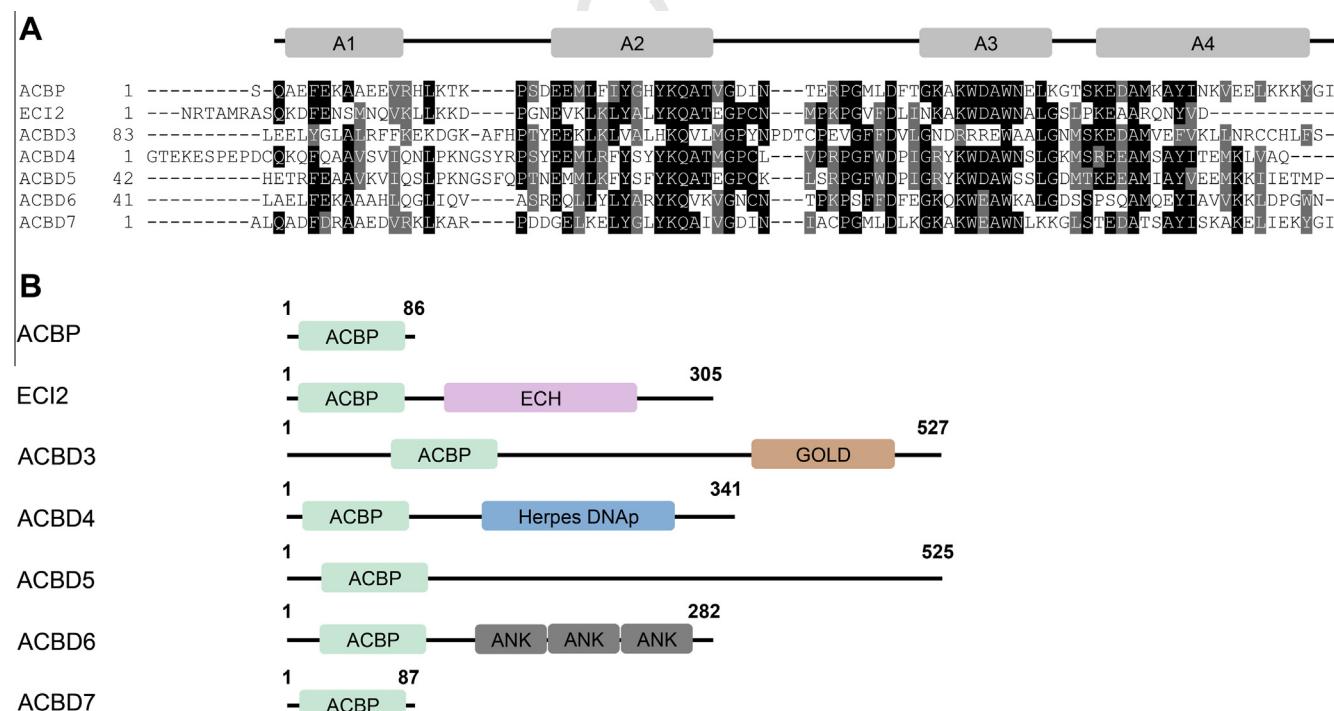


Fig. 1. Alignment and protein domain structure of human ACBDs. (A) Multiple alignment of human acyl-CoA binding domain proteins. Single-letter abbreviations for amino acid residues are used. Identical and similar amino acids are identified by black and gray shading, respectively. The α-helices (A1–A4) are indicated by light gray boxes above the alignment. (B) Linear domain structure of human ACBDs. The ACBP domain is colored green, the enoyl-CoA hydratase/isomerase domain in ECI2 is pink, the GOLD domain in ACBD3 is orange, the Herpes DNA replication accessory factor domain (Herpes DNAp) in ACBD4 is blue, and the ankyrin repeats in ACBD6 are gray.

due to the presence of acyl-CoA binding domain proteins, the protein formerly known as ACBP or DBI is also synonymous with ACBD1. In the present review we will use the standard nomenclature for acyl-CoA binding protein and for acyl-coenzyme A binding domain proteins defined by the National Center for Biotechnology Information. However, when we describe the acyl-CoA binding protein as the independent single-domain protein known as ACBP, ACBD1 or DBI we will solely use the term ACBP (Fig. 1).

ACBP is an approximately ~10 kDa protein, which binds LCACoA esters with high specificity and affinity (K_D , 1–10 nM). The gene encoding *Acbp* and the single domain ACBP protein have been detected in all eukaryotic species, in some prokaryotes including *Archaea*, and in one virus (Table 1). Thus, its evolutionary conservation and the fact that it is expressed in all tissues and cell types, argue that the function of ACBP is associated with one or more fundamental cellular functions. However, as mentioned above, ACBP not only functions as an independent single-domain protein, the ACBP domain architecture is also found in a total of 48 different protein structures of varying length encompassing all eukaryotic species and numerous prokaryotes (Table 1).

Despite several studies the precise biochemical function of ACBP still remain unclear, however, due to the generation of various knockout models, its functions are beginning to emerge. Several *in vitro* studies have shown that ACBP is able to act as an intracellular acyl-CoA transporter and pool former [7,8]. Furthermore, recent studies strongly imply that ACBP is required for fatty acid chain elongation and sphingolipid synthesis in eukaryotes. In the present review we will shortly highlight the most important aspects of the structure, folding, binding properties and phylogeny of acyl-CoA binding domain proteins, but the emphasis will be on the role of LCACoA esters and ACBP in cell function and metabolism.

2. Long-chain acyl-CoA esters at the center stage of lipid metabolism

Multiple regulatory circuitries and networks play central roles in adjusting the concentrations of membrane components, and are increasingly recognized to be regulated by metabolites

Table 1

Protein entities containing ACBP as a domain. Human ACBP (ACBD-1) transcript variant 3 (87 aa) was used as query to identify proteins containing the ACBP structural fold.

# Of sequences	Taxonomy	Taxid	Accessions	Names of domains found in ACBDs
3288	Cellular organisms	131567	cl00221	ACBP
473	Eukaryota	2759	cl00221 ~ cl02529	ACBP ~ ANK
352	Eukaryota	2759	cl00221 ~ cl21466	ACBP ~ crotonase-like
296	Opisthokonta	33154	cl00221 ~ pfam13897	ACBP ~ GOLD_2
47	Embryophyta	3193	cl00221 ~ cl02701 ~ cl21468	ACBP ~ Kelch_3 ~ Tropomyosin_1
29	Magnoliophyta	3398	cl00221 ~ cl02701 ~ cl09111	ACBP ~ Kelch_3 ~ Prefoldin
24	Eukaryota	2759	cl00221 ~ cl02701	ACBP ~ Kelch_3
21	Francisella	262	cl00221 ~ cl17173 ~ pfam00725 ~ cl21466	ACBP ~ AdoMet_MTases ~ 3HCDH ~ crotonase-like
19	Oomycetes	4762	cl00221 ~ cl17764 ~ cl17171	ACBP ~ FYVE ~ PH-like
16	Oomycetes	4762	cl00221 ~ pfam07064	ACBP ~ RIC1
11	Oomycetes	4762	cl00221 ~ pfam04707	ACBP ~ PRELI
11	Homininae	207598	cl00221 ~ cl00464	ACBP ~ URO-D_CIMS_like
8	<i>Homo sapiens</i>	9606	cl00221 ~ pfam04929	ACBP ~ Herpes_DNAp_acc
8	Burkholderiales	80840	cl09109 ~ cl00221	NTF2_like ~ ACBP
7	<i>fabids</i>	91835	cl00221 ~ cl02701 ~ pfam09304	ACBP ~ Kelch_3 ~ Cortex-L_coil
7	Boreoeutheria	1437010	cl00221 ~ cl00506	ACBP ~ Haemolytic
6	Sarcocystidae	5809	cl00221 ~ cl15787	ACBP ~ SEC14
6	Papilioioidae	3814	cl00221 ~ cl02701 ~ cl03951	ACBP ~ Kelch_3 ~ CDC37_N
5	BEP clade	359160	cl00221 ~ cl02701 ~ cl21548	ACBP ~ Kelch_3 ~ PRK10698
4	Andropogoneae	147429	cl00221 ~ cl02701 ~ pfam13874	ACBP ~ Kelch_3 ~ Nup54
4	Percomorphaceae	1489872	cl00221 ~ pfam00755	ACBP ~ Carn_acyltransf
4	<i>Danio rerio</i>	7955	cl00221 ~ PHA03249	ACBP ~ PHA03249
4	Cyanobacteria	1117	cl21496 ~ cl00221 ~ cl09936	2OG-Fell_Oxy ~ ACBP ~ PP-binding
4	rosids	71275	cl00221 ~ cl02701 ~ cl21468 ~ cl09111	ACBP ~ Kelch_3 ~ Tropomyosin_1 ~ Prefoldin
4	<i>Myotis</i>	9434	cl00221 ~ pfam12258	ACBP ~ Microcephalin
4	Saprolegniaceae	4764	cl00117 ~ cl00221 ~ pfam07064	PDZ ~ ACBP ~ RIC1
4	Cercopithecinae	9528	cl00221 ~ pfam13900	ACBP ~ GVQW
3	Chromadorea	119089	cl00386 ~ cl00221 ~ cl02529	Bola ~ ACBP ~ ANK
3	Bilateria	33213	cl00221 ~ cl05946	ACBP ~ PspB
3	Endopterygota	33392	cl17173 ~ cl00221 ~ cl02529	AdoMet_MTases ~ ACBP ~ ANK
3	<i>Rattus norvegicus</i>	10116	pfam12517 ~ cl00221 ~ pfam13897	DUF3720 ~ ACBP ~ GOLD_2
3	melanogaster subgroup	32351	cl00221 ~ pfam07889	ACBP ~ DUF1664
3	<i>Emilia huxleyi CCMP1516</i>	280463	cl00221 ~ cl02563	ACBP ~ PX_domain
3	<i>Ochotona princeps</i>	9978	cl00221 ~ cl00084 ~ pfam04812	ACBP ~ homeodomain ~ HNF-1B_C
3	Cellular organisms	131567	cl21454 ~ cl00221	SDR ~ ACBP
3	<i>Populus trichocarpa</i>	3694	cl00221 ~ cl02701 ~ cl14654 ~ cl09111	ACBP ~ Kelch_3 ~ V_Alix_like ~ Prefoldin
3	Betaproteobacteria	28216	cl21494 ~ cl00221	Esterase_lipase ~ ACBP
3	Saprolegniaceae	4764	cl00221 ~ cl17764 ~ cl02495	ACBP ~ FYVE ~ RabGAP-TBC
3	<i>Plasmodium falciparum</i>	5833	cl00221 ~ TIGR01639 ~ pfam09687	ACBP ~ P_fal_TIGR01639 ~ PRESAN
3	Agaricomycetes incertae sedis	355688	cl21454 ~ cl00221 ~ cl02529	SDR ~ ACBP ~ ANK
2	<i>Emilia huxleyi CCMP1516</i>	280463	cl00221 ~ pfam08549 ~ cl02701	ACBP ~ SWI-SNF_Ssr4 ~ Kelch_3
2	<i>Capsaspora owczarzaki ATCC 30864</i>	595528	cl00221 ~ cl07029 ~ pfam13897	ACBP ~ SPT2 ~ GOLD_2
2	<i>Aedes aegypti</i>	7159	cl00221 ~ PRK01194 ~ cl15454 ~ pfam13897	ACBP ~ PRK01194 ~ HrpJ ~ GOLD_2
2	<i>Camelus ferus</i>	419612	cl17070 ~ cl00221	AMPKA_C_like ~ ACBP
2	<i>Phytophthora parasitica</i> P10297	1317064	cl00221 ~ cl17764	ACBP ~ FYVE
2	Astrotheria	311790	cl00221 ~ pfam11804	ACBP ~ DUF3325
2	<i>Eucalyptus grandis</i>	71139	cl12038 ~ cl00221	ZipA ~ ACBP
2	Basidiomycota	5204	cl00221 ~ pfam13908	ACBP ~ Shisa

including lipids, which inhibit biosynthetic and degrading pathways or promote their own storage, export, metabolism and degradation.

Besides being at the central hub of lipid metabolism serving as intermediates in all lipid biosynthetic pathways and of fatty acid degradation, LCACoAs are recognized as critical regulators of metabolic enzymes and signaling pathways. A network of proteins, which sequester and direct the LCACoAs toward specific metabolic pathways, determines the differing metabolic routes and functions of LCACoAs. Long-chain fatty acids derived from *de novo* synthesis, dietary sources, or degradation of triacylglycerol (TAG), glycerophospholipids, and cholesterol esters must be activated to LCACoAs prior to degradation, incorporation into complex lipids and protein acylation. The synthesis of LCACoAs is carried out by one of 13 acyl-CoA synthetases, which not only differ in substrate preference but also in their tissue expression patterns and subcellular locations (reviewed in [9]). The multiplicity of such closely related acyl-CoA synthetases implies that each isoform serves distinctive roles in acyl-CoA metabolism. Although evidence for this is only beginning to emerge, knockout and overexpression studies indeed show that some ACSs are involved in targeting of fatty acids to degradation [10–12] and TAG synthesis [10,13], to ceramide synthesis [14,15], eicosanoid metabolism [16] or for regulation of transcription [17].

Fatty acids serve as a major energy source. Upon decreasing energy supply, long chain fatty acids are activated on the cytosolic

side of the mitochondrial outer membrane, and transported into the mitochondrial matrix by carnitine palmitoyl transferase 1 (CPT1), where they are oxidized via β -oxidation (Fig. 2). While mitochondrial β -oxidation is the major route of fatty acid degradation, very-long chain fatty acids and branched-chain fatty acids are degraded in peroxisomes. Peroxisomal β -oxidation terminates at medium- and short-chain acyl-CoA esters, which are transported out of the peroxisome and successively oxidized in the mitochondria [18,19].

2.1. Acyl-CoA utilization in glycerolipid biosynthesis

The initial and committed step in *de novo* synthesis of TAG and all glycerophospholipids is catalyzed by glycerol-3-phosphate acyltransferase (GPAT), which acylates *sn*-glycerol-3-phosphate using a fatty acyl-CoA to form 1-acyl-*sn*-glycerol-3-phosphate. Like acyl-CoA synthetases, multiple GPAT isoforms have been identified and found to localize in the outer mitochondrial membrane (GPAT1 and -2) and in the ER (GPAT3 and -4) and to differ in their tissue expression patterns [20,21]. While the endoplasmic reticulum (ER)-resident GPATs have been proposed to promote TAG synthesis [22], the mitochondrial GPATs are thought to divert the acyl-CoA esters away from degradation and towards synthesis of glycerolipids [23].

The second step in the glycerol phosphate pathway involves the acylation of the *sn*-2 position of lysophosphatidic acid to form

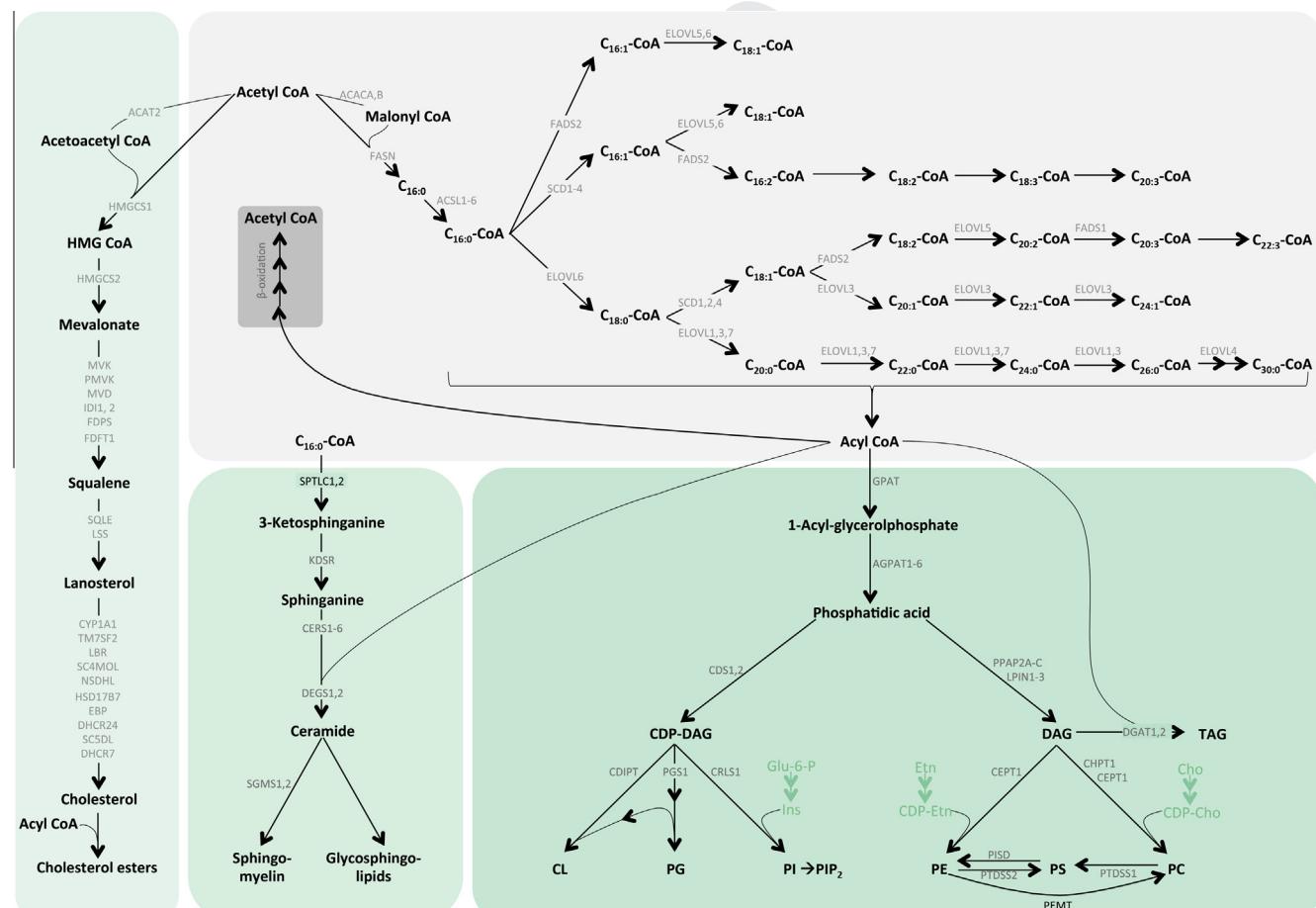


Fig. 2. Primary pathways for trafficking and utilization of LCACoAs. Lipid metabolites are marked in bold. Acronyms in light gray indicate genes encoding reaction-catalyzing enzymes. Some pathways utilizing LCACoAs have been omitted for clarity including acyl-chain remodeling of phospholipid. Abbreviations: CoA, coenzyme A; HMG, 3-hydroxy-3-methylglutaryl; PA, phosphatidic acid; DAG, diacylglycerol; CDP-DAG, cytidine diphosphate-diacylglycerol; PI, phosphatidylinositol; PIP, PI phosphate; PG, phosphatidylglycerol; CL, cardiolipin; PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine; Glu-6-P, glucose-6-phosphate; Ins-3-P, inositol-3-phosphate; Ins, inositol; Etn, ethanolamine; CDP-Etn, cytidine diphosphate ethanolamine; Cho, choline; CDP-Cho, cytidinediphosphate choline.

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phosphatidic acid, which is catalyzed by acylglycerophosphate acyltransferases (AGPATs). The AGPAT family comprises 11 members with distinct subcellular localization, tissue expression patterns and acyl-CoA specificities [21]. Overexpression studies and genetic defects have shown that AGPAT1 and AGPAT2 promote TAG synthesis [21]. AGPAT3 function has also been associated with glycerophospholipid synthesis, and AGPAT3 has, along with other family members, also been suggested to be involved in remodeling of glycerophospholipids (reviewed in [21]). Some AGPAT members have even been functionally linked to protein acylation [24].

Prior to the final acylation step phosphatidic acid is dephosphorylated by lipins/phosphatidic acid (PA) phosphohydrolases to produce diacylglycerols. Two membrane-bound ER residing acyl-CoA:diacylglycerol acyltransferases, DGAT1 and DGAT2, catalyze the acylation of diacylglycerol to produce TAG. Both enzymes are conserved and expressed in numerous tissues, particularly those, which make large amounts of TAG including liver, adipose tissue and mammary gland (see [25,26] for reviews). Both DGAT1 and DGAT2 have broad fatty acyl-CoA substrate specificity, however, DGAT2 has a higher affinity for both LCACoAs and diacylglycerols than DGAT1. On the other hand, DGAT1 is able to utilize a broader range of diacylglycerol acceptors than DGAT2 [27].

2.2. Acyl-CoA utilization in sphingolipid biosynthesis

De novo synthesis of sphingolipids is initiated at the cytosolic face of the ER membrane, where the rate-limiting enzyme serine palmitoyltransferase (SPTLC) catalyzes the condensation between serine and palmitoyl-CoA producing 3-ketosphinganine (Fig. 2). However, although SPTLC primarily utilizes palmitoyl-CoA, its substrate specificity can be altered by two small subunits, ssSPTa and ssSPTb (reviewed in [28]). Subsequently, 3-ketosphinganine is reduced to produce sphinganine, which along with sphingosine, comprises the backbone of sphingolipids. Sphinganine and sphingosine are also commonly known as sphingoid bases or sphingoid base-like compounds. Although 18-carbon sphingoid bases constitute the majority of long-chain bases found in most cells and organisms, this group of compounds encompasses a very diverse set of structural entities varying in chain length, and number, position, and stereochemistry of double bonds and hydroxyl groups [29]. Ceramide synthases (CERS) catalyze the N-acylation of sphingoid bases resulting in synthesis of ceramides. Ceramides can also be generated from sphingosine or upon recycling of glycosylceramide, sphingomyelin and ceramide-1-phosphate [30]. While the acyl-CoA specificity of the serine palmitoyl transferase seems to be dictated by the subunit composition, the specificity of ceramide synthases are conferred by specific ceramide synthases. Six ceramide synthases have been identified in mammals [28]. CERSs are integral membrane proteins residing in the ER. Despite numerous biochemical and *in silico* studies, the orientation of the protein is still unclear, however, the active site has been suggested to face the cytosol. By generation of chimeric proteins between CERS2 and CERS5, Futerman and co-workers recently identified a minimum region of 150 residues in the TLC domain of the ceramide synthases, which defines the acyl-CoA specificity [31]. Each ceramide synthase exhibits a characteristic substrate specificity, such that CERS1 uses C₁₈-CoA, CERS2 uses C₂₂- and C₂₄-CoA, CERS3 uses C₂₆-CoA and higher, CERS4 uses C₁₈ to C₂₂-CoA, while CERS5 and CERS6 primarily use C₁₆-CoA [28]. Each ceramide synthase exhibits a characteristic tissue distribution pattern [32] and differences in ceramide and sphingolipid composition among tissues can be explained, at least partly, by the differential expression of the CERS. For example, the abundance of C₂₄ ceramides and sphingolipids in liver and kidney correlates with high expression of CERS2 mRNA [33]. Similarly, C₁₈ ceramides and sphingolipids are

abundant in brain and muscle, where the expression of CERS1 mRNA is high [33]. Ceramides and sphingolipids containing very-long acyl chains (\geq C26) are abundant in the skin, where they are required for normal epidermal barrier function, and in testis, where CERS3 is expressed at high levels [34].

2.3. Acyl-CoA utilization in fatty acid elongation

Long-chain fatty acids comprised of 12–20 carbon atoms make up the majority of fatty acids found in eukaryotic cells, while very long-chain FAs (VLCFA) containing 22 or more carbon atoms typically constitute a minor fraction. The primary end product synthesized *de novo* by FA synthase is palmitic acid, which together with dietary fatty acids, can be elongated to other fatty acid species. Subsequent to their conversion to LCACoAs, fatty acids are elongated by an ER-membrane embedded fatty acid elongase, which catalyze the rate-limiting condensation between an acyl-CoA and malonyl-CoA (reviewed in [35]). Seven fatty acid elongases (ELOVL1–7) have been identified and characterized by distinctive tissue distribution patterns and substrate specificities towards acyl-CoAs. ELOVL1, 3, 4, 6 and 7 are involved in the elongation of saturated and mono-unsaturated fatty acyl-CoAs, while ELOVL2 and ELOVL5 strictly elongate polyunsaturated fatty acyl-CoAs [35].

2.4. Control of intracellular acyl-CoA partitioning: general considerations

The amphiphatic nature of fatty acids and LCACoAs not only promote micelle formation but also unanticipated membrane partitioning. The critical micelle concentration for oleic acid is 6 μ M [36], while the critical micelle concentrations for LCACoAs like palmitoyl-CoA, stearoyl-CoA and oleoyl-CoA have been determined to 70, 12 and 33 μ M, respectively [37,38]. LCACoAs partition into glycerophospholipid vesicles and cells by insertion of the hydrophobic acyl chain into the bilayer. The partition constant for palmitoyl-CoA in glycerophospholipid vesicles is $1.5 - 5 \times 10^5 \text{ M}^{-1}$ [39,40], and found to be highly dependent of the acyl chain length [39,40] but nearly independent of the polar head group [39].

Partitioning of LCACoAs into membranes markedly affects membrane integrity. Palmitoyl-CoA has been shown to decrease the latency of β -glucuronidase and UDP-glucuronosyltransferase in rat liver microsomes and to increase the permeability of microsomes to sucrose and citrate [41]. Moreover, both palmitoyl- and myristoyl-CoA potently inhibit ER assembly *in vitro* with an IC₅₀ of approximately 100 nM, whereas neither palmitate nor free CoA was found to affect membrane assembly [42].

Collectively, unbound long-chain fatty acids and LCACoAs can disrupt membrane integrity and impair protein functions. However, most of the enzymes, which generate or utilize LCACoAs reside in a hydrophobic membrane environment. Thus, to prevent them from forming micelles and from partitioning non-specifically into membranes, the total level and the availability of both fatty acids and LCACoAs must be carefully controlled, still being selectively targeted to utilizing enzymes to accommodate the cellular requirements of fatty acids and LCACoAs.

2.5. Control of intracellular LCACoA concentration

One of the most fundamental challenges for all living organisms is to coordinate metabolic fluxes in order to adapt their metabolism and physiology to promote survival and achieve balanced growth. Thus, import, transport, synthesis, and metabolism of a large variety of metabolites including fatty acids and LCACoAs must be strictly coordinated to meet the nutritional requirements

of cells and organisms. The total cellular concentration of LCACoA esters is in the range of 5–160 μM , depending on the tissue and its metabolic state [1], however, the concentration of free unbound LCACoA available for metabolism and regulatory purposes is not known. The size of the intracellular pool of LCACoA esters is determined by the rates of fatty acyl-CoA synthesis and utilization. Synthesis of LCACoA esters is to a large extent determined by the rate of activation by acyl-CoA synthetases of either imported fatty acids, endogenously synthesized fatty acids, or fatty acids from lipolysis of cellular lipids, whereas utilization is determined by the rate of degradation by β -oxidation, incorporation into cellular lipids, acyl-CoA hydrolysis and protein acylation.

It has previously been assumed that the ratio of LCACoA to ACBP was close to one [1,43,44]. Based on the reported *in vitro* binding affinity and the cellular level of ACBP, the concentration of unbound LCACoA would be in the low nM range as long as the acyl-CoA/ACBP ratio is below one [1]. However, since LCACoA can bind to numerous other intracellular proteins including acyl-CoA binding domain proteins (see below), high affinity-binding sites on acyl-CoA synthetases, acyl-CoA-utilizing enzymes and organelle-specific binding proteins like the peroxisomal SCP2, the level of unbound LCACoA esters must be even lower. Nevertheless, the presence of multiple acyl-CoA binding domain proteins, which also bind LCACoA, may target LCACoA and consequently orchestrate the intracellular flux of activated fatty acids to utilizing pathways.

Acyl-coenzyme A thioesterases (ACOTs) are also believed to play important cellular roles in mammalian fatty acid metabolism by modulating the cellular and subcellular concentrations of LCACoAs [45]. To date the ACOT family comprises 13 members, which each reside in the cytosol, mitochondria or peroxisomes and include short-, medium-, and LCACoA thioesterases [45]. The K_m values for acyl-CoA thioesterases are typically in the low μM range for LCACoA esters [46,47], and interestingly, conditions that increase the level of LCACoA and ACBP in rat liver [43,48,49] also increase the level of some of the ACOTs [50–53]. ACOTs may therefore act as scavengers, preventing LCACoA pools from rising to detrimental levels. This notion is supported by the observation that the psychrophilic Antarctic yeast *Rhodotorula aurantiaca* is not viable at non-permissive temperatures due to inactivation of an LCACoA thioesterase and the resulting accumulation of myristoyl-CoA [54].

However, despite playing a role in maintaining the intracellular availability of LCACoAs, ACOTs also control intracellular and intra-organelle concentrations of coenzyme A. Both in yeast and in mammals, peroxisomal acyl-CoA thioesterases hydrolyze acyl-CoA esters and are suggested to prevent depletion of the peroxisomal CoA pool required for oxidation of fatty acids [55,56]. Similarly, the presence of ACOTs in mitochondria prevents depletion of the mitochondrial CoA pool, ensuring mitochondrial β -oxidation [57–59]. Likewise, the presence of ACOTs in the nucleus [60] may ensure adequate levels of CoA to support synthesis of acetyl-CoA for histone acetylation and, ultimately, transcriptional activation. Collectively, ACOTs may not only prevent the accumulation of LCACoA, but also function as metabolic regulators of fatty acid degradation and of post-translational protein modifications.

The ubiquitous presence of ACOTs requires either high affinity acyl-CoA binding proteins to act as LCACoA pool formers and transporters or direct channeling of LCACoA from acyl-CoA synthetases to acyl-CoA utilizing enzymes. This ensures low concentrations of unbound LCACoA and adequate amounts of LCACoA for metabolic and regulatory purposes. To this end it is interesting that fatty acid synthesis occurs despite the fact that the K_i for acetyl-CoA carboxylase, the rate-limiting enzyme in fatty acid synthesis, is 5.5 nM [61–64]. This clearly implies that the cytosolic concentration of unbound LCACoAs is very low.

3. Regulatory functions of acyl-CoA; evidences from *in vivo* and *in vitro* studies

3.1. Regulation of carbohydrate metabolism, citric acid cycle and energy metabolism

Rat liver glucokinase is allosterically and specifically inhibited by LCACoAs [65,66]. The K_i for palmitoyl-CoA, stearoyl-CoA and oleoyl-CoA was found to be 1.8, 0.32 and 0.77 μM , respectively, well below the critical micelle concentration. Inhibition of glucokinase by LCACoAs could be reversed by LCACoA sequestration by albumin or by α - or β -cyclodextrins, and was specific, as non-esterified fatty acids, short-chain acyl-CoAs, free CoASH or similar amphipathic compounds did not affect enzyme activity [65,66]. Interestingly, inhibition of glucokinase is competitive with both ATP and glucose but does affect the positive cooperativity, which glucokinase normally displays with glucose. Consistently, Zhang et al. found that LCACoAs also inhibited glucokinase in pancreatic islets from rats [67].

The enzyme catalyzing the dephosphorylation of glucose, glucose-6-phosphatase, is also inhibited by LCACoAs [68,69]. Fulceri et al. found that both 50 μM palmitoyl-CoA and a non-hydrolysable analog inhibited enzyme activity to 50% in rat liver microsomes and that inhibition was reversed by albumin addition, arguing that the inhibitory effect is mediated by direct binding [68]. On the other hand, Mithieux and Zitoun found that the K_i for acyl-CoA inhibition of glucose-6-phosphatase activity was approximately 1 μM in untreated microsomes while in detergent-treated microsomes the K_i was approximately 30 μM [69].

In addition, low μM concentrations of palmitoyl-CoA also inhibit the activity of glucose-6-phosphate dehydrogenase from yeast and *Torulopsis utilis*, which can be reversed by removal of palmitoyl-CoA [70,71]. In either case, palmitoyl-CoA dissociated the tetrameric and dimeric forms into the dimeric or monomeric forms of the enzymes, respectively [70].

Free glycogen synthase from rat liver is inhibited by palmitoyl-CoA with a EC_{50} of 3–4 μM , in a reversible manner [72]. As above, palmitoyl-CoA dissociated the tetrameric enzyme into monomers, and was found to bind to the monomers. Furthermore, glycogen-bound glycogen synthase is also inhibited by palmitoyl-CoA, which also dissociated the enzyme into monomers and released the enzyme from glycogen [72].

Recently, Jenkins et al. demonstrated that purified rabbit muscle phosphofructokinase 1 (PFK-1), is inhibited by low μM concentrations of LCACoAs including a nonhydrolyzable palmitoyl-CoA analog in the absence of albumin or other acyl-CoA buffering systems [73]. Interestingly, PFK-1 was only inhibited in the presence of MgATP and not MgAMP and MgADP, arguing that regulation of PFK1 by LCACoAs is highly specific and only occurs during high cellular energy phosphate conditions [73]. PFK-1 was also found to be acylated in the presence of palmitoyl-CoA, however, this was not required for the inhibitory effect. Both palmitoyl-CoA and the nonhydrolyzable palmitoyl-CoA analog increased membrane association of PFK1 and interaction with one of its regulatory proteins, calmodulin [73].

LCACoAs inhibit rate-limiting and highly regulated mitochondrial dehydrogenases [74,75]. Erfle and Sauer found that inhibition of α -ketoglutarate dehydrogenase was due to irreversible binding of acyl-CoA to the CoASH site via an interaction between the acyl-chain and hydrophobic regions in the enzyme, which was found to dissociate the lipoamide dehydrogenase subunit from the enzyme complex [74]. Lai et al. found that palmitoyl-CoA inhibits both pyruvate dehydrogenase and α -ketoglutarate dehydrogenase in bovine heart mitochondria with a $K_i < 25 \mu\text{M}$ [75]. To this end LCACoAs have also been found to inhibit glutamate

dehydrogenase from bovine liver and mitochondrial malate dehydrogenase from pig heart with inhibitor constants of 0.15 and 1.8 μM , respectively [71]. LCACoA inhibition of glutamate dehydrogenase was only partly reversed by methylated cyclodextrins, whereas inhibition of malate dehydrogenase was fully reversible. Moreover, palmitoyl-CoA dissociated the native hexameric form of glutamate dehydrogenase to dimeric subunits, but did not affect the structure and subunit composition of malate dehydrogenase [71].

Degradation of fatty acids to acetyl-CoA via mitochondrial β -oxidation is a major energy source in animals, which mainly is inhibited by feedback mechanisms. When flux through the tricarboxylic acid cycle is limited, the acetyl-CoA level increases and inhibits β -ketothiolase. Consequently, 3-keto acyl-CoA intermediates accumulate and inhibit chain-length-specific acyl-CoA dehydrogenases [76], e.g. the long-chain acyl-CoA dehydrogenase is inhibited by 3-ketopalmitoyl-CoA with a K_i of 0.2 μM when free palmitoyl-CoA is the substrate [77], while free trans-2-enoyl, trans-3-enoyl-, and 3-ketoctanoyl-CoA inhibit medium-chain acyl-CoA dehydrogenase with a K_D in the 50–90 nM range [76].

The mitochondrial adenine nucleotide translocase (ANT) catalyzes the exchange of ADP and ATP across the inner mitochondrial membrane and is considered to be the overall rate-limiting step in energy metabolism [78]. LCACoAs potently inhibit ANT in both intact mitochondria and in submitochondrial particles in a chain-length-dependent manner with a K_i of 1–5 μM , depending on the experimental conditions [79–82] and when reconstituted in liposomes [83]. Consistently, a photoreactive LCACoA can label bovine mitochondrial ANT, indicating that LCACoAs bind ANT directly [84,85]. ANT has extensive sequence identity with the brown-adipose-tissue mitochondrial uncoupling protein. Accordingly, UCP is also photoaffinity labeled by a photoreactive LCACoA and regulated by long-chain acyl-CoAs [85,86]. In contrast, LCACoAs inhibit (K_i of 2.4 μM) the pH-dependent anion-conducting channel in the inner membrane of rat liver mitochondria [87]. However, the effects of LCACoA on ANT and the pH-dependent anion-conducting channel were all determined in the absence of albumin or another acyl-CoA buffering system, thus questioning the physiological relevance of these observations.

489 3.2. Regulation of lipid metabolism

490 3.2.1. Regulation of lipid biosynthetic pathways

491 Acetyl-CoA carboxylase (ACC) catalyzes the initial and rate limiting step in the biosynthesis of long-chain fatty acids [88]. 492 LCACoAs inhibit rat liver ACC directly by forming an equimolar 493 complex with the enzyme [61,64,89]. In the absence of albumin 494 the K_i for palmitoyl-CoA has been determined to 5.5–6.5 nM while 495 the K_i for stearoyl-CoA and arachidoyl-CoA was found to be 1.3 nM 496 and <1 nM, respectively [64,89]. Citrate, an activator of ACC, not 497 only prevents binding of LCACoAs, but when added to the inactive 498 equimolar enzyme-inhibitor complex in the presence of a long- 499 chain acyl-CoA acceptor, LCACoAs inhibition is relieved and the 500 enzyme polymerizes and forms a catalytically active form 501 [64,90]. LCACoAs also inhibit ACC activity in mammary gland 502 [91], in adipose tissue [92], in chicken liver [93], and in yeast 503 [94]. Consistently, long-chain fatty acids must be activated to 504 LCACoAs to repress ACC activity in *Saccharomyces cerevisiae* (*S. 505 cerevisiae*) [95]. ACC is not only inhibited directly by LCACoAs, 506 but supplementation of long-chain fatty acids also reduces the 507 cellular level of ACC in *S. cerevisiae* [95], *Candida lipolytica* [96], 508 human skin fibroblasts [97], and cultured rat hepatocytes [98].

509 Fatty acid synthesis is catalyzed by the fatty acid synthase (FAS) 510 complex. In *S. cerevisiae* synthesis is terminated by the malonyl- 511 CoA transferase component of FAS, which transfers the acyl chain 512 from the acyl carrier protein domain of FAS to CoA to form acyl- 513

CoA esters as final products. Free LCACoA has also been shown to inhibit yeast FAS *in vitro*, by serving as a competitive inhibitor with malonyl-CoA [99]. Citrate synthase, which catalyzes the synthesis of citrate from acetyl-CoA and oxaloacetate, is also inhibited by LCACoA esters [100–102]. Citrate not only promotes the polymerization, and hence activation of ACC, but also indirectly provides acetyl-CoA and reducing power for fatty acid synthesis. Control of citrate synthase activity may therefore be an important regulatory step in biosynthesis of fatty acids. These effects suggest that acyl-CoA esters can function as important regulators of fatty acid biosynthesis. However, the inhibitory effect was prevented by addition of albumin, questioning its physiological importance.

In the absence of an acyl-CoA buffering protein like albumin both saturated and unsaturated LCACoAs, particularly arachidonoyl-CoA, inhibit incorporation of [^{14}C]-acetate and [^{14}C]-acetyl-CoA but not [^{14}C]-mevalonate incorporation into sterols in a postmitochondrial supernatant from rat liver [103]. In support of these findings, both microsomal and purified hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activities are inhibited by oleoyl-CoA in the absence of albumin, suggesting that under certain conditions LCACoAs can inhibit cholesterol biosynthesis [104].

535 3.2.2. Regulation of lipid degradation pathways

The adipocyte triglyceride lipase (ATGL) efficiently mobilizes fatty acids stored in triglycerides in both adipose and non-adipose tissues (reviewed in [105]). Recently, it was shown that long- but not medium-chain acyl-CoA esters bind and inhibit ATGL activity in the presence of excess albumin with a K_i between 16 and 19 μM , without perturbing its interaction with one of its regulatory proteins CGI-58 [106]. Similarly, hormone-sensitive lipase (HSL), the rate-limiting enzyme for diacylglycerol and cholesterol ester breakdown in adipose tissue, is also inhibited by LCACoAs, albeit with a much lower K_i (K_i approximately 100 nM) [106,107]. Hu et al. also showed that LCACoAs inhibit HSL from adipocytes, but stimulated lipase activity in pancreatic islets from both normal and HSL-null mice [108]. Unexpectedly, ACBP potentiated the inhibitory effect of LCACoAs on HSL from adipocytes [108].

550 3.3. Regulation of ion fluxes and insulin secretion

Elevation of LCACoA levels upon long-term exposure of β -cells to non-esterified fatty acids activates K_{ATP} channel activity, resulting in hyperpolarization of the β -cells and inhibition of insulin secretion [109]. In the absence of albumin both saturated and unsaturated LCACoA (10 nM to 1 μM), but not short-chain and medium-chain acyl-CoAs or free CoASH, were found to induce a rapid opening of ATP-sensitive K^+ channels [109,110]. Accordingly, it has been shown that LCACoA esters induce a distinct and prolonged open state of the pancreatic K_{ATP} channel, Kir6.2/SUR1, leading to increased channel activity, and that this effect is mediated by LCACoA binding to both C- and N-terminal domains of cytosolic portion of the Kir6.2 subunit [111–117]. Moreover, overexpression of ACSL1 in cultured β -cells also increased K_{ATP} channel activity [118], supporting the idea that an intracellular increase in LCACoA esters modulates channel activity. Interestingly, polymorphisms in Kir6.2, which have been associated with an increased risk of developing type 2 diabetes, impaired glucose-stimulated insulin secretion, increased BMI, reduced glucagon secretion response during hyperglycemia, and increased K_{ATP} channel sensitivity to LCACoA esters [116].

Consistently, it has been demonstrated that LCACoA esters facilitate the opening of K_{ATP} channels by reducing their ATP sensitivity in cardiomyocytes [119]. Notably, despite the fact that the pancreatic SUR1/Kir6.2 and the cardiac SUR2A/Kir6.2 K_{ATP} channel both respond to free acyl-CoA esters, their sensitivities towards medium- and long-chain acyl-CoA esters differ

[117,119], arguing that acyl-CoA esters affect channel activities via different subunits in pancreatic β -cells and in cardiomyocytes. This may not only impair insulin secretion in response to the accumulation of acyl-CoA esters after long-term elevations of free fatty acids in the blood, but also decrease the contractility and energy expenditure of cardiac muscle cells during the initial phase of ischemia, when the total concentration of acyl-CoA esters increases [120]. To this end, unbound nanomolar concentrations of LCACoAs have been found to inhibit potassium fluxes through reconstituted mitochondrial potassium ATP-sensitive channels in a Mg^{2+} -dependent manner [121], suggesting that LCACoAs are important regulators of energy metabolism in the mitochondria.

That LCACoAs are important regulators in the heart is substantiated by recent observations showing that LCACoAs regulate the sodium–calcium exchanger NCX1, a critical mediator of calcium homeostasis in the heart [122]. NCX1 primarily operates in forward mode to extrude Ca^{2+} , but in the reverse-mode, NCX1 activity during ischemia/reperfusion can contribute to Ca^{2+} loading. In the absence of albumin saturated and monounsaturated LCACoAs (1 μ M) were able to activate reverse-mode NCX1 activity, while oleoyl-CoA was found to inhibit forward-mode NCX1 activity [122]. To this end, it is interesting that forward mode activity of NCX1 splice variants present in pancreatic β -cells are differentially affected by LCACoAs [123], indicating that LCACoAs may affect Ca^{2+} homeostasis in a tissue specific manner. LCACoAs also potently activate TRPV1 Ca^{2+} -channels, presumably via direct binding to the receptor [124], substantiating a regulatory function of LCACoAs on ion fluxes across membranes.

3.4. Regulation of vesicular trafficking

Regulation and co-ordination of membrane fusion and fission events play a fundamental role in secretion, endocytosis, cell division and transport between intracellular compartments. The molecular mechanisms, by which vesicles bud off from a donor compartment and transport, recognize and fuse to a target membrane have been studied intensively during the past decades. Numerous studies have not only led to the identification of a vast number of proteins required for vesicular trafficking, but have also recognized that various lipids serve fundamental functions in these pathways, including phosphoinositides, phosphatidic acid, diacylglycerols, fatty acids and LCACoA esters [125]. Glick and Rothman initially found that free palmitoyl-CoA can act as a co-factor for the Golgi-associated protein NSF and is required for transport between cisternae of the Golgi stack [126]. Soon after, it was shown that palmitoyl-CoA stimulated budding of non-clathrin coated transport vesicles from Golgi cisternae and that budding is inhibited by the nonhydrolyzable analog, arguing that acylation of proteins or lipids is involved [127]. Similarly, fusion of transport vesicles with Golgi cisternae also requires LCACoAs to support acylation of at least one component of the transport machinery [128]. In addition, LCACoAs and not short- or very-long chain acyl-CoA esters have been shown to support homotypic vacuole fusion *in vitro* [129]. Moreover, a protein complex consisting of TIP30, ACSL4 and Endophilin B1 promotes the fusion of endocytic vesicles with Rab5a positive vesicles, which transport endosomal acidification enzymes to the early endosomes [130]. Interestingly, synthesis of arachidonyl-CoA from arachidonic acid and coenzyme A was required for fusion of the two vesicles, perhaps by being incorporated into phosphatidic acid, which is known to support SNARE-mediated fusion events [131].

3.5. Regulation of gene expression

Peroxisome proliferator-activated receptors (PPAR) are recognized as ligand-activated transcription factors belonging to the

steroid/thyroid nuclear hormone receptor superfamily, controlling the transcription of a variety of genes involved in lipid metabolism and cell differentiation [132]. Besides primarily binding long-chain unsaturated fatty acids, PPAR α also binds LCACoAs with high affinity (K_D of 1–14 nM), which induce a conformational change and enhance recruitment of co-activators [133]. Yet, LCACoAs have also been shown to antagonize agonist induced DNA binding and recruitment of co-activators to both PPAR α and PPAR γ [134,135].

Hepatocyte nuclear factor 4 α (HNF4 α) is a member of the nuclear hormone receptor superfamily of transcription factors, which is involved in hepatocyte differentiation and metabolism. A number of studies show that HNF4 α binds LCACoAs with dissociation constants ranging from 1.7 to 4.4 nM, depending on acyl-chain length and degree of unsaturation [136]. Palmitoyl-CoA enhances DNA binding of HNF4 α . Polyunsaturated fatty acyl-CoA (PUFA-CoA) esters antagonize this effect by displacing palmitoyl-CoA from the ligand-binding site of HNF4 α [137]. Consistently, PUFAs and PUFA-CoAs inhibit binding of HNF4 α and activation of the glucose-6-phosphatase promoter activity in HepG2 hepatoma cells [138]. Moreover, ACBP and HNF4 α have been shown to interact *in vitro* and to co-localize in the nucleus and in the perinuclear region in rat hepatoma cells and in COS-7 cells [139]. Furthermore, ACBP overexpression enhanced HNF4 α dependent transactivation, while knock down inhibited transactivation. Collectively, LCACoA esters may therefore be bona fide ligands for HNF4 α .

Collectively, the binding affinities towards LCFA and LCACoAs are in the range of the unbound intracellular concentration of LCFA and LCACoAs, and both FABP and ACBP can localize in the nucleus and modulate the effects of ligands binding to the PPARs [140–143], supporting the idea that LCFA and LCACoAs can modulate PPAR and HNF4 α functions *in vivo*. Despite their ability to bind LCACoAs, it is not known in molecular detail how LCACoAs affect DNA binding and the transcriptional activities of PPAR and HNF4 α . In *Escherichia coli* the fatty acid responsive transcription factor FadR binds, in the absence of exogenous fatty acids, to specific DNA sequences as a homodimer and represses the transcription of genes involved in fatty acid degradation and import, and activates transcription of fatty acid biosynthetic genes [144,145]. Addition of fatty acids results in a concomitant increase in intracellular LCACoAs, which bind to and induce rearrangements of the DNA binding domains in FadR, resulting in loss of DNA binding [146,147].

In summary, LCACoAs can modulate the activity of numerous enzymes involved in intermediary metabolism, ion channels, vesicular trafficking, and transcription factors. Most of the regulatory effects require μ M levels of LCACoA, however, many of these findings have been obtained in the absence of proteins capable of buffering the LCACoA concentration or preventing membrane partitioning like acyl-CoA binding proteins. The presence of high affinity binding proteins and acyl-CoA thioesterases make it unlikely that the intracellular concentration of LCACoAs exceeds 1 μ M [1]. Some of the reported effects are therefore less relevant, unless the total intracellular pool of LCACoAs exceeds the binding capacity of intracellular acyl-CoA binding proteins. However, it is interesting that some transcription factors and ion channels bind are regulated by nM concentrations of LCACoAs, arguing that LCACoA may serve physiologically relevant regulatory functions. Yet, the existence of local intracellular pools of LCACoAs might also exist, which can be of regulatory importance.

4. Structure of the acyl-CoA binding domain scaffold

During the early 1990s the structures of both apo- and holo bovine ACBP were solved by NMR spectroscopy [148,149]. Later, the apo-structure of the yeast homolog (Acb1) from *S. cerevisiae* was resolved by NMR spectroscopy [150], and the structures of

both apo and holo forms of ACBP from both *Plasmodium falciparum* (*P. falciparum*) [151] and of human ACBP [152] were resolved by X-ray crystallography. Overall, independent of the methods used to resolve their structures, the structures of bovine, yeast, human and *P. falciparum* ACBP are very similar (Fig. 3). The structures show ACBP as an up-down-down-up four- α -helix bundle protein, with a 13-residue loop insertion between the parallel helix pair of A2 and A3. [148–152]. The bundle arrangement of ACBP is skew as helix A3 is disjoint to helices A1 and A4, resulting in four helix-helix interfaces, and not six commonly found in well-known super-coiled four-helix bundles, making it unique among known four-helix folds. In bovine ACBP the four α -helices A1 (Glu4-Leu15), A2 (Asp21-Val36), A3 (Gly51-Lys62) and A4 (Ser65-Tyr84) primarily interact through hydrophobic interactions and are organized in a shallow bowl-like structure with a highly polar rim and a predominantly non-polar interior [148,149]. By isothermal microcalorimetry ACBP was shown to bind saturated and unsaturated C₁₄–C₂₂ acyl-CoA esters with very high affinity (K_D of 1–15 nM) in a one-to-one binding mode, while CoA is bound with significantly lower affinity (K_D of 2 μ M) [153–155]. ACBP does not bind fatty acids, acyl carnitines, cholesterol or nucleotides [156].

The ligand-binding site of ACBP can conceptually be divided into three sub-sites: one for the 3'-phosphate, one for the adenine ring, and one for the acyl-chain of the ligand [149]. The 3'-phosphate group of the CoA part contributes with 40% of the total binding energy [153], and interacts strongly with ACBP through a network of two salt bridges to residues Lys32, and Lys54, and a hydrogen bond to Tyr28 [149]. Moreover, non-polar interactions also exist between ACBP and the CoA part of the ligand, which are produced by stacking of the aromatic ring of Tyr31 and the adenine ring. Furthermore, the aromatic rings of Phe5 and Tyr73 structurally support the aromatic ring of adenine. The non-polar ω -end of the acyl-chain is anchored in a hydrophobic cleft between helix A2 and A3, where it makes several van der Waals contacts to the non-polar side chains of Met24, Leu25 and Ala53 [149,154]. Similarly, the adenine ring also stacks on Tyr31 in human ACBP and forms hydrogen bonds with Arg13 and Tyr73, while the 3'-phosphate of the CoA hydrogen bonds to Tyr28, Lys32 and Lys54 and residues Ala53, Lys54, Met24, and Leu25 interacts with the acyl-chain [152].

Despite the structural similarities between bovine, yeast, *P. falciparum* and human ACBP some differences exist. Generally, two loop regions are loosely defined in all structures being the loop

between helix A1 and A2 and the incoming loop just before the start of helix A3. Compared to human ACBP and *P. falciparum* ACBP the N-terminus of helix A3 in bovine ACBP moves toward the binding pocket, making the bovine ACBP structure more compact [151]. Moreover, in *P. falciparum* ACBP two unique residues have been inserted between A1 and A2. This insertion and the alterations A53K, K50I and D21N change the binding pocket and close the tunnel at the end of the acyl-chain, resulting in a preference for shorter ligands compared with bovine ACBP. The preference of PfACBP for myristoyl-CoA may have occurred in order to accommodate the synthesis of massive amounts of the di-C_{14:0}-GPI-anchored protein, which coats and protects the parasite [151]. This also indicates that minor changes in ligand preference may have major biological significance.

Interestingly, X-ray crystallography reveals the liganded human ACBP as a dimer in an asymmetric unit comprised of two ACBP proteins and one myristoyl-CoA ligand, which binds with its adenine part to one of the proteins, whereas the ω -end of its acyl chain is bound to the other molecule of this dimer. This is not unique among acyl-CoA binding proteins, as ACBP from *Moniliophthora perniciosa* also forms dimers upon ligand binding [157]. ACBP from *M. perniciosa* is a 104 residues protein, which contains an extra helix at its C-terminus resulting in a total of five α -helices. The fifth helix is unique to the *M. perniciosa* ACBP and its packing with helices 1, 2 and 4 forms the classical four-helix bundle of ACBPs.

Δ^3,Δ^2 -enoyl-CoA isomerases (ECIs) catalyze the isomerization of unsaturated fatty acids with cis/trans double bonds at odd positions to allow them to enter β -oxidation. Recently, the structure of ECI2 including its N-terminus ACBP domain was resolved by X-ray crystallography [158]. This reveals that its ACBP domain is structurally highly similar to human ACBP (Fig. 4) and that it protrudes out of the central isomerase trimer via a linker region. Moreover, the ACBP domains extend away from the ECI2 core and is positioned at the corners of a triangle in the same plane as the flattened isomerase domain trimer [158]. Moreover, the ACBP domain does not only bind ligands with significantly higher affinity than a truncated form of the enzyme lacking the ACBP domain, but also contributes to the stability of ECI2 and its catalytic activity. The ACBP domain may therefore capture the acyl-CoA substrate and delivers it towards the active site of ECI2. Alternatively, the ACBP domain could also promote release of the product.

A number of cytoskeletal proteins such as erythrocyte membrane protein 4.1R, talin, and the ezrin-radixin-moesin protein

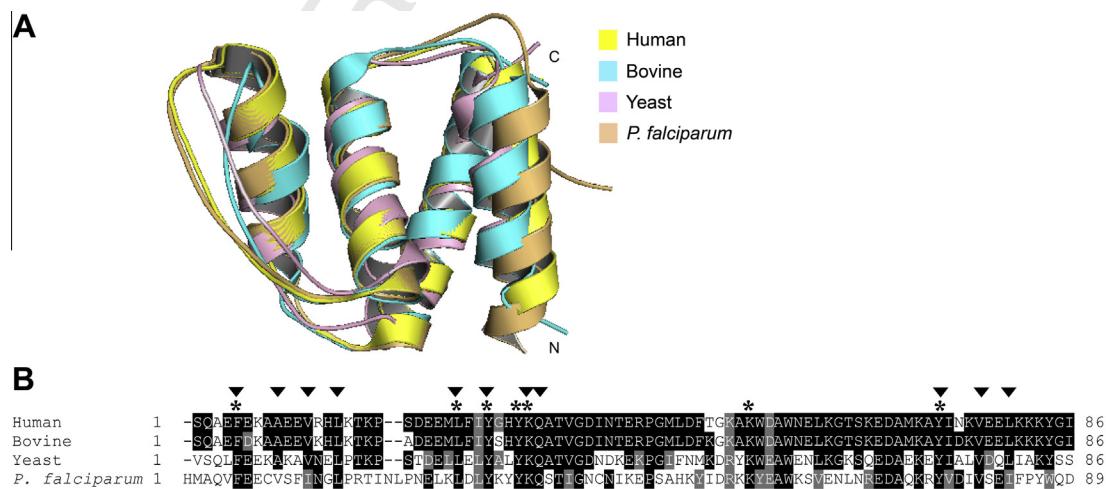


Fig. 3. Superimposition and alignment of human, bovine, *S. cerevisiae* and *P. falciparum* apo-ACBP structures. (A) A three-dimensional alignment of the backbone was performed using PyMOL. Human ACBP is marked in yellow, Bovine ACBP in light blue, *S. cerevisiae* Acb1 in pink and *P. falciparum* ACBP in light brown. (B) Multiple alignment of the ACBPs shown in (A). Single-letter abbreviations for amino acid residues are used. Identical and similar amino acids are identified by black and gray shading, respectively. Residues marked with a star and triangle are important for ligand binding and stability of bovine ACBP, respectively.

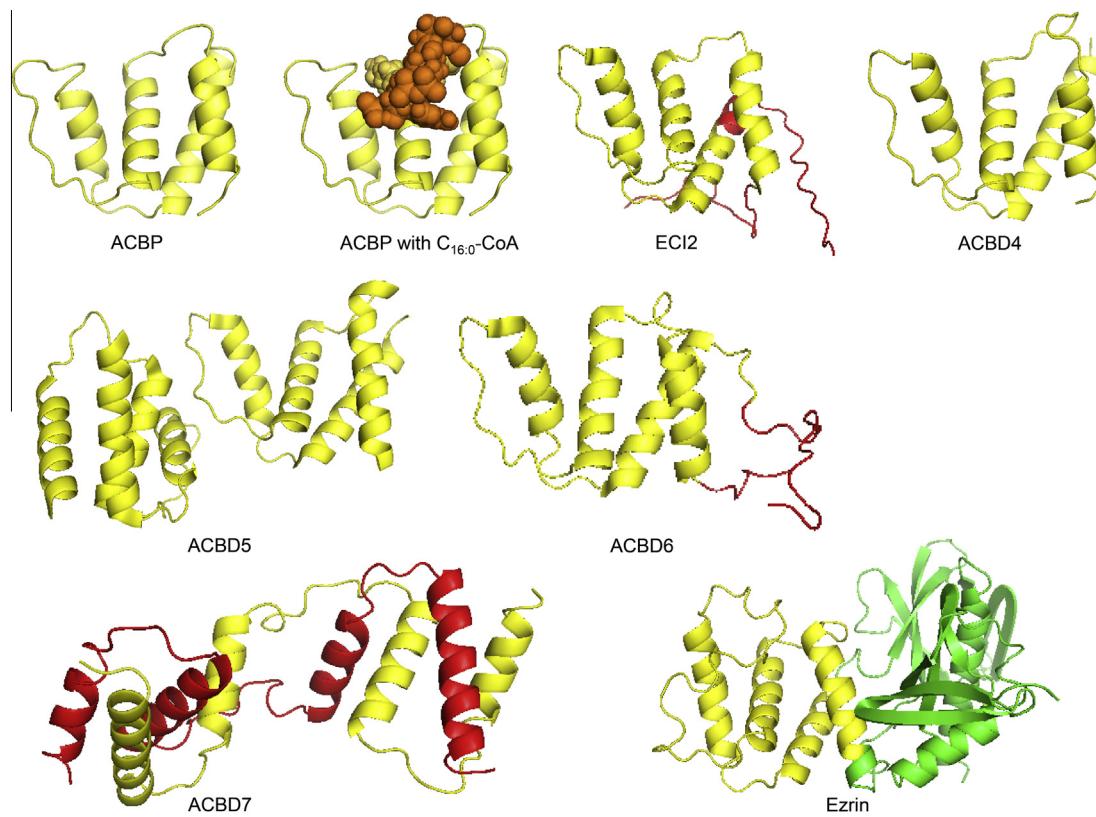


Fig. 4. Three-dimensional structures of ACBP domains in human ACBDs. Crystal structures of human ACBP both in its apo- (2FJ9) and holo (2CB8) form, of ACBP in ECI2 (4U18), of ACBD4 (2WH5), of ACBD5 (3FLV), of ACBD6 (2COP), and of ACBD7 (3EPY). ACBP domains are marked in yellow and for ACBD7 the monomers are shown in yellow and red, respectively. The FERM domain in human EZRIN (1NI2) is marked in yellow.

family, as well as several protein tyrosine kinases and phosphatases, and the neurofibromatosis 2 tumor suppressor protein Merlin contains a so-called band F ezrin-radixin-moesin (FERM) homology domain. The FERM domain contains three structural folds, of which one is structurally very similar to the ACBP structural fold (Fig. 4), despite the fact that the FERM domain and ACBP share no sequence similarity [159]. Consistently, the FERM domain has not been reported to bind LCACoAs, however, the FERM domain has such a structure that functions as a common membrane-binding module involved in localizing proteins to the plasma membrane [160], suggesting that ACBP may also associate with membranes *in vivo*.

5. Tissue expression and intracellular localization

5.1. Tissue expression in vertebrates

ACBP is expressed in all peripheral tissues studied and is ubiquitously expressed from early stages of mammalian embryogenesis [161]. However, the level of ACBP differs markedly among different cell types. Hepatocytes contain moderate amounts of ACBP, however, the total amount of ACBP in liver is relatively high due to the diffuse presence of ACBP in all hepatocytes [162]. High concentrations are found in steroid producing cells (glomerulosa and fasciculate cells of adrenal cortex, Leydig cells of testis) [162–166], keratinocytes and cells from sweat and sebaceous glands [167,168]. Intermediate concentrations are found in epithelial cells involved in water and electrolyte transport (intestinal mucosa, distal convoluted tubules of kidney) [162,165,166,169]. ACBP is found throughout the intestinal tract with highest concentration in the duodenum and antrum. The protein was detected in goblet cells

and enterocytes of the epithelial layer of the intestine. In the stomach ACBP was found to be restricted to the deep layer of epithelial cells [170]. Interestingly, ACBP has been found to be co-expressed with fatty acid binding proteins throughout the intestine and in glial cells [171,172]. In skeletal muscle expression of ACBP is about three fold higher in slow twitch, oxidative fibers compared to fast twitch glycolytic white gastrocnemius skeletal muscle fibers [173], and that level increases by approximately 30% in muscles from obese Zucker rats [174]. The expression of ACBP increases dramatically during differentiation of murine 3T3-L1 adipocytes [175,176]. Anti-sense mediated knock down of *Acbp* expression impaired the adipogenic potential, arguing that ACBP is important for adipocyte differentiation [176]. In the human preadipocyte cell line SGBS, the *ACBP* gene expresses various high- and low-abundant transcript variants encoding ACBP protein isoforms. Interestingly, one transcript variant was downregulated while other variants were upregulated during differentiation, indicating that ACBP isoforms encoded by such variants may play different roles in human adipocytes [177].

Due to its reported function as a neuromodulator, expression of ACBP has been widely examined in brain tissue. Numerous studies using *in situ* hybridisation or immunohistochemistry have shown that ACBP is heterogeneously distributed in rat brain, particularly in the area of postrema, the cerebellar cortex, and epidyma of the third ventricle. Intermediate levels were found in the olfactory bulb, pontine nuclei, inferior colliculi, arcuate nucleus and pineal gland. Relatively low but significant levels were observed in mesencephalic and telencephalic areas [165,166,178–185]. In mice ACBP is mainly found in astrocytes with high expression levels in the mediobasal hypothalamus [186]. A number of external cues have been shown to increase ACBP expression in selected areas of rat brain, including acute stress [187,188], psychological but

not physical stress [189], alcohol intake [190–193], nicotine [194–196], diazepam [197,198], continuous treatment with morphine [199], butanol [200] and N-methyl-D-aspartate (NMDA) [201]. Moreover, ACBP levels are also increased in liver, ovarian and brain tumors [202–205] and extraordinarily found in urine after chemotherapy of gall bladder cancer patients [206].

The expression level of the mammalian ACBP is also affected by feeding status. Fasting rats for 24 h resulted in a 33% decrease in liver ACBP levels [207] and in reduced *Acbp* mRNA level as well [43], whereas the level in heart and kidney was unaffected. High fat diet for 48 h increased liver ACBP levels by 36%. Hepatic levels of ACBP continued to increase and remained elevated with

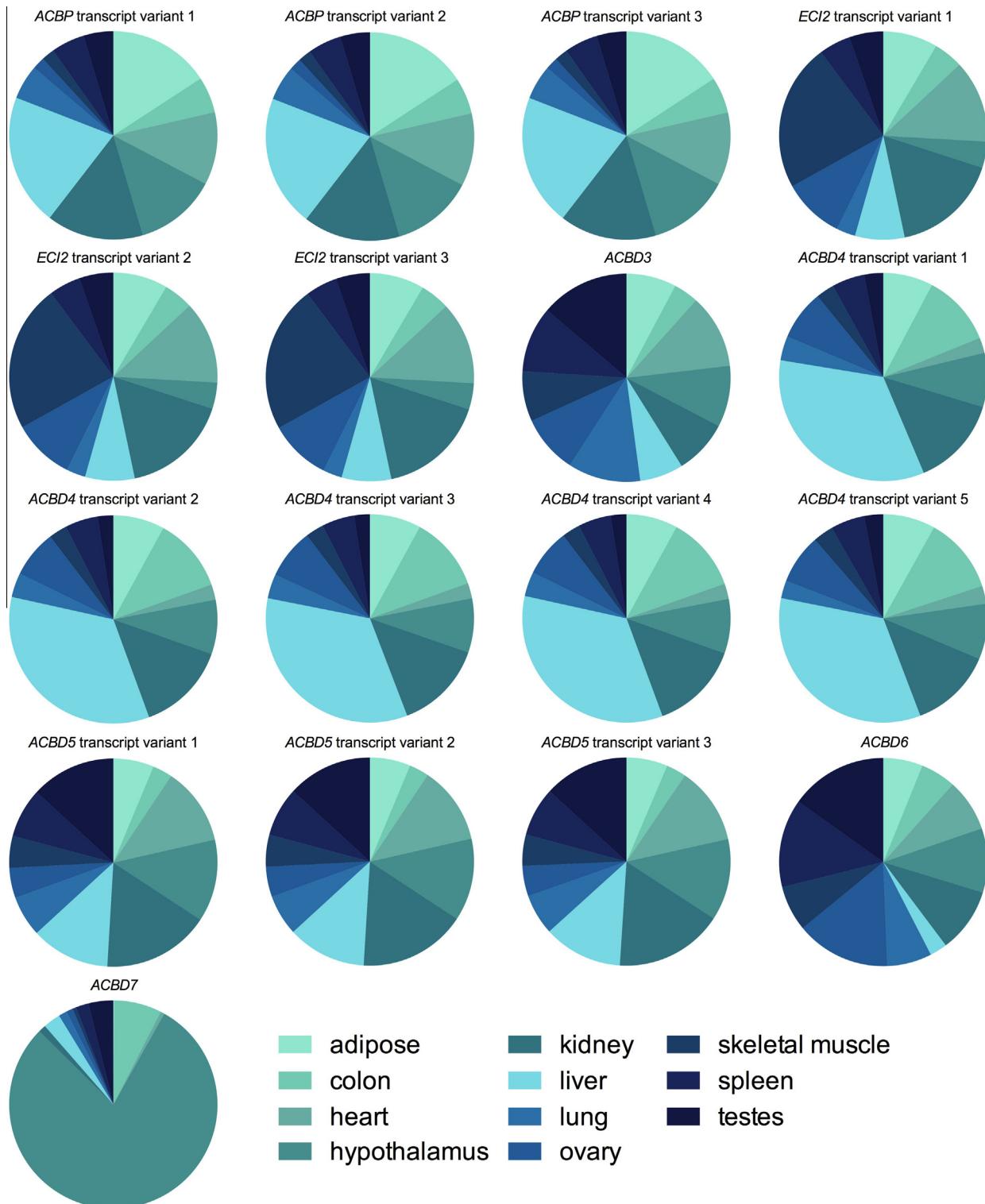


Fig. 5. Tissue distribution of human ACBD transcripts. Pie charts representing relative mRNA expression of each ACBD in different tissues shown as percentage of the total levels of the individual transcript. The distribution is based on recent RNAseq data reported in [217]. The ACBDs are ubiquitously expressed in humans, except for ACBD7, which is primarily expressed in the hypothalamus.

prolonged exposure to high fat (28 days), which likely is mediated by PPARs and the sterol regulatory element binding protein 1 (SREBP-1) [207]. Heart ACBP does not respond to short term fat feeding but was increased after prolonged exposure [207]. Transcription of the gene encoding ACBP is activated by both PPARs and by SREBPs [208–211]. While PPAR γ has been suggested to bind to an intronic enhancer and hence mediate induction of *Acbp* expression during differentiation of murine adipocyte [175,211], PPAR α has been found to activate *Acbp* expression in mouse liver [209].

Androgens, which stimulate growth of the human prostate cancer cell line LNCaP [212], also stimulate *de novo* fatty acid synthesis, cholesterol synthesis and lipid accumulation and induce ACBP

expression in this cell line [213], possibly via a sterol regulatory element in the proximal promoter of the *ACBP* gene. Similarly, androgens induce ACBP expression in several accessory sex organs in the male rat [214]. Peroxisome proliferators, perfluorodecanoic acid and 3-thia fatty acids have been shown to induce expression of *ACBP* in the liver [43,211,215,216]. This could indicate that ACBP expression is linked to lipid metabolism. However, growing LNCaP cells and differentiating 3T3-L1 cells undergo dramatic structural and functional changes, and it is therefore possible that other functions besides general lipid synthesis require increased expression of ACBP in these cells.

Since the majority of these studies were performed prior to identification of ACBD proteins and of transcript variants of each

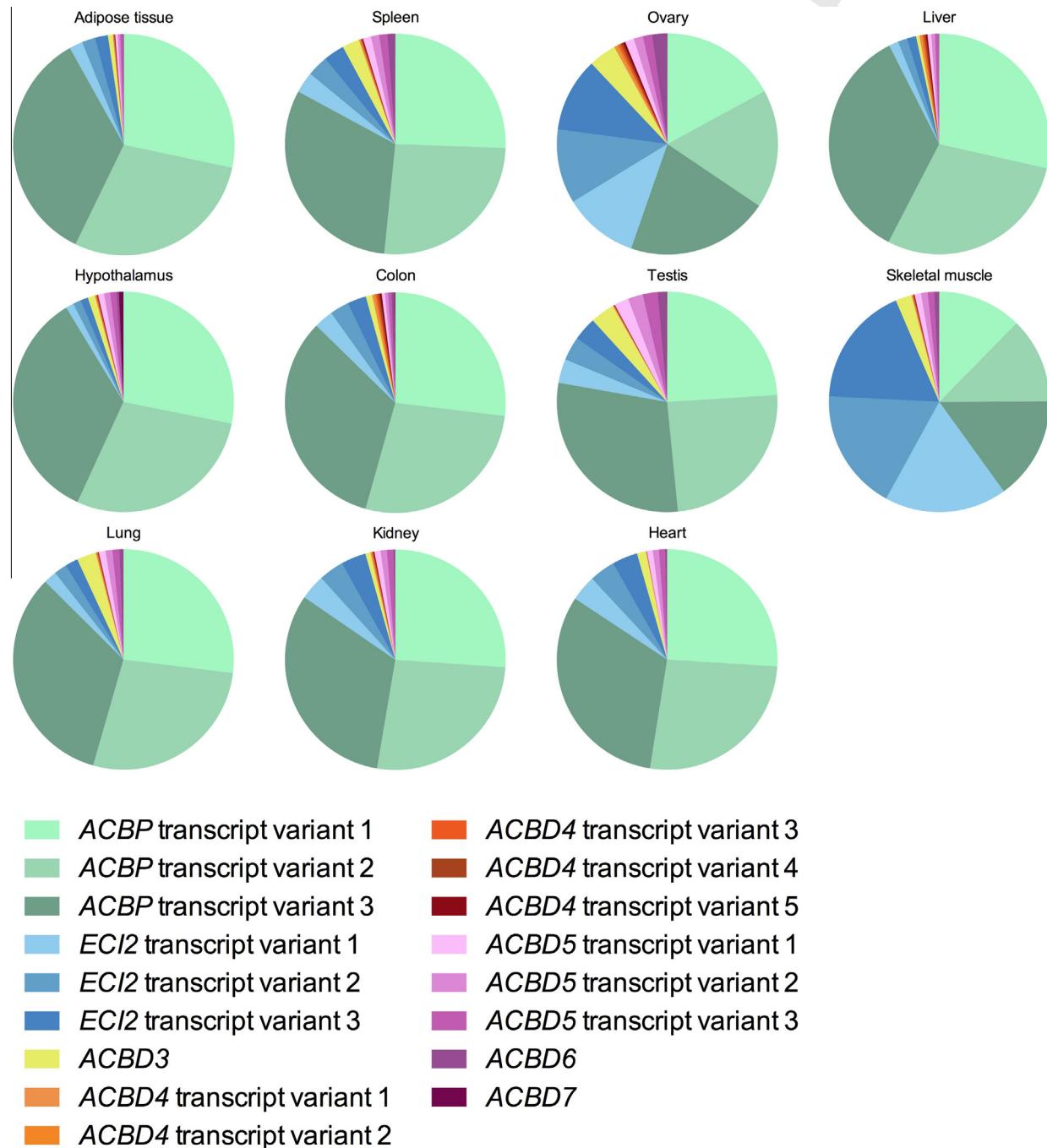


Fig. 6. Relative expression levels of ACBD mRNA in human tissues. Pie charts representing the relative mRNA expression level of ACBDs in individual tissues. The distribution is based on recent RNAseq data [217] showing that *ACBP* is the most abundant among all the ACBDs.

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isoform, these studies may not represent the precise tissue expression and distribution of ACBP. However, recent high-throughput RNA sequencing analyses [217] show that expression levels of ACBP in humans, to a large degree, are consistent with previous reports. These analyses confirm that ACBP is ubiquitously expressed but also show the presence of ACBP transcript variants (Figs. 5 and 6), which only recently have been recognized [177,218]. Moreover, the analyses also show that ACBP indeed is the most abundantly expressed parologue (Fig. 5), but also that the other ACBDs are expressed ubiquitously. Interestingly, among the tissues examined ACBD7 is primarily expressed in hypothalamus (Fig. 5) [217], suggesting that ACBD7 may serve tissue specific functions.

5.2. Expression in other eukaryotic organisms

The genome of *Caenorhabditis elegans* encodes seven highly conserved proteins containing an acyl-CoA-binding domain, which all contain the amino acids required for protein stability and ligand binding [219]. ACBP-1, ACBP-3, ACBP-4 and ACBP-6 contain only the ACBP domain, while ACBP-2, ACBP-5 and MAA-1 have an N-terminal ACBP domain as well as other functional domains. In the L1 larvae stage ACBP-3 is the most abundant ACBP-family member, while ACBP-1 is the most abundantly expressed family member during the L4 stage [219]. Transgenic animals expressing GFP fusions show that ACBP-1 is expressed in the intestine, which is consistent with a role in fat storage in the intestine. ACBP-2 is not only expressed in the intestine, but also in the hypodermis, the two major lipid-storing tissues [219]. ACBP-3 is expressed in muscle and hypodermis, while ACBP-4 and ACBP-5 are expressed in the intestine, where ACBP-4 specifically localizes to granular structures. Interestingly, ACBP-6 is only expressed in a few neurons in the head, body and tail of *C. elegans*. MAA-1 is expressed in the intestine, hypodermis and oocytes, and in the intestine is found to localize to the Golgi apparatus and to the endosomal compartment, which is consistent with a role in endosomal trafficking [220].

Immunohistochemical studies in *Drosophila melanogaster*, using antibodies against rat ACBP, detected high levels of immune reactivity in specific tissues including potassium-transporting cells in urine-secreting Malpighian tubules [221]. However, the presence of multiple ACBD paralogues including two acyl-CoA domain proteins obviously affect the interpretation of these studies as the antibodies used potentially could recognize more than one of these paralogues. ACBP expression in tobacco hornworm (*Manduca sexta*) increased about 15-fold in ecdysteroidogenic cells from day 1 to day 7 of the fifth instar. In the larval midgut ACBP expression was highest during times of active feeding [222,223]. Consistently, ACBP in the female mosquito *Aedes aegypti* increased significantly in ovaries during peak ecdysteroid production [224]. The silk worm *Bombyx mori* synthesizes the pheromone bombykol ((E,Z)-10,12-hecadecadiene-1-ol) from palmitoyl-CoA as a precursor in its pheromone gland. In *B. mori* two ACBP isoforms are expressed, one isoform in the midgut and another in the pheromone gland [225].

5.3. Intracellular localization of acyl-CoA binding protein

Several studies show that ACBP primarily localizes to the cytosol. In rat liver ACBP has been found to localize predominately to the cytosol by differential centrifugation, and less to the nucleus and the endoplasmic reticulum [226]. In primary rat liver hepatocytes ACBP localizes to the cytosol, however, upon induction of apoptosis ACBP is phosphorylated at its N-terminal end and concentrates in the proximity of the plasma membrane, including apoptotic buds, indicating that the intracellular localization of ACBP can be modulated by post-translational modifications [227]. Immuno-electron microscopy studies also show that ACBP

localizes to the cytosol in Leydig cells and in sweat and sebaceous gland cells from rat [163,167]. Similarly, ACBP is evenly distributed throughout the cytoplasm and not specifically associated with any organelles in primary astrocytes and C-6 cells [186,228] and in rat ovary cells [164]. Consistent with these observations ACBP was also found to localize in the cytosol of Leydig cells, Sertoli cells and in seminiferous tubules cells in rat testis, but was also found in the smooth endoplasmic reticulum, Golgi apparatus and the outer membrane of the mitochondria [229]. In L-cell fibroblast and McA-RH777 hepatoma cells ACBP was found in the cytosol and in the nucleus, but also to co-localize with acyl-CoA cholesteryl acyltransferase in the endoplasmic reticulum [90]. ACBP immunoreactivity was also found to be evenly distributed in the cytosol in frog adrenal chromaffin cells and to localize the periphery of large cytoplasmatic vesicles in Stilling cells [230]. Immunogold electron microscopy confirmed the cytosolic localization of ACBP in intestinal columnar cells and prothoracic gland ecdysteroidogenic cells in the tobacco hornworm *M. sexta*, but also revealed that ACBP localizes to the nucleolus and mitochondria, depending on its developmental stage [72]. That the intracellular localization of ACBP is dynamic has also been observed in murine fibroblasts. During the early stages of adipocyte differentiation of murine 3T3-L1 cells, ACBP predominantly localized to the nucleus, but was found in both the cytosol and nucleus in fully differentiated cells [140]. Moreover, Hansen et al. recently showed that fluorescently labeled bovine ACBP targets to the cytosol, the endoplasmic reticulum and Golgi when injected into HeLa and bovine mammary gland epithelial cells [231]. Interestingly, a variant of ACBP incapable of binding fatty acyl-CoA localized in the cytosol and nucleus and did not associate with the ER and the Golgi. In keeping with this observation, cellular depletion of fatty acids decreased its membrane association, whereas fatty acid overloading increased membrane association [231], arguing that the localization of ACBP dynamically may be regulated by ligand binding.

Differential- and density gradient centrifugation studies have shown that ACBP is highly enriched in synaptosomes and associates to synaptic vesicles in rat brain [185]. Immunohistochemical studies using an antibody against an ACBP octadecapeptide (residue 33–50) combined with electron microscopy found immunoreactivity in the neuronal perikarya, processes, terminals and synaptic vesicles. Interestingly ACBP could be released from neurons by depolarization, but not from astrocytes, liver and other peripheral tissues, arguing that ACBP specifically is released from neurons, and that ACBP both can serve as an intracellular lipid-binding protein and is secreted to serve extracellular functions in neurons [185].

Collectively, the reported observations show that ACBP predominantly is a cytosolic protein, but associates with or localizes in specific subcellular compartments including the endoplasmic reticulum, the Golgi apparatus, the nucleus and secretory vesicles. The observations also imply that its localization is dynamic and can be regulated by ligand binding and by the acyl-CoA level. This implies that the function of ACBP may depend on cellular metabolic fluxes of fatty acids, which could regulate its interaction with specific membrane lipid entities or proteins residing in the membrane, and hence control of substrates for acyl-CoA utilizing processes.

6. Acyl-CoA binding domain proteins; multiple; variations of a common theme

Sequencing of numerous prokaryotic and eukaryotic genomes has identified multiple, independent genes encoding a number of acyl-CoA binding domain containing proteins, which as the name indicates, comprise the ACBP structural fold as a subdomain.

Using the human ACBP (ACBD-1) transcript variant 3 (87 aa) as query, the ACBP structural fold can be found in 46 different protein architectures ranging from simple prokaryotes to mammals (Table 1). A total number of 3288 sequences have been identified that solely contain the ACBP domain are distributed throughout various kingdoms, while 473 sequences have been found to contain Ankyrin repeats besides the ACBP domain (Table 1). Overall, the ACBP domain can be found in proteins containing 46 other protein domains including Kelch-, SEC14-, FYVE-, PH-, and Crotonase-like/Enoyl-CoA isomerase domains.

In humans the ACBP domain is present in a total of 7 different proteins. These include ACBP (ACBD1/DBI), ECI2 (ACBD2), ACBD3 (GCP60/PAP7), ACBD4, ACBD5 (membrane associated diazepam binding inhibitor), ACBD6 and ACBD7 (Fig. 1). In addition, multiple transcript variants of human ACBP have been identified, including three high abundant and eleven low-abundant transcript variants (Figs. 5 and 6) [177,218,232], which collectively encode seven different ACBP protein isoforms, with specific tissue expression. The transcript variants are generated by a consequent use of alternative promoters and alternate use of the first or first two exons. Moreover, these transcripts are differentially regulated by SREBPs, HNF-4 α , NF- β B, and PPARs [218]. Three abundant transcripts encoding ACBP protein isoforms contain the common acyl-CoA binding protein domain but also carry alternative N-termini [218,232]. A fourth isoform identified by Ludewig et al. has an extended C-terminal domain of 81 amino acid residues, which is required for its localization to the ER/Golgi surrounding the nucleus [177]. Most of the low abundant transcripts contain an extended N-terminal end, except for one specific ACBP isoform, which has an N-terminal truncated ACBP isoform [218]. Localization analyses of the various isoforms in HepG2 cells show that most of the ACBP isoforms localize to the nucleus and cytosol, however, two of the low abundance isoforms are primarily found in vesicular structures enriched around the nucleus [218,232].

6.1. ACBD2/ECI2

By virtue of its enoyl-CoA isomerase (ECI) domain, ECI2 (ACBD2) belongs to the hydratase/isomerase superfamily, which is also called the crotonase superfamily of enzymes. CoA-dependent enzyme members of this family have been shown to catalyze a wide range of reactions that includes dehalogenase, hydratase, hydrolysis and isomerase activities. ECIs, specifically known as Δ^3,Δ^2 -enoyl-CoA isomerases, catalyze the degradation of unsaturated fatty acids with cis/trans double bonds at odd positions, like oleic acid and linoleic acid, which then can be further degraded in the classical β -oxidation pathway. In mammals a number of ECI isozymes have been identified. ECI1 is located only in the mitochondria, while ECI2 is located in both mitochondria and peroxisomes by virtue of an N-terminal mitochondrial targeting domain and a C-terminal SKL-peroxisomal targeting motif, respectively. ECI1 and ECI2 have recently been shown to be functionally redundant [233]. Besides ECI2, the multifunctional enzyme MFE1 also localizes to peroxisomes. In contrast to ECI1, ECI2 contains a 80 amino acid residue long four-helix bundle ACBP domain in its N-terminus, which is connected to the isomerase domain by a linker region [158]. At least 4 transcript variants of ECI2 have been identified, of which transcript variants 1, 2 and 3 are predicted to encode proteins of 364, 394 and 364 amino acid residues, respectively. Transcript variant 2 has a 30 amino acid long N-terminal extension, with an unknown function. Each variant has been identified in most tissues, however, each variant appear to be most abundantly expressed in skeletal muscle (Fig. 5).

Recent structural-functional analyses have shown that the ACBP domain contributes to the stability of ECI2 and is required for its catalytic activity [158]. This is in keeping with the fact that

the ECI2 paralog in *C. elegans*, ACBP-2, is required for β -oxidation of unsaturated fatty acids [219]. Interestingly, the ACBP domain of ECI2 binds medium-chain acyl-CoA esters with much lower affinity than previously reported for rodent- or bovine ACBP [153,155,234].

6.2. ACBD3/GCP60/PAP7

ACBD3 is a 528 amino acid residue protein, which besides an ACBP domain also contains a GOLD domain, a putative nuclear localization signal (NLS) located between the ACBP- and GOLD-domains, and a coiled-coil domain in its C-terminus (Fig. 1). ACBD3 was initially identified by its ability to interact with the mitochondrial peripheral benzodiazepine receptor (PBR) and the regulatory subunit of Protein Kinase A, PKAR1 α . ACBD3 is therefore also known as the PBR-associated protein 7 (PAP7) [235]. In the steroidogenic MA-10 Leydig tumor cell line, ACBD3 was found to stimulate mitochondrial cholesterol transport and steroidogenesis in response to human chorionic gonadotropin (hCG). However, a truncated form of ACBD3 comprising the PBR and PKA-RI binding domains but not the ACBP domain was found to suppress hCG-induced transport and steroidogenesis, indicating that the ACBP domain is required for hormone-induced steroid production by increasing transport of cholesterol to the mitochondria [236].

ACBD3 co-localizes and interacts with the Giantin in the Golgi complex, and is therefore also termed Golgi complex-associated protein GCP60 [237]. Interestingly, overexpression of GCP60 in COS-1 cells impaired Golgi structure as well as transport from ER to the Golgi complex, which required the coiled-coil domain in its C-terminus but was independent of the presence of the ACBP domain. Moreover, Golgi localization and Giantin interaction of GCP60 did not require the ACBP domain. This implies that the role of ACBD3 in maintaining ER-Golgi trafficking, Golgi structure and in Giantin interaction is not dependent on acyl-CoA binding.

ACBD3 also interacts with golgin-160, a Golgi residing protein which functions in the stacking of Golgi cisternae and in vesicular transport [238]. When overexpressed, ACBD3 can, via direct interaction, prevent nuclear accumulation of a golgin-160 fragment, which is generated by caspase activity upon induction of apoptosis. However, this interaction and effect on nuclear localization was independent of the ACBP domain [239].

ACBD3 furthermore interacts with m-Numb, a Numb homolog that is required for neuronal differentiation [240]. ACBD3 associates with the Golgi complex in neurons and interphase progenitor cells, but is released to the cytosol after Golgi fragmentation during mitosis, when Numb is asymmetrically distributed and required to distinguish the two daughter cells. Interestingly, ACBD3 acts synergistically with Numb to specify cell fates, e.g. with a cytosolic presence of ACBD3 during the progenitor cell cycle, Numb inhibits neuron production, while when localized to the Golgi complex ACBD3 and Numb promoted neuronal differentiation [240]. However, the interaction between ACBD3 and Numb does not require the ACBP domain of ACBD3, implying that LCACoA binding is not required for ACBD3 function in neuronal differentiation.

Furthermore, ACBD3 interacts with Dextras1, a 30 kDa G protein member in the Ras subfamily, and the divalent metal transporter (DMT1). Although these interactions mediate uptake of iron [241], it is doubtful that LCACoA binding to ACBD3 plays a role, as the interaction between ACBD3 and Dextras1 occurs independent of the ACBP domain in ACBD3 [241].

Besides the above-mentioned interactions, numerous studies have shown that ACBD3 also interacts with phosphatidylinositol 4-kinase IIIb (PI4KB), and that this interaction is involved in replication of picornaviruses [242–248]. Sasaki et al. have shown that ACBD3 interacts with PI4KB and several viral non-structural proteins, and hence promote synthesis of PI4P at viral replication sites to support viral RNA replication [242,247]. Similarly, Greninger

et al. also showed that ACBD3 interacts with numerous picornavirus 3A non-structural proteins as well as PI4KIII β and that poliovirus RNA replication is reduced upon silencing of ACBD3 or PI4KIII β [243]. In contrast to these findings, Teoule et al. demonstrated that suppression of ACBD3 expression resulted in increased poliovirus RNA replication despite that ACBD3 also was found to interact with Polio virus 3A proteins and PI4KIII β at viral RNA replication sites [244]. Recently, it was shown that coxsackievirus and rhinovirus recruit PI4KIII β to replication sites independently of ACBD3 [245,246] and that ACBD3 depletion enhances coxsackievirus RNA replication [246] but had no effect on rhinovirus replication [245].

Collectively, ACBD3 functions have been associated to a wide range of biological functions including steroidogenesis, apoptosis, neurogenesis, and embryogenesis. The part of ACBD3 containing the GOLD domain appears to be responsible for its localization to the Golgi and to mediate its biological functions. Albeit the function of the ACBP domain in ACBD3 remains largely unresolved, it is noteworthy that three tyrosine residues, which are required for binding of LCACoAs [154] have been substituted with valine, histidine (both in helix 2) and phenylalanine (in helix 4) (Fig. 1). Therefore it could be speculated that acyl-CoA binding is not be essential for its function. However, functional loss of Acb1 inhibits Brome mosaic virus RNA replication in the yeast *S. cerevisiae*, arguing that the presence of a functional acyl-CoA binding protein is required for viral RNA replication [249].

6.3. ACBD5/membrane associated diazepam-binding inhibitor

One of the best evolutionarily conserved cellular responses to dietary restriction is the activation of autophagy, a lysosomal degradation pathway in which the cell self-digests its own components, which provides nutrients to support crucial cellular functions. Autophagy was originally considered to be a non-selective process, however, during the past decade several studies have proven that autophagy can be highly selective. This selectivity is accomplished by autophagy receptors, such as the autophagy-related protein 30 (Atg30), which controls the selective autophagy of peroxisomes (pexophagy) via the assembly of a specific receptor protein complex. In the yeast *Pichia pastoris* Atg37, the yeast ortholog of ACBD5, has recently been found to be a peroxisome membrane-associated acyl-CoA-binding protein, and specifically required for formation of phagophores during pexophagy. Nazarko et al. found that Atg30 recruits Atg37 to the pexophagic receptor-protein complex, where it promotes the interaction between Atg30 and the scaffold protein Atg11 [250,251]. Interestingly, binding of palmitoyl-CoA to Atg37 completely inhibits interaction with Atg30. The role of Atg37 in pexophagy proved not only to take place in yeast, but also in human cells lines, strongly implying that its function is evolutionary conserved. Moreover, these observations also argue that the level of LCACoAs can regulate specific forms of autophagy. Consistent with a role in vesicular trafficking, Larsen et al. found that membrane-associated acyl-CoA binding protein, MAA-1, the ACBD5 ortholog in *C. elegans*, localizes to intracellular membrane organelles in the secretory and endocytic pathway and is required for endosomal recycling in a ligand-binding dependent manner [220] (see below).

As for some of the other ACBDs, numerous transcript variants of ACBD5 have been identified, which are predicted to encode proteins ranging from 416 to 525 amino acid residues (Table 2), and to be ubiquitously expressed. Hence, this may suggest that ACBD5 carries out a fundamental functional and that each transcript variant may carry out different, and specific functions in endosomal trafficking and autophagy.

Table 2

ACBD transcript variants identified by RNAseq in human tissues by Krupp et al. [217].

Gene symbol	Transcript	Transcript length (bp)	Amino acids
ACBP transcript variant 1	NM_020548	312	104
ACBP transcript variant 2	NM_001079863	264	88
ACBP transcript variant 3	NM_001079862	261	87
ECI2 transcript variant 1	NM_006117	1092	364
ECI2 transcript variant 2	NM_206836	1182	394
ECI2 transcript variant 3	NM_001166010	1092	364
ECI2 transcript variant 4	NR_028588	1463 non coding	
ACBD3	NM_022735	1584	528
ACBD4 transcript variant 1	NM_001135704	1023	341
ACBD4 transcript variant 3	NM_001135706	1023	341
ACBD4 transcript variant 2	NM_024722	915	305
ACBD4 transcript variant 4	NM_001135705	915	305
ACBD4 transcript variant 5	NM_001135707	801	267
ACBD5 transcript variant 1	NM_145698	1575	525
ACBD5 transcript variant 2	NM_001042473	1470	490
ACBD5 transcript variant 3	NM_001301251.1	1248	416
ACBD6	NM_032360	846	282
ACBD7	NM_001039844	264	88

6.4. ACBD6

Human acyl-coenzyme A binding domain protein 6 (ACBD6) is 282 amino acid residue long protein, which comprises an acyl-CoA binding domain at its N terminal end and 3 ankyrin motifs in its C terminus. Only a single transcript variant of ACBD6 has been found. ACBD6 is expressed in multiple tissues but has low abundance compared to the expression of the other ACBD transcripts (Figs. 5 and 6).

ACBD6 preferentially binds unsaturated LCACoAs over saturated acyl-CoA species independent of the ankyrin repeats [252]. The affinity constant for oleoyl-CoA is 3.5 μ M, much lower than the binding affinity reported for human and bovine ACBP, however, the binding constant was determined by Lipidex-1000 binding assays, which previously has been found to underestimate binding affinities of acyl-CoA esters to ACBP [153,253]. Despite the fact that ACBD6 transcript has been detected in multiple tissues (Fig. 5) [217], Soupene et al. found that expression of the protein is restricted to tissues and progenitor cells with functions in blood and vessel development [252]. In embryonic-like stem cells derived from placenta ACBD6 has been found to localize throughout the cell and is not restricted to a particular subcellular structure. In HeLa cells ACBD6 localizes to the cytosol and to the nucleus, but translocates exclusively to lipid droplets upon invasion of the human pathogen *Chlamydia trachomatis*, where it modulates the activity of the bacterial lysophosphatidylcholine acyltransferase (LPCAT) and hence synthesis of PC from lyso-PC [254]. In placenta, ACBD6 has only been detected in CD34 progenitor cells present in blood and in CD31 endothelial cells surrounding the blood vessels, which probably constitute hemangiogenic stem cells [252].

6.5. ACBD7

ACBD7 is a 88 amino acid residue long protein, which has been detected in multiple tissues, but primarily in the hypothalamus (Fig. 5). Hence, ACBD7 was previously named brain ACBP (B-ACBP) [255]. Although ACBD7 contains all the residues shown to be important for ACBP stability and acyl-CoA binding, ACBD7 appears to form a dimer in which one half from each monomer forms a four-helix bundle protein structure (Fig. 4). How and to what extent ACBD7 binds LCACoA esters is therefore yet to be determined.

6.6. Endozepine-like peptide

Endozepine-like peptide (ELP), previously also termed testis specific ACBP (T-ACBP) [255,256], is exclusively expressed in the late spermatid stages of spermatogenesis in the testes of sexually mature mice. During the elongation process and spermatozoa formation, the spermatid undergoes dramatic morphological changes, which also involve lipid remodeling that may involve ELP. The transcript encoding full-length ELP has been detected in rats, cows, marmosets and macaques, however, the orthologous gene in humans and other higher primates, is present only as an inactivated pseudogene [257].

7. In vitro functions and properties of ACBP

Soon after its discovery as an acyl-CoA binding protein in 1987 [6], the effect of ACBP on functions either utilizing or being regulated by LCACoAs was examined *in vitro*. ACBP effectively prevents the inhibitory effects of LCACoAs on mitochondrial acyl-CoA synthetase activity by sequestration of the synthesized acyl-CoA esters [44]. When added in equimolar or in excess to LCACoAs, ACBP has a strong attenuating effect on the inhibition of acetyl-CoA carboxylase and of the mitochondrial adenine nucleotide translocase by LCACoAs, and able to protect LCACoAs against microsomal acyl-CoA hydrolases [44]. ACBP can desorb LCACoAs immobilized in multilamellar liposomes and transport and donate them to mitochondrial β -oxidation and to microsomal glycerolipid synthesis [258]. Numerous studies also show that ACBP can stimulate or inhibit the activity of red cell acyl-CoA-lysophospholipid acyltransferase [259], GPAT [260–262] and acyl-CoA/cholesterol acyltransferase (ACAT) [263] depending on substrate concentrations and the experimental conditions. Accordingly, overexpression of ACBP in mice increases total and membrane-bound LCACoA levels and the enzymatic activity of liver microsomal GPAT. Consistently, the mass of phospholipid and TAGs in liver nearly doubled. Thus, these observations argue that ACBP play a role in glycerolipid biosynthesis *in vivo* [264].

ACBP completely opposes LCACoA mediated Ca^{2+} release by the ryanodine-sensitive Ca^{2+} release channel in longitudinal tubules and terminal cisternae of sarcoplasmic reticulum from rabbit skeletal muscle [76] and duckling sarcoplasmic reticulum [77,78]. However, ACBP in complex with LCACoA potently augments the sensitivity of the rabbit muscle terminal cisternae ryanodine receptor Ca^{2+} release channel to caffeine by several fold.

Purified native and recombinant rat ACBP has been shown to potently decrease the $[\text{Ca}^{2+}]$ required for activation of calpain [265]. Consistently, Shulga and Pastorino have shown that siRNA mediated knock down of ACBP in HeLa cells inhibits t-Bid-induced activation of mitochondrial μ -calpain, release of apoptosis-inducing factor from the mitochondrial intermembrane space and cleavage of full-length Bid to t-Bid. The activating effect of ACBP μ -calpain was ablated upon knock down of expression of the peripheral benzodiazepine receptor, arguing that interaction between ACBP and PBR is required for this effect [266].

8. In vivo functions of acyl-CoA domain proteins, lessons from *S. cerevisiae*, *C. elegans* and from *Arabidopsis thaliana*

8.1. Acb1 functions in the yeast *S. cerevisiae*

The yeast *S. cerevisiae* ACB1 gene encodes the only acyl-CoA binding protein in yeast belonging to the ACBD family [89]. Acb1 is an 86 amino acid protein, which not only shares 48% sequence identity but is also structurally very similar to human ACBP (Fig. 1). The most pronounced phenotypes caused by Acb1p depletion are impaired

growth, multi-lobed vacuoles, invaginations of the plasma membrane and accumulation of a large number of vesicles of variable sizes, autophagocytotic like bodies, membrane fragments as well as multi-layered plasma membrane structures [267–269].

Deletion of ACB1 results in accumulation of stearoyl-CoA rather than palmitoyl-CoA, whereas the levels of myristoyl-CoA, palmitoleoyl-CoA and oleoyl-CoA were reduced [267]. This implies that Acb1 is involved in termination of fatty acid synthesis in yeast, which is supported by the fact that Acb1 facilitates removal of newly synthesized acyl-CoA esters from the yeast fatty acid synthase (FAS) *in vitro* [267], and that overexpression of Acb1 can also increase the production of fatty acid ethyl esters [270,271]. Moreover, overexpression of either bovine or yeast ACBP markedly increases the intracellular acyl-CoA ester pool, showing that Acb1 can act as an acyl-CoA pool former *in vivo* [272,273]. Lipidomic analyses have shown that the total level of phosphatidylinositol (PI) and phosphatidylglycerol (PG) is increased in Acb1 depleted cells. The increased level of PI may be due a massive induction of the *INO1* gene, encoding inositol 3-phosphate synthase, in Acb1 depleted cells [274]. The level of *INO1* expression could be normalized by supplementation of exogenous fatty acids or increased *de novo* fatty acid synthesis arguing that Acb1 links fatty acid metabolism to transcriptional regulation of glycerophospholipid biosynthesis [274]. Moreover, in Acb1 depleted cells glycerophospholipids, particularly PI and phosphatidylcholine (PC), contain shorter and more unsaturated acyl-chains [269,275,276]. The increased unsaturation may be a result of increased transcription of *OLE1*, encoding the $\Delta 9$ -desaturase [267,274], while acyl-chain shortening could be due to reduced fatty acid elongation as observed in Acb1 depleted cells [268].

Besides a small reduction in the total content of fatty acids and an increased level of unsaturated fatty acids in Acb1 depleted cells compared to wild type cells, the most dramatic change in the fatty acid composition was a strong reduction in $\text{C}_{26:0}$ suggesting that Acb1 is involved synthesis of $\text{C}_{26:0}$ very-long chain fatty acids (VLCFA) [268]. Supplementation of exogenous palmitic acid did not complement the reduced level of VLCFAs in Acb1p depleted cells, indicating that Acb1p is also required for elongation of fatty acids. To this end, Acb1 depleted cells contain 50–60% less of the long-chain bases phytosphingosine (PHS) and dihydrosphingosine (DHS) (our unpublished results), and less ceramide and inositol phosphorylceramide (IPC), mannosylinositol phosphorylceramide (MIPC), [268,269], substantiating the role of Acb1 in sphingolipid synthesis. Accordingly, inactivation of the serine palmitoyl-transferase Lcb1, the rate-limiting enzyme in ceramide synthesis, renders Acb1p depleted cells nonviable [268].

The pronounced effects of Acb1 depletion on organelle morphology and membrane assembly suggest that Acb1 is involved in vesicular trafficking. In keeping with this, protein trafficking and maturation of vacuolar proteins is delayed in Acb1 depleted cells [269]. Moreover, vacuoles isolated from Acb1p depleted cells are unable to undergo homotypic vacuole fusion *in vitro*, which is likely due to loss of SNARE proteins from the vacuolar membrane, a consequence of the absence of ceramide in vacuoles isolated from Acb1p depleted cells [269]. Furthermore, both macroautophagy and the unfolded protein response pathways are induced in Acb1 depleted cells (our unpublished results), underlining its role in membrane and vesicular trafficking. Interestingly, in a genomic screen for genes affecting chronological life span, loss of *ACB1* was identified to increase life span and to induce resistance towards heat shock and acid [277].

8.2. Acbd functions in the nematode *C. elegans*

As mentioned above seven ACBD paralogues have been identified in the nematode *Caenorhabditis elegans* (*C. elegans*). ACBP-1,

ACBP-3, ACBP-4 and ACBP-6 only contain the ACBP domain and no other known domains [219]. Although their ligand binding properties have not been elucidated, each of the *C. elegans* isoforms can function as acyl-CoA-binding proteins *in vivo* as they can rescue the slow growth phenotype when ectopically expressed in *Acb1*-depleted yeast cells. ACBP-1 is considered to be the ortholog with highest similarity to mammalian ACBP/ACBD-1 and to *Acb1* in *S. cerevisiae*. Deletion of *acbp-1* in *C. elegans* significantly decreases lifespan and results in a dramatic increase in lipid droplet size but in a reduction in lipid droplet number and in TAG abundance. As β -oxidation of both saturated and unsaturated fatty acids is increased in *acbp-1* mutant worms, these observations imply that ACBP-1 regulates lipid storage and degradation [219]. Besides an N-terminal ACBP domain, ACBP-2 also contains an enoyl-CoA hydratase/isomerase domain and an N-terminal mitochondrial-targeting signal. Functional loss of ACBP-2 results in impaired β -oxidation of unsaturated fatty acids with a compensatory upregulation of genes encoding enzymes involved in peroxisomal and mitochondrial β -oxidation. Consistent with its function in degradation of unsaturated fatty acids, loss of ACBP-2 function also increases unsaturation of acyl-chains in lipid droplets. Moreover, ACBP-2 is transcriptionally regulated by the nuclear hormone receptor NHR-49, the PPAR α ortholog in *C. elegans* [219]. Deletion of *acbp-3*, *acbp-4*, or *acbp-6* results in 35–40% reduction in lipid chain unsaturation in intestinal lipid droplets, while only loss of ACBP-3 affects the number of intestinal lipid droplets and the level of TAG. However, since ACBP-3 is expressed in hypodermis and muscles and ACBP-6 only is expressed in a few head and tail neurons, this suggests that the role ACBP-3 and ACBP-6 in lipid droplet composition is indirect. ACBP-5 contains two ankyrin repeats and is therefore considered to be the closest ortholog of ACBD-6 in mammals. Besides a slight increase in the number of intestinal lipid droplets, *acbp-5* mutants do not have any marked phenotypes. Besides an N-terminal ACBP domain, membrane-associated acyl-CoA binding protein (MAA-1) also contains a coiled-coil and a transmembrane domain. Accordingly, MAA-1 is membrane-associated and localizes to intracellular organelles in the secretory and endocytic pathway. Deletion of *maa-1* or loss of its ability to bind LCACoAs changes the morphology of endosomal organelles and reduces the rate of endosomal recycling, arguing that MAA-1 may mediate the regulatory effects of LCACoAs on vesicular trafficking as mentioned above [220]. Consistently, Atg37, its ortholog in *P. pastoris*, binds LCACoAs, which is required for its role in pexophagy [250,251].

8.3. *Acbd* functions in the plant *A. thaliana*

The plant *Arabidopsis thaliana* (*A. thaliana*) probably represents the model system in which the functions of acyl-CoA binding domain proteins have been studied most extensively. However, as their characteristics and functions recently have been reviewed in detail [278], they will only be described in general terms here. The *A. thaliana* family of acyl-CoA binding proteins comprises six members, AtACBP-1 to AtACBP6, which vary from 92 to 668 amino acid residues in size. Based on the presence of other domains, AtACBPs have been categorized into four different classes. Class I holds AtACBP-1 and AtACBP-2, which both have ankyrin repeats in their C-terminus and an N-terminal transmembrane domain that targets the protein to the plasma membrane and the ER. AtACBP3 and AtACBP6 are single members of Class II and Class III, respectively, and are both characterized by not having other domains than the ACBP domain. Yet, AtACBP3 also has an N-terminal signal peptide/transmembrane motif, which is essential for its localization to intracellular membranes, ER/Golgi and the extracellular space. AtACBP4 and AtACBP5 belong to Class IV, both containing an N-terminal ACBP domain and five kelch motifs and

localize primarily to the cytosol. Finally, AtACBP6 only contains an ACBP domain and is therefore considered as the prototype ACBP. Consistently, AtACBP6 localizes to the cytosol and binds LCACoAs with nM affinity [278,279].

Knockout of *Atacbp1* results in increased tolerance to freezing while AtACBP1-overexpression results in enhanced sensitivity to freezing [280]. In contrast, AtACBP6 has been reported to be involved in freezing tolerance as *Atacbp6* knockout mutants are more sensitive to freezing than wild type, while AtACBP6 overexpression lines are more resistant to freezing [281]. AtACBP1 has also been found to bind Pb²⁺ and consistent with this property, overexpression of AtACBP1 increases tolerance to Pb²⁺, while knockout of *Atacbp1* resulted in increased sensitivity to Pb²⁺ [282]. Recently, Xue et al. found that AtACBP1 binds very long-chain acyl-CoAs (VLCACoAs) and that deletion of AtACBP1 decreases the VLCFA in cuticular waxes, suggesting that AtACBP1 is involved in biosynthesis of VLCFA [283]. AtACBP2 also binds heavy metals like Cd²⁺ and Cu²⁺, and in line with this, overexpression of AtACBP2 confers increased tolerance to Cd²⁺. Thus, AtACBP2 has been suggested to be involved in glycerophospholipid repair at the plasma membrane after heavy metal-induced lipid peroxidation [284,285]. AtACBP3 function has been linked to leaf senescence putatively via its interaction with phosphatidylethanolamine (PE), PE-modification of autophagy-related protein ATG8 and hence its regulation of formation of autophagosomes [286]. Moreover, AtACBP3 is also involved in the plant response to hypoxia, possibly via modulating the metabolism of VLCFA [287]. Finally, the roles of AtACBP4 and AtACBP5 in *A. thaliana* are yet to be fully resolved, however, recent studies show that these two proteins are involved in seed germination [279] and pollen development [288].

Albeit with different affinities and specificities, all the AtACBPs bind LCACoAs [278,279]. However, in contrast to common belief, the AtACBPs also bind glycerophospholipids, yet with different specificities, arguing that these proteins can interact and associate with membranes and of obviously contribute to their localization in a dynamic manner [278].

9. Functions of ACBP in mammals

Several studies using genetically modified mice and rats have aimed at elucidating the role of ACBP in mammalian physiology. These studies include overexpression of ACBP in both mice and rats. Overexpression of ACBP in mice to levels resembling the levels found during high fat feeding does not affect body and liver weights compared to wild type mice. However, LCACoAs levels are increased in livers from transgenic mice compared to wild type [264]. In keeping with this, transgenic rats overexpressing ACBP using the endogenous mouse ACBP promoter results in the accumulation of acyl-CoA esters in the liver and adipose tissue [289], supporting that ACBP can function as an acyl-CoA pool former in mammalian liver and that ACBP relieves product inhibition of ACS and protects LCACoAs from hydrolysis. The LCACoAs accumulated in the microsomal compartment at the expense of the level in the cytosol, leading the authors to suggest that ACBP can donate LCACoAs to membranes and especially to lipid synthesizing systems. In keeping with this, GPAT activity as well as the TAG and phospholipid content are increased in livers from ACBP transgenic mice [264]. Moreover, overexpression of ACBP in McA-RH7777 rat hepatoma cells also resulted in increased TAG levels [290]. Collectively, these studies argue that ACBP can deliver LCACoAs for TAG synthesis.

Recently, Landrock et al. reported that ACBP depletion in mice results in early pre-implantation embryonic lethality at the 8-cell stage [291]. This contradicts the reported findings by Lee and colleagues, who have characterized mice carrying a 400 kb spontaneous pleiotropic deletion (nm1054), which among other

sequences includes the *Acbp* gene [292]. These mice are easily distinguishable from their wild type littermates shortly after birth due to the smaller body size and a pale appearance due to anemia. On a C57BL/6J background the nm1054 mutation is prenatally lethal; only 15 mutants (7.3%) out of 220 births in total survived. Of the live born homozygous mutants only few survive beyond day 7 and very few were successfully weaned [293]. However, when bred on a mixed background (129S6/SvEvTac × C57BL/6J), the nm1054 mice survive to adulthood and are characterized by having sparse, matted, reddish hair with a greasy appearance along with sebocyte hyperplasia in skin of thorax and nose, but not paws and tail [292].

Neess et al. recently reported that mice with a targeted disruption of the *Acbp* gene (*Acbp*^{−/−}) are viable and fertile and born in a Mendelian ratio, demonstrating that ACBP is dispensable for essential cellular functions [294]. However, the ability of *Acbp*^{−/−} mice to survive weaning is significantly decreased unless special care is taken e.g. by supplying soaked food and water in the bottom of the cage. Consistent with the above-mentioned observations in the nm1054 mice, targeted deletion of *Acbp* also results in touselled and greasy fur, development of alopecia and scaling of the skin with age [168]. The wild type mice display a marked induction of the SREBP-regulated lipogenic as well as the cholesterogenic gene programs in the liver during weaning. Interestingly, *Acbp*^{−/−} mice show a delayed hepatic upregulation of these gene programs at weaning, which is accompanied by a markedly lower hepatic *de novo* cholesterol synthesis [294]. The observed suppression of SREBP activity may be due to a transient accumulation of triacylglycerol (TAG) and cholesteryl esters (CE) in the liver of *Acbp*^{−/−} mice around weaning. Surprisingly, SREBP target gene expression is not impaired in liver-specific ACBP knockout mice but only impaired in mice lacking ACBP in keratinocytes (K14-*Acbp*^{−/−}) [295]. K14-*Acbp*^{−/−} mice display a macroscopic phenotype similar to that of mice with complete knockout of *Acbp*. Accordingly, transepidermal water loss is significantly increased in both K14-*Acbp*^{−/−} and full body *Acbp*^{−/−} mice suggesting an important role for ACBP in establishing and/or maintaining the epidermal barrier. To this end, both Vaseline and Latex could establish an artificial barrier on the skin and thereby rescue SREBP target gene expression in the liver [295]. The impaired barrier function leads to increased lipolysis and elevated phosphorylation of HSL in the white adipose tissue of both K14-*Acbp*^{−/−} and full body *Acbp*^{−/−} mice. Consequently, the increased flux of non-esterified fatty acids from the white adipose tissue lead to hepatic lipid accumulation and could lead to suppression of the hepatic lipogenic gene program in ACBP deficient mice at weaning [295].

The impaired epidermal barrier of *ACBP*^{−/−} mice has been suggested to be due to decreased levels of very long chain free fatty acids in the stratum corneum [8,168], which is consistent with the role of ACBP in fatty acid elongation in yeast [268]. However, considering the importance of ceramides in epidermal barrier function, and the fact that ACBP also is required for ceramide synthesis in yeast [269], it cannot be excluded that ACBP might be involved in the synthesis of epidermal ceramides in mice.

Acbp^{−/−} mice have an increased water intake and diuresis compared to wild type mice and exhibit increased diuresis, reduced urine osmolality, elevated hematocrit, and higher relative weight loss compared with wild type mice after 20-h of water deprivation [169]. Whether this is linked to the impaired epidermal barrier function or caused by loss of ACBP in the kidneys is not known.

10. Other and extracellular functions of acyl-CoA binding protein

Prior to the identification of ACBP as an acyl-CoA binding protein, the protein was identified as a 104 amino acid brain

polypeptide capable of inhibiting binding of benzodiazepines to GABA_A receptors, and was therefore termed diazepam-binding inhibitor, DBI [2,296,297]. Accordingly, when injected intraventricularly ACBP can block the anticonflict effect of benzodiazepines and to facilitate the behavioral suppression induced by punishment [2]. Moreover, ACBP has also anti-nociceptive effects in the central nervous system [298]. To this end, ACBP polymorphisms have been linked to anxiety disorders with panic attacks [299]. Furthermore, plasma levels of ACBP are also much higher in adults suffering from generalized epilepsy and in drug-resistant pediatric and adult patients [300]. Interestingly, endogenous potentiation of GABAergic synaptic transmission and responses to GABA uncaging in the thalamic reticular nucleus is absent in nm1054 mice [292]. As viral transduction of ACBP into the thalamic reticular nucleus can rescue the endogenous potentiation of GABAergic transmission in nm1054 mice, these observations support the notion that ACBP indeed can modulate GABAergic transmission *in vivo* [301]. However, overexpression of ACBP in mice neither causes anxiety or proconflict behavior, nor affects drug-induced seizure activity, but results in hydrocephalus, decreased plasticity in excitatory synapses, and impaired hippocampus-dependent learning [302].

Diazepam and other benzodiazepines can also bind to the peripheral benzodiazepine receptor to stimulate mitochondrial conversion of cholesterol into pregnenolone. Thus, stimulation of PBR by ACBP via direct interaction has been suggested to promote cholesterol influx into mitochondria and the subsequent formation of pregnenolone, the precursor for endogenous steroid production, which not only takes place in peripheral steroidogenic tissues, but also in glial cells, steroidogenic cells in the brain [303,304].

Herzig et al. have demonstrated that ACBP is secreted into the duodenum of rats to potently stimulate release of cholecystokinin (CCK), to ultimately stimulate release of amylase from pancreas [305]. Soon after it was shown that ACBP also could stimulate CCK release from the STC-1 murine tumor cell line, which was mediated by ACBP-induced Ca²⁺ oscillation via L-type Ca²⁺ channels [306]. Furthermore, the fact that the peptone-induced CCK release and pancreatic secretion can be blocked by an ACBP-antibody underlines the role of ACBP in the regulation of CCK release and pancreatic secretion [307].

ACBP and proteolytic fragments of ACBP inhibit glucose induced insulin secretion in isolated pancreatic islands and in *in vivo* perfusion experiments in rats [308–310], while an antibody directed against ACBP augments glucose-stimulated insulin secretion from isolated islets [309].

Collectively, these observations suggest that ACBP also serves extracellular functions, which obviously requires that ACBP is secreted in a regulated manner. Interestingly, in both *Dictyostelium discoideum* and the yeasts *S. cerevisiae* and *P. pastoris*, Acb1 orthologs are secreted via an unconventional pathway, which depends on functional autophagy, multivesicular body pathways and the Golgi-associated protein GRASP [311–314]. Moreover, depolarization, induction of autophagy, as well as pregnenolone sulfate and cortisol treatment of astrocytes have been shown to stimulate secretion and proteolytic processing of ACBP [315,316], suggesting that secretion of ACBP indeed can be regulated.

11. Conclusion and future perspectives

As depicted above, fatty acids derived from *de novo* synthesis, dietary sources, or the turnover of membrane or storage lipids must be activated to acyl-CoA esters before they can be targeted for degradation, incorporation or reincorporation into glycerolipids and various complex lipids, or used for protein acylation. Besides these basal metabolic functions, acyl-CoAs are also recognized as important regulators of transcription factors, intracellular signalling pathways, and numerous metabolic pathways.

Acyl-CoAs are generated by activation of fatty acids, which are catalyzed by multiple acyl-CoA synthases. These enzymes comprise a large family of enzymes, which not only localize to different cellular compartments and are expressed in different tissues, but also generate acyl-CoAs that vary in chain length and degree of saturation. Moreover, acyl-CoA utilizing enzymes including acyl-CoA thioesterases also show varying chain length specificity and differ in tissue expression and intracellular localization. Therefore, the intracellular pool of acyl-CoAs, including LCACoAs and VLCACoAs, is likely to be very heterogeneous in its composition and its intracellular distribution. Considering the complexity of intracellular acyl-CoA pools, it makes sense that they are protected against hydrolysis and are targeted to utilizing enzymes by numerous intracellular lipid-binding proteins including FABP1, SCP2 and ACBDs. Since only the liver-type FABP binds acyl-CoAs and SCP2 specifically localizes to peroxisomes, the acyl-CoA binding domain proteins are considered to be the most predominant class of intracellular proteins which bind acyl-CoAs. Although it is ACBP (synonymous with ACBD1 and DBI), which has been studied most extensively, the presence of multiple ACBDs in different cellular compartments suggests that multiple intracellular acyl-CoA pools can exist. Numerous transcript variants of many of the ACBDs are ubiquitously expressed and found to encode ACBD isoforms that differ in their intracellular localization, suggesting that transcript variants may serve functional roles.

A key question is still how acyl-CoAs are specifically channeled to utilizing processes under varying physiological conditions. One obvious answer might be that ACBP sequesters and binds acyl-CoAs to subsequently interact with downstream utilizing enzymes. Although ACBP primarily is considered to be a cytosolic protein, ACBP dynamically localizes to different cellular compartments depending on the cellular conditions and cell type. The fact that the ability of ACBP to bind its ligand can determine its intracellular localization [231] favors the hypothesis of such an interacting mechanism. To date, neither ACBP nor any of the other ACBDs have been shown to physically interact with enzymes utilizing acyl-CoAs. However, such interactions are likely to be transient and therefore difficult to experimentally detect. On the other hand, it seems unlikely that a single protein interacts with multiple acyl-CoA utilizing systems, suggesting alternative and yet unknown mechanisms to how ACBP affects partitioning and fluxes of acyl-CoA esters. The most pronounced phenotypes seen after functional loss of ACBP in yeast and mice [168,268,269,295] suggests an important role for ACBP in the synthesis of very-long chain fatty acids and ceramides, indicating that ACBP may specifically target and affect the activity of enzymes utilizing very-long chain acyl-CoA esters like e.g. the ELOVLs and CERSS. Since ACBP has been found to form dimers, it may simply interact and heterodimerize with an ACBP domain in other ACBDs in various compartments, which then passes the acyl-CoA on to a utilizing enzyme in its vicinity. Alternatively, ACBP might mediate its effects by interacting with membranes, which it is able to do *in vitro* [317]. Such mechanisms do not exclude that ACBP can protect acyl-CoAs from hydrolysis and can serve as a general pool former of acyl-CoAs *in vivo*.

Independent of the function of ACBP another key question is how the ligand is released once it is bound to ACBP. ACBP binds LCACoAs with nM affinity, and intuitively, its binding affinity must be lowered to release the ligand. Specific interactions with other proteins could potentially induce structural changes, which might favor ligand release. Alternatively, post-translational modifications of specific residues may affect its binding affinity. Depending on the experimental conditions, ACBP can be phosphorylated at its N-terminal end and at residues critical for ligand binding [227,315,318], suggesting that ligand binding might also be regulated in a dynamic manner.

Further loss-of-function studies in genetic tractable model systems will aid identifying specific functions of ACBP and of proteins with an ACBP domain. Such studies should focus on how metabolic fluxes are affected in these models using isotopic-labeling studies combined with global lipidomic and metabolomic methodologies to further elucidate their specific roles in acyl-CoA metabolism and partitioning.

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