JPP JOURNAL OF PROTEINS AND PROTEOMICS
3(1), January-June 2012, pp. 61-71

STRUCTURAL AND FUNCTIONAL ASPECTS OF ACYL-COENZYME A BINDING PROTEINS (ACBPs): A COMPREHENSIVE REVIEW

Richa Arya[1], Monica Sundd[2*] and Suman Kundu[1*]

[1]*Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi 110021, India*

[2]*National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110067, India*

Abstract: ACBP was originally identified as a mammalian diazepam binding inhibitor – a neuropeptide that has the ability to inhibit diazepam binding to the γ-aminobutyric acid (GABA) receptor (Guidotti *et al.*, 1983). Typically, ACBPs are small (~10 kDa) cytosolic proteins (Burton *et al.*, 2005). However, a number of hybrid ACBPs are reported that are fused with ankyrin repeats, such as ACBP1 and ACBP2 in *Arabidopsis thaliana* (Chye *et al.*, 1999; Li and Chye, 2003). Other functional domains, such as the human peroxisomal δ3/ δ2-enoyl-CoA isomerase (Geisbrecht *et al.*, 1999), or any non-functional/ uncharacterized domain are also cited. ACBP predominantly functions as an intracellular acyl-CoA transporter and pool former, and is critical to lipid metabolism in cells (Gossett *et al.*, 1996; Knudsen *et al.*, 2000; Schroeder *et al.*, 1998). Impaired lipid metabolism and other cellular functions in humans arising out of ACBP defects thus need to be explored. ACBP has only been reported in eukaryotes, not in prokaryotes, except for a few pathogenic eubacteria that might have acquired ACBP from eukaryotic hosts via lateral gene transfer (Burton *et al.*, 2005). Whole genome sequences of several prokaryotes and pathogens being available currently, it is worthwhile to extend search for ACBPs beyond eukaryotes as well, to explore their potential as drug targets, given their essential role in lipid metabolism. As a prelude to such investigations, the current review summarizes available knowledge of ACBPs and outlines the scope of future research.

Keywords: ACBP; Long chain fatty acyl coenzyme-A; Acyl Co-A esters; β-oxidation.

Introduction

Acyl-coenzyme A binding proteins (ACBP), named after their ability to bind thiol esters of long fatty acids and coenzyme A, are a group of highly conserved proteins found in animals, plants, protists and a number of pathogenic bacteria (Burton *et al.*, 2005). They are typically small, cytosolic biomolecules of approximate molecular mass of 10 kDa. However, a number of larger ACBPs (e.g. >73 kDa) have also been identified in many species like mammals, plants and parasites. In fact, mammals possess multiple ACBPs that are differentially expressed in various tissues. It has been shown that ACBP binds long chain acyl-CoA esters (C_{12}–C_{22}) with high specificity and affinity (K_d, 1–10 nM); however, specific molecular mechanism of action of ACBP remains largely unknown. Many *in vitro and in vivo* experiments have suggested that ACBP functions as an intracellular acyl-CoA transporter and contributes to the formation of the acyl-CoA pool. ACBP effectively acts as potent protector of acetyl-CoA carboxylase and mitochondrial adenine nucleotide translocase by preventing their inhibition by long chain acyl-CoA esters. It also protects acyl-CoA against hydrolysis by microsomal hydrolases. ACBP is able to transport and donate acyl-CoA to mitochondria for β-oxidation, to peroxisomes for ω-oxidation and to microsomes for glycerolipid synthesis. ACBP stimulates the mitochondrial long chain acyl-CoA synthetase as well. Long chain Acyl-CoA esters are an intermediate in lipid metabolism and modulate a wide range of cellular functions, such as regulation of energy metabolism, lipid synthesis, signal transduction, ion flow regulation and gene expression. In *Trypanosoma brucei*, an

Corresponding Author: **Monica Sundd, Suman Kundu**
E-mail: monicasundd@nii.ac.in, suman.kundu@south.du.ac.in

ACBP was found to be involved in the synthesis of glycosylphosphatidylinositol (GPI) anchor in variant surface glycoproteins (Milne and Ferguson, 2000; Milne *et al.*, 2001). In summary, ACBPs play significant role in wide range of cellular activities and demands heightened attention.

Isoforms of ACBP

Different organisms have been shown to have various isoforms of ACBP, some of which are tissue specific. In mammals, the prominent group is the commonly expressed ACBP isoform, first isolated from bovine liver (**l-ACBP**). It contains no cysteine and is 86–92 residues long (~10KDa). This basic isoform is expressed in almost every tissue of all the eukaryotes studied for this purpose, including yeast and is most likely the ancestor of more specialized ACBP isoforms. The second group is the testis specific isoform (**t-ACBP**) also called endozepine-like protein (ELP). t-ACBP have now been isolated from three different species and they all contain three cysteine amino acid each (Pusch *et al.*, 1996). A putative third group, deduced from gene sequences from duck and frog brain, constitutes brain specific isoform of ACBP (**b-ACBP**). The fourth group of ACBP is a group of longer sequences with up to 999 amino acids, which usually have ACBP domain at N-terminal and some other functional domains like 3 δ or 2 δ enoyl Co-A isomerase or ankyrin repeats at the C-terminal. Some members of this group contain non-functional (or uncharacterized) domains at the C-terminal as well. Some of these longer sequences are suggested to be membrane-bound ACBP domain proteins, whereas others remain to be isolated as proteins. Although ACBPs are capable of binding medium to long-chain fatty acyl-CoA esters, they may vary in their substrate preference and binding affinities. For example, the highest affinities of ACBPs from bovines (liver) and trypanosomes (or *P. falciparum*) are for C18 stearoyl- and C14 lauroyl-CoA, respectively (Milne and Ferguson, 2000; van Aalten *et al.*, 2001).

Structure of *ACBP*

Typically, ACBP is folded into a four α-helix–bundle architecture (Andersen and Poulsen, 1992, 1993; Kragelund *et al.*, 1993). The binding site is located in a hydrophobic groove on the surface of ACBP. The acyl chain is buried in the binding pocket and is completely protected from the outside aqueous environment by the acyl-CoA head group, which forms a cap over the binding pocket by interacting with specific residues on the rim of the binding cavity.

Conserved residues and their role in structure, function and stability of ACBP: Sequence alignment of a set of 31 ACBP sequences we could identify in public domain are shown in Figure 1 for reference. In the family of eukaryotic acyl-coenzyme A binding proteins, around 24 known sequences were compared (Kragelund *et al.*, 1999b); their identity was found to be >25%, and they contain a subset of 26 sequence positions that are identical in >90% of all sequences. The subset of 26 residues, conserved throughout evolution

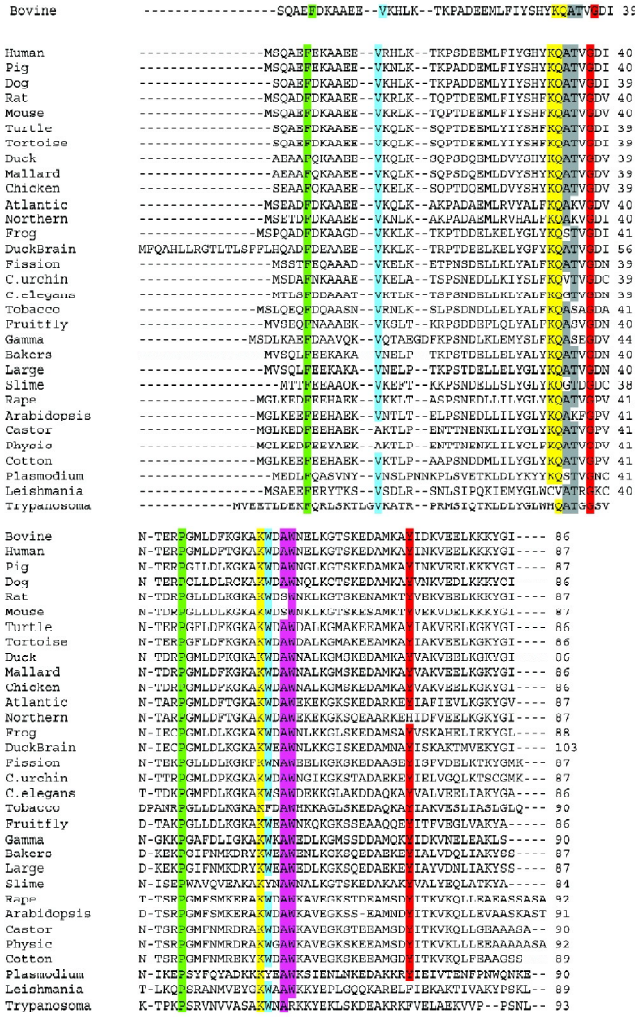

Figure 1: **Alignment of 31 ACBP sequences from different species. The conserved residues are colour coded**

of the eukaryotic kingdoms, is mostly hydrophobic or involved in electrostatic binding of an acyl-coenzyme A ester (acyl-CoA). In the context of bovine protein, the importance of these 26 sequence positions for structure, function, stability, and folding has been analyzed using single-site specific mutations (Kragelund *et al.*, 1999b). The structure is a skewed four α-helix bundle and the folding process has been thoroughly studied and characterized using various fast-mixing spectroscopic techniques. ACBP has thus proven to be an excellent model system for studies of both protein dynamics (Kragelund *et al.*, 1995b; Rischel *et al.*, 1994) and completely reversible protein folding (Kragelund *et al.*, 1996; Kragelund *et al.*, 1995a; Kragelund *et al.*, 1999a; Kragelund *et al.*, 1999b). The fully reversible folding processes of the bovine, rat, and yeast proteins have all been shown to occur in apparent two state reactions without any observable intermediate states (Kragelund *et al.*, 1996).

In the three-dimensional structure of bovine ACBP, most of the conserved residues were found to form three separate mini-cores, which partly include the helix interfaces (Kragelund *et al.*, 1996). This is represented in Figure 2. The four α-helices of ACBP were named as A1–A4 and span the sequences Ala 3–Leu 15 (A1), Asp 21–Val 36 (A2), Lys 52–Glu 62 (A3) and Ser 65–Tyr 84 (A4). The bundle arrangement of the helices is skewed, since helix A3 has contacts only to helix A2. The residues in closest contact at the four interfaces are Ala 9 and Tyr 31 (A1–A2), Phe 5 and Ile 74 (A1–A4), Ser29 and Trp 58 (A2–A3) and Ala 34 and Ala 69 (A2–A4). To fully analyse and characterize the role of the conserved residues in the ACBP family, a total of 28 mutant variants of bovine ACBP were constructed (Kragelund *et al.*, 1999b). The set of variants covers 20 sequence sites of both hydrophobic and polar/charged residues at both conserved and non-conserved positions. The mutations introduced in the sequence of bovine ACBP resulted in changes of both the thermodynamics of ligand binding and protein stability. Residues Phe5, Ala9, Val12, Leu15, Tyr28, Lys32, Gln33, Ala34, Tyr73, Val77, and Leu80, showed a significant effect on the stability of the protein. These positions are highlighted in Figure 2a. Mutations in the conserved residues

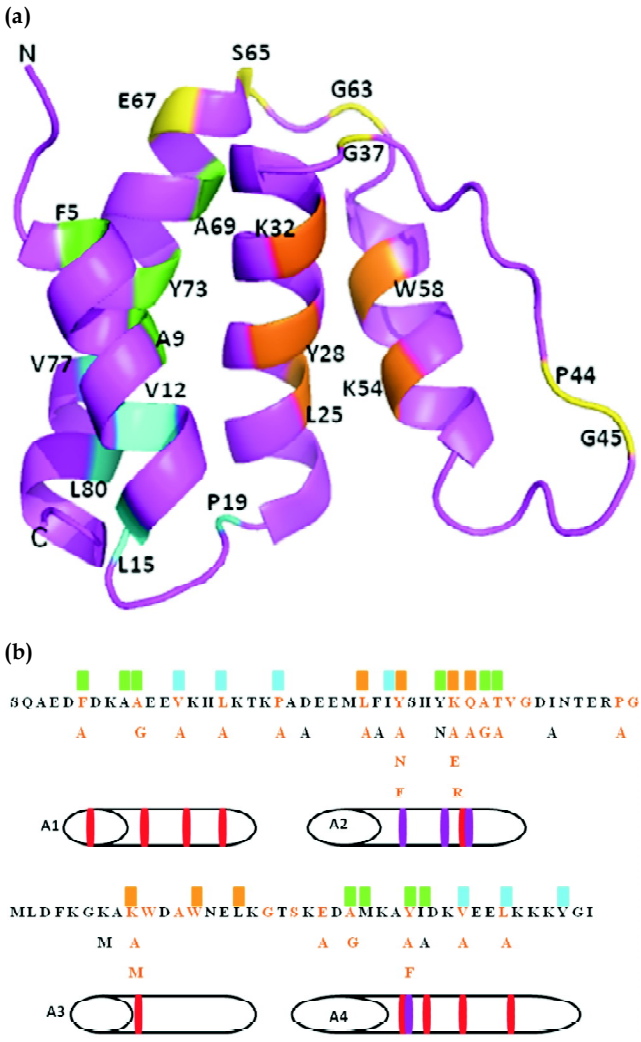

Figure 2: **(a) Three-dimensional structure of ACBP (PDB accession code 2ABD). The 26 conserved residues are colored (yellow, green, cyan and orange), and residues shown in green form mini-core 1, cyan form the mini-core 2 and orange form the mini-core. (b) Mutations introduced in the sequence of bovine ACBP and conserved sequence positions in orange (Kragelund et al., 1999a). The mutations analyzed are listed below the sequence. Colored boxes above the sequence indicate residues involved in the mini-cores (green, mc1; cyan, mc2; orange, mc3). Red areas denote sequence positions where mutations decrease folding rates significantly, and purple areas indicate positions where mutations increase folding rates**

thus could be pivotal in impaired cellular function related to ACBPs and presents scope for further investigations.

Dodecanoyl-CoA (C_{12}-CoA) was used (Kragelund *et al.*, 1999b) to probe the binding profile of bovine ACBP and its 28 mutant proteins. From the structure of the complex between bovine ACBP and hexadecanoyl-CoA it is evident that

the four methylene groups in the ω-end of the chain make specific hydrophobic interactions with the protein. The affinity for the ligand C_{12}-CoA resides therefore, mostly in the electrostatic interactions between the Co-A head and ACBP, especially with the 3'-phosphate group which has been shown to be responsible for around 40% of the binding energy. Seven residues for which mutations affected ligand binding affinity and energy dramatically are Phe5, Leu25, Tyr28, Tyr31, Lys32, Lys54, and Tyr73. Six of these seven residues are involved directly in binding of the adenosine 3'-phosphate part of the Co-A.

Function of ACBPs and their Interacting Partners

ACBPs display a number of important physiological and biochemical functions (Figure 3). Originally, it was shown to inhibit diazepam binding to benzodiazepine binding site of γ-aminobutyric acid-ergic (GABA)-receptor (Gray *et al.*, 1986; Guidotti *et al.*, 1983). Its other functions include regulation of glucose-induced insulin secretion from pancreatic beta-cells (Borboni *et al.*, 1991; Chen *et al.*, 1988), release of cholecystokinin (CCK) in intestine (Herzig *et al.*, 1996), stimulation of steroidogenesis through peripheral-type benzodiazepine receptor (PBR) (Besman *et al.*, 1989) and modulation of cell proliferation (Garnier *et al.*, 1993). ACBP seems also to be part of the calcium-dependent proteolytic system through the activation of calpains (Melloni *et al.*, 2000). In this respect, lately it was proposed that ACBP is involved in pro-apoptotic processes and cell death (Shulga and Pastorino, 2006). As ACBP is known to bind and sequester long chain acyl-CoAs (LCFA CoA) with high affinity, it suggests its function as intracellular carrier and pool former of LCFA CoAs. In doing so, ACBP protects membranes as well as enzymes against detergenic and inhibitory effects of LCFA CoAs. Furthermore, ACBP mediates intermembrane transport of acyl-CoAs by displacing them from membranes and donating them to utilizing enzymes, i.e. β-oxidation in mitochondria, microsomal glycerolipid synthesis and cholesteryl ester acylation (Cohen Simonsen *et al.*, 2003; Rasmussen *et al.*, 1994; Rasmussen *et al.*, 1993). ACBP is found to be localized throughout the cytosol and in special cell organelles as Golgi apparatus or endoplasmic reticulum (Hansen *et al.*, 2008), in nucleus and in the perinuclear area (Petrescu *et al.*, 2003). Except for a distinct nuclear localization during adipocyte differentiation (Helledie *et al.*, 2000), a functional relevance for nuclear abundance of ACBPs is not known. However, it has also been shown (Petrescu *et al.*, 2003) that ACBP in the nucleus physically and functionally interacted with hepatocyte nuclear factor-4α (HNF-4α), a nuclear binding protein that regulates transcription of genes involved in both lipid and glucose metabolism. Following lines of evidence have shown that ACBP is bound to HNF-4 α *in vitro* and in the nucleus of intact cells. (i) ACBP interaction with HNF-4α elicited significant changes in secondary structure. (ii) ACBP and HNF-4α were co-immunoprecipitated by antibodies to each protein. (iii) Double immunolabeling and laser scanning confocal microscopy (LSCM) of rat hepatoma cells and transfected COS-7 cells significantly colocalized ACBP and HNF-4 α within the nucleus (Figure 3) and in the perinuclear region close to the nuclear membrane. (iv) LSCM fluorescence resonance energy transfer determined an intermolecular distance of 53 Å between ACBP and HNF-4α in rat hepatoma cell nuclei. (v) Immunogold electron microscopy detected ACBP within 43 Å of HNF- 4α.

Other than mammals, ACBP has been reported and investigated in various lower organisms and plants. Following are a few examples.

African **trypanosomes** are shielded from their hosts' defenses by a coat of variant surface glycoprotein molecules, each of which is attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor (Milne and Ferguson, 2000). During the later stages of GPI anchor biosynthesis, myristic acid is incorporated into the anchor from the donor myristoyl-CoA by a series of unique fatty acid remodeling and exchange reactions. It has been shown that acyl-Co-A binding protein (ACBP) of trypanosome has preference for binding relatively short chain acyl-CoAs and that has a high affinity for binding myristoyl-CoA (Milne and Ferguson, 2000). Also, this protein enhances fatty acid remodelling of GPI precursors in the

trypanosome cell-free system (Figure 3). It has been speculated that the trypanosome acyl-CoA-binding protein plays an active role in supplying myristoyl-CoA to the fatty acid remodeling machinery in the parasite. Indeed, target disruption of ACBP in the parasite ***Trypanosoma brucie*** leads to lethality.

Cryptosporidium parvum is a globally important parasitic protist that infects both humans and animals (Okhuysen and Chappell, 2002; Thompson *et al.*, 2005; Tzipori and Widmer, 2000). *C. parvum* belongs to the phylum Apicomplexa that contains many important human and animal parasites (e.g. *Plasmodium, Babesia, Toxoplasma and Eimeria*) (Zhu *et al.*, 2000). ACBP gene from this opportunistic protist *C. parvum* has been identified (Zeng *et al.*, 2006). The CpACBP1 gene encodes a protein of 268 amino acids that is three times larger than typical ACBPs (i.e. 90 amino acids) and this protein contains a C-terminal ankyrin repeat sequence (170 amino acids), which is speculated to be involved in protein-protein interaction. For functional analysis and characterization, this CpACBP1 gene fused with MBP tag was recombinantly expressed. Acyl-CoA-binding assays clearly revealed that the preferred binding substrate for CpACBP1 is palmitoyl-CoA. RT-PCR, Western blotting and immunolabelling analyses clearly showed that the CpACBP1 gene is mainly expressed during the intracellular developmental stages and that its level increases during parasite development.

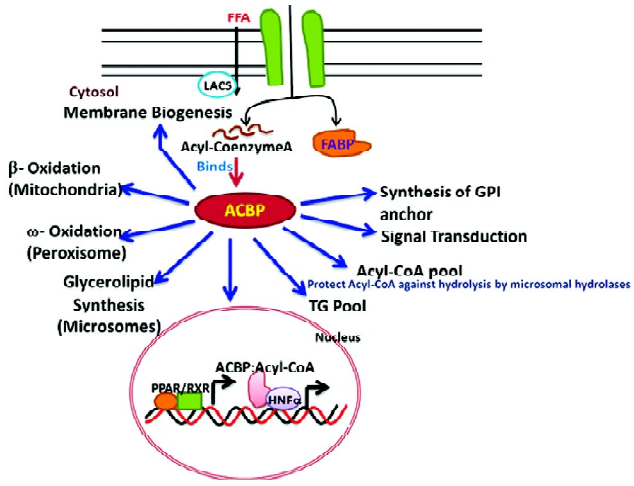

Figure 3: **Role of ACBP in various cascades involved in the lipid metabolism**

Immunofluorescence microscopy showed that CpACBP1 is associated with the parasitophorous vacuole membrane (PVM), which implies that this protein may be involved in lipid remodelling in the PVM, or in the transport of fatty acids across the membrane.

In ***Saccharomyces cerevisae*** it was observed that deletion of gene *ACB1* encoding Acb1p, the yeast homologue of the acyl-CoA-binding protein (ACBP), resulted in a slower growing phenotype that adapted into a faster growing phenotype with a frequency >1:10^5 (Gaigg *et al.*, 2001). The plasma membrane of the Acb1p-depleted strain contained increased levels of inositol-phosphoceramide and mannose-inositol-phosphoceramide and lysophospholipids. Also, Acb1p-depleted cells accumulated 50- to 60-nm vesicles and autophagocytotic like bodies and showed strongly perturbed plasma membrane structures. These results strongly suggest that Acb1p plays an important role in fatty acid elongation and membrane assembly and organization. These observations stated a role of ACBP in sphingolipid synthesis, membrane assembly and organization and organelle structures (Gaigg *et al.*, 2001). These observations suggest that Acb1p may be directly or indirectly involved in vesicular trafficking and in the maintenance of the integrity of cellular compartments. It is shown that depletion of acyl-CoA-binding protein, Acb1p, in yeast affects ceramide levels, protein trafficking, vacuole fusion and structure (Faergeman *et al.*, 2004). Vacuoles in Acb1p-depleted cells are multi-lobed and contain drastically less number of SNAREs (soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptors). It is also suggested that the reduced ceramide synthesis in Acb1p-depleted cells leads to severely altered vacuole morphology, perturbed vacuole assembly and strong inhibition of homotypic vacuole fusion.

Insects have developed various systems of chemical communication during the course of evolution. Many lepidopteran insects have their own pheromone blends that play a significant role as a mode of communication in mating behaviour; structural and compositional variations of the pheromone blends being responsible for species

specificity. Various fatty acyl-CoAs are involved as intermediates or precursors of sex pheromone components in the biosynthetic pathway of the pheromones in many lepidopteran insects. A ~10kDa protein from the cytosolic fraction of ***Bombyx mori*** pheromone glands was purified and on amino acid sequence analysis of its fragment peptides obtained from the purified protein, and a homology search, revealed that this protein was a member of acyl CoA-binding proteins (ACBPs)(Matsumoto *et al.*, 2001). MALDI-TOF mass spectral analysis of the purified protein and cloning of the gene from a pheromone gland cDNA library confirmed *B. mori* ACBP to be a 90 amino acid protein with 78.9% identity to that of ***Manduca sexta*** ACBP. The secondary structure of the recombinant *B. mori* ACBP was determined by NMR spectroscopy. Northern blot analysis demonstrated that *B. mori* ACBP was predominantly expressed in the pheromone gland and the corresponding transcript was expressed from the day before adult eclosion. Their results suggested that ACBP play an important role in the production of sex pheromones regulated by the neurohormone, pheromone biosynthesis activating neuropeptide (PBAN).

In insects, it has been demonstrated that expression of *M. sexta* ACBP is highest during times of active feeding (Snyder and Antwerpen, 1997) and lipid transport by the larval midgut, suggesting a role of lipid uptake from the midgut. An ACBP gene sequence has also been identified in the genome of ***Rhodnius prolixus*** (Alves-Bezerra *et al.*, 2010). This ACBP gene (RpACBP-1) was expressed in all analyzed tissues and quantitative PCR showed that expression was highest in posterior midgut. In this tissue, ACBP gene expression increased with the intake of blood meal (~10-fold) and then decreased to unfed levels on the seventh day after meal. Also, here RpACBP-1 levels are regulated by serotonin, which acts as a neuropeptide.

C. elegans expresses seven functional ACBPs; four basal forms and three ACBP domain proteins. In a recent study (Elle *et al.*, 2011), mutants with functional loss were obtained of each of six of the ACBPs and their macroscopic and biochemical phenotypes were characterized. The seventh paralogue membrane-associated ACBP-1 has been shown to be involved in endosomal vesicle transport. It was found that each of the six paralogues is capable of complementing growth of ACBP-deficient yeast cells, and that they exhibit distinct temporal and tissue expression patterns. All ACBP single mutants display only subtle phenotypes, likely due to compensatory mechanisms and some extent of functional redundancy. ACBP-1 is shown to be involved in triglyceride storage and lipid droplet morphology. ACBP-2, containing an enoyl-CoA hydratase domain, is necessary for β-oxidation of unsaturated fatty acids, and ACBP-3 is needed for normal skin barrier function. Further, quadruple mutant was generated (Elle *et al.*, 2011), which is being currently investigated. This mutant is developmentally delayed compared to N2 and preliminary data suggest that it increases its β-oxidation of exogenous fatty acids, suggesting that lack of all basal ACBPs elicits a starvation-like phenotype.

Six-membered ACBP family in ***Arabidopsis thaliana*** consists of ACBP1 to ACBP6, with ACBP6 being the smallest member (10.4 kDa) and ACBP4 the largest (73.2 kDa). These six ACBPs map to four distinct classes based on the presence of other protein domains besides the conserved acyl-CoA-binding domain. ACBP1 and ACBP2 each possess a C-terminal domain of ankyrin repeats, which is important for protein-protein interactions. ACBP4 and ACBP5 show conservation in a domain of five kelch motifs, which also helps in interaction with different protein partners. ACBP3 lacks both ankyrin repeats and kelch motifs but it is a larger (39.3 kDa) isoform than ACBP6 (10.4 kDa), which is the smallest member and exists in free form as it consists of only the acyl-CoA-binding domain. The six *Arabidopsis* ACBPs are subcellularly located in various compartments. The *N*-terminal transmembrane domain of ACBP1 and ACBP2 targets each protein to the endoplasmic reticulum (ER) and the plasma membrane while ACBP3 is targeted extracellularly. The three other ACBPs (ACBP4, ACBP5 and ACBP6) are cytosolic proteins. These observations on the distribution of ACBPs to a variety of locations are not unexpected if ACBPs were to cater for different functions in plant lipid metabolism. Several

ACBPs are responsive to stress treatments. ACBP1 and ACBP2 displayed ability to bind linoleoyl-CoA and linolenoyl-CoA *in vitro* suggesting that they are probably involved in phospholipid membrane repair following lipid peroxidation arising from heavy metal and oxidative stress. ACBP1 and ACBP2 play overlapping roles in lipid transfer during *Arabidopsis* embryogenesis. ACBP3, an extracellularly targeted ACBP plays an important role in the regulation of leaf senescence by modulating both phospholipid metabolism and the stability of an ubiquitin-like protein ATG8 which conjugates with phosphatidylethanolamine (PE) to which recombinant ACBP3 binds *in vitro*. *ACBP4* and *ACBP5* mRNAs are up-regulated in the light and down-regulated in constant darkness. The ethylene-responsive element binding protein AtEBP was reported to interact with ACBP4 via its *C*-terminal kelch motifs. Both *ACBP4* and *AtEBP* mRNAs are induced by the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid, methyl jasmonate, and *Botrytis cinerea* infection, supporting a possible role for ACBP4 in plant defence. *ACBP6* mRNA and protein are cold (4 °C)-inducible, and that the *acbp6* mutant showed enhance sensitivity to freezing (-8 °C) in comparison to wild type. The ability of recombinant ACBP6 in binding phosphatidylcholine (PC) suggests that it is a potential candidate in the intracellular binding and trafficking of PC in plant phospholipid metabolism.

ACBP and Apoptosis

Activation of calpain (calcium-dependent cysteine proteases) has been shown to occur in some contexts to cell injury and to be essential for loss of cell viability as these have been implicated in performing a role in apoptosis and necrosis. Part of this may be mediated at the mitochondrial level. It has been demonstrated that the calpain activity is necessary for the complete discharge of apoptosis-induced factor from the mitochondrial intermembranous space and can cause the cleavage of full-length Bid to a more potent truncated form.

Acyl-CoA-binding protein, a 20-kDa homodimer from rat skeletal muscle, exerts many physiological functions. It thus promotes activation of the classic calpain forms, most markedly that of the m-isozyme. Both native and recombinant acyl-CoA-binding proteins show the same molecular properties and an identical capacity to decrease the [Ca^{2+}] required for m-calpain activity (Melloni *et al.*, 2000). The binding of long-chain acyl-CoAs to ACBP does not modify the activating effect on calpains. ACBP seems to be involved in the m-calpain regulation process. Acyl-CoA-binding protein is proposed as a new component of the Ca^{2+}-dependent proteolytic system. A comparative analysis among levels of classic calpains and their activator proteins is also reported.

It was also reported that a liver specific protein phosphatase inhibiting toxin nodularin (from *Nodularia spumigena*) rapidly induces hepatocyte apoptosis. Incubation of freshly isolated hepatocytes with this toxin results in hyperphosphorylation of cellular proteins before any morphological signs of apoptosis appear. These phosphorylated proteins may play key roles in the early stage of apoptosis. One of the phosphoproteins, ACBP has been identified (Solstad *et al.*, 2008). Phosphorylation-site analysis by MALDI MS/MS revealed that the observed phosphorylation is positioned on Ser1 in the N-terminal tryptic peptide Ac-SQADFDKAAE EVKRLK of the rat liver protein. Additionally, it was observed that ACBP translocate towards the cellular membrane in the apoptotic hepatocytes. Moreover, nodularin-induced apoptosis was highly dependent on calpain activation, an event previously shown to be regulated by ACBP. These findings introduce the possibility that reversible phosphorylation of ACBP regulates its ability to activate calpain in phosphatase inhibitor-induced apoptosis and controls the cellular accessibility of long-chain fatty acid-CoAs for cellular signaling.

ACBP - a Modulated Housekeeping Gene

ACBP is expressed in most cell types at low levels; however, expression differs markedly between different cell types with expression being particularly high in cells with a high turnover of fatty acids. In mammalian cells induction of ACBP expression appears to be correlated at least to some extent with increased lipogenesis. Thus, the expression of ACBP is moderately up-

regulated in the liver by feeding and significantly upregulated during adipocyte differentiation.

Relatively high basal promoter activity of the rat ACBP gene in fibroblasts and hepatoma cells has been shown (Neess *et al.*, 2006) to rely on sequences between -331 to -182 and on the Sp1 and NF-Y sites at -172 and -143, respectively (Figure 4). The basal transcription is modulated by members of the PPAR and SREBP families. In adipocytes, PPARγ is in part responsible for the induction during adipocyte differentiation, but other transcription factors appear to play a role as well. In hepatocytes, SREBP-1c is the main regulator of ACBP in response to changes in insulin levels during fasting/refeeding. PPARα counteracts this effect by stimulating ACBP expression during fasting. In addition, PPARγ mediates the induction of ACBP expression in response to peroxisome proliferators. PPARα and PPARγ do not require sequences upstream of -182 for transactivation; however, SREBP-1c requires the synergistic action of sequences in intron 1 for transactivation of the ACBP promoter. The high basal promoter activity is in keeping with the ubiquitous expression and the house keeping nature of the ACBP gene.

ACBP Regulated Gene Expression

As an intracellular pool former and carrier of acyl-CoAs, ACBP influences overall lipid metabolism (Figure 3). Its nuclear abundance and physical interaction with hepatocyte nuclear factor 4α suggested a gene regulatory function of ACBP. In order to identify ACBP target genes, genome-wide transcript profiling under si-RNA-mediated ACBP knockdown in human liver HepG2 cells was performed (Vock *et al.*, 2010), where, 256 down-regulated and 198 up-regulated transcripts with minimal fold change were identified. Gene annotation enrichment analysis revealed ACBP-mediated down-regulation of 18 genes encoding key enzymes in glycerolipid (i.e. mitochondrial glycerol-3-phosphate acyltransferase), cholesterol (i.e. HMG-CoA synthase and HMG-CoA reductase) and fatty acid (i.e. fatty acid synthase) metabolism. Integration of these genes in common pathways suggested decreased lipid biosynthesis. Accordingly, saturated (16:0) and monosaturated (16:1, 18:1) fatty acids were significantly reduced to 75% in ACBP-depleted cells. Taken together, evidence was obtained that ACBP functions in lipid metabolism at the level of gene expression. This effect seems to be translated into certain metabolites. The identified 454 ACBP regulated genes present a first reference for further studies to define the ACBP regulon in mammalian cells.

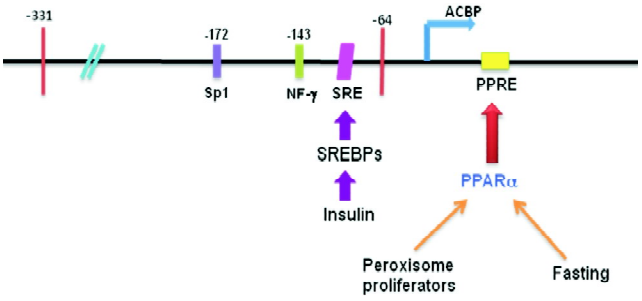

Figure 4: **Transcription factors regulating expression of the Rat ACBP gene. The upstream region -331 to -64 is important for basal transcriptional activity of the ACBP promoter**

It is still debatable whether ACBP mostly sequesters acyl-CoA esters, or whether ACBP is solely responsible in the transport of acyl CoA esters and donates them to metabolic reactions involving specific enzymes. It is very important to determine these properties of ACBP because that would bring ACBP in a position to regulate lipid metabolism and lipid signaling. The ubiquitous expressed multifunctional protein ACBP is a well-known intracellular transporter of acyl-CoAs thereby determining profoundly their intracellular fate. Through binding and directing acyl-CoAs towards nucleus and nuclear factors ACBP may influence gene expression as well (Helledie *et al.*, 2000).

Summary

ACBP is a highly conserved N-acetylated polypeptide expressed in a wide variety of species ranging from yeast to mammals including insects and parasites. The broad range of ACBP distribution throughout the eukaryotes and its high degree of similarity across different tissues and species indicate that ACBP is a pivotal player in the trafficking and transfer of long chain acyl-CoA esters (Faergeman *et al.*, 2007). ACBP is an essential protein (Mandrup *et al.*, 1992) whose function is associated with one or more basic function(s) common to all cells.

Through binding and directing acyl-CoAs towards nucleus and nuclear factors ACBP influence gene expression. Disruption of ACBP expression via RNAi has shown significant growth arrest, cell detachment and apoptosis induction (Faergeman and Knudsen, 2002) in HepG2, HeLa and Chang cells and homologous recombination experiments have shown that ACBP is required for the viability of *Trypanosoma brucei* (Milne *et al.*, 2001). Depletion of ACBP in *Saccharomyces cerevisiae* results in severe growth retardation and changes in vacuoles and the plasma membrane and, affects vesicular trafficking, organelle biogenesis and membrane assembly, reduces both the levels of very-long-chain fatty acids (C_{26}) and sphingolipid synthesis (Gaigg *et al.*, 2001), and affects ceramide levels and protein trafficking. Specifically it is seen that a mutation within the ACBP locus in mice resulted in sparse hair, male infertility, failure to thrive, hydrocephaly and anemia. Data supports the fact that ACBP has a gene regulatory function as well as supply coenzyme A derivatives in cholesterol and fatty acid biosynthesis. This effect seems to be translated into relevant metabolites. ACBP thus play a significant role in various cellular functions and need to be investigated further.

Fatty acids are essential to all organisms. Recently, fatty acid metabolism has been considered as a promising target for drug development against cryptosporidiosis and other important pathogenic apicomplexans as well as few parasites (Gornicki, 2003; Kuo *et al.*, 2003; Ralph *et al.*, 2001; Roberts *et al.*, 2003; Waller *et al.*, 2003; Zhu *et al.*, 2004). Since ACBP plays a critical role in fatty acid metabolism, it is reasonable to speculate that ACBPs from pathogenic organisms may be explored as new drug targets for the control of cryptosporidiosis or other apicomplexan (parasite)-based diseases.

Acknowledgement

Financial support through grants from University of Delhi, National Institute of Immunology and Department of Biotechnology, Government of India are acknowledged. Help from University Grants Commission (SAP programme) is also appreciated. Richa Arya is supported by Junior Research Fellowship from UGC, Government of India.

Abbreviations

ACBP, Acyl Coenzyme-A binding protein; CCK, Cholecystokinin; Co-A, Coenzyme A; HNFα, Hepatocyte nuclear factor α; PPAR, Peroxisome proliferator-activated receptor; SREBP, sterol regulatory element-binding protein; HMG, 3-hydroxy-3-methyl-glutaryl; LCFA Co-A, long chain fatty acyl coenzyme-A; LSCM, Laser scanning confocal microscopy; GPI, glycophosphatidylinositol; MALDI TOF, matrix-assisted laser desorption ionization time-of-flight; MBP, Mannose Binding Protein; PVM, Parasitophorous vacuole membrane; PBR, Peripheral type Benzodiazepine Receptor.

References

Alves-Bezerra, M., Majerowicz, D., Grillo, L.A., Tremonte, H., Almeida, C.B., Braz, G.R., Sola-Penna, M., Paiva-Silva, G.O., and Gondim, K.C. (2010). Serotonin regulates an acyl-CoA-binding protein (ACBP) gene expression in the midgut of Rhodnius prolixus. *Insect Biochem Mol Biol 40*, 119-125.

Andersen, K. V., and Poulsen, F. M. (1992). Three-dimensional structure in solution of acyl-coenzyme A binding protein from bovine liver. *J Mol Biol 226*, 1131-1141.

Andersen, K. V., and Poulsen, F. M. (1993). The three-dimensional structure of acyl-coenzyme A binding protein from bovine liver: structural refinement using heteronuclear multidimensional NMR spectroscopy. *J Biomol NMR 3*, 271-284.

Besman, M. J., Yanagibashi, K., Lee, T. D., Kawamura, M., Hall, P. F., and Shively, J. E. (1989). Identification of des-(Gly-Ile)-endozepine as an effector of corticotropin-dependent adrenal steroidogenesis: stimulation of cholesterol delivery is mediated by the peripheral benzodiazepine receptor. *Proc Natl Acad Sci U S A 86*, 4897-4901.

Borboni, P., Condorelli, L., De Stefanis, P., Sesti, G., and Lauro, R. (1991). Modulation of insulin secretion by diazepam binding inhibitor and its processing products. *Neuropharmacology 30*, 1399-1403.

Burton, M., Rose, T. M., Faergeman, N. J., and Knudsen, J. (2005). Evolution of the acyl-CoA binding protein (ACBP). *Biochem J 392*, 299-307.

Chen, Z. W., Agerberth, B., Gell, K., Andersson, M., Mutt, V., Ostenson, C. G., Efendic, S., Barros-Soderling, J., Persson, B., and Jornvall, H. (1988). Isolation and characterization of porcine diazepam-binding inhibitor, a polypeptide not only of cerebral occurrence but also common in intestinal tissues and with effects on regulation of insulin release. *Eur J Biochem 174*, 239-245.

Chye, M. L., Huang, B. Q., and Zee, S. Y. (1999). Isolation of a gene encoding Arabidopsis membrane-associated acyl-CoA binding protein and immunolocalization of its gene product. *Plant J 18*, 205-214.

Cohen Simonsen, A., Bernchou Jensen, U., Faergeman, N.J., Knudsen, J., and Mouritsen, O.G. (2003). Acyl-coenzyme A organizes laterally in membranes and is

recognized specifically by acyl-coenzyme A binding protein. *FEBS Lett 552*, 253-258.

Elle, I.C., Simonsen, K.T., Olsen, L.C., Birck, P.K., Ehmsen, S., Tuck, S., Le, T.T., and Faergeman, N.J. (2011). Tissue- and paralogue-specific functions of acyl-CoA-binding proteins in lipid metabolism in Caenorhabditis elegans. *Biochem J 437*, 231-241.

Faergeman, N. J., Feddersen, S., Christiansen, J. K., Larsen, M. K., Schneiter, R., Ungermann, C., Mutenda, K., Roepstorff, P., and Knudsen, J. (2004). Acyl-CoA-binding protein, Acb1p, is required for normal vacuole function and ceramide synthesis in Saccharomyces cerevisiae. *Biochem J 380*, 907-918.

Faergeman, N. J., and Knudsen, J. (2002). Acyl-CoA binding protein is an essential protein in mammalian cell lines. *Biochem J 368*, 679-682.

Faergeman, N. J., Wadum, M., Feddersen, S., Burton, M., Kragelund, B.B., and Knudsen, J. (2007). Acyl-CoA binding proteins; structural and functional conservation over 2000 MYA. *Mol Cell Biochem 299*, 55-65.

Gaigg, B., Neergaard, T.B., Schneiter, R., Hansen, J.K., Faergeman, N.J., Jensen, N.A., Andersen, J.R., Friis, J., Sandhoff, R., Schroder, H.D., *et al.* (2001). Depletion of acyl-coenzyme A-binding protein affects sphingolipid synthesis and causes vesicle accumulation and membrane defects in Saccharomyces cerevisiae. *Mol Biol Cell 12*, 1147-1160.

Garnier, M., Boujrad, N., Oke, B.O., Brown, A.S., Riond, J., Ferrara, P., Shoyab, M., Suarez-Quian, C.A., and Papadopoulos, V. (1993). Diazepam binding inhibitor is a paracrine/autocrine regulator of Leydig cell proliferation and steroidogenesis: action via peripheral-type benzodiazepine receptor and independent mechanisms. *Endocrinology 132*, 444-458.

Geisbrecht, B. V., Zhang, D., Schulz, H., and Gould, S. J. (1999). Characterization of PECI, a novel monofunctional Delta(3), Delta(2)-enoyl-CoA isomerase of mammalian peroxisomes. *J Biol Chem 274*, 21797-21803.

Gornicki, P. (2003). Apicoplast fatty acid biosynthesis as a target for medical intervention in apicomplexan parasites. *Int J Parasitol 33*, 885-896.

Gossett, R.E., Frolov, A.A., Roths, J.B., Behnke, W.D., Kier, A.B., and Schroeder, F. (1996). Acyl-CoA binding proteins: multiplicity and function. *Lipids 31*, 895-918.

Gray, P.W., Glaister, D., Seeburg, P.H., Guidotti, A., and Costa, E. (1986). Cloning and expression of cDNA for human diazepam binding inhibitor, a natural ligand of an allosteric regulatory site of the gamma-aminobutyric acid type A receptor. *Proc Natl Acad Sci U S A 83*, 7547-7551.

Guidotti, A., Corda, M.G., Wise, B.C., Vaccarino, F., and Costa, E. (1983). GABAergic synapses. Supramolecular organization and biochemical regulation. *Neuropharmacology 22*, 1471-1479.

Hansen, J.S., Faergeman, N.J., Kragelund, B.B., and Knudsen, J. (2008). Acyl-CoA-binding protein (ACBP) localizes to the endoplasmic reticulum and Golgi in a ligand-dependent manner in mammalian cells. *Biochem J 410*, 463-472.

Helledie, T., Antonius, M., Sorensen, R.V., Hertzel, A.V., Bernlohr, D.A., Kolvraa, S., Kristiansen, K., and Mandrup, S. (2000). Lipid-binding proteins modulate ligand-dependent trans-activation by peroxisome proliferator-activated receptors and localize to the nucleus as well as the cytoplasm. *J Lipid Res 41*, 1740-1751.

Knudsen, J., Neergaard, T.B., Gaigg, B., Jensen, M.V., and Hansen, J.K. (2000). Role of acyl-CoA binding protein in acyl-CoA metabolism and acyl-CoA-mediated cell signaling. *J Nutr 130*, 294S-298S.

Kragelund, B.B., Andersen, K.V., Madsen, J.C., Knudsen, J., and Poulsen, F.M. (1993). Three-dimensional structure of the complex between acyl-coenzyme A binding protein and palmitoyl-coenzyme A. *J Mol Biol 230*, 1260-1277.

Kragelund, B.B., Hojrup, P., Jensen, M.S., Schjerling, C.K., Juul, E., Knudsen, J., and Poulsen, F.M. (1996). Fast and one-step folding of closely and distantly related homologous proteins of a four-helix bundle family. *J Mol Biol 256*, 187-200.

Kragelund, B. B., Knudsen, J., and Poulsen, F. M. (1995a). Local perturbations by ligand binding of hydrogen deuterium exchange kinetics in a four-helix bundle protein, acyl coenzyme A binding protein (ACBP). *J Mol Biol 250*, 695-706.

Kragelund, B. B., Osmark, P., Neergaard, T. B., Schiodt, J., Kristiansen, K., Knudsen, J., and Poulsen, F. M. (1999a). The formation of a native-like structure containing eight conserved hydrophobic residues is rate limiting in two-state protein folding of ACBP. *Nat Struct Biol 6*, 594-601.

Kragelund, B. B., Poulsen, K., Andersen, K. V., Baldursson, T., Kroll, J. B., Neergard, T. B., Jepsen, J., Roepstorff, P., Kristiansen, K., Poulsen, F. M., *et al.* (1999b). Conserved residues and their role in the structure, function, and stability of acyl-coenzyme A binding protein. *Biochemistry 38*, 2386-2394.

Kragelund, B. B., Robinson, C. V., Knudsen, J., Dobson, C. M., and Poulsen, F. M. (1995b). Folding of a four-helix bundle: studies of acyl-coenzyme A binding protein. *Biochemistry 34*, 7217-7224.

Kuo, M. R., Morbidoni, H. R., Alland, D., Sneddon, S. F., Gourlie, B. B., Staveski, M. M., Leonard, M., Gregory, J. S., Janjigian, A. D., Yee, C., *et al.* (2003). Targeting tuberculosis and malaria through inhibition of Enoyl reductase: compound activity and structural data. *J Biol Chem 278*, 20851-20859.

Li, H. Y., and Chye, M. L. (2003). Membrane localization of Arabidopsis acyl-CoA binding protein ACBP2. *Plant Mol Biol 51*, 483-492.

Mandrup, S., Hummel, R., Ravn, S., Jensen, G., Andreasen, P.H., Gregersen, N., Knudsen, J., and Kristiansen, K. (1992). Acyl-CoA-binding protein/diazepam-binding inhibitor gene and pseudogenes. A typical housekeeping gene family. *J Mol Biol 228*, 1011-1022.

Matsumoto, S., Yoshiga, T., Yokoyama, N., Iwanaga, M., Koshiba, S., Kigawa, T., Hirota, H., Yokoyama, S., Okano, K., Mita, K., *et al.* (2001). Characterization of acyl-CoA-binding protein (ACBP) in the pheromone gland of the silkworm, Bombyx mori. *Insect Biochem Mol Biol 31*, 603-609.

Melloni, E., Averna, M., Salamino, F., Sparatore, B., Minafra, R., and Pontremoli, S. (2000). Acyl-CoA-binding protein is a potent m-calpain activator. *J Biol Chem 275*, 82-86.

Milne, K. G., and Ferguson, M. A. (2000). Cloning, expression, and characterization of the acyl-CoA-binding protein in African trypanosomes. *J Biol Chem 275*, 12503-12508.

Milne, K. G., Guther, M. L., and Ferguson, M. A. (2001). Acyl-CoA binding protein is essential in bloodstream form Trypanosoma brucei. *Mol Biochem Parasitol 112*, 301-304.

Neess, D., Kiilerich, P., Sandberg, M.B., Helledie, T., Nielsen, R., and Mandrup, S. (2006). ACBP—a PPAR and SREBP modulated housekeeping gene. *Mol Cell Biochem 284*, 149-157.

Okhuysen, P. C., and Chappell, C. L. (2002). Cryptosporidium virulence determinants—are we there yet? *Int J Parasitol 32*, 517-525.

Petrescu, A. D., Payne, H. R., Boedecker, A., Chao, H., Hertz, R., Bar-Tana, J., Schroeder, F., and Kier, A.B. (2003). Physical and functional interaction of Acyl-CoA-binding protein with hepatocyte nuclear factor-4 alpha. *J Biol Chem 278*, 51813-51824.

Pusch, W., Balvers, M., Hunt, N., and Ivell, R. (1996). A novel endozepine-like peptide (ELP) is exclusively expressed in male germ cells. *Mol Cell Endocrinol 122*, 69-80.

Ralph, S.A., D'Ombrain, M.C., and McFadden, G.I. (2001). The apicoplast as an antimalarial drug target. *Drug Resist Updat 4*, 145-151.

Rasmussen, J. T., Faergeman, N. J., Kristiansen, K., and Knudsen, J. (1994). Acyl-CoA-binding protein (ACBP) can mediate intermembrane acyl-CoA transport and donate acyl-CoA for beta-oxidation and glycerolipid synthesis. *Biochem J 299 (Pt 1)*, 165-170.

Rasmussen, J. T., Rosendal, J., and Knudsen, J. (1993). Interaction of acyl-CoA binding protein (ACBP) on processes for which acyl-CoA is a substrate, product or inhibitor. *Biochem J 292 (Pt 3)*, 907-913.

Rischel, C., Madsen, J.C., Andersen, K.V., and Poulsen, F.M. (1994). Comparison of backbone dynamics of apo- and holo-acyl-coenzyme A binding protein using 15N relaxation measurements. *Biochemistry 33*, 13997-14002.

Roberts, C. W., McLeod, R., Rice, D. W., Ginger, M., Chance, M. L., and Goad, L.J. (2003). Fatty acid and sterol metabolism: potential antimicrobial targets in apicomplexan and trypanosomatid parasitic protozoa. *Mol Biochem Parasitol 126*, 129-142.

Schroeder, F., Jolly, C.A., Cho, T.H., and Frolov, A. (1998). Fatty acid binding protein isoforms: structure and function. *Chem Phys Lipids 92*, 1-25.

Shulga, N., and Pastorino, J. G. (2006). Acyl coenzyme A-binding protein augments bid-induced mitochondrial damage and cell death by activating mu-calpain. *J Biol Chem 281*, 30824-30833.

Snyder, M.J., and Antwerpen, R.V. (1997). Cellular distribution, levels, and function of the diazepam-binding inhibitor/acyl-CoA-binding protein in last instar Manduca sexta midgut. *Cell Tissue Res 288*, 177-184.

Solstad, T., Fismen, L., Garberg, H., and Fladmark, K.E. (2008). Identification of a novel phosphorylation site of acyl-CoA binding protein (ACBP) in nodularin-induced apoptotic hepatocytes. *Exp Cell Res 314*, 2141-2149.

Thompson, R. C., Olson, M. E., Zhu, G., Enomoto, S., Abrahamsen, M. S., and Hijjawi, N. S. (2005). Cryptosporidium and cryptosporidiosis. *Adv Parasitol 59*, 77-158.

Tzipori, S., and Widmer, G. (2000). The biology of Cryptosporidium. *Contrib Microbiol 6*, 1-32.

van Aalten, D. M., Milne, K. G., Zou, J. Y., Kleywegt, G. J., Bergfors, T., Ferguson, M. A., Knudsen, J., and Jones, T. A. (2001). Binding site differences revealed by crystal structures of Plasmodium falciparum and bovine acyl-CoA binding protein. *J Mol Biol 309*, 181-192.

Vock, C., Biedasek, K., Boomgaarden, I., Heins, A., Nitz, I., and Doring, F. (2010). ACBP knockdown leads to down-regulation of genes encoding rate-limiting enzymes in cholesterol and fatty acid metabolism. *Cell Physiol Biochem 25*, 675-686.

Waller, R. F., Ralph, S. A., Reed, M. B., Su, V., Douglas, J. D., Minnikin, D. E., Cowman, A. F., Besra, G. S., and McFadden, G. I. (2003). A type II pathway for fatty acid biosynthesis presents drug targets in Plasmodium falciparum. *Antimicrob Agents Chemother 47*, 297-301.

Zeng, B., Cai, X., and Zhu, G. (2006). Functional characterization of a fatty acyl-CoA-binding protein (ACBP) from the apicomplexan Cryptosporidium parvum. *Microbiology 152*, 2355-2363.

Zhu, G., Keithly, J.S., and Philippe, H. (2000). What is the phylogenetic position of Cryptosporidium? *Int J Syst Evol Microbiol 50 Pt 4*, 1673-1681.

Zhu, G., Li, Y., Cai, X., Millership, J. J., Marchewka, M. J., and Keithly, J. S. (2004). Expression and functional characterization of a giant Type I fatty acid synthase (CpFAS1) gene from Cryptosporidium parvum. *Mol Biochem Parasitol 134*, 127-135.

xenith

xenith marketing services, a reputed and leading supplier & stockiest of consumables & accessories for Analytical Instrumentation covering Spectral Studies in UV-VIS Spectro photometry, Fluorescence, NMR, IR AA,GC, LC, MS; Reference standards & calibration standards.

Specialization in spectrophotometric and fluorimetric cuvettes & all types of sample holders including Deuterium, Xenon, UV, WI Laser Lamps and sources, Also Quartz Products, NMR tubes and accessories.

Contact us at following address:

xenith marketing services
634, pocket 5, mayur vihar phase 1
delhi 110091 INDIA
TEL: +91 11 2275 0434, FAX: 2275 4874
E-Mail: xenith@rediffmail.com, xenith.marketing@gmail.com